A STUDY OF
OIL BEARING SEEDS
FROM SOME INDIGENOUS PLANTS.

Thesis

Presented for the Degree of Doctor of Philosophy

by

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CHAPTER III.

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GENERAL INTRODUCTION.

The everyday use of fats and oils can roughly be divided into the following two classes. Firstly, they are one of the basic ingredients, directly and indirectly, in the diet of the human being. Secondly, they are used for soap-making, for the manufacture of paints and other drying vehicles, and also for making greases and other miscellaneous, industrial products. For each purpose a different type of oil is needed, so that it is necessary not only to know the origin of an oil, but also its properties and composition before it can be classed for use in any one of the above-mentioned categories.

For human consumption an edible oil with a pleasant taste and flavour is required, preferably an oil with a low iodine value or a fat with a low melting point. For soapmaking a fat or oil is required which contains a relatively small proportion of fatty acids with more than eighteen carbon atoms in the chain. For the drying oil industry an oil is required with an iodine value higher than 170 (castor oil is an exception in this category), while the different industrial users lay down very rigid standards for the oils which they require.

When isolating a new oil, it is thus very important to determine not only its physical and chemical constants, but also the nature and composition of its fatty acids.

The annual consumption of fats and oils has increased tremendously during the past twenty years, and production has not been able to keep pace with demand. Not only is it therefore necessary to increase the present production of fats and oils from established resources, but it is also

desirable /....

desirable to investigate the possibilities of producing fats from lesser known resources. The study of oil bearing seeds from a variety of indigenous plants is thus of some significance.

In the course of this investigation, the seeds and fruit of the following species have been investigated:

(1) Three members of the genus Ximenia viz., X. caffra Sond., X. caffra var. natalensis Sond. and X. americana var. microphylla Welw., commonly known as "sour plum".

(2) Sclerocarya caffra Sond., commonly known as Maroola.

(3) Three members of the Strychnos family viz. Strychnos innocua, Strychnos dysophylla and Strychnos coccoloides.

The oil content of the kernels of the Ximenia species and of Sclerocarya caffra Sond. has been determined, as well as the oil content of the fruit pulp and kernels of the Strychnos species. As the oil content of the fruit of the Strychnos species was found to be extremely low, work on these species was not carried further. The composition of the kernel oils of the other species was, however, determined. The absorption spectra of the oils from the Ximenia species revealed the presence of a conjugated acid. This acid has been isolated and characterised as Octadeca-11-ene-9-ynoic acid; the common name Ximenynic acid, is proposed for it. Two other unsaturated acids, Ximenic acid and Lumeniqueic acid were isolated in 95-97% purity. The composition of oils are listed in Tables XXXIII - XLV.

The composition of the kernel oil of Sclerocarya caffra Sond. has been determined. It showed no distinctive characteristics and is typical of the other oils from the Anacardiaceae family.

THE CHEMICAL INVESTIGATION OF THE FRUIT AND KERNELS OF THE XIMENIA SPECIES.

Introduction.

The genus Ximenia belongs to the Olacaceae or hog plum family, and is represented in almost all parts of the tropical world e.g., in the tropics of America, Africa and Asia. The most widely spread and best known species is Ximenia americana which is considered to be of some economic importance, the wood is said to make a fair timber, the kernels contain a high percentage of oil, while the fruit, which is extremely tart, is used for jams and jelly making.[1] Other species are found in Australia, Africa, India and America, and in South Africa three species are known to exist, viz:-

Ximenia caffra Sond., Ximenia caffra var. natalensis Sond. and Ximenia americana var. microphylla Welw., all are commonly known as the "Sour plum" and as "Ma-gwenya" by the Zulus.[2]

The natives of the low-veld (Bushveld) realise the potentialities of the Ximenia fruit; not only do they pick the fruit for its juice but the seeds are also dried and the oil pressed from the kernels with the aid of stones. The oil is used as a grease on the axles of the waggon wheels, and also applied to the skin in winter. They maintain that the oil keeps the skin soft and prevents the chapping of the hands and feet, but they do not appear to use it for frying or cooking. In Rhodesia the natives use the oil in leather tanning, and an extract from the roots of the tree is used as a medicine for calves.[3] The kernels of the seeds taste like filberts, and when roasted

could /...

could replace roasted nuts or almonds. In Southern India the oil is extracted and is used as a substitute for Ghee[4] (Ghee is a clarified butterfat, which is used in India for cooking).

Ximenia species were first investigated chemically by Heckel,[5] who described the species and reported the oil content of the seeds; other workers who investigated the seeds were Suzzi,[6] Grimme,[7] Schroeder,[8] Pieraerts,[9] Margaillan[10] and Freise,[11] all these workers described the Ximenia species in general and reported on the high oil content of the kernels. Kernels from local species were investigated by the Imperial Institute[12] in 1917, 1924 and in 1934. The three samples have been recorded as Ximenia americana, and although the exact origins of two samples are unknown, it is now known that the sample analysed in 1934 and which was sent from Ixopo in Natal, was most probably the Ximenia caffra var. natalensis Sond.

All these workers reported the physical and chemical constants of the oil; and remarked on the high viscosity of the oil as pressed, or extracted with acetone; the oil extracted with petroleum or carbon disulphide was also viscous and a rubbery material was separated from the petroleum-extracted oil. They also remarked on the relatively low saponification number of the oils and whereas Schroeder postulated the presence of C_{20} acids, Pieraerts (very rightly) postulated the presence of cerotic acid (C_{26} acid).

The first attempts at a more complete chemical investigation of the oil were made by Puntambekar and Krishna,[4] and Boekenoogen;[13] the first named authors discovered the presence of a new unsaturated acid having 26 carbon atoms in the chain, while Boekenoogen confirmed the results of these workers and (in addition) isolated a C_{30} unsaturated acid. Puntambekar and Krishna worked on Ximenia americana, Linn from India, while Boekenoogen studied "Lumeque" nuts from Western Africa, which were later identified as Ximenia seeds. Both these groups of workers reported on the presence of a rubbery material in the petroleum ether extracted oil; the oil extracted with acetone was also somewhat cloudy and more viscous than the other vegetable oils.

The results of analyses by previous workers are summarised in Table I while the fatty acid compositions of the oils are given in Table XIV.

According to the literature nothing has been done on the isolation and characterisation of the acidic principle in the fruit juice and also on the characterisation of the rubbery material which was extracted from the kernels with light petroleum. In the course of this investigation, the attention was therefore not only focussed on the oil in the kernels, but also on the characterisation of the acidic principle in the juice and the rubbery material in the kernels.

Table I. /....

TABLE I.

Analysis of Ximenia species and Constants of the Oils.

	Ximenia americana ex S. India.[4]	*Ximenia americana* ex West Africa.[13]	*Ximenia americana*.[14]
Percentage kernel	45	-	-
Oil content of kernel (%)	49	53	62.7
Moisture content of kernel (%)	3.6	-	-
Constants of Oil:-			
Specific gravity	0.9262	-	0.9195
Refractive index	1.4710	-	1.4725
Iodine value	82.5	84.3	93.4
Saponification value	169.5	167.3	171.0
Acid value	2.3	10.0	-
Reichert Meissel value	Nil	-	-
Unsaponifiable matter (%)	1.7	-	1.5
Viscosity (Hoppler)	-	200 cps.	-
Molecular weight of acids	319.8	314	-
Saturated acids of total	31.0%	6.0%	-

Discussion. /....

DISCUSSION.

Fruit Juice.

The composition of the fruits of two Ximenia species, collected in the Groblersdal district , was determined and the results are listed in Table II.

The fruit juice had a light reddish colour and a pronounced sour taste. An attempt was made to determine the acid content of the juice by titration with standard alkali, using the standard indicators, but the colour of the solution changed to a purple blue and finally the acidity was determined by potentiometric titration. This change in colour on addition of the alkali, may be due to the presence of an orthocyanine dye,[15] but no attempt to isolate and characterise the pigment responsible for the colour changes was made. From the chemical tests on the juice, the presence of citric acid was suspected and this was estimated quantitatively by the penta-bromo-acetone method.[16]

It will be noticed that there is a difference of 0.26% in the citric acid content of the juice by the two methods, and it appears that some of the citric acid is present in the form of a salt.

Table II. /....

TABLE II.

(1) Composition of the Fruit of the Ximenia Species.

	Ximenia caffra Sond.	*Ximenia caffra* var. *natalensis* Sond.	Reported previously.[12]
Colour of fruit	Orange red	Purple red	-
Average weight of fruit (gr.)	12.4	11.8	3.2
Weight of seeds (gr.)	2.4	2.1	1.1 (Kernel)
Seed as % of fruit	19	18	35 (Kernel)
Moisture content of pulp (%)	77.6	77.8	-
Moisture content of fresh kernels (%)	26.2	22.5	3.1
Oil content of fruit pulp (%, wet)	0.43	0.42	-
Oil content of fruit pulp (%, dry)	1.92	1.88	-
Oil content of kernels (%, dry)	65.7	64.1	67.4

TABLE III.

(2) Analysis of the Fruit Juice.

Normality of juice	1.337
Percentage volatile acids	negligible
Citric acid (%, by penta-bromo-method)	8.76
Equivalent citric acid (from acidity %)	8.5
Thus citric acid in salt form	0.26%

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The citric content of the fruit juice is remarkably high. It is somewhat higher than lemon juice and much higher than that of most other fruit.[16]

Kernels.

The Ximenia seeds, which were analysed, were collected in the different areas mentioned in Table IV. The fruit was picked when fully ripe, the flesh and pulp removed and the seeds dried in the sun. A soft hull protected the kernel, and this could easily be broken by rubbing it between the fingers. This is in agreement with other reports on the seeds: the Bulletin of the Imperial Institute[12] suggested that for large scale extraction of the oil, the shells could be broken in a Miller's palm nut cracking machine and the hulls and kernels separated by means of sieves and an air-blast. For the analyses of the kernels the hulls were removed, and the kernels minced in a household meat mincer and analysed according to the A.O.C.S. methods,[17] these results are given in Table IV.

TABLE IV.

Composition and Analysis of Ximenia Seeds.

	Ximenia caffra Sond.	Ximenia caffra var. natalensis Sond.	Ximenia americana var. microphylla
Seeds collected at	Bechuanaland	Ixopo, Natal	Pondoland
Average weight (g.)	1.46	2.27	1.06
Average weight of kernel (g.)	1.15	1.63	0.68
Percentage kernel in seed	78.8	71.8	64.1
Oil content of kernels (%)	65.0	60.1	65.0
Oil content of total seeds (%, wet)	51.1	43.2	41.6
Moisture content of kernels (%)	4.1	4.1	5.0
Percentage "sweet material" extracted with acetone (%)	0.85	0.5	0.9

Both /....

Both acetone and light petroleum were used in the preliminary experiments for the extraction of the oil from the kernels: the different solubilities of the different constituents of the kernels in these two solvents, resulted in somewhat different results. Thus, the acetone extract contained a polysaccharide (which separated out from the extract during the extraction) and a crystalline material insoluble in light petroleum. The latter tended to separate from the extracted oil on standing and could be precipitated therefrom by dilution with light petroleum. On the other hand, the petroleum extracted oil contained a rubbery constituent. This was insoluble in acetone and therefore not present in the acetone extracted oil, and was precipitated by addition of acetone to the oil; the presence of this rubbery material in the petroleum extract, but not in the acetone extract probably accounted for the difference in viscosity of the freshly extracted oils (E and Y on the Gardner-Holt viscosity scale[18]). The compounds precipitated by addition of petroleum to the acetone extracted oil and vice versa, will be described in greater detail in the section on the unsaponifiable matter in the oil (see p. 34). The acetone extracted ~~extracted~~ petroleum treated oil and the petroleum extracted acetone treated oil were similar in viscosity and appearance and both oils deposited only a small "break" on storage.

For the determination of the chemical and physical constants of the oil and for studies on the component acids, bulk oil supplies were obtained by acetone extraction of the minced kernels, followed by petroleum precipitation of the solid crystalline material from the extracted oil. The chemical and physical constants of the oil are given in Table V and were determined by the methods outlined in the experimental section.

Table V. /....

TABLE V.

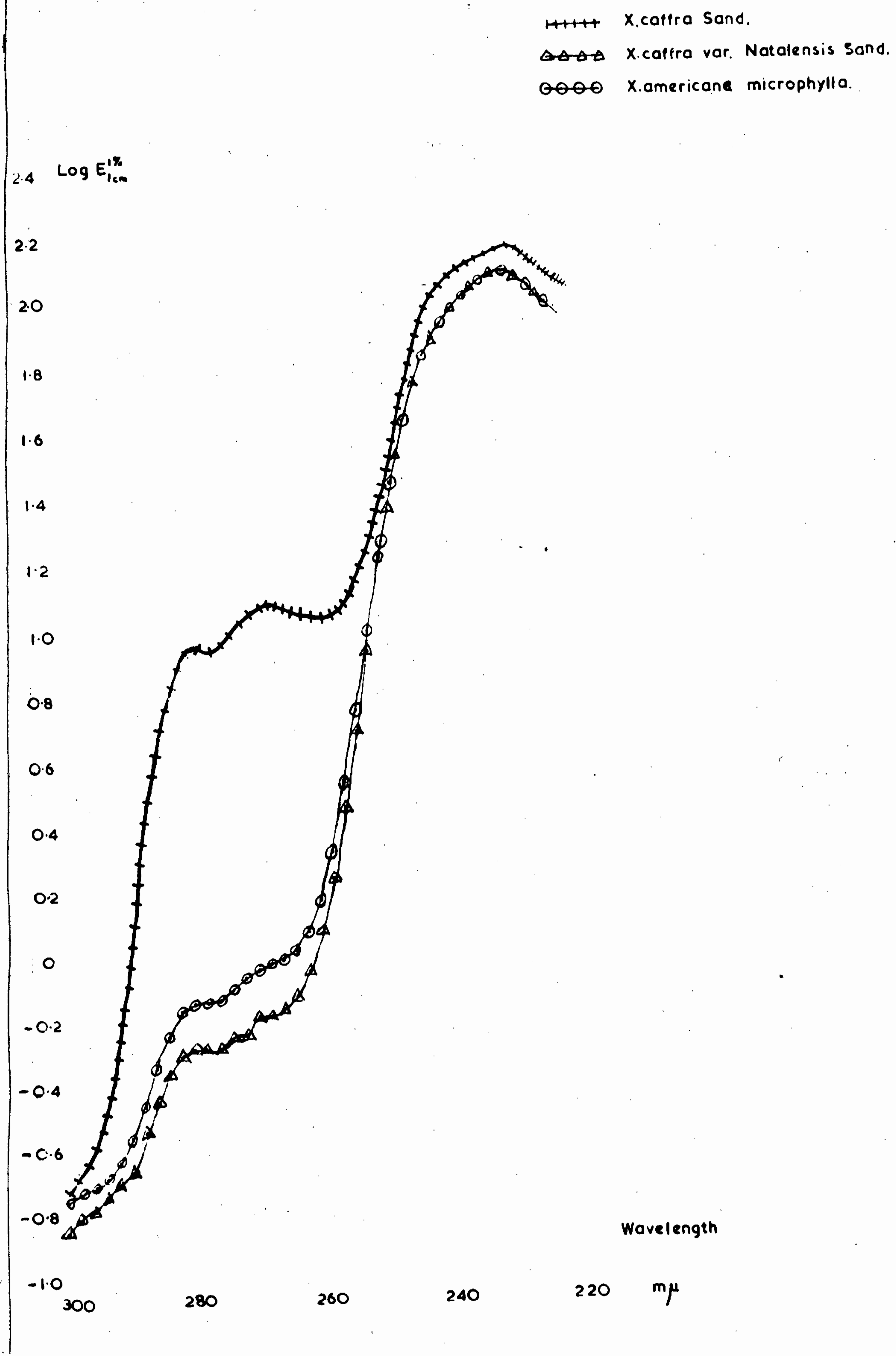
Chemical and Physical Constants of Ximenia Oils.

	Ximenia caffra Sond. ex Bechuanaland.	*Ximenia caffra* var. *natalensis* Sond. ex Ixopo.	*Ximenia americana* var. *microphylla* ex Pondoland.
Specific gravity 25°/25°	0.9190	0.9152	0.9154
Refractive index at 25°	1.4734	1.4710	1.4712
Acid value of oil	2.52	0.99	1.49
Iodine value of oil	83.03 %	79.66 %	77.36 %
Saponification value	170.4	172.8	165.2
I.V. from hydrogen absorption	129.0	-	-
Reichert Meissl value	0.22	0.40	0.19
Polenske value	0.31	nil	nil
% Non-saponifiable material	1.36	1.34	1.35
% Rubbery material	0.75	1.0	1.1
% Gelatinous material	0.3	0.39	0.5
E(1%, 1 cm.) at 268 mμ	11.78	0.67	0.93
E(1%, 1 cm.) at 229 mμ	142.3	121.5	123.7

The iodine value calculated from the hydrogen absorption was higher than that obtained by conventional methods and reflected the presence of compounds whose unsaturation could not be measured by the iodine value reagent (for discussion of this see p. 29). The absorption spectra of the oils between 300 and 220 mμ are given in Fig. 1. (For the light coloured Ximenia oils it was only necessary to determine the absorption spectra between 220 and 340 mμ.) The spectra showed a well-defined maximum in the 230 mμ region and an inflection in the 270 mμ region: the fatty acids of the oils had the same spectra and it is evident that the components which caused the maxima were part of the fatty acids.

Fig. 1.

Absorption Spectra of *Ximenia* Oils.



It is a known fact that conjugated dienes show a maximum in the 234 mμ region, and that trienes show a maximum in the 270 mμ region;[19] furthermore, if a double bond is conjugated with a carboxyl group the maximum is between 10-20 mμ lower than the maximum of the corresponding number of double bonds. It is also known, that if a triple bond forms part of the conjugated system then the extinction coefficient of the compound is about $^1/_3$ to $^1/_2$ of that of a corresponding compound containing only double bonds.[20]

As the absorption spectra of the oils had a well-defined maximum at 229 mμ and an inflection at 268 mμ, the oils apparently contained a relatively large percentage of an acid having one of the following structures as part of the molecule:-

$$-C=C-C=C- \quad \text{or} \quad -C=C-C=O,$$

or a larger percentage of an acid having the following structure:-

$$-C=C\cdot C\equiv C \quad \text{or} \quad -C\equiv C-C=O;$$

and a small percentage of an acid having three double bonds (or two double bonds and one triple bond) in conjugation.

Before the final composition of the oils could be effectively studied, it was thus necessary to isolate and characterise the diene (or enyne) acid and to determine its iodine value, neutralisation equivalent and extinction coefficient.

The presence of two other diene acids has been reported in the literature: in the alkali isomerisation of linoleic acid, a conjugated acid is formed, having λ max. at 232 mμ with an E(1%, 1 cm.) of 1190[21] (Later work by Nicholls[22] *et al*. reported the isolation of an acid with E(1%, 1 cm.) of 950 and an acid with E(1%, 1 cm.) of 870), while a C_{18} conjugated diene acid was isolated from the dehydrohalogenation

of /...

of mono-bromo-oleic acid, having a $\lambda_{\text{max.}}$ at 230.5 mμ, $\mathcal{E}$ = 28.840, which is equivalent to an E(1%, 1 cm.) of 1028.[23]

During attempts to isolate the unsaturated acid, the probability that it would have either an E(1%, 1 cm.) of about 1000-1200 or an E(1%, 1 cm.) of about 500-600 (for enyne system) was borne in mind.

Owing to the complexity of the fatty acid mixture derived from natural fats, no single method is universally applicable to the separation of an unknown acid. In most cases several methods must be combined to get reasonable separation from the other acids present.

Regardless of the method to be followed for the separation and identification of the fatty acids of an oil, it is always essential to obtain the free fatty acids by saponification: several methods have been recommended for the saponifications of fats and oils, e.g. the use of different alkalis, and of solvents with higher boiling points to facilitate saponification of the glycerides. During the investigation of the Ximenia oils, the method recommended by the S.P.A.[24] for the saponification of the oil and extraction of the unsaponifiable matter was used with the following modification: after saponification, the alcohol solution containing the soaps and unsaponifiable matter, was poured into double its volume of distilled water (with tap-water, a precipitate formed at the interfacial layer which tended to enhance emulsion-formation).

There are generally three methods for the separation of fatty acids into fractions with similar properties or molecular weight. (1) Distillation, (2) Crystallisation, (3) Adsorption. Usually a combination of these methods is applied for the concentration of a particular acid or for the quantitative study of the composition of the total acids,

and /....

and usually the fatty acids are first separated into "solid" and "liquid" fatty acid fractions by crystallisation. Various methods are available in this category:-

(a) Differences in the relative solubilities of the metal salts of the fatty acids may be exploited, e.g. the Twitchell's[26] or leadsalt method for the separation of the liquid and solid fatty acids.

(b) Differences in the relative solubilities of the fatty acids in various solvents may be exploited, this is the basis of the low temperature crystallisation of acids, which was developed by Brown[27] et al.

(c) Crystallisation of the bromo derivatives of the unsaturated acids.[28]

(d) Conversion of the acids (or methyl esters) into the urea complexes and the fractional crystallisation of these urea complexes.[29]

After the different fractions have been obtained from the above mentioned processes, the fatty acids are converted into the methyl esters and fractionally distilled under vacuum. The fractions containing the highest percentages of the unknown or wanted acid, are then combined and fractionally crystallised or else subjected to chromatographic adsorption on various adsorbents.

Twitchell's leadsalt method for the separation of solid and liquid fatty acids has slowly been replaced by the simpler but more efficient low temperature crystallisation method of Brown et al., and this method was also applied in the concentration of the enyne acid as well as in the quantitative fatty acid studies of the Ximenia oils.

Several trial experiments were carried out to determine the most suitable solvent, concentration and temperature for the low temperature crystallisation of the total fatty acids, and only the results of the following three have been given:-

(1) Crystallisation of total acids from ether at -40° and crystallisation of the liquid acids from acetone at -60°, according to the method used by Schwartz *et al.* for the fatty acid studies of *Acacia cyclops*.[30]

The results of this experiment have been summarised in Table VI.

TABLE VI.

Low Temperature Crystallisation from Ether and Acetone.

Fraction		% of Total Acids	I.V.	N.E.	E(1%, 1cm.) 268 mμ	E(1%, 1cm.) 229 mμ
A_1	Insoluble in ether -40°	27.2	40.14	384.5	-	-
A_2	Soluble in ether -40°	7.15	106.1	292.9	13.2	268.4
B_1	Insoluble in acetone -60°	59.5	102.1	291.8	9.5	175.0
B_2	Soluble in acetone -60°	6.16	151.7	290.4	58.2	342.8

(2) Crystallisation of the total fatty acids from acetone at -25°C, followed by cryatallisation of the liquid fatty acid from light petroleum at -22°C; the results of which have been summarised in Table VII.

Table VII. /....

TABLE VII.

Low Temperature Crystallisation from Acetone and Light Petroleum.

Fraction		Percentage of total acids.	E(1%, 1cm.) 229 mμ
A	Insoluble in acetone at -25°	36.8	55
B	Soluble in acetone at -25°	63.2	180
B_1	Insoluble in light petroleum at -22°	14.7	457 *

* The neutralisation equivalent of this fraction was 294.

It will be noticed that by using ether and acetone as solvents, the E(1%, 1cm.) at $\lambda_{max.}$ 229 mμ could be increased from about 140 to 342.8, but that two other fractions were obtained with E(1%, 1 cm.) values of 268 and 175 respectively (see Table VI). By using acetone and light petroleum as solvents, however, the E(1%, 1 cm.) of the acids could be increased from 140 to 457, so that this was a better method for the concentration of the conjugated unsaturated acid.

It was noticed, however, that after each drying process for the isolation of the fatty acids, that the liquid fatty acids developed a redder colour. To avoid any unnecessary heating and oxidation, the fatty acids for the quantitative studies of the composition of the oils, were crystallised only once from acetone. The results and analyses of the "solid" and "liquid" fatty acids in this experiment have been summarised in Table VIII (i), (ii) and (iii).

Table VIII. /....

TABLE VIII.

(i) Low Temperature Crystallisation of the Fatty Acids of *Ximenia caffra* Sond.

	Weight (g.)	%	I.V.	N.E.	E(1%,1cm.) 268 mμ	E(1%,1cm.) 299 mμ
Total fatty acids	--	-	-	312.0	11.63	148.3
(A) Insoluble in acetone at -25° (10 ml/g. acid)	140.8	34.65	47.5	372.8	2.71	42.09
(B) Soluble in acetone at -25°	265.7	65.35	97.0	288.5	13.45	196.9

(ii) Low Temperature Crystallisation of the Fatty Acids of *Ximenia caffra* var. *natalensis* Sond.

	Weight (g.)	%	I.V.	N.E.	E(1%,1cm.) 268 mμ	E(1%,1cm.) 229 mμ
Total fatty acids	--	-	78.6	310.6	0.75	137.6
(A) Insoluble in acetone at -25° (10 ml./g. acid)	94.9	29.16	47.3	371.8	0.26	32.8
(B) Soluble in acetone at -25°	230.5	70.84	93.9	286.9	0.91	178.3

(iii) Low Temperature Crystallisation of the Fatty Acids of *Ximenia americana* var. *microphylla* Welw.

	Weight (g.)	%	I.V.	E(1%,1cm.) 268 mμ	E(1%,1cm.) 229 mμ
Total fatty acids	--	-	76.9	1.04	132.9
(A) Insoluble in acetone at -25° (10 ml./g.)	101.8	38.63	54.4	0.35	38.9
(B) Soluble in acetone at -25°	161.7	61.37	96.0	1.51	189.2

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The acid fractions were then converted into the methyl esters and fractionally distilled through an electrically heated and packed column and collected with the aid of a Perkin triangle.[31] For the bulk isolation of the unsaturated acid, the fractions were cut according to the boiling point; for the acid studies, successive fractions of 5-6 g. were collected: these fractions were then accurately weighed and analysed without delay. The weights, iodine values (Wijs, one hour) and saponification equivalents of the fractions from the three oils are summarised in Tables XXXIV - XLI. The results of a fractionation for the bulk extraction of the unsaturated acid are given in Table IX.

TABLE IX.

Distillation of the Methyl Esters with E(1%, 1cm.) of 350.

Fraction	B.P. (top)	Column Temp.	Oil Bath (°C)	Pressure mm's	Weight (g.)	E(1%,1cm.)	S.E.
1	155	187	247	0.5	23.1642	59.1	296
2	161	194	258	0.5	27.7243	191.6	295.5
3	161	194	258	0.6	29.5822	231.0	295.3
4	160	193	258	0.4	30.7202	425.0	293.9
5	162	198	258	0.45	30.2100	543.0	292.8
6	162	198	258	0.45	29.4100	484.0	294.1
7	158	198	258	0.1	31.5588	450.0	295.4

During these distillations an unexplainable variation in the E value of successive fractions was observed. Instead of finding a steady increase in the E value of the fraction, an irregular rise and drop was observed with every third fraction, also a wide variation in the duplicate determinations if different cells were used for the absorption determination

of /....

of one sample. It was later found that the silica cells adsorbed the diene ester very strongly and it was therefore necessary to determine the cell corrections after each series of analyses.

From Tables IX and XXXIV it will be noticed that there was a gradual drop in the saponification equivalents with the increase in the E value. This was observed in the distillation of all the methyl esters of the liquid fractions of the Ximenia oils: the saponification equivalents reached a minimum with the maximum E(1%, 1 cm.) and this indicated that the unknown acid had a lower molecular weight than the accompanying oleic acid.

Chromatography was subsequently used in an attempt to purify the methyl esters of the conjugated diene acid.[32] In general the procedure was carried out as follows: a weighed quantity of the adsorbent of desired activity was placed in the column (a glass tube), constricted at the lower end and containing a small perforated disc and a cotton wool pad. The adsorbent was tightly packed and the solution containing the methyl esters poured onto the adsorbent. This was followed by more solvent and care was taken that the column did not run dry and that a regular flow of liquid was obtained through the column. The fractions were collected at the bottom and analysed. Due to the complication introduced by the lack of visual means of differentiating between the adsorbed bands, appropriate experimentally determined conditions were employed, and in doing so the experience gained by previous workers in the field was borne in mind.

Using this method Manunta[33] succeeded in separating a mixture of palmitic, stearic and oleic acids on hydrated magnesium sulphate with light petroleum as solvent. Swift[34] <u>et</u>. <u>al</u>. prepared 98% pure methyl linoleate from a mixture of

esters /...

esters containing 95% linoleic acid; these esters were chromatographed on an alumina column and eluted with hexane; Dutton[35] separated a synthetic mixture of oleic and stearic acids on a silica-gel-active-carbon column, using light petroleum and ether as solvent. Riemenschneider[36] et. al. obtained pure methyl linoleate and linolenate by chromatographic adsorption on silica gel, using light petroleum containing 1% ether as solvent; while Brown and White[37] obtained pure methyl arachidonate by adsorption on an alumina column, using light petroleum and ether as solvents. Separation of long chain saturated, branched chain and unsaturated fatty acids was achieved by Claesson[38] using a flowing chromatographic method with heptane as solvent and silica as adsorbent.

Howard and Martin[39] separated C_{12} to C_{18} acids by reversed phase partition chromatography, using Hyflo Supercel containing dichloro-dimethyl-silane as adsorbent, with liquid paraffin as the stationary phase and aqueous acetone as the moving phase. Marvel and Rands[40] accordingly used partition chromatography with silica gel (wetted with water) as adsorbent and variable mixtures of butanol and chloroform as solvents for the separation of slightly water-soluble acids.

In the attempted purification of the conjugated diene acid, alumina and silica gel were independently used as adsorbents and in the course of the work about 20 experiments were conducted in a search for the best solvent combination. Results of the initial experiments indicated too that the activity of the alumina was a very critical factor; the use of highly active alumina caused large losses of methyl esters (due to hydrolysis) while slightly active alumina was completely ineffective for the separation. The results of two experiments with alumina and three with silica gel have been summarised in Tables XV - XIX.

It will be noticed that with the aid of chromatographic adsorption, the E(1%, 1 cm.) of the methyl ester fraction could be increased by about 20-25%, however, the yields were of the order of 45-50%. Comparing the results of the adsorption using alumina and silica gel, it can be concluded that the percentage total yield from the latter experiments was higher and less hydrolysis took place on the column. However, it was evident that chromatography could not be used to purify the acid any further and it was decided to use low temperature crystallisation again for the attempted isolation and purification of the acid. By crystallising the liquid acids from light petroleum, a fraction was obtained with E(1%, 1cm.) of 457. The neutralisation equivalent of the fraction, however, was over 290 and as the neutralisation equivalent of the acid was evidently lower than 280 (see Tables VI and VIII), it meant that this fraction still contained a large percentage of higher molecular weight acids. For the isolation of the unsaturated acid, these acids were converted into the methyl esters and fractionally distilled (see Table IX.) and the fractions with the highest E values and saponification equivalents of 292-294 combined; these were resaponified and the free fatty acids extracted and dried.

Several other unsaturated fatty acids have been isolated in high purity by low temperature crystallisation: Brown and Shinowara[41] isolated a relatively pure oleic acid from olive oil by crystallising the mixed fatty acids from acetone at -60°, similarly a relatively pure linoleic acid[27] was obtained by crystallising the mixed fatty acids from acetone at -20° and removing the solid acids; the filtrate was cooled to -50° and a second batch of solid acids removed. The filtrate was then cooled to -70° and the solid acids collected. These were then recrystallised from light petroleum at -40° and the solid acids again recrystallised from light petroleum at -62°; this yielded practically pure linoleic acid. The isolation of pure linolenic acid was attempted on the same basis, but low temperature crystallisation followed by distillation failed to yield pure linolenic acid.[42] Arachidonic acid of 97% purity was prepared by the crystallisation of the mixed methyl esters from acetone at -65° and -75°, followed by distillation.[43] Elaeostearic acid of 90% purity was prepared by crystallising the total fatty acids of tung oil from acetone at -30°;[44] the insoluble acids were recrystallised from acetone at -60°, this portion constituted 70% of the acids and contained 90% of elaeostearic acid. Crystallisation of the soluble portion from light petroleum at -60°, followed by recrystallisation from light petroleum at -40° yielded another fraction rich in elaeostearic acid. Ricinoleic acid[45] of over 99% purity was obtained by the low temperature crystallisation of the total fatty acids of castor oil. The fatty acids were fractionally crystallised from acetone and the portion insoluble at -40° and -50° was recrystallised at -70°; seven recrystallisations yielded ricinoleic acid of over 99% purity.

From the foregoing it was probable that recrystallisation from acetone, followed by recrystallisation from light petroleum would be the most effective means for the purification of the unsaturated fatty acid.

The Isolation of Ximenynic Acid.

The acid fraction, isolated from the fractional distillation and richest in the conjugated diene acid, was accordingly dissolved in acetone and crystallised at -30° (at this temperature the crystal-cake was so heavy that it was decided not to cool any further), and the insoluble acids

recrystallised /..

recrystallised from acetone at -30°, followed by recrystallisations from light petroleum at -10° and -5°. This yielded flat partly transparent crystals, m.p. 39-40°; E(1%, 1 cm.) 583 at $\lambda_{max.}$ 229 mμ. The melting point and the E value could not be increased by repeated recrystallisations and the acid, with these constants was considered to be the pure conjugated unsaturated acid. During the purification procedure the E(1%, 1cm.) at $\lambda_{max.}$ 268 mμ was reduced from 15 to 2.5, and the absorption in this region could thus not be attributed to the conjugated unsaturated acid. From this value (E(1%, 1 cm.) = 2.5), the conjugated diene acid contained about 0.3% of the conjugated triene acid. The absorption spectrum of the acid is given in Fig. 2.

The acid was recrystallised from light petroleum for analysis; it showed a neutralisation equivalent of 278.9 and the elementary analysis fitted a C_{18} acid; on hydrogenation 3.05 moles of hydrogen were absorbed and the resulting saturated acid proved to be stearic acid; keto groups were absent and the new acid was beyond any doubt a C_{18} acid having threefold unsaturation. The methyl, ethyl, p-phenyl-phenacyl and the p-bromo-phenacyl esters, the amide, the alcohol and the chloride of the acid were prepared. The alcohol (by reduction of the acid with lithium aluminium hydride) showed the same spectrum as the acid (Fig 2), from which it was concluded that one double bond was not in conjugation with the carboxyl group. Oxidation of the acid with alkaline potassium permanganate yielded heptanoic, oxalic and azelaic acids, which meant that only two unsaturated centres could be present; this suggested the following structures for the acid:

$CH_3.(CH_2)_5.CH{=}CH.C{\equiv}C.(CH_2)_7.COOH$ ..... (1)

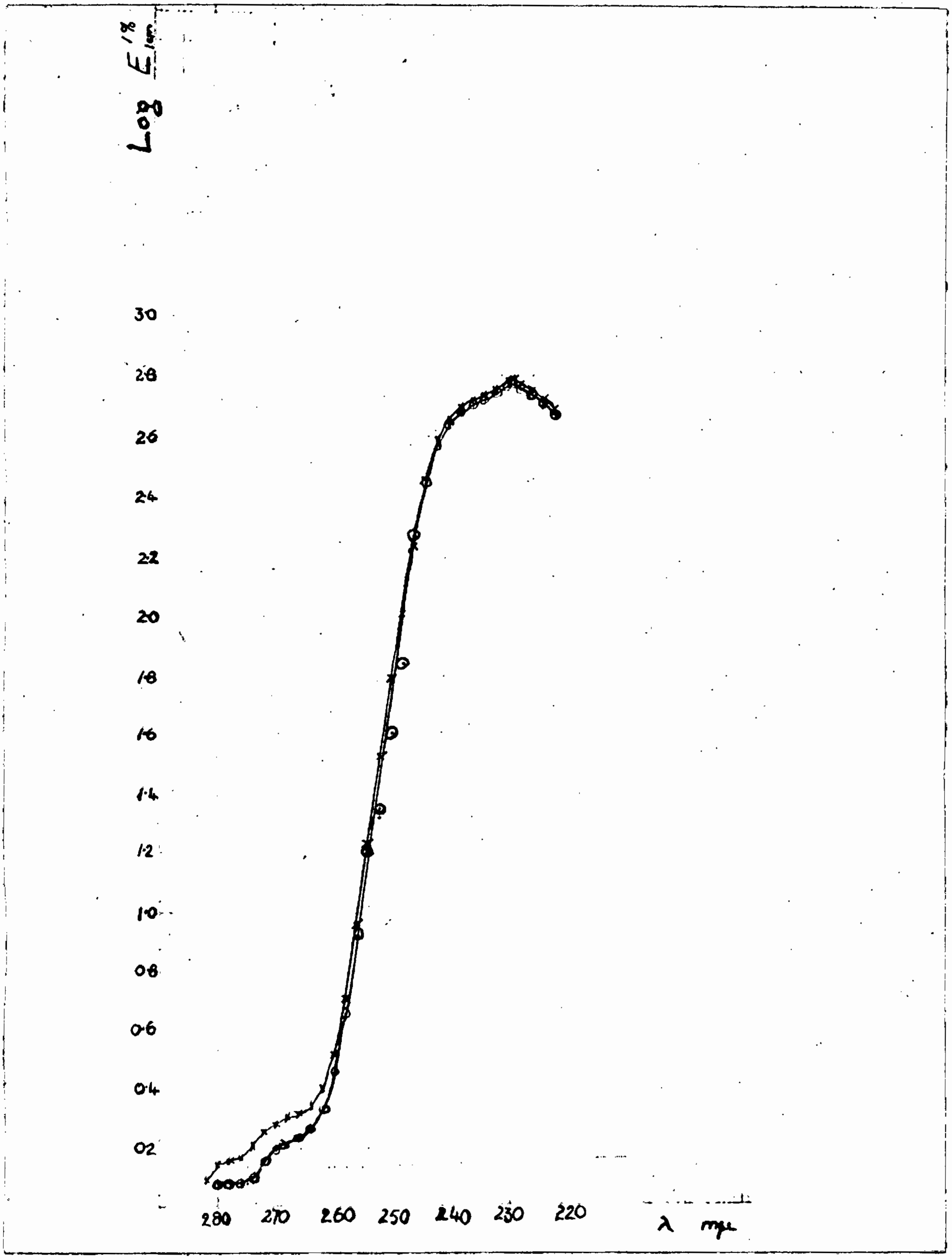
or $CH_3.(CH_2)_5.C{\equiv}C.CH{=}CH.(CH_2)_7.COOH$ ..... (2)

Treatment /....

Fig. 2.

oooo Absorption Spectra of Ximenynic Acid.

xxxx Absorption Spectra of Ximenynyl Alcohol.



Treatment of the acid with ozone, followed by reduction of the ozonide with zinc and acetic acid, yielded heptaldehyde and azelaic acid together with a very small quantity of azelaic half aldehyde. Structure (1) was consequently accepted as the most possible structure for the acid.

The name Ximenynic acid (Heptadeca-10-ene-8-yne-1-carboxylic acid or Octadeca-11-ene-9-ynoic acid) was proposed for it. From the absorption spectrum of the acid, it was suspected to contain about 0.3% of a conjugated diene acid (see above); the presence of azelaic half aldehyde in the split products of ozonolysis, confirmed the presence, in small quantities, of the trienoic acid.

Attempts to elaidinise ximenynic acid with selenium metal were unsuccessful and only the unchanged starting material and some polymerised material were isolated.

Attempts to prepare the Diels-Alder adduct of ximenynic acid and maleic anhydride were also unsuccessful. Reaction of these two compounds at 120° for one hour, yielded only the starting materials.

Attempts were made to reduce ximenynic acid catalytically and chemically to the dienoic acid. Several experiments were carried out on the acid, using a poisoned palladium on charcoal catalyst of Isler[46] et al.

As the initial experiments of the attempted catalytic reduction of the acid were proved to be negative, and as the experimental detail on the use of the poisoned catalyst was somewhat scanty, more information was requested from the originators of this method. In a private communication Dr. Isler stated that the presence of acids were deleterious to the poisoned catalyst, and consequently another experiment was carried out on ximenynyl alcohol. In this experiment, however, the hydrogen absorption was still rapid (theoretical

volume /....

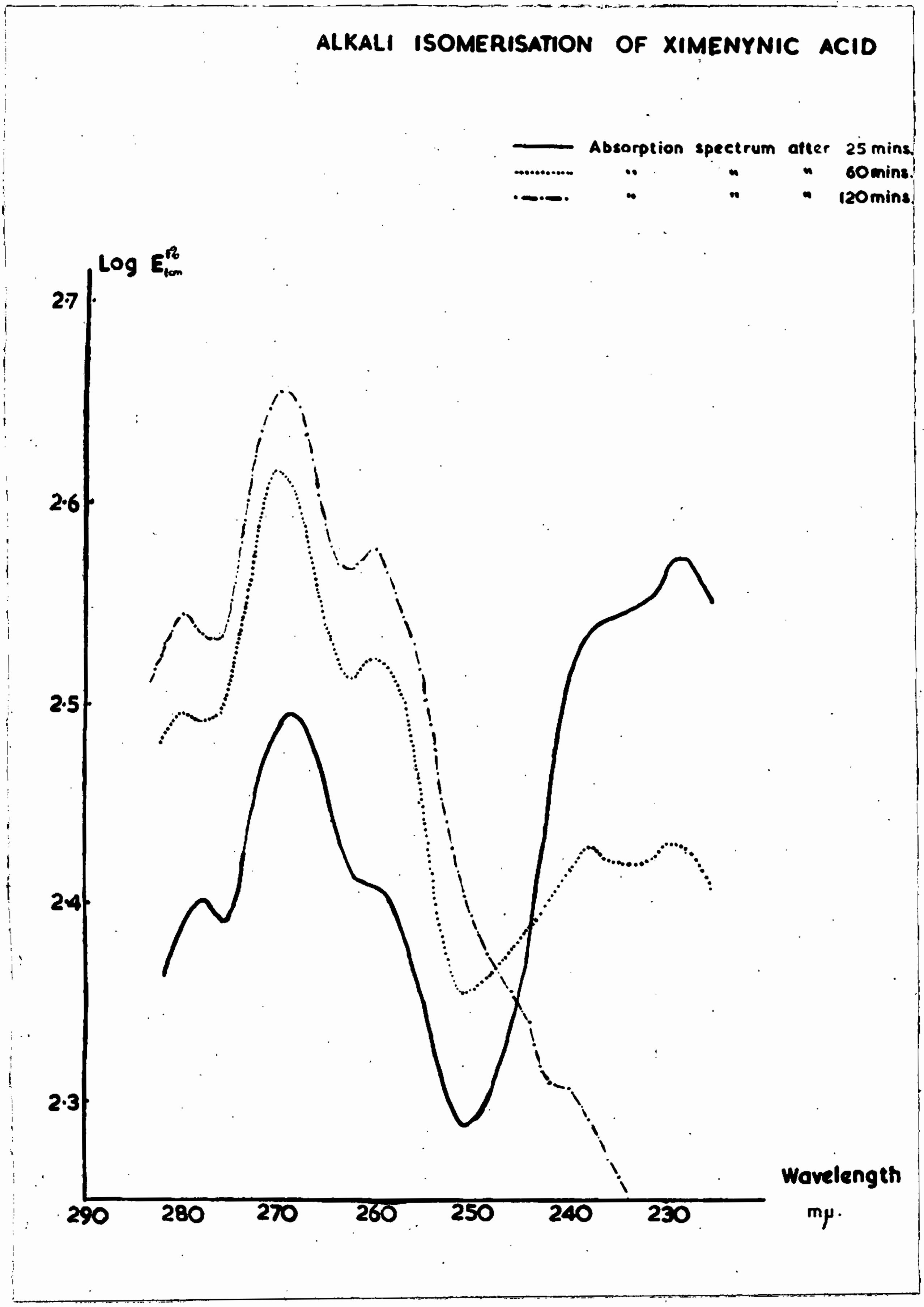
volume of hydrogen to saturate one unsaturated linkage, was absorbed within five minutes) and yielded a product with E(1%, 1 cm.) of 670 at $\lambda_{max.}$ 229 mμ. As the dienoic acid should have an E(1%, 1 cm.) of 1000-1400, the reductions proved to be unselective.

Attempted reduction of methyl ximenynate with sodium and absolute alcohol gave a product with E(1%, 1 cm.) of about 465 at $\lambda_{max.}$ 232 mμ, and as this value was of the same order as the results of the catalytic reduction, further research work was not continued.

The behaviour of ximenynic acid in the presence of potassium hydroxide at 180° was studied with the intention of determining the influence of this acid on the quantitative determination of linoleic and linolenic acids. The method employed was essentially the same as that proposed by Mitchell, Kraybill and Zschiele:[47] the acid was isomerised with potassium hydroxide in ethylene glycol reagent for 25 min. at 180°C, and the absorption spectrum determined between 300 and 220 mμ. Similar spectra were determined after 60 mins. and 120 mins. isomerisation. The general tendency in the spectra was for the disappearance of the maxima at 229 mμ and the formation of a well-defined maximum at 269 mμ, with two subsidiary maxima at 260 and 280 mμ. The maximum after 120 mins. was higher than the maximum for 60 mins. isomerisation, which in turn was higher than that after 25 mins. isomerisation. Similar spectra were also determined at 190°. The acids were isolated and the absorption spectra determined in cyclohexane as solvent; the absorption spectra of the free acids, after 25, 60 and 120 minutes isomerisation have been given in Fig. 3, while the E(1%, 1 cm.) values are summarised in Table X.

Table X. /....

Fig. 3.



ALKALI ISOMERISATION OF XIMENYNIC ACID
Absorption spectrum after 25 mins.
" " " 60 mins.
" " " 120 mins.
Log $E^{1\%}_{1cm}$
2·7
2·6
2·5
2·4
2·3
290
280
270
260
250
240
230
Wavelength
mμ.

TABLE X.

The E(1%, 1 cm.) of Ximenynic Acid on Alkali Isomerisation (Free Acids.).

	E(1%, 1 cm.) 229 mμ	E(1%, 1 cm.) 268 mμ
Ximenynic Acid	583	215
After 25 mins. at 180°	376	315
After 60 mins. at 180°	251	388
After 90 mins. at 180°	195	420
After 120 mins. at 180°	170 (no maximum)	455
After 60 mins. at 190°	155 (.. ..)	450
After 90 mins. at 190°	115 (.. ..)	425
After 120 mins. at 190°	98 (.. ..)	385

It is evident from the table that the alkali isomerisation at 190° for one hour gave about the same conversion as that at 180° for two hours; longer isomerisation caused polymerisation and lowering of the E values.

Crystallisation of the isomerised acids (at 190° for one hour) from light petroleum at -60° yielded a small quantity of an acid with E(1%, 1 cm.) of 1705 at $\lambda_{max.}$ 269 mμ; the bulk of the material was polymerised syrup. Reduction of the total isomerised acids with lithium aluminium hydride gave a syrupy liquid with the same absorption spectrum, and the unsaturation was consequently situated in the middle of the chain.

The split products of the oxidation of the isomerised acids (E(1%, 1cm.)= 1705) with alkaline permanganate could not be identified and it appeared that the isomerisation reaction was accompanied by polymerisation, and that the product which was isolated was not the simple triene acid:

$$CH_3.(CH_2)_x CH{=}CH.CH{=}CH.CH{=}CH.(CH_2)_y COOH.$$

What /....

What would be the mechanism of the isomerisation reaction? Nichols, Herb and Riemenschneider[48] stated the following about the alkali isomerisation of linoleic acid to the conjugated acid: "From the essentially ionic character of the prototropic shift mechanism, it would appear that the system $CH_3.(CH_2)_4.CH{=}CH.CH_2CH{=}CH.(CH_2)_7.COOH$ has an important property, namely, when a proton is removed from the centre methylene there remains a negative ion, which can be represented as a resonance hybrid of the structures:

$$\begin{array}{l}
CH_3.(CH_2)_4.\overset{13}{CH} = \overset{12}{CH} \;.\; \overset{11}{\overset{-}{CH}} \;.\; \overset{10}{CH} = \overset{9}{CH}.(CH_2)_7.COOH \\
CH_3.(CH_2)_4.\overset{-}{CH} \;.\; CH = CH \;.\; CH = CH.(CH_2)_7.COOH \\
CH_3.(CH_2)_4.CH = CH \;.\; CH = CH \;.\; \overset{-}{CH}.(CH_2)_7.COOH
\end{array}$$

Resonance involved with the negative ion should have two important effects; firstly, the carbon atoms numbered would lie in a coplanar configuration, and secondly, resonance stabilisation would promote the tendency of coplanar configurations to ionise to such an extent that the prototropic shift would occur predominantly through configurations of this type."

Furthermore, the following mechanism was proposed for the reaction which takes place during the alkali isomerisation reaction:[49]

$$\begin{array}{c}
CH_3.(CH_2)_4.CH = CH \;.\; CH_2 \;.\; CH = CH.(CH_2)_7.COOH \\
\Big\downarrow \; OH^- \\
[CH_3.(CH_2)_4.CH = CH \;.\; \overset{-}{CH} \;.\; CH = CH.(CH_2)_7.COOH] + H_2O \\
\Big\downarrow \; H_2O \\
CH_3.(CH_2)_4.CH_2.CH = CH.CH = CH.(CH_2)_7.COOH + OH^-
\end{array}$$

It /....

It is also known that the acetylenic bond has less resonance abilities (or resonance energy) than a conjugated diene structure (the structure $C \equiv C - C \equiv C -$ has only a small absorption band, and the extinction coefficient of the system $-C = C - C \equiv C -$ is about one half of that of the diene system), and it is thus possible that the acetylenic bond will tend to migrate to the system with more resonance energy, namely two conjugated double bonds. It would appear though that this is an equilibrium reaction, as previous workers have reported the isomerisation of conjugated diene systems to an acetylenic bond (Maruyana and Suzuki[50] reported the isomerisation of sorbic acid to 2-pentine-1-carboxylic acid, and the isolation of stearolic acid from the treatment of 9:10-dichlorostearic acid with KOH at 170°C).

In the case of ximenynic acid the equilibrium, however, appears to lie on the side of the conjugated triene compound.

In the alkali isomerisation of ximenynic acid the negative ion could form on the methylene group on either side of the unsaturated centres, to give the negative ion, which can be represented as a resonance hybrid of structures (1) and (2). It is, however, also possible that the triple bond will just migrate in the presence of the alkali at 180°, and that the compound represented by structure (3) may also form:-

$$CH_3.(CH_2)_4.CH_2.CH{=}CH.C \equiv C.CH_2.(CH_2)_6.COOH$$

$$\Big\downarrow \; OH'$$

$$CH_3.(CH_2)_4.\overset{-}{C}H.CH{=}CH.C \equiv C.CH_2.(CH_2)_6.COOH$$

and $$CH_3.(CH_2)_4.CH_2.CH{=}CH.C \equiv C.(\overset{-}{C}H).(CH_2)_6.COOH \quad + \quad H_2O$$

$$CH_3.(CH_2)_4.HC{=}CH.CH{=}CH.CH{=}\overset{-}{C}.(CH_2)_6.COOH \;\ldots\ldots\; (1)$$

$$CH_3.(CH_2)_4.\overset{-}{C}{=}CH.CH{=}CH.CH{=}CH.(CH_2)_6.COOH \;\ldots\ldots\; (2)$$

$$\Big\downarrow \; H_2O$$

$$CH_3.(CH_2)_4.CH{=}CH.CH{=}CH.CH{=}CH.(CH_2)_6.COOH$$

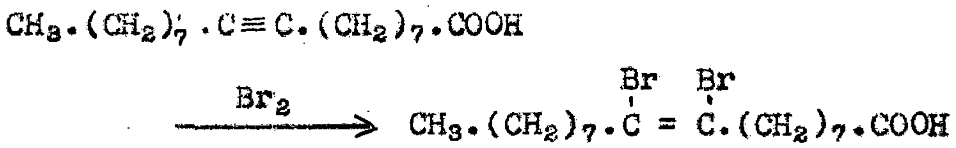
$$CH_3.(CH_2)_5.CH{=}CH.C \equiv C.(CH_2)_7.COOH$$

$$180^\circ \Big\downarrow KOH$$

$$CH_3.(CH_2)_5.CH{=}CH.CH{=}CH.CH{=}CH.(CH_2)_5.COOH \;\ldots\ldots\; (3)$$

As intermolecular dimerisation and polymerisation can also take place, the complexity of the resulting acid mixture is readily concievable.

The iodine values of ximenynic acid and its methyl ester were determined with the following reagents: (a) Wijs reagent,[51] (b) Woburn reagent,[52] (c) modified Rosemund-Kuhnhenn reagent.[53] It is a known fact that addition of halogens to double bonds are only complete in the case of isolated double bonds, incomplete addition takes place in the case of double bonds in close proximity to carbonyl groups, in the case of conjugated double bonds or acetylenic bonds.[54] In such cases, the iodine values found experimentally are lower than the theoretical values, e.g. in the case of elaeostearic acid, rapid addition takes place in the case of two of the three double bonds, but with considerable difficulty or not at all to the third double bond. In the case of stearolic acid, only one mole of halogen adds according to the following equation:[55]



$$CH_3.(CH_2)_7.C\equiv C.(CH_2)_7.COOH \xrightarrow{Br_2} CH_3.(CH_2)_7.\overset{Br}{C} = \overset{Br}{C}.(CH_2)_7.COOH$$

Reagents (b) and (c) have been recommended for the determination of the iodine values of conjugated unsaturated compounds, while bromine vapour[56] was recently proposed as a tool for the determination of the degree of unsaturation of organic compounds. The results obtained with these reagents have been summarised in Table XI, to complete this table the values found by Hawke _et_ _al_. with the bromine vapour method have been included.

Table XI. /...

TABLE XI.

Iodine Values of Ximenynic Acid and Methyl Ximenynate.

	Ximenynic Acid	Methyl Ximenynate.
Theoretical Iodine Value:	273.6	260.6
Theoretical for two double bonds:	182.5	173.8
Woburn - One hour	151.9	140.9
Woburn - Two hours	148.1	137.6
Woburn - 10 hours	157.4	141.7
Woburn - 24 hours	153.7	142.6
Woburn - 48 hours	161.0	144.2
Woburn - 96 hours	189.2	168.7
Wijs - one hour	130.3	124.8
Wijs - two hours	131	125.5
Wijs - 10 hours	129.7	124.5
Wijs - 24 hours	127.5	120.5
Wijs - 48 hours	127.5	120.5
Rosenmund Kuhnhenn - one hour	193.3	174.9
Rosenmund-Kuhnhenn - two hours	196.6	178.9
Bromine vapour	187.1	170.3

As incomplete addition took place with most reagents, longer reaction times were used, but it will be noticed from the Table XI that none of the reagents gave a quantitative iodine value; the Rosenmund-Kuhnhenn reagent added to about two of the three double bonds, while the Wijs and Woburn reagents added only to about $1^1/_2$ of the three double bonds. The addition products were, however, not investigated any further.

The phenomenon that normal aliphatic straight chain compounds form complexes with urea was applied to ximenynic acid and its derivatives: this phenomenon was first reported

by /....

by Bengen,[57] while detailed investigations on the structure and composition of these complexes were published later,[58,59] the method was also applied on the pilot-plant scale for the separation of fatty acids.[29] Schlenk and Holman[60] also reported to have prepared methyl oleate of 98% purity in 40% yield and pure methyl linoleate in 40% yield from corn oil. Furthermore, these authors found that the urea complexes of the unsaturated acids do not absorb oxygen and that this is a very convenient way of stabilising and storing unsaturated compounds.

The urea compounds of ximenynic acid, ximenynic alcohol and methyl ximenynate were prepared and as the "yield value" of the methyl ester was lower than that of the acid, the reaction was also applied to the total methyl esters of Ximenia caffra Sond. By fractional crystallisation of the urea complexes of the total esters, a methyl ester fraction was obtained with E(1%, 1 cm.) of 379 (229 mμ), which could not be purified any further through the urea complexes.

Other acids of Ximenia oil which were isolated in 95-97% purity were ximenic, lumeniqueic and cerotic acids, while oleic acid was identified as the dihydroxy compound. Erucic acid could not be identified as the dihydroxy compound, and it will be reported as being absent.

Isolation and Characterisation of the High Molecular Weight Acids.

The presence of Ximenic acid, the C_{26}-mono-unsaturated acid and cerotic acid, a saturated C_{26} acid, in the kernel oil of the Ximenia species, was initially proposed by Puntambekar and Krishna.[4] These workers failed to isolate the C_{26}-2H acid, but prepared and characterised dihydroxy-cerotic acid from ximenic acid. Boekenoogen[13] investigated

the /....

the kernel oil of Ximenia americana Linn. from West Africa, and reported the isolation of ximenic and cerotic acids of high purity. He characterised the unsaturated acids and determined the position of the ethylenic linkage, but did not report a melting point for it. Boekenoogen also reported the isolation of relatively pure lumeniqueic acid, a C_{30}-mono-unsaturated acid. The mono-unsaturated acids were reported to be isolated by the mercury-salt method of Bertram.[61]

The presence and isolation of other C_{26}-mono-unsaturated acids was reported by Bernhardt and Albrecht[62] and Klenk and Schumann[63]: Bernhardt and Albrecht isolated small quantities of a hexacosenoic acid (C_{26}-2H) from the lipids of Phycomyces blakesleeanus, melting point 45° - 45.5°C, iodine value 63.5, neutralisation equivalent 395.6 (Theory: I.V. 63, N.E. 394.7). The acid was isolated as follows: the methyl esters from the fractional distillation was saponified and the fatty acids separated into the saturated and unsaturated acids through the magnesium salts; the soluble fraction was further purified by crystallisation through the sodium salt. Oxidation of this pure acid with osmium tetra-oxide, gave a dihydroxy-cerotic acid, melting point 124° - 125°C; quantities of the acid were too small to establish the position of the double bond. Klenk and Schumann isolated a hexacosenoic acid from brain cerebrosides, melting point 45°C; details of the isolation and structure of the acid were, however, not reported.

As the saponification equivalents of the fractions from the fractional distillation were equal to and over 400, and even exceeded 440 in some cases (see Table XXXVIII) and as the fractions had an iodine value of over 40, the presence of C_{26} acids (S.E. 410, I.V. 64) and C_{30} acids were suspected. The methyl esters with saponification equivalent

of /....

of 408-410 and 450-455 were combined and used for the isolation of ximenic and lumeniqueic acids respectively; these combined fractions were saponified and the free fatty acids extracted with ether.

Attempted isolation of ximenic and lumeniqueic acids through the mercury salt, gave acids of about 80-90% purity; as it was, however, rather difficult to free the fractions of the adhering mercury salts and as the sodium salt method yielded a gelatinous mass, it was decided to attempt to purify the acids by crystallisation from a solvent. Trial experiments with light petroleum, acetone and alcohol indicated the superiority of the first-named solvent, and the final experiments for the isolation of ximenic and lumeniqueic acids were based on the fractional crystallisation of the mixed acids from light petroleum; final crystallisation from acetone yielded acids of 95-97% purity. From the constants, ximenic acid was contaminated with lumeniqueic acid and *vice versa*, and as the physical properties of these acids are closely related, it was not possible to purify them any further.

The dihydroxy acids were prepared by oxidation of the acids with hydrogen peroxide and osmium tetraoxide catalyst;[64] oxidation of the acids with potassium permanganate in acetone gave pelargonic acid and pentadecane-1,15-dicarboxylic acid, and pelargonic and nona-decane-1,19-dicarboxylic acid, from ximenic and lumeniqueic acids, respectively. These split products confirmed the structures for the acids proposed by Boekenoogen, (e.g.), $CH_3(CH_2)_7CH{=}CH(CH_2)_{15}COOH$ and $CH_3(CH_2)_7CH{=}CH(CH_2)_{19}COOH$.

Cerotic acid[65] has been isolated before as a substitute of several waxes, and also as a minor constituent of several oils; the following melting points have been reported for cerotic acid: 78°, isolated from waxes(a); 80°, (methyl ester, 62°C;(b) amide 105-107°)(c) from butter fat(c); 78°, from wool

fat /...

fat;(d) 78°, from an insect wax(e); 86.9°, from degras (wool fat)(f) (amide 112.1°); 77.5-78°, from beeswax as ceryl alcohol which was oxidised to the acid(g); 82°, from Artocarpus Hirsula Lamk(h); 78-79°, from the lipids of Phycomyces blakesleeanus (i). Piper, Chibnall *et al.*(j) prepared cerotic acid from the C_{26} alcohol, m.p. 87.5-87.7°, while Francis and Piper(k) reported a melting point of 87.7° for this acid and a melting point of 62.9° for the methyl ester; synthetic cerotic acid was reported to have a melting point of 87.5-87.7°(l). From the foregoing it can be concluded that the product with melting point of 87.5-87.7° can be regarded as pure cerotic acid.

The saturated fatty acids obtained from the isolation of the unsaturated acids were combined and after conversion into the methyl esters fractionally distilled through an efficient still. The methyl esters with melting point 63.5° were combined and saponified, and yielded cerotic acid with melting point 86-87°.

Investigation of the Unsaponifiable Material.

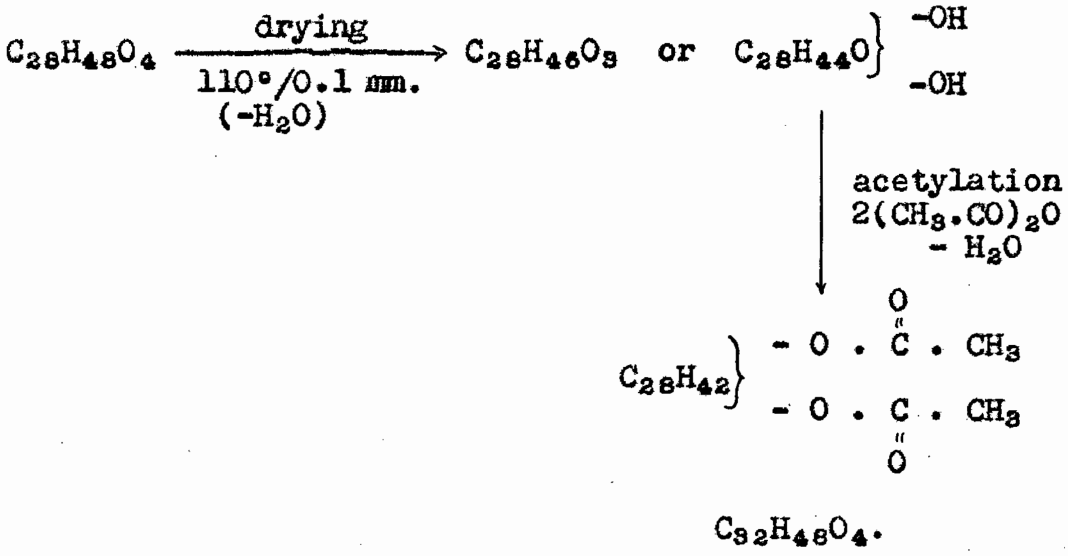
The isolation of the crystalline material (residue B) which gave the acetone extracted oil its gelatinous nature, was discussed earlier (pp. 10 and 92).

The gelatinous cakes of the three *Ximenia* oils were freed of oil and dissolved in 90% alcohol; yielding white feathery crystals which were recrystallised from 90% alcohol. After drying at 110°C in an air oven, these analysed for $C_7H_{12}O$; they did not give a precipitate with digitonin[66], and did not give the Liebermann-Burchard test for cholesterol[67].

The compounds isolated from *Ximenia caffra* Sond. var. *natalensis* Sond. appeared to be the same (mixed melting point determination) /...

determination) but both gave a lowering in melting point when mixed with that from Ximenia americana var. microphylla Welw.

The compound ex Ximenia caffra Sond. formed an acetate and benzoate, and thus has free hydroxyl groups; the acetate analysed for $C_{32}H_{48}O_4$ and had molecular weight 512. This indicated that the original compounds might have the formulae $C_{28}H_{48}O_4$ or four times that of the empirical formula. The compound from Ximenia caffra Sond. after drying at 110° and 0.01 mm. for one hour analysed for $C_{28}H_{46}O_3$, and it appeared that one mole of water of crystallisation was lost on drying. From the available information, it would appear that the compounds have three hydroxyl groups. On acetylation, two of these hydroxyl groups are acetylated, while the third is lost by the elimination of water (this third hydroxyl group is most probably a tertiary hydroxyl group - see ref. 82). Thus:



$$C_{28}H_{48}O_4 \xrightarrow[\substack{110°/0.1\text{ mm.}\\(-H_2O)}]{\text{drying}} C_{28}H_{46}O_3 \text{ or } \left.C_{28}H_{44}O\right\}\begin{matrix}-OH\\-OH\end{matrix}$$

$$\Big\downarrow\ \begin{matrix}\text{acetylation}\\2(CH_3.CO)_2O\\-H_2O\end{matrix}$$

$$\left.C_{28}H_{42}\right\}\begin{matrix}-O.\overset{O}{\overset{\|}{C}}.CH_3\\-O.\underset{O}{\underset{\|}{C}}.CH_3\end{matrix}$$

$$C_{32}H_{48}O_4.$$

The original mother liquor of the compound from Ximenia caffra Sond. yielded two more compounds (Nos. 2 and 3) with different melting points; these compounds also analysed for $C_{28}H_{46}O_3$ (after drying). Due to the lack of raw material these compounds could not be characterised.

If these compounds are phyto-sterols, they are the sterols with the highest melting points ever reported. Two other /....

other sterols which do not give precipitates with digitonin are Calosterol[68] and Cerevisterol[69]: Calosterol was isolated by Basu and Nath from the milky juice of Calotropis Gigantea; it was reported to have three double bonds, melting point 202-203°, $[\alpha] = 100.6$, and the formula $C_{28}H_{44}O$ was proposed for it. Cerevisterol was isolated as one of the minor sterols from yeast, melting point 265.3° and it was reported to have two double bonds, at least 2-OH groups and the formula $C_{28}H_{46}O_3$ was proposed for it.

The rubbery material (Residue C - see p. 95) was freed of any adhering oil and dried. Although the analysis did not agree with that of natural rubber (poly-isoprene) the ratio of carbon to hydrogen and the high iodine value indicated that the compound was natural rubber which autoxidised during the isolation.

A partial examination of the composition of the unsaponifiable fraction of the kernel oil was also made. The unsaponifiable matter was isolated by the S.P.A. method (see Appendix C). Squalene, saturated hydrocarbons and α-glyceryl ethers were determined by methods described by Karnovsky and Rapson et al.[70] and previously applied in this laboratory; the sterol content was determined colorimetrically[71]. The results of the analysis of the unsaponifiable fraction are reported in Table XII. The components of the unsaponifiable material which were determined only account for 44% of the total. Due to the lack of raw material it was not possible to determine the nature of the remaining 56%.

Table XII. /...

TABLE XII.

Analysis of the Unsaponifiable Material.

Unsaturated Hydrocarbon content:	2.70%
Saturated Hydrocarbons:	4.4%
Sterols:	37.0%
α-Glyceryl Ethers:	nil
Difference:	55.90%

Analysis of the Meals:

The residual meals, after extraction of the oils as described earlier, were dried and re-extracted with light petroleum to remove the last traces of oil and fat. The meals were then air dried and stored in stoppered bottles. The meals were analysed according to the methods of the Association of Official Agricultural Chemists[72], the results of which are reported in Table XIII.

TABLE XIII.

Analysis of Ximenia Meals.

	Ximenia caffra Sond. (Kernel)	*Ximenia caffra* var. *natalensis* (Total seed)	*Ximenia americana* (Total Seed)	*Ximenia americana* (Kernel)	Reported for kernel meal.
Protein (%)	56.5	46.9	23.54	52.5	44.6
Moisture and Volume	8.74	6.78	6.00	6.5	5.2
Ash (%)	4.4	5.00	2.39	5.17	4.7
Fibre (%)	10.62	10.39	48.8	11.46	-

It will be noticed that the residual meals are rich in proteins; feeding trials will, however, have to be carried out to test their value as feeding stuff.

Computation /....

Computation of the Composition of the Fractions.

Introduction.

For the calculation of the weights of ximenynic and elaeostearic esters, it was assumed that neither these esters nor the presence of the other components cause any irrelevant absorption at $\lambda_{max.}$ 229 and 268 mμ and that the E values at 229 and 268 mμ are a measure of the "true content" of these esters in the fractions.

The iodine value of methyl ximenynate with the Wijs reagent was found to be 124.8, and that of methyl elaeostearate 160.0 (one hour exposure); both esters have saponification equivalent of 292.4. The E(1%, 1 cm.) of methyl ximenynate at $\lambda_{max.}$ 229 mμ was found to be 549.2. The E(1%, 1 cm.) of elaeostearic acid at $\lambda_{max.}$ 268 mμ has been quoted as 2161[73] while Devine[74] assumed an E(1%, 1 cm.) of 2100 at 268 mμ. In the calculation of the weight of the methyl elaeostearate a value of E(1%, 1 cm.) = 2000 at $\lambda_{max.}$ at 268 mμ was used as the extinction coefficient of that ester.

Say a = Weight of the fraction

and $x = \dfrac{E(1\%, 1\text{cm.}) \times a}{549.2}$ = Weight of methyl ximenynate

and $y = \dfrac{E(1\%, 1\text{cm.}) \times a}{2000}$ = Weight of methyl elaeostearate

and say that E' and I represent the saponification equivalent and iodine value of the fraction, respectively, and a' the weight of the other esters in the fraction.

Then
$$x + y + a' = a \quad \text{............} \quad (1)$$

$$\frac{x + y}{292.4} + \frac{a'}{E'} = \frac{a}{E} \quad \text{............} \quad (2)$$

$$x \times 124.8 + y \times 166.8 + a' \times I' = a \times I \quad \text{.......} \quad (3)$$

where E' and I' represent the saponification equivalent and iodine value of the other esters

(residual /....

(residual esters - other than methyl ximenynate and methyl elaeostearate) in each fraction.

By solving equations 1, 2 and 3, a', E' and I' for each fraction could be calculated, which represent the weight, Saponification Equivalent and Iodine Value of the components other than methyl ximenynate and methyl elaeostearate in each fraction, see Tables XXXIV - XLI.

By using the simplified formulae of Hilditch[31] the composition of these "residual esters" could be calculated.

Say p, q, and r are the weights of a saturated and say two unsaturated esters of the "residual esters" in the fraction of weight s'; and E_p, E_q, E_r and E' are the corresponding Saponification Equivalents; and I_q, I_r and I' are the corresponding Iodine Values of these constituents, then:

$$p + q + r = a' \quad \ldots\ldots\ldots\ldots\ldots (4)$$

$$\frac{p}{E_p} + \frac{q}{E_q} + \frac{r}{E_r} = \frac{a'}{E'} \quad \ldots\ldots\ldots\ldots\ldots (5)$$

and

$$q \times I_q + r \times I_r = a' \times I' \quad \ldots\ldots\ldots\ldots (6)$$

By solving the three equations 4, 5 and 6 the values for p, q, and r could be obtained.

The other equations used in the calculation of the composition of the ester fractions are those proposed by Rapson _et al_.[75]

Calculation of the Component Esters of Liquid Fraction
(Fraction B)

The calculations involved in the evaluation of the liquid fractions will be illustrated by using the data obtained in the case of the _Ximenia caffra_ oil only, the calculations of the composition of the other two oils were carried /....

carried out in the same way:

Fraction L_1:

Weight: 9.5553 g.; S.E., 284.5; I.V., 75.56;
$E^{1\%}_{229}$ 12.12; $E^{1\%}_{268}$ 0.34.

Using the equations 1, 2 and 3 (see under introduction) and the absorption data, Saponification Equivalent and Iodine Value of methyl ximenynate and elaeostearate, the following weights and constants were calculated for the residual esters:

Weight (a'), 9.3428 g.; S.E. (E'), 284.3;
I.V. (I'), 74.46; Wt. X. ester, 0.2107 g.;
Wt. El. ester, 0.0018 g.

From the saponification equivalent and iodine value, this fraction is a mixture of C_{16} and C_{18} saturated and unsaturated esters. The composition was calculated with the aid of the Rapson equations, and the final composition for the fraction is as follows:

Weight of hexa-decenoic ester:	3.1742 g.
Weight of methyl oleate:	4.6074 g.
Weight of palmitic ester:	0.7254 g.
Weight of stearic ester:	0.8358 g.
Weight of methyl ximenynate:	0.2107 g.
Weight of methyl elaeostearate:	0.0018 g.

The composition of Fraction L_2 was calculated on the same basis; the weights of the different esters are given in Table XXXIV.

Fraction L_3 - L_7:

From the spectroscopic data, the weights of ximenynic and elaeostearic esters were calculated, and by using equations 1, 2 and 3 the constants and weight of the residual esters in the fraction were evaluated. From the saponification equivalents and iodine values these fractions may be mixtures of C_{16} and C_{18} saturated and unsaturated esters. On

calculating /....

calculating the composition on this basis, however, the hexadecenoic esters came to a negative quantity. The composition was consequently calculated on the assumption that C_{16} saturated, C_{18} saturated and C_{18}-2H esters only are present, and the weights of the esters are given in Table XXXIV.

Fractions L_8 -L_{11}:

The weights of methyl ximenynate and elaeostearate in the fraction were calculated in the normal way from the spectroscopic data. According to the saponification equivalents these fractions may be mixtures of C_{16} saturated, C_{18} saturated and C_{18}-2H esters; on calculating the composition on this basis, however, the weight of the C_{16} ester came to a negative quantity. The composition was consequently calculated on the basis of C_{18} saturated and C_{18}-2H esters only. To speed the calculation, the weights were combined and the average iodine value and saponification equivalent (average E' and I') calculated by multiplying the weight of each fraction (a') with its iodine value (I') and saponification equivalent and dividing the total of these values by the total weight.

The weight of methyl oleate was calculated from the iodine value, and the difference designated as the weight of C_{18} saturated ester. The composition is given in Table XXXIV.

Fraction L_{12}:

Weight of residual esters, 1.7468 g.; S.E'., 297.1; I.V'.,95.92.

The saponfication equivalent of this fraction is between that of C_{18} saturated and C_{18} unsaturated ester. The average unsaturation of the fraction was calculated with the Charnley equation[76]

$$a_2 = \frac{\text{I.V. x S.E.}}{25400}$$

The /...

The sharp rise in the saponification equivalent of the following fractions, however, indicated the presence of higher molecular weight esters (higher than C_{18}). The composition of these fractions (L_{12} - L_{14}) could be computed on one of the following assumptions;

(1) that these fractions are mixtures of C_{18} and C_{26} esters

or (2) calculating the composition of these fractions on the basis of two successive homologues, e.g., mixtures of C_{18} and C_{20} esters.

The weights of these fractions are relatively small, and whatever method is adopted will not affect the total composition considerably. Furthermore, due to polymerisation and oxidation the iodine values of these fractions do not appear to be a measure of the true unsaturation of the fractions. It was therefore assumed that these fractions consisted of unsaturated esters only; the unsaturation was calculated (with the Charnley equation) and the composition computed on the basis of the saponification equivalents assuming, furthermore, that these fractions are mixtures of C_{18}-2H and C_{20} esters, only. Fractions 13 and 14 were calculated on the same basis, but assuming that C_{18}-2H ester is the one unsaturated ester.

Fraction 15.

The average unsaturation of the residual esters was calculated with the Charnley equation, and the composition was calculated on the basis that C_{20} and C_{22} unsaturated esters only are present. The results are tabulated in Table XXXIV.

Fractions 16 and 17.

The weights of ximenynic and elaeostearic esters were calculated from the spectroscopic data and the weights, saponification equivalents and iodine values of the residual esters calculated as usual. From the saponification equivalents these fractions are mixtures of C_{22} and C_{24} methyl esters. The average unsaturation was calculated with the Charnley equation and the composition calculated on the basis of the saponification equivalents only, the results are given in Table XXXIV.

Fraction 18.

From the spectroscopic data the weights of ximenynic and elaeostearic esters were calculated, also the weight, saponification equivalent and iodine value of the residual esters. The average unsaturation was calculated with the Charnley equation and the composition calculated on the basis that only C_{24} and C_{26} esters are present. The results are summarised in Table XXXIV.

Fraction 19.

This is the residual or pot fraction. Due to polymerisation the fraction contains some unsaponifiable matter. The fraction was consequently saponified and the non-sap. extracted; the fraction was then analysed as the acids. From the spectroscopic data, the weights of the ximenynic and elaeostearic acids were calculated and expressed as the methyl ester. The value of the saponification equivalent and iodine value was also expressed as that of the methyl ester; by using the standard equations (1, 2 and 3), the weights, saponification equivalent and iodine value of the residual esters were calculated. The saponification equivalent is higher than that of the C_{26} ester, and it was decided to compute the composition on the basis of C_{26} and C_{30} unsaturated esters, using only the equations for the saponification equivalents. The results are given in Table XXXIV.

The Calculation of the Composition of the Solid Fractions.

The solid acids as obtained from the acetone crystallisation at -25° should contain most of the stearic acid and all the higher molecular weight saturated acids, also most of the unsaturated acids, having 22 carbons and more in the chain.

The presence of erucic acid was a key problem in the calculation of the composition of the fatty acids; it will be noticed that the chain length of the unsaturated fatty acids increases with 4 carbon atoms, but Boekenoogen failed to establish the presence of erucic acid in *Ximenia* oil (*loc. cit.*). To test for the presence of erucic acid, the fraction with S.E. 364-366 was oxidised with potassium permanganate to the dihydroxy acids[77], (see Section V(m)) the dihydroxy acids are less soluble than the corresponding saturated acids and could be seperated from them. Dihydroxy behenic acid could not be identified, however, and erucic acid was considered to be absent.

For the calculation of the composition of the ester fraction with saponification equivalent between 298-410 it was assumed that C_{18}, C_{26} saturated and C_{26} unsaturated esters only are present. Methyl oleate is more soluble in acetone than methyl ximenynate, which is present in small quantities, and it would be expected that the quantities of this ester should be smaller, with C_{16}-2H ester present only in trace quantities.

All fractions were examined spectrophotometrically and it will be noticed that there is a rise in the E value of the higher boiling fractions; this may be due to

(1) the presence of higher molecular weight enyne of diene acid or

(2) the hydroperoxide or polymerised acids, which are known

to /...

to absorb in the ultraviolet region.[87]

For the calculation of the composition of the fractions the equations of Rapson[75] *et al*. have been used in conjunction with the equations forwarded by the author for the calculation of the liquid fractions (1, 2 and 3). From the absorbtion data, the weights of ximenynic and elaeostearic esters were calculated, and their combined weight of the fraction subtracted from the weight of the fraction, to give the weight of the residual esters.

The fractions with saponification equivalents between 411-465 were calculated on a basis that only C_{26}-2H, C_{26} saturated and C_{30}-2H esters are present (see Boekenoogen[13]). This will be explained more fully when the different calculations of each oil are discussed. To speed the calculation these fractions were combined and the "weight average" of the saponfication equivalents and iodine values calculated. The feasibility of this step was checked in the case of the esters of *Ximenia americana* var. *microphylla* Welw. (S7 - S14). The composition of the esters by combining the fractions was calculated as

C_{26} saturated: 7.7071 g.; C_{26}-2H, 15.7430 g.; and C_{30}-2H, 26.9543 g.

while the composition for the separate fractions was calculated as:

C_{26} saturated, 7.7760; C_{26}-2H, 16,2538; C_{30}-2H, 26.3746 g.

The difference in the final composition of the total fatty acids between these two calculations is of the order of 0.3%. With this small variation between the two methods, the composition of these fractions was calculated from the total weight of the fractions, using the "average weight", saponification equivalent and iodine value.

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The saponification equivalents of several fractions were found to be higher than 465 (theoretical saponification of C_{30}-2H ester), from the relative high iodine value of these fractions it is evident that an unsaturated acid is present having a chain length of more than 30 carbons. By analogous reasoning on the way in which the chainlength increases in going from one unsaturated acid to the next higher homologue, it was postulated that the C_{34}-2H acid would be the next higher unsaturated acid in this series. The composition of these fractions was accordingly calculated on the basis of C_{30} and C_{34} acids, although no proof is forwarded for the presence of this acid. The isolation of ximenic and lumemiqueic acids of 95-97% purity will be described in a later section.

The calculations involved in the evaluation of the solid fractions will again be illustrated by using only the data obtained in the case of the <u>Ximenia caffra</u> oil.

By applying the equations presented under the liquid fractions, the constants were obtained for the weights and constants of the "residual esters" in the fraction.

Say x = Weight of methyl ximenynate

$$= E^{1\%}_{229} \times \frac{\text{Wt. of fraction}}{549.2}$$

y = Weight of methyl elaeostearate

$$= E^{1\%}_{268} \times \frac{\text{Wt. of fraction}}{2000}$$

a = Total weight of fraction

and a' = Weight of residual esters

$$= a - x - y \qquad \ldots\ldots\ldots\ (4)$$

From the following equations the saponification equivalent and iodine value of the residual esters were calculated.

$$\frac{x + y}{292.4} + \frac{a'}{E'} = \frac{a}{E} \qquad \ldots\ldots\ldots\ldots\ (5)$$

$$x \times 124.8 + y \times 166.8 + a' \times I' = a \times I \quad \ldots\ldots\ (6)$$

where /....

where E and I are the saponification equivalent and iodine value of the total fraction and E' and I' are the saponification equivalent and iodine value of the "residual esters".

<u>Fraction</u> S_1:

From the spectroscopic data the weights of methyl ximenynate and elaeostearate were calculated, also the weight, saponification equivalent and iodine value of the residual esters (see Table XXXV). From the saponification equivalent and iodine value (E' and I') the residual esters are mixtures of C_{16} and C_{18} saturated and unsaturated esters, and the composition was calculated on this basis, giving the weights of the different esters tabulated in Table XXXV.

<u>Fraction</u> S_2 - S_6:

From the spectroscopic data the weights of ximenynic and elaeostearic esters were calculated. From the saponification equivalents these fractions are mixtures of C_{18} and C_{26} saturated and unsaturated esters (see under introduction). The results of the calculations on this basis are given in Table XXXV.

<u>Fraction</u> S_7 - S_9:

The saponification equivalents indicate that these fractions may consist of C_{18} and C_{26} unsaturated and saturated esters. Methyl oleate distils at about 150°-160°/ 0.5 mm., however, and with the boiling point of these esters over 210°, it was postulated that only C_{18} saturated, C_{26}-2H and C_{26} saturated esters could be present. The results of the calculations are given in Table XXXV.

<u>Fractions</u> S_{10} - S_{19}:

From the spectroscopic data the weights, saponification

equivalents /....

equivalents and iodine values of the "residual esters" were calculated. The composition of these fractions was calculated on the basis that these fractions are mixtures of ximenic, lumeniqueic and cerotic (C_{26} and C_{30} unsaturated and C_{26} saturated) esters. To accelerate the calculations the average "weight saponification equivalent" and average "weight iodine value" of the residual esters were calculated.

Total weight (residual esters): 61.8161;

Average S.E. (E'): 435.1; Average I.V. (I') 44.62.

Fraction S_{20}:

The saponification equivalent and iodine value of the residual esters were calculated from the spectroscopic data. The S.E. (E') of these esters are higher than C_{30} esters, and the composition was calculated on the assumption that C_{26} saturated, C_{30}-2H and C_{34}-2H esters are present. (The saturated acids, after the removal of the lumeniqueic acid had a N.E. of the order of 400, and it appears that C_{30} saturated acids are absent.) The results of the calculation on this basis are given in Table XXXV.

Fraction S_{21}:

The pot fraction (or residue) was weighed as the esters, but as unsaponifiable material was present, the fraction was saponified, the non-sap. extracted and the acids obtained after acidification. The fatty acids were dried and analysed, but the different constants were recalculated for the corresponding esters. From the spectroscopic data the weights of ximenynic and elaeostearic esters were calculated and also the weights, saponification equivalent and iodine value of the residual esters. It was further postulated that the iodine value is not a true measure of unsaturation of the fraction and the composition was calculated on the basis that only C_{30}-2H and C_{34}-2H esters are present. The

results /...

results are tabulated in Table XXXV.

Further Details of the Calculation.

The weights of the esters of the "solid" and of the "liquid" fractions were added, and are referred to as the total esters of each fraction. This weight is usually about 1-2% lower than the weight of the esters used for distillation; the difference being due to the formation of volatile pyrolysis products as well as the presence of a low percentage of solvents in the esters, which distilled off.

The weights of the individual esters of each acid were also added and converted to the weights of the corresponding acids, with the following formula:

$$W_a = \frac{W_e \times E_a}{E_e}$$

where W_a = weight of corresponding acid of N.E. E_a

W_e = weight of ester of S.E. E_e

To facilitate the calculations, the factor

$$\frac{\text{Molecular Weight of Acid}}{\text{Molecular Weight of ester}}$$

of each acid was calculated, and this used for the conversion of the weight of the ester to acid.

The total weights of the individual acids (say C_{18}-2H) of each fraction (liquid or solid), were then expressed in terms of the total acids of the oil. This was done by multiplying the weight of each acid by the percentage weight of that fraction obtained in low-temperature crystallisation of the total acids and dividing by 100, e.g., the percentage of ximenynic acid in the liquid fraction was calculated as follows (see Table XXXIV):-

$$= \frac{47.9050 \times 65.35}{100}$$

$$= 21.554\ \%$$

The total of all the percentages of the acids in each fraction

should /....

should add up to the percentage of the fraction and the total of the two fractions to 100. In Table XXXIII the total fatty acids of the oils are combined to give the total of each acid in the oil.

Discussion of the Results.

The results obtained by subjecting the fatty acids of the *Ximenia* oils to low temperature crystallisation, and fractional distillation of the methyl esters will be discussed and the results compared with some of the other species of the Olacaceae family. The seeds and nuts of several other members of the Olacaceae family (Hilditch, Monograph on Fats and Oils, p. 158) have been investigated for the oil content and the composition of the oils. Jurgel and de Amorim[78] isolated an oil in 35% yield from the seeds of Ivory wood (*Agonandra brasiliensis*, Miers); the following composition was calculated for the oil: ricinoleyl, 44.85%; linoleyl, 34.65%; oleyl, 11.45%; myristyl, 2.2 and palmityl, 1.32. Steger and van Loon[79] investigated the seeds of the Coula plant and calculated the following composition for the fatty acids: 9-oleic acid, 87.1; linoleic, 2.7; sat. acids, 1.6. The seed fat of Onguekora Gore Engler (syn. *Ongokea klaineana*, Pierre) was investigated by Steger and van Loon[80a], Boekenoogen[80b], and Castillé[80c]. Although the investigation on the composition was not completed, it was found that erythrogenic acid (C_{18} acid) is the chief component acid of the oil; it contains one double and two acetylenic bonds. On ozonolysis of the ethyl ester the following fractions were obtained: 1 mole of formaldehyde, oxalic acid, adipic acid and ethyl hydrogen azelate, so that the acid can have one of the following structures:

$$CH_2 = CH.C{\equiv}C\ (CH_2)_4.C{\equiv}C.(CH_2)_7.COOH$$

or $$CH_2 : CH(CH_2)_4C{\equiv}C.C{\equiv}C.(CH_2)_7.COOH$$

The /...

The other constituents are: oleic, linoleic and saturated acids. In a later publication Steger and van Loon[80d] showed that the latter of the two structures was correct and this was later supported by Castille[80e].

The work on the investigations of the composition of _Ximenia_ oils has been discussed (Puntambekar and Krishna[4], and Boekenoogen[13]), Puntambekar and Krishna reported the presence of ximenic and cerotic acids, while Boekenoogen confirmed the presence of these acids and in addition isolated a C_{30}-2H acid; these workers, however, did not detect the presence of the enyne acid. In the following table the composition of the three local oils are compared with the composition of the _Ximenia_ _americana_ oils reported by the above mentioned workers.

It will be noticed that there is a remarkable agreement between the percentages oleic and linoleic acids as reported by the previous workers and the total of the percentages of oleic and ximenynic acids in the local oils. It would appear that these workers attributed the increase of iodine value to the presence of linoleic acid, without taking into consideration the possibility of having any other unsaturated acids present in the oil.

Table XIV. /...

TABLE XIV.

Component Fatty Acids of Ximenia Oils.

	Ximenia americana ex India[4]	*Ximenia americana* ex West Africa[13]	*Ximenia caffra* Sond.	*Ximenia caffra* var. *natalensis*	*Ximenia americana* var. *microphylla*.
Palmitic	-	-	0.4	0.3	0.3
Stearic	1.2	4.0	4.1	4.5	2.0
Cerotic	15.2	0.2	6.4	5.7	5.5
Hexadecenoic	-	-	1.5	1.0	0.5
Oleic	60.8	54.0	34.8	41.6	37.1
Linoleic	6.7	10.0	-	-	-
Ximenynic	-	-	24.3	22.1	21.9
Elaeostearic	-	-	0.4	0.2	0.2
C_{20} unsat.	-	-	0.5	1.3	0.6
C_{22} unsat.	-	-	1.8	0.7	0.1
C_{24} unsat.	-	-	3.6	4.0	1.0
C_{26}-2H	14.6	25.0	10.3	12.8	15.9
C_{30}-2H	-	5.0	11.3	6.0	14.4
C_{34}-2H	-	-	6.8	-	0.6
Total Oleic and Ximenynic (or Linoleic) acids	67.5	64.0	59.1	63.7	59.0

The iodine value is not a measurement of the true unsaturation of compounds containing acetylenic bonds[54], and in the case of ximenynic acid it measures only 50% of the unsaturation; without any other means of measuring the unsaturation of such compounds, it would be impossible to detect their presence.

The presence of two acids containing acetylenic acids is exceptional and in the light of these findings, the oils of

the /...

the other members of this family should be re-investigated and the total unsaturation of the oils checked with both the iodine value and hydrogenation, and especially the absorption spectra between 340 and 220 mμ.

There is also a noticeable difference in the composition of the three local *Ximenia* oils; the saturated fatty acids varied between 7.6 to 10.3%, while the oleic acid content varied between 35.5-41.2% and the ximenynic acid content varied between 22-24%. A somewhat unusual variation is incountered in the content of the high molecular weight unsaturated acids, from 11 to 13.4% for ximenic acid and from 6 to 14.4% for lumeniqueic acid; this fact is also reflected in the saponification values of the three oils, e.g., 170.4, 172.8 and 165.2 (see Table XII (2)).

It is impossible to say at this stage whether these variations are due to difference in variety or due to the locality in which the seeds of these species have been collected. (See Hilditch, Monograph on Fats and Oils, p. 163 for discussion of variation due to varieties and localities in linseed oils.) The oils extracted from the two species collected in the Transvaal have very similar constants:

	Sap. Value	I.V.	E(1%,1cm.) 229 mμ	E(1%,1cm.) 268 mμ
Ximenia caffra Sond.	169.1	76.0	124.1	6.1
Ximenia caffra var. *natalensis* Sond.	168.5	76.1	122.6	9.1

The complete composition of these two oils was, however, not determined.

Apart from *Ximenia*, only one member of the Olacaceae family is represented in South Africa, namely, *Olax dissitiflora* Oliv.[81] This is a shrub or small tree found in Zululand, Portuguese East Africa and Northern Transvaal. As the nuts of this species are difficult to obtain, they could not be investigated.

EXPERIMENTAL.

(A) Investigation of the fruit.

The fruit of Ximenia caffra Sond. and Ximenia caffra var. natalensis Sond. analysed, were collected near the Loskop Dam in the Middelburg District, in the summer of 1949-1950. Average samples of each species were picked and dispatched to this Department, where the fruit was analysed without delay.

About 100 fruits of each species were accurately weighed to determine the average weight of the fruit. The skin and fruit-pulp was then removed and the seeds weighed to determine the composition of the fruit and the average weight of the seed.

The moisture content of the fruit-pulp and the fresh kernels was determined according to the standard A.O.C.S. methods (Appendix B.).

The oil content of the fruit-pulp, dried by vacuum desiccation, was determined by extraction with acetone for ca. 24 hours. The acetone was removed by distillation, leaving a brown residue. This was extracted with light petroleum and the petroleum also removed by distillation yielding a very small quantity of oil. This was too small for further investigation.

To obtain the juice, the skins of a large number of fruits were removed, the fruits hand-pressed and the resulting turbid juice cleared by filtration through a Buchner filter under vacuum. The juice had a slight reddish colour and a pronounced sour taste, reminiscent of that of a lemon. On addition of alkali the colour changed into a purple blue and it was impossible to see the end-point of the titration with any of the standard indicators (e.g., phenol phthalein and alkali blue); on titration with N/10 alkali, using a fluorescent indicator, there was no improvement. Finally the acidity in the juice was determined by potentiometric titration and the end-point of the titration taken at pH = 9.2.

Treatment of the juice with calcium hydroxide did not give any precipitate in the cold; on heating, however, a white precipitate formed which indicated the presence of citric acid. The citric acid was finally determined by penta-bromo-acetone method according to the method of Gray[16].

The composition and analysis of the fruit have been given in Table II, while the results of the investigation of the fruit juice have been summarised in Table III.

(B) Investigation of the Seeds.

The seeds of *Ximenia caffra* Sond. analysed during this investigation, were collected in Bechuanaland; the seeds of *Ximenia caffra* var. *natalensis* Sond. came from Ixopo, Natal; while seeds of *Ximenia americana* var. *microphylla* were collected in Pondoland.

The fruit was picked when fully ripe, the flesh and the pulp removed and the seeds dried in the sun, the hulls of the seeds were left intact to prevent exposure of the kernel to the air. When completely dry, the seeds were packed and sent to this Department and analysed without delay. The composition of the seeds was determined by weighing about 100 seeds (choosing more or less representative samples), the hulls were removed and the residual kernels reweighed.

The seeds had an oblong shape, were light brown to dark brown in colour and the relatively soft hulls could easily be broken by rubbing the seeds between the fingers.

The kernels were minced in a household meat mincer and the moisture and volatile content (see Appendix C) and the oil content determined according to the standard A.O.C.S. methods (see next paragraph).

The results of the analysis of the seeds have been summarised in Table IV.

(C) The Kernel Oil.

(1) Acetone Extraction.

A weighed quantity of the minced kernels was extracted with dried acetone in a Soxhlet apparatus for 10-12 hours. The extract was replaced by fresh solvent and the extraction continued for another 10-12 hours. During the extraction, a white crystalline solid separated, and after the extraction was completed, the extract was decanted therefrom; the solid (residue A) was washed with small volumes of fresh acetone and the washings added to the extract. The acetone was then removed by distillation through a tared distilling flask. Residual volatile material and moisture were removed by heating on a water bath under vacuum of a water pump for one to two hours: gentle swirling was necessary to avoid foaming which otherwise proved troublesome. The residual oil was cooled and weighed. The average of duplicate determinations have been given in the table.

The oil, slightly turbid and yellow in colour, was stored under carbon dioxide. The viscosity of the oil, when freshly extracted, was about E on Gardner-Holt standards. The turbidity tended to increase on standing and some solid material began to separate after a few days, the rate of precipitation being increased at low temperatures (0°C).

The separation of the crystalline material gave the oil a thixotropic nature and increased the viscosity to about Y on the Gardner-Holt standards.

Examination of the oil microscopically showed that the solid was crystalline and dilution of the oil with various solvents showed that it was soluble in acetone, alcohol, chloroform and ether, but was insoluble in light petroleum (b.p. 40°-60°) and benzene.

Accordingly, its separation was achieved by dilution with 3-4 volumes of light petroleum, leaving the solution at 0°C for a few hours, and filtration on a carefully packed Buchner filter under slight vacuum. This yielded a gelatinous cake (Residue B) on the filter and a clear petroleum solution of the oil. Experiments on the nature of the precipitate are described in connection with the unsaponifiable matter of the oil. Removal of the petroleum from the filtrate left a light yellow oil, which was dried in vacuum at 80°-100°C. The viscosity (about E on the Gardner-Holt standards) now no longer increased on standing and only a small amount of precipitate formed on standing several weeks.

<u>Residue A</u>.

The white solid material, which separated from the acetone extract, had a sweet taste and was water soluble.

The material did not reduce Fehling's solutions,[82] and did not give a positive test with 2,4-dinitrophenyl hydrazine. After hydrolysis with dilute hydrochloric acid, however, the compound reduced Fehling's solution and gave a positive test with 2,4-dinitrophenylhydrazine. As the amount of material isolated was very small, it could not be identified completely; from the reactions it appeared to be a polysaccharide.

(2) Petroleum Extraction.

Extraction of the minced kernels with light petroleum (b.p. 40°-60°) was performed in a similar manner to the acetone extraction described above, but in this case, no crystalline material separated in the extract. Removal of the solvent, volatile matter and moisture as described above, left a light yellow, slightly turbid oil with a viscosity of about Z (Gardner-Holt standards). Dilution of the oil with acetone or alcohol resulted in the precipitation of rubber-like material: this was collected by filtration (Residue C) and dried under vacuum at about 80°C and will be dealt with in the section on the unsaponifiable material. The acetone was distilled from the filtrate and the oil dried under vacuum on the water bath: it was a light yellow, clear oil and had a viscosity of E on the Gardner-Holt standards.

(3) Bulk Extraction of the Oil.

Acetone was used for the bulk extraction of the oil from the minced kernels, using a large copper Soxhlet. The solvent was removed from the extract by distillation and the residual oil dried under vacuum. The oil, cooled to room temperature, was diluted with 3-4 volumes of light petroleum (b.p. 50°-60°) and the solution stored overnight in the refrigerator. After addition of a few grams of filter aid, the gelatinous precipitate was collected on a Buchner filter.

The petroleum was removed from the filtrate by distillation, the residual oil dried under vacuum and stored under carbon dioxide in a brown bottle.

(4) The Chemical and Physical Constants of the Ximenia Oils.

The constants of the bulk extracted, purified oil were determined by the standard A.O.C.S. methods (see Appendices B - H); /...

B - H); the percentage unsaponifiable matter was determined by the S.P.A. method (Appendix C).

The iodine value from the hydrogen absorption was determined as follows: the hydrogen absorption was measured in a Towers Microhydrogenation Apparatus, using cyclohexane as solvent and palladium on calcium carbonate catalyst[83]. The moles of hydrogen absorbed (total unsaturation) were expressed in terms of the iodine value of the oil (grams of iodine per 100 grams of oil).

These results have been summarised in Table V, and include the percentages gelatinous material (Residue B) and rubbery material (Residue C). The Villavechia test[84] (see Appendix M) for sesamol and sesamolin (compounds isolated from sesame oil) was negative; the total tocopherols of Ximenia oil were also determined, Ximenia oil contains only 0.25 mg. of total tocopherols per gram of oil. The experimental details of these determinations are given in the section on Maroola oil.

The absorption spectra of the oils were determined in a Bechman spectrophotometer, model D.U., using a hydrogen lamp as the light source and spectroscopically pure cyclohexane as solvent; the absorbent was contained in quartz cells (silica) of 1 cm. width. The concentration of the solutions was regulated in such a way as to give readings between 0.3 and 0.8 on the scale. The absorption intensity (extinction coefficient) was expressed in terms of E (1%, 1cm.) and the logarithm of this value was plotted against the wavelength (measured in $m\mu$); these spectra are given in Fig. 1 and the E (1%, 1cm.) values at $\lambda_{max.}$ 229 and 268 $m\mu$ have been given in Table V.

(5) Component Acid Studies.

(a) Saponification of the Oil.

The method recommended by the Society of Public Analysts (see Appendix C) was used for the saponification of the kernel oil. This method is essentially the same as that used for the determination of the percentage unsaponifiable matter in an oil: fifty grams of oil were accurately weighed into a 1 litre round-bottomed flask and refluxed for one hour with 500 ml. N/2 alcoholic KOH. The alcoholic solution was slightly cooled and added to $^1/_2$ litre of distilled water, contained in a 4 litre separating funnel. The flask was rinsed with another $^1/_2$ litre of distilled water and added to the alcoholic solution in the separating funnel. The flask was then rinsed a few times with ether and the soap solution extracted with a total of 1 litre of ether. During the extraction, the contents of the separating funnel were strongly swirled but not shaken, so as to avoid emulsion formation. The contents of the separating funnel was allowed to stand for a short while and the aqueous phase run into a second 4 litre separating funnel. This was similarly extracted with another 1 litre of ether, followed by two more extractions of 750 ml. of ether each. The ether extracts were combined and washed with 250 ml. of water and this water solution added to the original aqueous soap solution, and then extracted with two portions of 250 ml. N/2 aqueous KOH solution; these solutions were also added to the soap solution; and the ether solution washed free of alkali. With all the extractions, care was taken to avoid the formation of emulsions. The water was separated as completely as possible and the ether distilled off through a tared distilling flask, and the residue dried under vacuum of the water pump on a boiling water bath. In the bulk saponification of the oil, the extracts of 8-10 lots of saponifications were combined and resaponified with N/2 alcoholic KOH (10 ml. of alkali per gram of residue) and the unsaponifiable matter extracted according to the abovementioned procedure. The material was then dried and stored under carbon dioxide; its analysis will be described in a later section

(b) Extraction of Fatty Acids.

The aqueous alcohol layer (together with one water wash and two alkali washes) was poured into a 4 litre separating funnel and 1 litre of ether added. The solution was then just acidified with concentrated hydrochloric acid and well shaken. The solution was allowed to separate and the aqueous layer taken off and successively extracted with 2 litres of ether. The ether volumes were combined and washed with successive portions of water to remove the excess hydrochloric acid. The ether was then distilled off through a distilling flask and the residual fatty acids dried under vacuum of a water pump on a boiling water bath.

The fatty acids from 8-10 lots of saponifications were combined, together with those from the re-saponification of the unsaponifiable matter and stored under carbon dioxide for further analysis.

(c) Low Temperature Crystallisation of the Fatty Acids.

Experiment 1: Crystallisation of Fatty Acids from Ether and Acetone.

The total fatty acids from *Ximenia caffra* Sond. were crystallised from dry ether at -40°, yielding the solid acids (Fraction A) and the liquid acids (Fraction B).

The solid fatty acids were recrystallised from ether at -40° to give an insoluble fraction A_1 and a soluble fraction A_2. The soluble fraction B was recrystallised from dried acetone at -60°, yielding an insoluble fraction B_1 and a soluble fraction B_2. The experimental procedure was briefly as follows: The acids were accurately weighed off in a 5 litre round-bottomed flask and the required volume of solvent added (10 ml./g. of acid), and the acids brought into solution by heating on a water bath. The flask was placed into the cooling bath, and with slow stirring the bath cooled to the desired temperature by the addition of dry ice - the temperature being taken down at a speed of about one degree per minute. The temperature was kept at the crystallisation temperature for about 4-5 hours, and the insoluble acids collected on a cooled Buchner filter. The filter cake was sucked dry and the crystals collected in a round-bottomed flask and dried. The solvent of the filtrate was removed by distillation and the residual acids dried under vacuum. The fractions were stored under CO_2 before analysis. The results of this experiment and the analysis of the fractions have been summarised in Table VI.

<u>Experiment 2</u>. Crystallisation from Acetone and Light Petroleum (b.p. 40°-60°).

The original fatty acids were dissolved in anhydrous acetone (10 ml. of solvent per gram of acid) and the solution cooled in the methylated spirits bath. At -25° the precipitate of crystals was so heavy that it was decided not to cool any further, and the solution was consequently kept at -25° for 4-5 hours. The crystals were collected on a cooled Buchner filter and the different fractions isolated as described in Experiment 1.

The liquid fatty acids were dissolved in light petroleum (b.p. 40°-60°, 5 ml. solvent/g. acid) and the solution cooled as before. At -22° the precipitate was relatively thick and the solution was kept at -22° for 4-5 hours and the crystals collected on a cooled filter and dried. The results of this experiment have been summarised in Table VII.

Experiment 3: Crystallisation of the Fatty Acids of the Three *Ximenia* Oils.

For the component acid studies of the oils of *Ximenia caffra* Sond., *Ximenia caffra* var. *natalensis* Sond. and *Ximenia americana* var. *microphylla* Sond. the free fatty acids were crystallised from dry acetone at -25°, and the soluble and insoluble fatty acids isolated as described in Experiment 1. The weights and analyses of the fractions have been summarised in Table VIII.

(d) Preparation and Isolation of the Methyl Esters.

The methyl esters of the acids were prepared according to the method outlined in Appendix L.

(e) Fractional Distillation of the Methyl Esters.

The fractionations of the different fractions were carried out in a two foot electrically heated column, packed with single and multiple turn glass helices. The column is a modification of the one described by Longenecker[85]. During the distillation any flooding of the column was avoided, and a temperature difference of about 100° was maintained between the bath temperature and that of the head of the column. An attempt was made to keep the initial fractions relatively small (4-5 g.), and the subsequent fractions were cut at about 5-7 g. The fractionation was continued until about 6-8 g. were left behind in the distilling flask; the distillation was then stopped and the apparatus cooled. After cooling, the column, side-arm and Perkin triangle were rinsed with small volumes of acetone. The acetone washed were combined with the residue in the flask and the solvent removed by distillation. The last

traces /....

traces of solvent were removed under vacuum and the flask cooled and weighed. This fraction was then resaponified according to the standard procedure and the non-sap. extracted. The free fatty acids were then extracted and analysed as such. The details of the fractional distillation (boiling points, pressures, etc.) have been summarised in the tables in the Appendix.

(f) Analysis of the Fractions.

In order to be able to compute the composition of each fraction, the saponification equivalent and iodine value of each fraction are required. Furthermore, as all the fractions contain considerable amounts of the conjugated diene and triene acids, the E (1%, 1cm.) values of each fraction were measured at 229 and 268 mμ.

Iodine Values: The iodine values of the fractions were determined by using the Wijs reagent (see Appendix A).

Saponification Equivalents: The saponification equivalents of all fractions were determined according to the method outlined in Appendix G.

The E (1%, 1cm.) Values: The E values were taken in a Beckman spectrophotometer, using a hydrogen lamp as light source, spectroscopically pure cyclohexane as solvent, and measuring the E values between 0.3 and 0.8 at the appropriate wavelengths.

The constants (iodine values, saponification equivalents and E (1%, 1cm.) values) were carried out in duplicate and where these values were not within 0.5 - 1% agreement, a third determination was carried out. The average of the two values was taken as iodine value, saponification equivalent and E (1%, 1cm.) for the calculation of the composition of each fraction.

(g) Attempted Purification of the Conjugated Diene Acid by Chromatography.

Experiment 1.

Chromatographic grade alumina ex Peter Spence was passed through 100 and 200 mesh screens and the portion -100+200 was used for the adsorption experiments.

By using Brockman's[86] dye test on a series of alumina of varying degrees of activity, an alumina of activity of grade IV-V was found to give the best results. The exact procedure for the preparation of the alumina is as follows: Hundred grams of alumina were placed in a small bottle and a few ml. of ethyl acetate and water added. (The ethyl acetate was added to de-activate the tendency of the alumina to hydrolyse the methyl esters during the adsorption.) The alumina was well shaken to ensure even distribution of the liquids and then heated at 145° for one hour. While hot, the alumina was poured into the adsorption tube (24 x 3.00 mm.) and the column well tapped to ensure good packing of the adsorbent; a small pad of cotton wool was placed on top of the alumina to prevent distortion of the surface on addition of the solvents. The methyl esters, dissolved in a small volume of light petroleum (b.p. 40°-60°) were then added, followed by the solvents listed in the table; in all cases care was taken not to allow the surface of the alumina to become dry. The fractions were collected in Erlenmeyers, the solvents distilled off through tared 25 ml. distilling flasks and the residues dried under vacuum. The results of the experiments are given in Table XV.

Table XV. /...

TABLE XV.

Fraction.	Eluant.	Weight.	E(1%,1cm.) *
Starting material	In 10 ml. light petroleum	1.0560	324.5
1	200 ml. light petroleum	0.0048	--
2	5 ml. ether and 95 ml. light petroleum	0.1991	277.8
3	60 ml. 10% ether in light petroleum	0.3848	527
4	80 ml. 10% ether in light petroleum	0.2623	523
5	60 ml. 10% ether in light petroleum	0.0555	387.5
6	50 ml. 50% ether in light petroleum	0.0081	487
7	60 ml. ether	0.0073	145.2
8	Mixture of benzene and alcohol	0.0948	111.7
	Recovery:	1.0167 (96.2%)	

* Note: Cell corrections were not applied.

Experiment 2. /...

Experiment 2.

The alumina was activated in the same way as in Experiment 1, but the weight of adsorbent was increased to 150 g.

TABLE XVI.

Fraction.	Eluant.	Weight.	E(1%,1cm.)
Starting material	Dissolved in 15 ml. light petroleum	1.0722	324.5
1	300 ml. light petfoleum	0.0035	--
2	100 ml. $2^1/_2$% ether in light petroleum	0.0024	--
3	100 ml. $2^1/_2$% ether in light petroleum	0.0075	--
4	100 ml. 5% ether in light petroleum	0.0576	275
5	100 ml. 5% ether in light petroleum	0.1299	367
6	100 ml. 10% ether in light petroleum	0.1662	522
7	100 ml. 10% ether in light petroleum	0.1775	500
8	100 ml. pure ether	0.0866	550
9	100 ml. pure ether	0.1382	529
10	100 ml. benzene and alcohol	0.0912	431
11	100 ml. absolute alcohol	0.0679	--
	Recovery:	0.9385 (87.4%)	

Note: Cell corrections have not been applied.

Experiment 3. /....

Experiment 3.

In this experiment silica gel (silica gel ex Davison Chemical Corporation, Baltimore, U.S.A. grade through 200) and Hyflo Supercel (ex John Mansville) in a ratio of 80:20 was used as adsorbent. The adsorbent (30 g.) was well mixed and activated by heating at 190-200° for $1^1/_4$ hours. The adsorbent was poured into the column and wetted with 60 ml. light petroleum (b.p. 40°-60°), followed by the solution of the mixed esters and the column developed as follows:

TABLE XVII.

Fraction.	Eluant.	Weight. (g.)	E(1%.1cm.) *
Starting material	In 15 ml. of light petroleum	0.9421	466
1	500 ml. of light petroleum	0.0072	-
2	70 ml. light petroleum	0.0025	-
3	70 ml. light petroleum	0.0044	-
4	70 ml. light petroleum	0.0050	-
5	70 ml. light petroleum	0.0064	-
6	70 ml. light petroleum	0.0054	-
7	70 ml. light petroleum	0.0068	-
8	70 ml. light petroleum	0.0087	-
9	100 ml. $1^1/_2$% ether in light petroleum	0.6040	475
10	70 ml. $1^1/_2$% ether in light petroleum	0.2501	542
11	70 ml. $1^1/_2$% ether in light petroleum	0.0028	-
12	75 ml. alcohol and benzene	0.0485	-
	Recovery:	0.9558	

* Cell corrections were not applied.

Note: The E value increased by about 20-25%, but the yield of the purified material was low. The increase in weight of the fraction indicated that the fractions were not well dried.

Experiment 4. /....

Experiment 4.

The adsorbent (50 g.) was activated by heating it at 200° for 2 hours. The residual methyl esters (after distillation of the solvent) were dried for at least half an hour under vacuum on the water bath. The column was wetted with 80 ml. light petroleum (b.p. 40°-60°) and the mixed esters added.

TABLE XVIII.

Fraction.	Eluant.	Weight. (g.)	E(1%,1cm.) *
Starting material	In 30 ml. light petroleum	1.0523	466
1	500 ml. light petroleum	0.0020	-
2	100 ml. $^1/_2$% benzene in light petroleum	0.0024	-
3	500 ml. $^1/_2$% benzene in light petroleum	0.0150	-
4	100 ml. 1% benzene in light petroleum	0.0235	14.3
5	100 ml. 1% benzene in light petroleum	0.0236	4.98
6	300 ml. 1% benzene in light petroleum	0.0293	-
7	75 ml. 2% benzene in light petroleum	0.0063	-
8	75 ml. 2% benzene in light petroleum	0.0068	-
9	75 ml. 5% benzene in light petroleum	0.0056	-
10	75 ml. 5% benzene in light petroleum	0.0026	-
11	75 ml. $^1/_2$% ether in light petroleum	0.0112	-
12	75 ml. $^1/_2$% ether in light petroleum	0.0042	-
13	75 ml. 10% ether in light petroleum	0.800	520
14	100 ml. pure ether	0.0948	-
	Recovery:	1.0079 (95.5%)	

* **Note:** Cell corrections have not been applied.

Experiment 5. /....

Experiment 5.

The mixed methyl esters of fraction 13 in the previous experiment were used. The adsorbent was activated at 200° for $2^1/_2$ hours, packed in the column and the column wetted with 60 ml. light petroleum (b.p. 40°-60°).

TABLE XIX.

Fraction.	Eluant.	Weight. (g.)	E(1%,1cm.)
Starting material in 25 ml. light petroleum		0.7672	520
1	200 ml. light petroleum		
2	200 ml. 1% benzene in light petroleum	0.0012	-
3	200 ml. 2% benzene in light petroleum	0.0045	-
4	100 ml. 5% benzene in light petroleum	0.0293	420
5	100 ml. 5% benzene in light petroleum	0.0429	440
6	100 ml. $7^1/_2$% benzene in light petroleum	0.0648	490
7	100 ml. $7^1/_2$% benzene in light petroleum	0.0584	-
8	100 ml. $7^1/_2$% benzene in light petroleum	0.0498	-
9	100 ml. $7^1/_2$% benzene in light petroleum	0.1077	560
10	100 ml. 10% benzene in light petroleum	0.0492	565
11	100 ml. 10% benzene in light petroleum	0.3496	558
12	120 ml. 15% ether in light petroleum	0.0071	-
	Recovery:	0.7645 (99.5%)	

(h) /....

(h) Isolation, Derivatives, Identification and Chemistry of Ximenynic Acid.

The methyl esters obtained from the fractional distillation of the liquid fatty acids, having saponification equivalent of 292-294 and E(1%, 1cm.) at $\lambda_{max.}$ 299 mμ of over 400, were combined and saponified, and the free fatty acids extracted according to the standard procedure (see Appendix C). The free fatty acids were dried under vacuum and crystallised from acetone as follows: A weighed quantity of acid (8.5 g.) was dissolved in 40 ml. dry acetone and the solution cooled in a methylated spirits bath. The solution was cooled to -30°C and kept there for three to four hours and the crystals collected on a cooled Buchner filter. The crystals were dried under vacuum, yielding 3.29 g. of solid acid with E (1%, 1cm.) of 508. This was again recrystallised from acetone (15 ml.) at -30°C, yielding partly transparent plates (1.5 g.) with E (1%, 1cm.) of 552. Recrystallisation of this material from 7.5 ml. light petroleum (b.p. 50°-60°) at -5°C yielded 0.759 g. of plates with E (1%, 1cm.) of 575, m.p. 38°-39°C.

Further recrystallisation from 4 ml. light petroleum (b.p. 50°-60°) at -5°C yielded partly transparent plates having E (1%, 1cm.) of 583 at $\lambda_{max.}$ 229 mμ and m.p. 39°-40°C (corrected).

Recrystallisation of this compound did not increase the E value or the melting point.

<u>Analysis</u>: Calculated for $C_{18}H_{30}O_2$:-C, 77.65; H, 10.86; Neutralisation equivalent, 278.4

Found: C, 77.68; H, 10.81; N.E., 278.9

Refractive Index: n_D^{40}, 1.4741, taken with an Abbe Refractometer.

Density: 1.045 (25°) using the modified Fryer and Weston method[17] with a salt solution and water.

The /....

The E (1%, 1cm.) of the compound at $\lambda_{max.}$ 268 mμ was 2.5 and indicated the presence of a small quantity of a conjugated triene acid (ca. 0.1%).

Ximenynic acid crystallised in flat, partly transparent plates. It was very soluble at room temperature in acetone, alcohol, petroleum ether, chloroform, benzene and also in weak aqueous sodium hydroxide and sodium carbonate solutions; it dissolved in the latter with evolution of carbon dioxide. With concentrated sulphuric acid it gave an intense red coloration. No precipitate formed on the addition of 2,4-dinitrophenylhydrazine solution to an alcoholic solution of the acid, and ketone groups are therefore absent.

Hydrogenation of Ximenynic Acid.

The hydrogenation was carried out in a Towers micro-hydrogenation apparatus, using palladium on calcium carbonate catalyst and alcohol as solvent. After reduction of the catalyst, the sample was added and the shaking continued. The following table summarised the results of the hydrogenation of three different samples of acid

	1	2	3
Wt. of sample (g.)	0.01240	0.01077	0.01393
Vol. of the H_2 absorbed (ml.)	3.537 at 751.5 mm. and 293 A°	2.910 at 756.1 mm. and 293.3 A°	3.666 at 754.6 mm. and 294 A°
Moles H_2 absorbed	3.09	3.10	3.01

The alcohol solution of the saturated acid was centrifuged to remove the catalyst, and reduced to small volume and left for crystallisation. White plates crystallised out, m.p. 68°-69°, which were recrystallised from methanol, yielding white plates, m.p. 69.3°-70°. Mixed melting point determination with pure stearic acid (m.p. 69°-69.5°) did not give a depression, and ximenynic is consequently a C_{18} acid.

Derivatives /....

Derivatives of Ximenynic Acid.

Methyl Ximenynate. The methyl ester was prepared and isolated according to the general procedure for the preparation and isolation of methyl esters of fatty acids (Appendix L).

From 20 g. of acid (0.072 mole) after refluxing with 100 ml. methyl alcohol and 10 drops of concentrated sulphuric acid, 21.00 g. of methyl ximenynate were isolated. (Yield, quantitative), b.p. 148°/0.2 mm; 163-165°/0.4 mm; 172-174°/0.8 mm; refractive index (25°), 1.4700; specific gravity (25°/25°), 0.9067; E (1%, 1cm.) at $\lambda_{max.}$ 229 mμ, 549.2. Analysis: Calculated for $C_{19}H_{32}O_2$: C, 78.02; H, 11.03; Sap. Eq. 292.4. Found: C, 77.52; H, 11.10; Sap. Eq., 292.9.

The methyl ester turned yellow on standing in the air, and from the analysis the oxygen uptake was more rapid than originally anticipated, because after half an hour's exposure to the air, the compound showed the following analysis: C, 76.45; H, 10.67.

Ethyl Ximenynate. The procedure for the preparation of the ethyl ester was essentially the same as that for the preparation of the methyl ester. From 5 g. of ximenynic acid (0.00179 mole), after refluxing with 25 ml. of absolute alcohol and 5 drops of concentrated sulphuric acid, there was isolated 5.4 g. (98.2%) of impure ester. The ethyl ester was fractionated through a pear-shaped column (and a pig adapter) and only the middle fraction collected, b.p. 168-169°C/0.2 mm.; refractive index (25°C) 1.4680. Analysis: Calculated for $C_{20}H_{34}O_2$: C, 78.25; H, 11.25; Sap. Eq. 306.6. Found: C, 78.44; H, 11.21; Sap. Eq. 306.9; E (1%, 1cm.) at $\lambda_{max.}$ 229 mμ, 525.

The Amide of Ximenynic Acid. The amide of ximenynic acid was prepared according to the procedure outlined by Shriner and Fuson[88]. The thionyl chloride used in the reaction was purified according to the method outlined by Fieser[89]. Ximenynic acid (2.2 g., 0.0008 mole) was refluxed in 20 ml. purified thionyl chloride for 15 minutes. The solution was cooled and cautiously poured into 50 ml. cold concentrated ammonia. The precipitated amide was collected on a Buchner filter, washed with cold water and crystallised from 50% aqueous solvent. Yield: 1.8 g. (82%), m.p. 104-5°-105°C; second crystallisation from 80% alcohol, m.p. 105°-105.5°; third crystallisation, 105°-105.5°; yielding small, yellow, nest-like crystals.
Analysis: Calculated for $C_{18}H_{31}ON$: C, 77.92; H, 11.26.
Found: C, 78.31; H, 11.29.

p-Phenyl-phenacyl and p-Bromo-phenacyl Esters of Ximenynic Acid. The p-phenyl-phenacyl and p-bromo-phenacyl esters of ximenynic acid were prepared according to the general method outlined by Shriner and Fuson[88]. About 1 g. of acid was dissolved in 5 ml. of water containing excess potassium hydroxide and the solution neutralised with N/3 HCl until just acid to phenol phthalein. A solution of 1 g. of the phenacyl bromide in 10-15 ml. alcohol was added to the potassium salt and the solution refluxed for 1-2 hours. The solution was allowed to cool and the precipitated ester recrystallised from 96% alcohol.

p-Phenyl-phenacyl Ester. Slightly yellow needles, m.p. 61.5°-62°. Analysis: Calculated for $C_{32}H_{40}O_3$: C, 81.31; H, 8.53. Found: C, 81.31; H, 8.66.

p-Bromo-phenacyl Ester. Slightly yellow needles, m.p. 53.75°-54.25°. Analysis: Calculated for $C_{26}H_{35}O_3Br$: C, 65.66; H, 7.44. Found: C, 65.84; H, 7.53.

Ximenynyl /....

Ximenynyl Alcohol. Ximenynic acid was reduced according to the method of Nystrom and Brown[90]. The experimental procedure was essentially the same as that outlined by the authors. The apparatus consisted of a 250 ml. round-bottomed ground glass flask, fitted with a dropping funnel and condenser, while moisture from the air was excluded by means of drying tubes containing silica gel.

Finely powdered lithium aluminium hydride (0.261 g., 0.0069 mole) was refluxed with specially dried ether for two hours. A solution of 1.39 g. (0.005 mole) of ximenynic acid in 20 ml. dried ether was slowly added to the lithium aluminium hydride and the solution finally refluxed for 30-40 minutes. The solution was cooled in an ice bath and the lithium complex decomposed by the addition of 10% sulphuric acid, and the reaction product isolated by extraction with ether. The ether extracts were combined, washed once with 10% sulphuric acid and once with cold water and a few times with a 5% potassium carbonate solution to remove any unreacted acid.

The ether extract was finally dried over anhydrous magnesium sulphate and the ether removed by distillation, yielding 1.35 g. (97%) of ximenynyl alcohol. The product was distilled (b.p. 172°-175°/1 mm.) and the main fraction was once crystallised from light petroleum, m.p. 26°-27°, which rose to 30°-31° on further crystallisation; refractive index (25°C), 1.4790; refractive index (40°C), 1.4726; E (1%, 1cm.) at $\lambda_{max.}$ 229 mμ, 591.5 (in cyclohexane).
Analysis: Calculated for $C_{18}H_{32}O$: C, 81.75; H, 12.2.
Found: C, 81.9; C, 12.2.

Reduction of ximenynyl alcohol with a larger excess of lithium aluminium hydride yielded a product with the same absorption spectrum.

Hydrogenation /....

Hydrogenation of Ximenynyl Alcohol. The hydrogenation of ximenynyl alcohol was carried out in a Tower's micro-hydrogenation apparatus, using ethyl alcohol as solvent and palladium on calcium carbonate catalyst.

0.01399 g. of alcohol absorbed 3.893 H_2 at 751.7 mm. and 290°A and 0.01666 g. of alcohol absorbed 4.397 ml. H_2 at 751.5 mm. and 290°A, this being equal to 3.05 and 2.89 moles of hydrogen respectively. The hydrogen absorption was faster than that of the acid; within five minutes 78% of the total amount of hydrogen was absorbed.

The catalyst was centrifuged off, and the alcohol reduced to a small volume, yielding white plates, m.p. 58.5°-60°, mixed m.p. with an authentic sample of octadecyl alcohol (octadecane-1-ol), m.p. 59°-60°, did not give any depression.

Urethanes. The naphthyl and phenyl urethanes of ximenynyl alcohol were prepared from the corresponding isocyanates according to the method described by Shriner and Fuson[88]. The products were recrystallised four times from light petroleum, yielding white plates.

Naphthyl urethane: m.p. 57°-57.5° Analysis: Calculated for $C_{29}H_{39}NO_2$: C, 80.32; H, 9.05. Found: C, 80.35; H, 9.05.

Phenyl urethane: m.p. 69°-69.5°. Analysis: Calculated for $C_{25}H_{37}NO_2$: C, 78.28; H, 9.72. Found: C, 78.45; H, 10.05.

Ximenynyl Chloride. Ximenynyl chloride was prepared by treatment of the alcohol (1 g.) with purified thionyl chloride (10 ml.) in chloroform in the cold. The chloroform solution was then refluxed for about fifteen minutes and cooled. The solution was poured into ice water (25 ml.) and then washed with water to remove any free acid. The

chloroform /...

chloroform solution was dried over anhydrous sodium sulphate and the residual oil fractionated under vacuum: b.p. 148°/ 1 mm; E (1%, 1cm.) at $\lambda_{max.}$ 229 mμ, 546.

<u>Analysis</u>: Calculated for $C_{18}H_{31}Cl$: C, 76.5; H, 11.06; Cl, 12.53. Found: C, 75.71; H, 11.88; Cl, 12.81.
From the analysis the compound was impure due to oxidation.

<u>Oxidation of Ximenynic Acid</u>. The acid was oxidised according to the method described by Brown and Farmer[91] for elaeostearic acid. <u>Ximenynic acid</u> (1.7 g., 0.0061 mole) was dissolved in 400 ml. distilled water, containing 1.7 g. hydrated sodium carbonate and 0.5 ml. potassium hydroxide. The solution was cooled to 5° and with good stirring a 7.5% solution of potassium permanganate in distilled water slowly added. Slight excess of permanganate solution was added and the solution was allowed to stand for about ten hours at room temperature, and the excess permanganate removed with sulphur dioxide. The manganese dioxide mud was filtered off and the filter cake washed several times with small volumes of hot water; the total filtrate was then concentrated to about 500 ml.

The resulting solution was just acidified with hydrochloric acid and then distilled until about 50 ml. of liquid was left in the flask; this distillate was allowed to drip into a solution of alkali. A further 150 ml. of water was added to the distillation flask and the distillation continued until a further 150 ml. of distillate had passed into the alkali solution. The distillate was concentrated to about 50 ml., allowed to cool to room temperature, just acidified with hydrochloric acid (10%) and the aqueous phase extracted four times with ether. The dried ethereal solution was distilled off through a 25 ml. distilling flask, yielding 0.5 g. (63% yield) of an acid with a b.p. of 219°-220° (micro-boiling point method); m.p. of <u>p</u>-phenyl-phenacyl ester 57°-58°; <u>p</u>-phenyl-phenacyl ester of heptanoic acid, m.p.

57.7° /...

57.7°-58°, with no depression on mixed melting point determination.

From the aqueous liquid in the distilling flask, white crystals were obtained which were combined with more material extracted with ether. Yield: 1.01 g. (87.5%). This material was crystallised once from a small volume of water, yielding white crystals m.p. 100°-104°, second crystallisation 104°-106°, third crystallisation 105°-106°; mixed melting point determination with azelaic acid - no depression.

The residual mother liquor (after extraction with ether) was heated to remove the excess ether, and the solution then cooled and half of this solution used for the determination of oxalic acid. Fifteen ml. of concentrated sulphuric acid were added to the solution and the oxalic acid titrated with N/10 permanganate.

Volume of 0.09 N $KMnO_4$ used = 15.5 ml.
≡ 31 ml. for the total solution
≡ 0.1758 g. of oxalic acid
(22.75% yield)

The acid, on oxidation, yielded therefore three acids: Isolation?

Heptanoic acid $CH_3(CH_2)_5COOH$,
Azelaic acid $HOOC.(CH_2)_7COOH$
and Oxalic acid $HOOC.COOH$, giving a fatty acid with eighteen carbons.

<u>Ozonolysis of Ximenynic Acid</u>. A stream of ozonised oxygen containing 2.5% of ozone was passed through a solution of ximenynic acid (3 g.) in dry glacial acetic acid (20 ml.) for 150 minutes, the solution being kept at 15°-20° to avoid excessive loss of acetic acid[92]. Zinc dust (6.5 g.) and a trace of water were added to the solution; the whole was refluxed with stirring for 90 minutes and then water (5 ml.) was added. The product was filtered through a Buchner funnel,

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the filter being well washed with ether; the combined filtrate was washed with water, dried over calcium chloride and the ether was removed by distillation through a column. The residue was fractionally distilled to give

(a) b.p. 100°-120°/650 mm. (ca. 3 g.),
(b) b.p. 120°-123°/650 mm. (ca. 2 g.),
(c) b.p. 123°/650 mm. - 60°/20 mm. (0.2 g.),
(d) b.p. 160°-175°/0.5 mm. (0.18 g.),
(e) b.p. 175°/0.5 mm. (0.5 g.).

(a) was chiefly acetic acid; (b) contained n-heptaldehyde, it yielded a semicarbazone (0.3 g.) giving m.p. 107°-109° after one recrystallisation from aqueous alcohol. (Found: C, 56.34; H, 10.55%. Calculated for $C_8H_{17}N_3O$: C, 56.1; H, 10.05%: the melting point being undepressed in admixture with an authentic sample of n-heptaldehyde semicarbazone of m.p. 108°-109°); (c) also contained n-heptaldehyde (oxime, m.p. 54°-55°; melting point undepressed in admixture with n-heptaldehyde oxime of m.p. 54°-55°); (d) consisted chiefly of azelaic acid which was obtained in quantity (0.40 g.) by recrystallisation of (e) from ethyl acetate and iso-heptane giving m.p. 106°-107°, undepressed in admixture with an authentic sample of m.p. 106°-107° (Found: C, 57.66; H, 8.88%; E.Wt. = 96.7. Calculated for $C_9H_{16}O_4$: C, 57.44; H, 8.56%; E.Wt. - 94.1).

The mother liquors from the recrystallisation of (e) were evaporated down and boiled with an excess of semicarbazide to give a precipitate (15 mg.) of m.p. 141°-140° which after four recrystallisations from aqueous alcohol, yielded ca. 1 mg. of material having m.p. 158°-160° (azelaic semi-aldehyde semicarbazone has m.p. 162°)[93].

The different fragments obtained through ozonolysis were therefore heptaldehyde and azelaic acid.

Reactions /...

Reactions of Ximenynic Acid.

(1) Attempted Elaidinisation.

Ximenynic acid was elaidinised according to Bertram's method described by Hilditch[94]. An oil bath was heated to 200°C and kept at that temperature during the reaction.

The Ximenynic acid (1.40 g.) and 0.007 g. selenium metal were placed in a 1 inch pyrex tube and placed in the oil bath for two hours; a control without any selenium metal was placed in the bath for the same time. During the reaction carbon dioxide was bubbled through the acid to prevent autoxidation. The tubes were cooled to room temperature and the partly solid material dissolved in a small volume of light petroleum (b.p. 40°-60°); part of the material, however, separated out as an oil, from which the solution was decanted. The petroleum solution was placed in the refrigerator, yielding 0.44 g. of white crystals, m.p. 37.5°-38°; the mother liquor yielded more crystals (0.01 g.), m.p. 38°-40°, while more crystals were obtained on evaporation of the solvent (0.029 g.). The control yielded crystals m.p. 39°-40°. These crystals did not give depression on mixed melting point determination with pure ximenynic acid.

Attempted Reaction of Ximenynic Acid with Maleic Anhydride:

Ximenynic acid was reacted with double distilled maleic anhydride in an attempt to prepare the Diels-Adler adduct[95].

One and a half grams (0.0054 mole) of ximenynic acid and maleic anhydride (0.47 g., 0.005 mole) were placed in a Pyrex test tube and heated at 120° under CO_2. The cooled residue was extracted several times with hot petroleum and the petroleum solutions combined and reduced to a small volume. On cooling in the refrigerator, 1 g. acid was obtained, m.p. 38.5°-40° (no depression on mixed melting

point /....

point determination with ximenynic acid). The residual material from the petroleum extraction was crystallised from a small volume of chloroform, yielding small white crystals m.p. 47°-54°, which melted at 54°-56° after one crystallisation; maleic anhydride melts at 56°; no depression on mixed melting point determination with pure maleic anhydride.

Attempted Reduction of Ximenynic Acid to the Dienoic Acid.

The attempted reduction of ximenynic acid to the dienoic acid was carried out using a poisoned palladium-charcoal catalyst, according to the method used by Isler *et al.*[46].

The acid (0.0411 g.) was dissolved in 15 ml. alcohol and 0.1 g. of poisoned catalyst added. After the absorption of about 3.43 ml. hydrogen (1 mole), the hydrogenation was stopped and the product isolated, showing an E (1%, 1cm.) of 232 at $\lambda_{max.}$ 229 mμ.

The hydrogenation was thus largely unselective, which was expected from the fact that the volume of gas was absorbed within 5 minutes.

In a private communication, Dr. Isler stated that the presence of any acid destroys the toxicity of the catalyst and another experiment was conducted on ximenynyl alcohol, using a poisoned catalyst obtained from the laboratories of Messrs. Hoffman-La Roche, Switzerland. The alcohol (0.056 g.) was reduced until 1 mole of hydrogen was absorbed (approx. 4.5 ml. of hydrogen); the hydrogen absorption took place within 5 minutes, after which the reduction was stopped and the product isolated. This material had an E (1%, 1cm.) 670 at $\lambda_{max.}$ 229 mμ, which indicated that the hydrogenation was still largely unselective.

(The E (1%, 1cm.) for $\Delta^{9:11}$-octa-deca-dienoic acid is approximately 1200[21,22] for $\lambda_{max.}$ 229-234 mμ, while Schmid

and /....

and Lehman[23] reported to have prepared an octa-dienoic acid with E (1%, 1cm.) of 1028 (log E = 4.46 at $\lambda_{max.}$230.5).

Methyl ximenynate was reduced with absolute alcohol and sodium according to the method of Adams and Marvel[96]. Methyl ximenynate (1.5 g., 0.005 mole) was dissolved in 20 ml. absolute alcohol contained in a 50 ml. round-bottomed flask fitted with a reflux condenser. Clean cut sodium (1.2 g., 0.05 g.-atom) was slowly added and the total solution refluxed for 2 hours. Extraction with ether, followed by extraction of the ether solution with a 5% potassium carbonate solution and evaporation of the ether, yielded 1.1 g. of material having E (1%, 1cm.) of 464.7 at $\lambda_{max.}$232.

Behaviour of Ximenynic Acid on Alkali Isomerisation.

Ximenynic acid was treated with potassium hydroxide in ethylene glycol at 180° according to the method outlined in Appendix K. A small quantity (about 0.1 g.), accurately weighed, was isomerised at 180° for 25 minutes and the solution quickly cooled. The solution was dissolved in purified 96% alcohol (see Appendix A) and made up to 100 ml. in a measuring flask; it was stored overnight and the absorption spectrum determined between 300 and 220 mμ.

Similar spectra were determined for the acid after one and two hours of alkali treatment at 180°. The isomerised acids were isolated as follows: After isomerisation at 180° the potassium hydroxide-ethylene glycol reagent was poured into 25 ml. of water and the solution just acidified with 10% hydrochloric acid. This solution was extracted with two portions of light petroleum (15 ml. each) and the petroleum solutions combined and freed of excess hydrochloric acid by shaking a few times with water. The petroleum solution was dried over anhydrous sodium sulphate and the petroleum

distilled /....

distilled off through a 25 ml. distilling flask.

The residual oily liquid was dried under vacuum and the absorption spectrum determined in cyclohexane. The E (1%, 1cm.) values at $\lambda_{max.}$ 268 and 229 mμ are listed in Table X, while the spectra are given in Fig. 3.

Isolation of the Isomerised Acid.

Ximenynic acid (25 g.) was treated with alkali in ethylene glycol at 190°C for 1 hour under nitrogen and the isomerised acids isolated as described above. The syrupy acids were dissolved in light petroleum (b.p. 40°-60°, 10 ml./g. of acids) and stored overnight in the refrigerator.

Some syrupy material, which separated out, was removed by decantation, and the clear solution cooled to -60°C. The white precipitate was collected on a cooled Buchner filter and recrystallised twice from a small volume of light petroleum at -10°C, yielding 0.6 g. of white crystals, m.p. 60°-61°C.

The isomerised acid was soluble in alkali and in most of the organic solvents. It autoxidised very rapidly; after 2-3 hours exposure to the atmosphere the melting point dropped to 57°C, and after 12 hours an amorphous oily solid formed.
Analysis: Calculated for $C_{18}H_{30}O_2$: C, 77.65; H, 10.86.
Found: C, 74.47; H, 10.47; E (1%, 1cm.) 1705 at $\lambda_{max.}$ 269 mμ, and E (1%, 1cm.) 130.1 at 229 mμ (no maximum).

The original petroleum solution was cooled to -75°C, but no more material crystallised out.

Oxidation of the Isomerised Acid.

The crystalline acids (0.4 g.) obtained by crystallisation from light petroleum (b.p. 40°-60°) with value of E (1%, 1cm.) 1705, were dissolved in 100 ml. water containing 1 g. of potassium /...

potassium carbonate and 1 ml. N/2 aqueous potassium hydroxide. The solution was cooled to below 5° and a 2% solution of potassium permanganate solution slowly added with stirring. Excess permanganate solution was added, the solution allowed to come to room temperature and left to stand overnight. The excess permanganate was removed with SO_2 and the manganese dioxide mud collected on a Buchner filter. The precipitate was washed a few times with hot water (2-3 times) and the filtrate collected, acidified and the volume of solution reduced by distillation. The distillate was collected in an alkali solution. About 500 ml. more water were added to the distilling flask and this volume again distilled off and the volume in the flask finally reduced to about 50 ml. This, on cooling to room temperature, yielded white crystals (0.3 g.), m.p. below 80°C., which were crystallised twice from benzene, yielding white crystals, m.p. 105.5°-106.5°. Mixed melting point determinations with pimelic acid and azelaic acid gave a lowering of 20°C. Found: C, 62.45 ; H, 9.39.

The distillate, which was reduced to a small volume, acidified and extracted with ether, yielded 0.15 g. of liquid fatty acids, b.p. 224°-226°C. p-Phenyl-phenacyl ester, m.p. 60·5°-62°C which gave a lowering on mixed melting point determination with that from heptanoic acid.

Iodine Value of Ximenynic Acid.

The following iodine value reagents have been used to determine the iodine values of ximenynic acid and its methyl ester:
(a) Woburn's reagent, (b) Modified Rosenmund-Kuhnhenn reagent, (c) Wijs reagent, (d) To complete the table, the results obtained with bromine vapour are included. The different reagents are described in Appendix A, and the results obtained have been summarised in Table XI.

Urea /....

Urea Complexes of Ximenynic Acid and Derivatives.

The urea complexes were prepared according to the method of Schlenk and Holman[60] (*loc. cit.*). A saturated solution of urea in methanol (16-20 g. per 100 ml.) was prepared by warming the solution on the water bath for a short while; the solution was allowed to stand for a few days and only the clear liquid was used in the experiments.

One gram of acid (or methyl ester or alcohol) was dissolved in 30 ml. of the urea-methanol solution (slight warming) and the solution cooled to room temperature for a few hours. The crystals were collected on a Buchner filter, washed with a little ice-cold methanol and dried under vacuum. The E value was determined in an alcoholic solution at 229 mμ. The "yield values" (see ref.), melting points and E values of the urea complexes are given in the following table:

TABLE XX.

Urea Complexes of Ximenynic Acid and Derivatives.

	Ximenynic Acid	Methyl Ximenynate	Ximenynyl Alcohol
Yield value	1.55	1.0	1.0
E (1%, 1cm.)	132	132.6	150.4
Melting point	130.5-131.5°	133-135°	132-133°
Mole ratio of urea to compound in complex	16.00	15.25	12.1

The urea complexes of the total fatty acids of ximenia oils (see Holman and Schlenk) were also prepared. The oil was saponified and the unsaponifiable material extracted. The fatty acids were isolated and converted to the methyl esters.

10 g. of esters were dissolved in 300 ml. of urea-methanol solution, and the total solution crystallised at 31°

for /....

for three hours. (Fraction A). The crystals were collected on a Buchner filter and the filtrate cooled to room temperature and a second batch of crystals collected after 8 hour's standing (Fraction B). Each fraction was dissolved in water containing a few ml. of concentrated hydrochloric acid, and the liberated methyl esters extracted with light petroleum (b.p. 40°-60°). The petroleum solutions were distilled through tared flasks and dried. The results of the experiment have been tabulated in Table XXI.

TABLE XXI.

Separation of the Methyl Esters of Ximenia Oil through the Urea Complexes.

Fraction.	Weight of Ester.	E(1%, 1cm.) at 229 mμ.
A	5.6	57.1
B	2.8	277
C	0.8	279

Fraction B was dissolved in 90 ml. of methanol solution, and left for crystallisation. The crystalline material, however, yielded a methyl ester with E (1%, 1cm.) of 279.

(i) Isolation of Ximenic Acid.

The methyl esters with saponification equivalent 410-412, iodine value 39-40 were saponified and the total acids (7.35 g.) dissolved in 180 ml. light petroleum (b.p. 40°-60°). The solution was allowed to cool to room temperature and the solid acids collected on a Buchner filter and dried. The petroleum in the filtrate was removed by distillation and the residual acids dried, yielding:

Insoluble acids: Wt. 3.00 g. I.V. 3.22, m.p. 75°-77°

Soluble acids: Wt. 4.35 g. I.V. 59.7, m.p. 45°-47°

The /....

The soluble acids were dissolved in light petroleum (10 ml. per g.) and the solution crystallised at 8° and the crystals collected on a Buchner filter; the mother liquor was cooled to 0°, the crystals again collected on a Buchner filter and dried, yielding 2.25 g. material, m.p. 45°-46.5°, I.V. 60. Recrystallisation from acetone gave a product m.p. 45°-45.5°, I.V. 62.3, N.E. 399.9 (Theory: N.E. 394.7, I.V. 64.3) <u>Analysis</u>: Calculated for $C_{26}H_{50}O_2$: C, 79.1; H, 12.77. Found: C, 79.04; H, 12.94. The neutralisation equivalent of this product indicated that it was contaminated with lumeniqueic acid, but it could not be purified by further crystallisation. Catalytic hydrogenation in acetic acid as solvent yielded an acid, m.p. 82°-83°, which increased to 83°-84° on recrystallisation from acetone (reported 87.5°-87.7°). <u>Analysis</u>: Calculated for $C_{26}H_{52}O_2$: C, 77.8; H, 13.18. <u>Found</u>: C, 78.13; H, 12.8; 0.02463 g. of acid absorbed 1.418 ml. H_2 at 762.6 mm. and 288°A, 0.03351 g. of acid absorbed 1.918 ml. of H_2 at 761.3 mm. and 288°A; which calculated to 0.965 and 0.958 moles of hydrogen, respectively. The melting point of cerotic acid indicated, too, that ximenic acid was still impure.

<u>Dihydroxy cerotic acid</u>.

Ximenic acid (1.0 g., 0.0025 mole) was oxidised with Milas's reagent[64] (hydrogen peroxide in t-butyl alcohol and osmium tetroxide catalyst) and the solution allowed to stand at room temperature for a few days. A solid material crystallised out, m.p. 109°-111°, which was extracted with light petroleum and crystallised twice from acetone, yielding small pearl-like crystals, m.p. 119°-120° (reported 118°-119°), yield 0.5 g. <u>Analysis</u>: Calculated for $C_{26}H_{52}O_4$: C, 73.0; H, 12.28. Found: C, 73.25; H, 12.10.

<u>Oxidation</u> /....

Oxidation of Ximenic acid.

Ximenic acid (1 g., 0.0025 mole) was dissolved in 200 ml. dry acetone and oxidised with excess potassium permanganate according to the method of Armstrong and Hilditch[97]. The oxidation was continued for 10-12 hours and the acetone removed by distillation. The brown residue was cooled and suspended in 250 ml. ice-cold water and the excess permanganate and manganese dioxide reduced with SO_2. The solution was then acidified and extracted several times with ether. The ether solutions were combined and shaken carefully with a 10% potassium carbonate solution. The potassium carbonate solutions (about 250 ml.) were combined, acidified with HCl and distilled to a small volume to remove the liquid volatile acids. More water was added to the distilling flask and the same volume of liquid collected in the distillate. The distillate was extracted several times with ether, and the ether distilled through a 25 ml. flask, yielding 0.25 g. of liquid acids with a pronounced cocoanut flavour, b.p. 240°-245°, refractive index 1.4385, amide m.p. 96°-98°. (Reported for pelargonic acid, b.p. 254.4°, refractive index 1.4345, amide m.p. 99°.) The residue from the flask was saponified and the fatty acids extracted with ether, yielding 0.6 g. of material, m.p. 80°-100°. On recrystallisation from alcohol this yielded acids with m.p. 105°-110°. (Reported for octadecane-1,15-dicarboxylic acid 110°-118°.)[98]

(j) Lumeniqueic Acid.

Lumeniqueic acid was isolated according to the same method as that described for ximenic acid. The methyl esters, with saponification equivalent over 450, were saponified, the acids (22 g., I.V. 47-48) dissolved in light petroleum (b.p. 40°-60°) (2000 ml.) and the insoluble acids (at room temperature) collected on a Buchner filter, yielding

10.3 g. /...

10.3 g. of material, I.V. 36. 17.5 g. of material, obtained by cooling the mother liquor to -10°, was recrystallised from light petroleum at room temperature, yielding crystals of weight 6.00 g., I.V. 53.6; further cooling to 0° yielded 9.1 g. of material, N.E. 452.6, I.V. 54.5, m.p. 56°-57°. The material was recrystallised from a small volume of acetone to give acids, m.p. 56°-57° <u>Analysis</u>: Calculated for $C_{30}H_{58}O_2$: C, 79.93; H, 12.97; N.E., 450.8; I.V., 56.31. Found: C, 79.81; H, 12.93; N.E., 452.6; I.V., 54.5. Hydrogenation with palladium calcium carbonate catalyst, yielded white, pearl-like crystals, m.p. 91°-92°. This material was recrystallised once from acetone.
Analysis: Calculated for $C_{30}H_{60}O_2$: C, 79.7; H, 13.37. Found: C, 79.73; H, 13.37. (Reported m.p. 93.4°-93.9°.) 0.03251 g. of acid absorbed 1.630 ml. of H_2 at 774.7 mm. and 287.8A°, 0.94 mole H_2. 0.03544 g. of acid absorbed 1.890 ml. of H_2 at 757.7 mm. and 288A°, 1.04 mole H_2.

Methyl Lumeniquate.

The methyl ester was prepared according to the general procedure outlined in Appendix L. The methyl ester was crystallised once from petroleum, yielding white crystals, m.p. 29.75°-30.5°. Calculated for $C_{31}H_{60}O_2$: C, 80.1; H, 13.01. Found: C, 79.76; H, 12.85.

Dihydroxy-mellisic Acid.

Lumeniqueic acid was oxidised according to the method of Milas *et al.*, using hydrogen peroxide in t-butyl alcohol and osmium tetroxide catalyst. From 0.5 g. of acid, 0.5 g. of impure acid was isolated, m.p. 80°-90°. This material was extracted with light petroleum, and the residue recrystallised from acetone, yielding 0.1 g. of material, m.p. 123°-124°. Analysis: Calculated for $C_{30}H_{60}O_4$: C, 74.5; H, 12.47. Found: C, 74.25; H, 12.55.

Oxidation /....

Oxidation of Lumeniqueic Acid.

Lumeniqueic acid was oxidised according to the method described for ximenynic acid. One gram of acid, gave 0.25 g. of liquid acid, having a pronounced cocoanut flavour, and 0.5 g. of solid acids. The liquid acids had b.p. 240°-245°, refractive index 1.4370, amide 97°-98°. Mixed melting point determination with amide of pelargonic acid did not show a depression.

The solid fatty acids (m.p. 80°-100°) were crystallised several times from acetone and yielded a few crystals, m.p. 108°-112° (reported for nonane-1,19-dicarboxylic acid, 112°-113°)[99].

(k) Cerotic Acid.

The saturated acids obtained from the isolation of ximenic and lumeniqueic acids, were combined and crystallised once from light petroleum (b.p. 60°-80°C) to give solid fatty acids, m.p. 78°-80°, I.V. 0.5-1.0, N.E. 390. The acids were converted into the methyl esters, by refluxing with five volumes of methyl alcohol and 2% concentrated sulphuric acid for four hours and extracting the methyl esters with ether.

After extraction with 5% of potassium carbonate to remove the excess acids, the ether was removed by distillation and the residue dried under vacuum. The methyl esters (20.5 g.) were then distilled through a Piros-Glover still under reduced pressure (1 mm. ± 0.05 mm.) and the fractions were cut on the basis of boiling point. The methyl esters with m.p. 63.5° were collected and analysed. Analysis: Calculated for $C_{27}H_{54}O_2$: C, 79.0; H, 13.2; S.E., 412. Found: C, 79.16; H, 13.16; S.E., 410.7.

The /....

The free fatty acids were crystallised twice from acetone, yielding white, pearl-like crystals, m.p. 86°-87°C (reported 87.5°-87.7).

(l) Identification of Oleic Acid.

The sodium salt of the acids (1.5 g.) from the methyl esters with S.E. 295-296, I.V. 84-85, from *Ximenia caffra* Sond. was oxidised with a 2.5% potassium permanganate solution below 5°, and the dihydroxy acids isolated as described for erucic acid. The solid acids were extracted with light petroleum (b.p. 40°-60°) and crystallised twice from acetone, yielding white plates, m.p. 130°-131°, no depression on mixed melting point determination with dihydroxy stearic acid prepared from pure oleic acid.

(m) The absence of Erucic Acid.

The methyl esters of fractions with saponification equivalent 364-366 from *Ximenia caffra* Sond. were saponified and the acids extracted with ether. The acids so obtained were oxidised according to the method of Kaufmann and Fiedler[100]: the acids (1.5 g.) were dissolved in 200 ml. water containing 2 g. of potassium carbonate and 2 ml. of N/2 potassium hydroxide. The solution was cooled to below 5° and, with stirring, a 1% solution of potassium permanganate slowly added until a permanent pink colour persisted. The excess permanganate and manganese dioxide were removed with SO_2, the solution acidified and the solid acids collected on a Buchner filter. These were extracted with hot petroleum (b.p. 60°-80°); and the acids crystallised from alcohol, yielding acids with m.p. below 100°; repeated crystallisations did not give dihydroxy-behenic acid (m.p. 130°). Erucic was consequently reported as being absent.

6. Investigation of the Unsaponifiable Material.

(a) Investigation of the Crystalline Material (Residue B).

The isolation of this material was described in the section on the extraction of the oil (see p. 57). Owing to the gelatinous nature of the material it was impossible to remove all the adhering oil. The material was dissolved in 100 ml. N/2 alcoholic KOH and refluxed for 20 minutes. The solution was cooled and the unsaponifiable material extracted according to the S.P.A. method (see Appendix C); the ether was removed by distillation, leaving a white crystalline solid. This was extracted with a few volumes of hot petroleum (b.p. 60°-80°) and the material collected on a Buchner filter, washed with a few ml. of light petroleum and dried. It was insoluble in hot and cold petroleum, benzene and water, but soluble in hot alcohol, ether, acetone, chloroform, acetic acid and acetic anhydride; it was also insoluble in 5% sodium hydroxide, sodium carbonate and hydrochloric acid solutions. It dissolved in concentrated sulphuric acid with the formation of a red colour; the material burned away completely in a hot flame and qualitative tests for nitrogen, sulphur and the halogens were negative. Tests for aldehyde and ketone groups were also negative. A solution of the material in acetic anhydride gave a purple coloration on addition of a few drops of concentrated sulphuric acid; on standing this colour changed into a reddish-brown.

A weak solution of the material in chloroform, did not give any colour on addition of a few drops of acetic anhydride and sulphuric acid (cholesterol gives a purple coloration which turns into green after a few minutes - Liebermann-Burchard test). The material was optically inactive (measured in chloroform) and its absorption spectra did not show any maximum in the 220-330 mμ region. It decolorised a bromine

solution /....

solution very slowly and the presence of double bonds remained doubtful.

The crystalline materials from the three oils (*Ximenia caffra* Sond., *Ximenia caffra* var. *natalensis* and *Ximenia americana*) were dissolved in 90% alcohol and the solutions allowed to crystallise: the first crop of crystals was recrystallised from 85% alcohol (twice) yielding white feather-like crystals (compounds 1) having the melting points 307°-308°, 308°-309°, 304.5°-305.5°, respectively; all three compounds started to sublime at 260°. Mixed melting point determinations indicated that the compounds from *Ximenia caffra* Sond. and *Ximenia caffra* var. *natalensis* Sond. were identical, but that both of them were different from that isolated from *Ximenia americana* var. *microphylla* Welw. (lowering on mixed melting point determinations). Owing to the lack of raw material, the compounds from *Ximenia caffra* var. *natalensis* and *Ximenia americana* could not be investigated any further and only the elementary analyses are reported.

The mother liquor from *Ximenia caffra* Sond. was evaporated and yielded, on cooling, more crystalline material, which was recrystallised twice from 80% alcohol, yielding white nest-like crystals, m.p. 276°-278° (sublimed at 230°-240°); recrystallisation of this compound did not raise the melting point (compound 2 ex *Ximenia caffra* Sond.).

The original mother liquor was further evaporated and yielded, on cooling, more crystals which were recrystallised twice from 75% alcohol, yielding crystals m.p. 267°-268° (sublimed at 230°-240°)(compound 3 ex *Ximenia caffra* Sond.). Recrystallisation from 75% alcohol did not raise the melting point. No depression was observed on the mixed melting point determination of compounds 1, 2 and 3.

The acetate of compound 1 was prepared according to the method outlined by Fuson and Shriner[88]: the compound was dissolved in a small volume of pyridine, cooled and a few ml. of acetic anhydride added and the solution then refluxed for 5 minutes. The solution was cooled and poured onto chopped ice and the crystals collected on a Buchner filter and dried: 0.15 g. of original material yielded 0.14 g. of acetate. This material was crystallised twice from 80% alcohol and dried, yielding small, partly transparent crystals, m.p. 283°-284° (mixed melting point determination with the original compound gave a lowering of 20°). The compound was dried at 110° and 0.01 mm. and analysed: Found: C, 77.02; H, 10.26; Molecular Weight (Rast): 512. Calculated for $C_{32}H_{48}O_4$: C, 77.4; H,10.72; Molecular Weight, 496.7.

The benzoate was prepared according to the method of Fuson and Shriner[88]: 0.5 g. of compound, 10 ml. of pyridine and 2 ml. of benzoyl chloride gave 0.25 g. of material, m.p. 286°-288°. Recrystallisation from methanol yielded crystals, m.p. 285°-286° (dec.); 2nd crystallisation ex methanol: crystals which turned slightly grey at 280°, almost black at 290° and melted with decomposition at 318°. <u>Analysis</u>: Found: C, 79.49; H, 9.40. Calculated for $C_{38}H_{54}O_4$ C, 79.6 ; H, 9.45.

Saponification of the benzoate with alcoholic potash yielded, on extraction with ether, a solid, m.p. 325°, which was crystallised once from 85% alcohol, yielding crystals m.p. 331°-332°. The material was dried at 110° and 0.01 mm. and analysed. Found: C, 79.56; H, 9.66 (after a few hour's exposure the material showed the following analysis: C, 78.98; H, 9.35; C, 78.29; H, 9.17, which was apparently caused by the absorption of moisture). Calculated for $C_{29}H_{43}O_3$: C, 79.21; H, 9.87.

Table XXII. /....

TABLE XXII.

Elementary Analysis of Crystalline Compounds from the Ximenia Oils.

	Ximenia caffra Sond.	*Ximenia caffra* var. *natalensis*	*Ximenia americana*.
Compound 1 (dried in oven 110°)	C, 75.46; H, 10.55	C, 75.15; H, 10.53	C, 74.99; H, 10.82
Calculated for $C_{28}H_{46}O_3.H_2O$	C, 74.92; H, 10.78		
Compound 1 (dried at 110°/0.01 mm.)	C, 78.08; H, 10.60		
Calculated for $C_{28}H_{46}O_3$	C, 78.1; H, 10.73		
Compound 2: Found	C, 77.9; H, 10.65		
Compound 3: Found	C, 77.92; H, 10.98		

Owing to the lack of raw material the investigation of these compounds could not be continued.

(b) Investigation of the Rubbery Material.

The rubbery material had the appearance of milled rubber. The impure material was purified as follows - it was dissolved in a small volume of benzene and precipitated, with stirring, in a large volume of acetone (repeated twice). The compound dissolved with difficulty in benzene, carbon tetrachloride, chloroform and was insoluble in alcohol, acetone and ethyl acetate; a chloroform solution decolorised a bromine solution very rapidly. The material was elastic and resembled raw rubber in viscosity. The compound was dried at 100° under high vacuum. Analysis: Calculated for $(C_5H_8)_n$: C, 88.2; H, 11.8. Found: C, 85.2; H, 11.55. However, the ratio (C/H) found (7.4/1) is close to that for $(C_5H_8)_n$ - 7.5/1. Iodine value: 346 (theory: 373), using the Wijs-Kemp method[101] (see Appendix A - C).

(c) /...

(c) Analysis of the Unsaponifiable Material of *Ximenia caffra* Sond.

The unsaponifiable material was extracted according to the standard S.P.A. method (see Appendix C). The method used for the determination of sterols, α-glyceryl ethers, unsaturated and saturated hydrocarbons in unsaponifiable matter have been described by Rapson *et al.*[70], and a short description of the procedure will be given later.

The unsaponifiable material had a yellowish colour, and gave a precipitate with digitonin; it was completely soluble in light petroleum and it was impossible to detect the presence of any crystalline material - Residue B.

The determination of the sterols, α-glyceryl ethers, saturated and unsaturated hydrocarbons in the unsaponifiable material was carried out as follows:

The hydrocarboh fraction was separated from the hydroxylic components by chromatographic separation, using alumina, activated at 140° for 12 hours. The non-sap. was accurately weighed out (1-1.5 g.), dissolved in 25 ml. light petroleum and chromatographed on the alumina column, 10 cm. in length and 2 cm. in diameter. The chromatogram was developed with a further 150 ml. light petroleum and this whole fraction was collected in one flask for the analysis of the hydrocarbon content of the non-sap. (Fraction 1). The chromatogram was further developed with 250 ml. of dry ether, and this volume collected in one flask and analysed for sterols (Fraction 2). The chromatogram was subsequently developed with 100 ml. absolute alcohol containing 5% acetic acid (Fraction 3); this solvent should remove all the material left on the column.

Analysis of Fraction 1: The light petroleum was removed by distillation through a 25 ml. tared distilling flask and the residual material dried under vacuum and the fraction weighed.

The /....

The iodine value of the fraction was determined by the Rosenmund-Kuhnenn method and the unsaturated material expressed as the "squalene" content of the fraction. The difference between this weight and the total weight of the hydrocarbon fraction was called the "saturated hydrocarbons".

Analysis of Fraction 2: The ether was distilled off through a tared distilling flask and the residue dried under vacuum and weighed. The fraction was analysed for its sterol content by the colorimetric method of Pijoan and Walter.[71]

Analysis of Fraction 3: The alcohol was removed by distillation through a tared distilling flask and the residue dried under vacuum and weighed. This fraction was then analysed for the sterol content by the above-mentioned method and also for its α-glyceryl ether content. The glyceryl ethers were determined as follows: The fraction was dissolved in ethyl acetate and oxidised with periodic acid according to the method described by Rapson et al.[102] and the formaldehyde determined as the dimedone derivative. The results of the analysis have been reported in Table XII.

(D) Analysis of the Meals of the Ximenia Species.

The meals have been analysed according to the methods outlined in the "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists[72].

The meals were re-extracted for 10 hours with light petroleum in a Soxhlet to remove the last traces of oil and fat, then air-dried for a few days and stored in stoppered bottles.

Moisture and Volatiles.

About 2 g. of accurately weighed meal were placed in a petri-dish and heated at 135°C for two hours. The petri-dish and contents were allowed to cool in a desiccator, and weighed. The heating process was repeated until constant weight was obtained; the loss in weight was calculated as moisture and volatiles.

Ash.

Two grams of meal were accurately weighed into a porcelain crucible and placed in a muffle furnace previously heated to 600°C. The sample was heated for 2 hours, and the crucible cooled in a desiccator.

Protein.

The protein content was calculated from the nitrogen content of the sample x 6.25.

The nitrogen was determined by the Kjeldahl method, and the ammonia collected in a measured volume of standard acid. The excess acid was backtitrated with standard alkali solution, using methyl red as indicator.

Fibre.

Two grams of a weighed sample of the meal was refluxed with sulphuric acid solution (1.25 g./100 ml.), followed by refluxing with sodium hydroxide solution (1.25 g./100 ml.). The residue was collected in a weighed Gooch crucible, and the residue washed with water followed by a few ml. alcohol, and the crucible dried at 110° to constant weight.

The contents of the crucible were then incinerated in a muffle furnace (approx. 600°C) and the loss in weight reported as fibre.

The results of the analysis of the meals from the *Ximenia* kernels and seeds are summarised in Table XIII.

THE CHEMICAL INVESTIGATION OF THE KERNELS FROM SCLEROCARYA CAFFRA SOND. ("MAROOLA").

Introduction.

The Sclerocarya genus (Phill. Gen., p. 381) is indigenous to tropical Africa and Madagascar. There are three or more species and all have been reported as having edible fruits. Sclerocarya caffra Sond. occurs abundantly in Africa south of the equator, in the lowveld of the Transvaal as far as the Magaliesberg, in the warmer regions of Natal, Zululand, in Swaziland, in Mozambique, in the two Rhodesias and also in Madagascar[103]. In South Africa it is commonly known as "Maroola" and in Madagascar as the "Sakoa Nut".[104]

The fruit, a drupe, is produced in large quantities, and drops from the trees before it is completely ripe. On ripening the green skin turns pale yellow and has numerous inconspicuous spots. The ripe fruit has a very pleasant smell, reminiscent of that of apples, which becomes overpowering where there are large quantities or when the fruit is brought indoors. A strong skin protects the yellowish flesh, which adheres firmly to the central stone. The fruit has a tart taste due to the presence of citric acid, it is very juicy and has a slight guava flavour. It is one of the favourite fruits of the low-veld, and from the juice and flesh an alcoholic drink can be brewed, or it can even be used to make a very delicious jelly[105].

The tree appears to grow relatively fast and, once it is brought to maturity, should be able to deliver a constant bulk of fruit (and seeds) under climatic conditions where very few other oil-bearing plants will thrive, and if a cheap extraction method can be developed, the "Maroola" should rank

amongst /....

amongst our oil producing trees.

The nuts or kernels of "Maroola" (or Sakoa) were previously investigated by Jones,[106] who described the seeds, and calculated the following composition for the fatty acids: stearic and palmitic acids 9%, oleic and linoleic 91% with linolenic absent; the physical and chemical constants have been tabulated in Table I. The Imperial Institute[107] re-examined the oil from the "Maroola", and the investigation of the oil from Madagascar nuts was reported at about the same time[104]. The "Maroola" as a potential source of a non-drying oil was discussed by Rindl[108], who came to the conclusion that the large scale extraction of the oil was limited due to the hardness of the shell.

In the course of the present investigation, the composition of the seeds was re-investigated and the oil content of the kernels and total seeds were determined. The composition of the total fatty acids of the kernel oil was calculated from the iodine values and saponification equivalents of the methyl ester fractions.

Discussion /....

DISCUSSION.

"Maroola" seeds have a very strong fibrous hull; when struck with a heavy hammer it tends to slip away and usually more than one blow is necessary to break it. It was found, however, that the hull could easily be broken by clamping it in a vice and applying pressure. The seed has three cavities, which contain the three separate kernels. The composition of the seeds, which was determined by breaking them in a vice and removing the kernels is given in Table XXIII.

TABLE XXIII.

Composition of the Seeds of the "Maroola".

	Present Investigation	Previously reported[106].
Average weight of seed (g.)	5.93	-
Average weight of kernel (g.)	0.82	-
Kernel as percentage seed	13.84	12.0

The kernels of a large number of seeds were removed, minced and analysed according to standard A.O.C.S. methods. These results are summarised in Table XXIV.

TABLE XXIV.

Analysis of the Kernels of the "Maroola".

	Present Investigation	Previously reported[106].
Oil content of kernel (Petroleum extraction %)	54.0	-
Oil content of kernel (Acetone extraction %)	53.5	-
Oil content of total seed (calculated)	7.45	5 - 6.3
Moisture content of kernels (%)	4.97	5.0

Both petroleum ether and acetone were used as solvents in the determination of the oil content of the kernels. Both solvents, after evaporation, yielded an oil with a light brown colour; trial experiments with several solvents and activated charcoal were carried out to find the most suitable solvent to decolorise the oil, and acetone was found to give the best results. The oil obtained from the bulk extraction of the total seeds was consequently decolorised by using acetone as solvent and adding a little filter-aid to enhance the filtration.

The physical and chemical constants of the oil were determined by the standard A.O.C.S. methods and these constants are listed in Table XXV, together with those reported in the literature by previous authors. The unsaponifiable matter in the oil was determined according to the standard S.P.A. method (Appendix C). The characteristics of the oil are summarised in Table XXV.

TABLE XXV.

Characteristics of "Maroola" Oil.

(Compared with those reported by previous investigators.)

	Present Investigation.	Previously reported[106].	Previously reported[107].
Specific gravity (25°/25°)	0.9051	0.9153(15.5°)	0.9167 (15°)
Refractive index at 25°	1.4630	-	1.460 (40°)
Acid value	14.8	-	3.7
Iodine value	74.4%	-	76.6%
Saponification value	190.0	-	193.5
Acetyl value	8.4	-	-
Reichert Meissel	0.35	-	0.1
Polenske value	0.39	-	0.45
% Unsaponifiable material	2.4%	0.93	0.6

To /...

To determine the presence of conjugated diene, triene or tetraene unsaturated compounds, the absorption curves of the impure and decolorised oils were determined between 340 and 220 mμ. The absorption curves of the oils which are given in Fig. 4 clearly indicate the absence of any conjugated unsaturated component in the oils.

As many vegetable oils contain the natural antioxidant - tocophenol - the tocopherol content of the kernel oil was also determined. The method used is based on the oxidation of tocopherols with ferric chloride in an alcoholic solution and the estimation of the ferrous ions with αα'-dipyridyl reagent[109]. The tocopherols were separated from the other interfering substances by molecular distillation, followed by hydrogenation of the interfering, unsaturated compounds.

The total tocopherols were then determined colorimetrically in an Evelyn photoelectric colorimeter[67]. It was recently reported that sesamol and sesamolin[110] also give a positive reaction with the ferric chloride-αα'-dipyridyl reagent and the absence of these compounds in the kernel oil had to be proved before the presence of tocopherols could be reported.

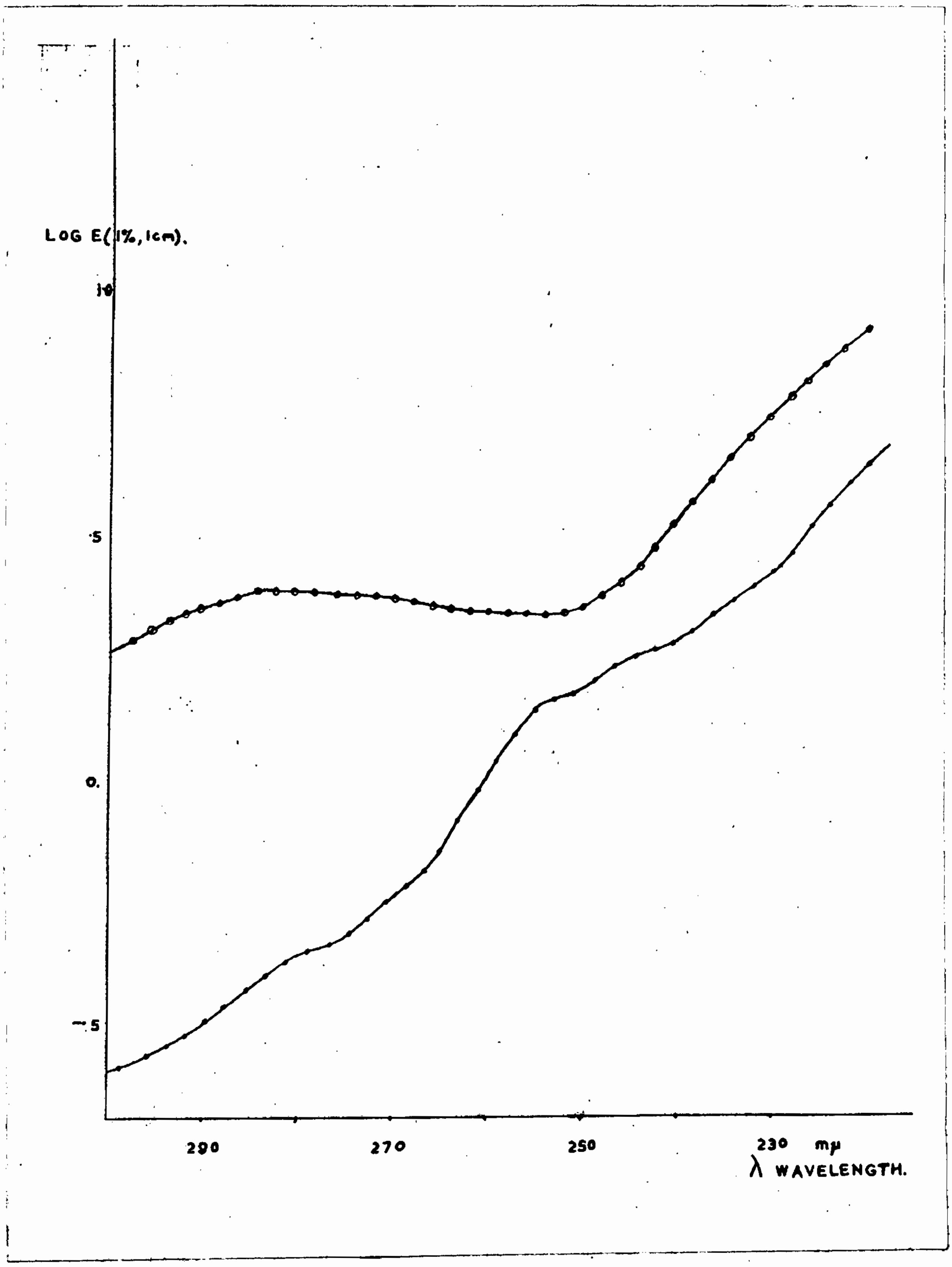
The modified Villavechia test[84] (see Appendix M) on the kernel oil was, however, negative and these compounds are consequently absent. (A control test for the presence of sesame oil and also sesamol and sesamolin in margarine was positive.) The impure oil contained 7.43 mg. and the decolorised oil 0.38 mg. of total tocopherols per gram of oil (see Table XXVIII).

For the quantitative determination of the composition of the total fatty acids, the oil was saponified and the unsaponifiable material extracted according to the standard S.P.A. method[24]. /...

Fig. 4.

• • • • Absorption Spectrum of Impure "Maroola" Oil.

o o o o Absorption Spectrum of Purified "Maroola" Oil.



It will be noticed that the total fatty acids, with an iodine value of 75, have been divided by low temperature crystallisation into three groups of acids, with iodine values 31.7, 88.9 and 136.3 respectively. The acids of these groups were then converted into the methyl esters and the esters fractionally distilled through an electrically heated and packed column under reduced pressure. The different fractions were weighed and analysed without delay (iodine value, saponification equivalent and alkali isomerisation of the appropriate fractions). The boiling points, the temperatures of the oil bath and column, and results of the analyses are given in Tables XLII - XLV.

A partial examination of the composition of the unsaponifiable fraction of the kernel oil was also made. The unsaponifiable matter was isolated by the S.P.A. method (see Appendix). Squalene, saturated hydrocarbons and α-glyceryl ethers were determined by methods described by Karnovsky, Rapson et al. and previously applied in this laboratory: the sterol content was determined colorimetrically. The analysis of the unsaponifiable fraction is reported in Table XXVII. The components of the unsaponifiable material which were determined only account for 48% of the total. Owing to the lack of raw material it was not possible to determine the nature of the remaining 52%.

TABLE XXVII.

Analysis of the Unsaponifiable Material.

Saturated hydrocarbons	7.51%
"Squalene"	0.94%
"Sterols"	37.53%
Glyceryl ethers	0.94%
Difference	53.08%

Computation /....

Computation of the Composition of the Fractions.

Introduction.

An exhaustive mathematical analysis of the data and calculations used in the determination of the composition of marine oils has been given by Charnley[76], and a simplified method was presented by Hilditch in his monograph on Fats and oils[31], while Rapson *et al.*[75] presented simplified computation forms for the calculation of the different fractions in marine oils. For the calculation of the component esters of "Maroola" oil, only the simplified equations of Hilditch (*loc. cit.*) were used.

If *x*, *y* and *z* are the weights of a saturated and, say, two unsaturated esters in a fraction of weight *w*, and E , E_y, E_z, E_w are the corresponding saponification equivalents, and I_y, I_z, I_w are the corresponding iodine values of these constituents, then

$$x + y + z = w \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$x/E + y/E_y + z/E_z = w/E_w \quad \ldots\ldots\ldots \quad (2)$$

$$y \times I_y + z \times I_z = w \times I_w \quad \ldots\ldots\ldots \quad (3)$$

(It can also be assumed that E represents the saponification equivalent of two saturated esters, and that the mean molecular weight of these two esters is the same as that of the fraction under the consideration. If this assumption is made, a fraction can be computed for four esters.)

Similarly, if the iodine value is too high for the presence of any saturated esters, or when calculation of the composition gives a negative result for the saturated esters, the presence of three unsaturated esters can be postulated and the components calculated with the aid of the following three equations: /....

equations:

$$p + q + r = w \quad \ldots\ldots\ldots \quad (4)$$

$$p/268 + q/295.8 + r/321.3 = w/E_W \quad \ldots \quad (5)$$

$$94.8\ p + 94.5\ q + 185.8\ r = w \times I_W \quad \ldots \quad (6)$$

where $\underline{p}$, $\underline{q}$ and $\underline{r}$ are the postulated weights of the three unsaturated esters, say C_{16}, C_{18}, C_{20} unsaturated esters having iodine values of 94.8, 94.5 and 185.8 respectively. From these three equations (having three unknowns) the weights of the postulated esters can be calculated.

Calculation of the Component Esters of Group A.

For the calculation of the components of the individual esters of this group, it was assumed that C_{16-2H} esters were absent.

Fractions $A_1 - A_3$: From the low iodine value these fractions have a low content of unsaturated esters. They were initially computed as consisting of the methyl esters of palmitic, stearic and oleic acids; when the composition was calculated on this bases, however, the weight of stearic acid came to a negative quantity. The composition was then calculated on the basis that only the methyl esters of palmitic and oleic acids could be present:

A_1 Wt. 6.8188 g., S.E. 270.9, I.V.6.41

If x is the weight of C_{16} saturated ester, having saponification equivalent 270 and iodine value 0, and $\underline{y}$ is the weight of C_{18-2H} ester, having saponification equivalent 296 and iodine value 85.74, then

$$x + y = 6.8188 \quad \ldots\ldots\ldots \quad (1)$$

$$85.74\ y = 6.41 \times 6.8188$$

$$\text{thus} \quad y = 0.5098 \quad \ldots\ldots\ldots \quad (2)$$

$$\text{and} \quad x/279 + y/296 = 6.8188/270.9 \quad \ldots \quad (3)$$

and substituting for the value of $\underline{y}$ obtained from (2) in

equation /...

equation (3):

$$x + 0.9122 \times 0.5098 = 6.797 \text{ (after multiplication)}$$

$$\text{thus} \quad x = 6.3320$$

On substituting the value of y from (2) in equation (1), however, a value of 6.3090 for x was obtained. The values of x, calculated from the two equations show fairly good agreement; for the final calculation the second value was taken as the weight of oleic acid ester. The composition of Fractions A_2 and A_3 was calculated on the same basis, and the weights have been summarised in Table XLIII.

<u>Fractions</u> A_4 to A_{14}: From the saponification equivalents, these fractions are mixtures of C_{16} and C_{18} saturated and unsaturated esters. It was, however, assumed that C_{16-2H} could not be present, and the composition of these fractions was calculated on the basis that only the methyl esters of C_{16} and C_{18} saturated and C_{18-2H} acids are present. The equations, which have been used are the following: (using the values of Fraction A_4)

Wt. 6.6895, S.E. 276.8, I.V. 21.79

$$x + y + z = 6.6895 \quad \ldots\ldots\ldots\ldots \quad (1)$$

$$\frac{x}{270} + \frac{y}{298} + \frac{z}{296} = \frac{6.6895}{276.8} \quad \ldots\ldots\ldots \quad (2)$$

$$\text{and} \quad 85.74\,z = 6.6895 \times 21.79 \quad \ldots\ldots\ldots \quad (3)$$

$$\text{thus} \quad z = 1.7001 \quad \ldots\ldots\ldots \quad (4)$$

Substituting this value for z in equations (1) and (2), and solving for x and y:

$$x = 4.8309 \quad \text{and} \quad y = 0.1585$$

where x, y and z are the weights of the methyl esters of C_{16} saturated, C_{18} saturated and C_{18-2H} methyl esters.

All the values calculated for the fractions have been summarised in Table XLIII.

<u>Fraction</u> /....

Fraction A_{15}:[x] Weight 3.8716, saponification equivalent 341.6, iodine value 12.84. From the saponification equivalent this fraction is a mixture of C_{18}, C_{20} and C_{22} esters.

The previous fraction still contained a high percentage of methyl oleate, and this fraction should consequently still contain some of it. It is only possible, however, to calculate the composition for the presence of three constituents, and it was decided to compute the composition of this fraction for the presence of the following methyl esters:

$$x = C_{18-2H}, \quad y = C_{20} \text{ sat. and } x = C_{22} \text{ sat.}$$

$$x + y + z = 3.8716 \quad \dots\dots\dots\dots (1)$$

$$\frac{x}{296} + \frac{y}{326} + \frac{z}{354} = \frac{3.8716}{341.6} \quad \dots\dots\dots\dots (2)$$

$$x \times 85.74 = 3.8716 \times 12.84$$

$$\therefore \; x = 0.5685 \quad \dots\dots\dots\dots (3)$$

Substituting this value for x in equations (1) and (2) and solving for y and z:

$$y = 0.3121 \quad \text{and} \quad z = 2.9797.$$

The /....

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[x] Owing to the small weight, this fraction was not re-saponified and the high saponification equivalent may not be the true value due to the presence of unsaponifiable material.

The Calculation of the Component Esters of Group B.

Group B_1: Weight 5.2342 g., saponification equivalent 287.6, iodine value 60.11. The saponification equivalent of the fraction indicates that it is a mixture of C_{16} and C_{18} esters, and the iodine value fits a postulation of the presence of C_{16} and C_{18} saturated and monoethenoid esters. From the results of the alkali isomerisation of the total fatty acids and Fraction C, however, there is some linoleic acid missing, and it was postulated that some linoleic acid remained in this fatty acid group (B). The component esters were thus calculated on the basis of the following:

$x = C_{16}$ sat.; $y = C_{18-2H}$ and $z = C_{18-4H}$

Thus $x + y + z = 5.2342$ . . . . (1)

$$\frac{x}{270} + \frac{y}{296} + \frac{z}{294} = \frac{5.2342}{284.5} \quad \text{. . . . . (2)}$$

and $y \times 85.74 + z \times 172.7 = 5.2342 \times 60.11$ . . . .(3)

The three equations have three unknowns, and solving for them gave:

$x = 2.1557$ $y = 2.4958$ $z = 0.5827$

Group B_2: Weight 6.7401 g.; S.E. 294.1; I.V. 85.15.

The components of this fraction were calculated on the same basis as that of group 1.

$x = 0.4260$, $y = 5.9397$, $z = 0.3784$.

Groups B_3 to B_{14}: Analysis of the constants of these fractions showed that they consist of the same components. The weights were added together, and the saponification equivalent and iodine value of the total fraction were calculated as follows: the saponification equivalent (or iodine value) of each fraction was multiplied by its weight, these values for all the fractions were added and divided by the total weight, /....

weight, giving the "average weight", saponification equivalent and iodine value. The constants for the combined fraction are as follows:

Total weight, 73.9481 g.; Av. S.E., 293.1; I.V., 86.74.

The composition was calculated on the same basis as that of the previous two fractions; after solving for x, y and z, the composition was calculated as:

C_{16} sat., 1.9278; C_{18-2H}, 69.0990; C_{18-4H}, 2.9213.

Group B_{15}: Weight, 5.0390 g.; S.E., 302.9; I.V., 76.94.

This fraction was analysed as the methyl esters. From the saponification equivalent and iodine value this fraction is a mixture of C_{18} and C_{20} methyl esters. Owing to the insolubility of C_{20} saturated methyl esters it was assumed that this ester could not be present in this fraction. The composition was thus calculated on the basis of the following methyl esters:

x, C_{18-2H}; y, C_{18} sat.; z, C_{20-2H}.

The standard equations were used and the composition was calculated as:

methyl stearate, 0.444; methyl oleate, 2.9403;
methyl gadoleate (C_{20-2H}), 1.6547.

The results of calculations are summarised in Table XLIV.

The Calculation of the Component Esters of Group C.

This is the most soluble acid group, and is usually referred to as the "liquid ester" fraction. The percentage solid esters are usually very low or absent (Schwartz, loc. cit.). In the calculation of the composition of this fraction, it was assumed that saturated esters are completely absent.

Fraction /...

<u>Fraction</u> C_1: Weight, 5.1943; S.E., 283.2; I.V., 130.7

The saponification equivalent of the fraction indicates a mixture of methyl esters of C_{16} and C_{18} acids, while the high iodine value indicates the presence of esters having more than one double bond. Fraction C, as a whole, contains 56% of linoleic acid, and as oleic and linoleic esters distil more or less in the same ratio, it was assumed that C_1 contained some linoleic ester. Throughout the calculation of the composition of these fractions, the weights of linoleic methyl ester were mathematically calculated from the known equations; the calculated weights are listed in Table XLII with the results obtained by alkali isomerisation.

Let x = Wt. of C_{16-2H}, y = Wt. of C_{18-2H} and z = Wt. of C_{18-4H}

$$x + y + z = 5.1943 \quad . . . . (1)$$

$$\frac{x}{268} + \frac{y}{296} + \frac{z}{294} = \frac{5.1943}{283.2} \quad . . . . (2)$$

$$x \times 94.71 + y \times 85.7 + z \times 172.7 = 5.1943 \times 130.7 \quad . . . (3)$$

These equations gave the following results:

Weight of hexadecenoic ester:	2.0884
Weight of oleic ester:	0.6357
Weight of linoleic ester:	2.4702

<u>Fraction</u> C_2: Weight, 4.7731; S.E., 289.7; I.V., 146.75.
E(1%, 1cm.) at 234 mμ, 562.4 (corrected);
E(1%, 1cm.) at 268 mμ, 5.46.

This fraction was calculated on the basis of the same composition as the first fraction.

Result:	Weight of hexadecenoic ester:	0.7799 g.
	Weight of oleic ester:	0.7252
	Weight of linoleic ester:	3.2680

(Weight of linoleic ester from alkali isomerisation 3.0834 g.)

<u>Fraction</u> /....

Fraction C_3: Weight, 4.4732 g.; S.E., 292.4; I.V., 145.6
E(1%, 1cm.) at 234 mμ, 554 (corrected);
E(1%, 1cm.) at 268 mμ, 6.55.

The composition was calculated on the same basis as that used for Fraction 1, the result of alkali isomerisation for linoleic acid is given in brackets:

Weight of methyl hexadecenoate: 0.2192 g.
Weight of methyl oleate: 1.1976 g.
Weight of methyl linoleate: 3.0564 g.
Weight of methyl linoleate: (2.8500 g.)

Fraction C_4: Weight, 4.7790; S.E., 294.8; I.V., 143.15.
E(1%, 1cm.) at 234 mμ, 520.2 (corrected);
E(1%, 1cm.) at 268 mμ, 7.7

In a trial calculation, the composition was computed on the same basis as that used for the previous fractions; the value for C_{16-2H} ester was found to be negative, however, and the composition was calculated for the presence of C_{18-2H}, C_{18-4H} and C_{18-6H} esters, thus:

$$x + y + z = 4.7790 \quad \ldots\ldots\ldots\ldots \quad (1)$$

$$\frac{x}{296} + \frac{y}{294} + \frac{z}{292} = \frac{4.7790}{294.8} \quad \ldots\ldots\ldots\ldots \quad (2)$$

$$x \times 85.74 + y \times 172.7 + z \times 260.8 = 4.7790 \times 143.15 \quad \ldots \quad (3)$$

which gave on solving for x, y and z:

Weight of methyl oleate (x): 1.9809 g.
Weight of methyl linoleate (y): 2.4478 g.
Weight of methyl linolenate (z): 0.3513 g.

From the results of alkali isomerisation:

Weight of methyl linoleate: 2.8530 g.
Weight of methyl linolenate: 0.0683.

(The lowering in iodine value from C_3 to C_4, indicated the presence of polymerised material, and the determined iodine value may not be a measure of the "true" iodine value.

This /....

This explains the low weight of methyl linoleate.)

<u>Fraction</u> C_5: Weight 7.0980; N.E. (of acids, 298.8;
Equivalent S.E. (for esters), 312.8;
I.V. (of acids), 111.2;
Equivalent I.V. (for esters), 106.2

This fraction was not analysed for the presence of linoleic and linolenic acids. In fact, previous workers Rapson *et al.* - *loc. cit.*) assumed that the polymerisation etc. made the iodine values of such fractions meaningless, and assessed the compositions of these fractions on the basis of the saponification equivalents only. As this fraction (total C) was relatively small, the degree of polymerisation and oxidation was considered small and the composition calculated on the basis of the methyl esters of the following acids:

$C_{18-2H}(\ )$; $C_{18-4H}\ (y)$; $C_{20-2H}\ (z)$.

$$\quad + y + z = 7.0980$$

$$\frac{\quad}{296} + \frac{y}{294} + \frac{z}{324} = \frac{7.0980}{312.8}$$

$$\times 85.74 + y \times 172.7 + z \times 78.34 = 7.0980 \times 106.2$$

Solving for the three unknowns give:

Weight of methyl oleate:	0.4860 g.
Weight of methyl linoleate:	2.0577 g.
Weight of methyl gadoleate:	4.5543 g.

The weights of the fractions of the total C fraction are summarised in Table XLV.

Further Details of the Calculation.

The weights of all the esters were added together and are referred to as the "Total Esters" of the group, this weight was usually about 1-2% lower than the original weight of esters used for the distillation, the difference being

due /...

due to the formation of volatile pyrolysis products as well as the presence of residual solvent. The weights of the esters were then converted to those of the corresponding acid, this was done according to the formula:

$$Wa = \frac{We \times Ea}{Ee} \qquad \text{(Ref. 20)}$$

where
Wa = weight of the corresponding acid.
Ea = neutralisation equivalent of the acid.
Ee = saponification equivalent of the ester.
We = weight of the methyl ester.

e.g., to convert methyl oleate to oleic acid (Group B):

$$\text{Corresponding weight of acid} = \frac{80.4748 \times 282}{296}$$

To facilitate the calculations, the factor

$$\frac{\text{Molecular weight of acid}}{\text{Molecular weight of ester}}$$

was calculated for each acid, and this was used for the calculations of the weights of the acids.

The percentages of acids (on the total mixed acids) were determined by the method outlined on p. 49 and these values are summarised in Table XLII.

Discussion of Results.

The results obtained by subjecting the fatty acids of *Sclerocarya caffra* Sond. ("Maroola") to low temperature crystallisation and fractional distillation will now be discussed and compared with some of the other species of the family and of vegetable oils in general.

The "Maroola" is a member of the sub-family *Sclerocarya* of the family *Anacardiaceae* or the Mango family (I.C. Verdoorn[103], loc. cit.). The seeds and nuts of

several /....

several members of the Anacardiaceae have been investigated for their oil content and composition of the fatty acids[31].

The oils and fats of this family consist primarily of esters of palmitic, oleic and linoleic acids (see Hilditch ref. 19, p. 153). Tsjimoto[111] analysed the fruit coat fat from Sumach Rhus succedanea and later the composition of the seed fat was also reported; the chief difference between these two fats is that the former consists mainly of palmitic (77%) and oleic acids, while the latter consists mainly of oleic and linoleic acids.

The following list (Table XXIX) summarised the composition of the fatty acids of several members of this family, together with the percentages of the main acids which constitute "Maroola" oil.

TABLE XXIX.

Composition of Fatty Acids of the Anacardiaceae.

Plant.	Common Name.	Habitat.	C_{16} sat.	C_{18} sat.	Oleic.	Linoleic
Anacardium occidentale	Cashew Nut	Tropics	6.4	11.3	74.1	7.7
.. ..	..	..	4.1	5.8	68.2	21.7
Anthrocaryon Nannani	Gonya Almond	Congo	13.5	10.5	45.5	24.5
Buchanania latifolia	Seed	India	28.9	8.1	57.4	5.5
Mangifera Indica	Mango	India	8.8	34.0	49.8	-
Pistacia lentiscus	Shinia nut	Levant	27.0	13.0	53.0	7
Pistacia vera	Pistachio nut	Asia	19	-	60	21
.. ..	..	India	8.2	1.6	69.6	20
Sclerocarya caffra	Maroola	South Africa	9.0		91 with linolenic absent.	
Present Investigation:	..	..	16.1	5.1	66.7	7.3

It /....

It will be noticed that the other species show a palmitic acid content between 4 to 29%, stearic acid content 2 to 34%, oleic acid content between 46 to 74%, and linoleic acid content between 5.5 to 24.5%. The composition of the fatty acids of the "Maroola" does not agree closely with any composition of the above mentioned species, and our analysis is quite different from the previous investigation of "Maroola" seeds. The main constituents of the oil, however, are palmitic, oleic and linoleic acids; and in that respect it is truly a vegetable oil and representative of the Anacardiaceae family.

The tocopherol content of the impure, kernel oil was high compared with the values reported for most oils[117], and this should make the oil particularly resistant to oxidation. The difference between the tocopherol content of the crude and the charcoal treated oils is striking, the greater part of the tocopherol content of the crude oil being removed by the charcoal treatment. This was unexpected since it has been shown that treatment of cottonseed and soyabean oils with bleaching earths does not reduce the tocopherol content of the oils appreciably[117]. The reason for this loss on charcoal treatment was, however, not investigated.

EXPERIMENTAL.

A. Origin and Investigation of Seeds.

The seeds were collected in the Groblersdal district, Transvaal, in April, 1949. Many of the hulls were covered with dried fruit, which was removed before the composition of the seeds was determined. The composition was determined as follows: Ten lots of ten seeds were weighed on a rough balance to 100 mg.; the seeds were then opened and the kernels carefully removed and weighed. The difference between these weights was taken as the weights of the hulls (it was rather difficult to remove the hull without losing some of it and the hulls were not weighed as such).

The average weight and composition of the seeds have been given in Table XXXIII.

B. Analysis of Kernels.

For the analysis of the kernels, the seeds were broken in a vice and the kernels removed as completely as possible. The kernels were then minced in a household meat mincer and analysed according to standard A.O.C.S. methods.

Moisture and Volatile content.

The moisture and volatile matter was determined according to A.O.C.S. method Aa 3-38 (see Appendix B).

C. The Kernel Oil.

(i) Results of Acetone Extraction.

A weighed quantity of the minced kernels was placed in the extraction thimble of a Soxhlet extractor and extracted for 10-12 hours with dry acetone, after which the acetone was replaced with fresh, dry solvent and the extraction continued for another 10-12 hours. After the extraction was complete, the solvent was removed by distillation through a tared distilling flask and the last residual traces of solvent and moisture removed under vacuum on a boiling water bath. The determination of oil content was carried out in duplicate, the average of which was 54.0%.

(ii) Petroleum extraction.

A quantity of minced kernels was similarly extracted in a Soxhlet apparatus and the solvent (light petroleum) subsequently removed by distillation through a tared distilling flask and the residual oil dried under vacuum on a boiling water bath. The determination was carried out in duplicate, the average of which was 53.5%.

(iii) Bulk Extraction and Purification of Oil.

For the bulk extraction of the oil, the seeds were broken in a vice, the fractured seeds placed in a cotton bag, weighed and placed in the upper container of a copper Soxhlet. About five gallons of acetone were poured in the lower container of the Soxhlet and the seeds extracted for about ten hours. After this period the acetone was run off and replaced with fresh acetone and the extraction continued for another ten hours. The whole volume of acetone was then removed by distillation through a tared $1^1/_2$ litre flask and the residual oil dried under vacuum on a boiling water bath. The oil, after drying, was cooled to room temperature under vacuum and decolorised as follows:

For each forty grams of oil, 150 ml. dried acetone were used as solvent and three grams of activated charcoal and five grams of filter-aid added and the mixture refluxed for five minutes. The solution was cooled and filtered through a carefully packed Buchner filter. The solvent was removed by distillation and the oil dried as before. The oil had a light straw colour and was considerably paler than the starting material which had a light brown colour. The purified oil was stored under carbon dioxide in a brown bottle for further processing.

(iv) Chemical and Physical Investigation of the Oil.

The chemical and physical constants of the oil (purified) were determined according to the standard A.O.C.S. methods. Abstracts of these methods are given in Appendices B - J.

The absorption spectra of the impure and decolorised oils were determined in a Beckman spectrophotometer, model DU, using silica cells and cyclohexane as solvent. The absorption curves between 300 and 220 mμ are given in Fig. 4. In all cases the dilutions were made in such a way as to give E values of 0.3 - 0.8 on the spectrophotometer.

The linoleic and linolenic acid contents of the total fatty acids from the oil were determined according to the method of Mitchell, Kraybill and Zschiele (Appendix K). According to this method a weighed quantity of the acid (0.1 - 0.12 g.) was treated with potassium hydroxide in ethylene glycol reagent at 180°C for 25 minutes and examined spectrophotometrically at 234 and 268 mμ. The results of the analyses of the oil have been summarised in Table XXV.

(v) Component Acid Studies.

(a) Saponification of Oil.

The method recommended by the Society of Public Analysts (see Appendix C) was used for the saponification of the kernel oil. This method is essentially the same as that used for the determination of the percentage unsaponifiable matter in an oil. Fifty grams of oil were accurately weighed into a 1 litre round-bottomed flask and refluxed for one hour with 500 ml. N/2 alcoholic KOH. The alcoholic solution was slightly cooled and added to $^1/_2$ litre of water, contained in a 4 litre separating funnel. The flask was rinsed with another $^1/_2$ litre of water and added to the alcoholic solution in the separating funnel.

The flask was then rinsed a few times with ether and the soap solution extracted with a total of 1 litre of ether During the extraction, the contents of the separating funnel were strongly swirled and not shaken to avoid emulsion formation. The contents of the separating funnel were allowed to stand for a short while and the aqueous phase run into a second 4 litre separating funnel. This was similarly extracted with another 1 litre of ether, followed by two more extractions of 750 ml. of ether. The ether extracts were combined and washed with 250 ml. of water and this water solution added to the original aqueous soap solution, and then extracted with two portions of 250 ml. N/2 aqueous KOH solution; these solutions were also added to the soap solution, and the ether solution then washed free of alkali. During all the extracts, care was taken to avoid the formation of emulsions. The water was separated as completely as possible and the ether distilled off through a tared distilling flask, and the residue dried under vacuum of the water pump on a boiling water bath. In the bulk saponification of the

oil /...

oil, the extracts of 8-10 lots of saponifications were combined and resaponified with N/2 alcoholic KOH (10 ml. of alkali per g. of residue) and the unsaponifiable matter extracted according to the above mentioned procedure. The material was dried and stored under carbon dioxide; its analysis will be described in a later section.

(b) Extraction of Fatty Acids.

The aqueous alcohol layer (together with 1 water wash and two alkali washes) was poured into a 4 litre separating funnel and 1 litre of ether added. The solution was then just acidified with concentrated hydrochloric acid and total solution well shaken. The solution was allowed to separate and the aqueous layer taken off and successively extracted with two litres of ether. The ether volumes were combined and washed with successive portions of water to remove the excess hydrochloric acid. The ether was then distilled off through a distilling flask and the residual fatty acids dried under vacuum of a water pump on a boiling water bath.

The fatty acids from 8-10 lots of saponifications were combined, together with those from the re-saponification of the unsaponifiable matter and stored under CO_2 before further analysis.

(c) Separation of the Fatty Acids by Low Temperature Crystallisation.

The fatty acids, obtained after saponification of the oil, were dissolved in pure ether (10 ml. of ether per gram of acid) contained in a round-bottomed flask. The flask was placed in an insulated bath filled with methylated spirits and with slow but continuous stirring, the temperature was taken down to -40°, and kept there for $4^1/_2$ hours. Crystals started to form at -20° and at the end of the crystallisation the heavy

precipitate /...

precipitate was collected on a cooled Buchner filter, and the crystal cake sucked dry. The crystals were dried under vacuum and cooled to room temperature. This fraction was again dissolved in purified ether and crystallised under the same conditions and at the same temperature as previously described. The insoluble acids were dried under vacuum on the water bath, weighed and stored under carbon dioxide (Group A).

In each case the solvent was removed from the filtrate by distillation through a tared distilling flask and the residual acids dried under vacuum of the water pump at 80-100°.

After cooling these fractions were weighed, combined and recrystallised from acetone (10 ml. acetone per gram of acid) at -60° for five hours; at the end of this period the insoluble acids were collected on a cooled Buchner filter, and the filter cake sucked dry. The crystals were dried under vacuum in a tared round-bottomed flask, cooled, and stored under carbon dioxide (Group B). The solvent was removed by distillation through a tared distilling flask and the last traces of solvent removed under vacuum on the water bath. After cooling, these acids were also stored under carbon dioxide (Group C). The weights of the different groups, as well as their percentage weight of the total fatty acids have been summarised in Table XXVI.

(d) Preparation and Isolation of the Methyl Esters.

Each of the above mentioned fractions was converted into the methyl esters by refluxing it with five times the volume of absolute methyl alcohol in the presence of 1 to 2 per cent of concentrated sulphuric acid for four hours (see Appendix L).

A STUDY OF OIL BEARING SEEDS FROM SOME INDIGENOUS PLANTS.

An Abstract of the Thesis Presented by

S. P. Ligthelm

for the

Degree of Doctor of Philosophy.

A survey was made of the oil content of seeds of the following indigenous plants:

(a) Three members of the Olacaceae family viz. *Ximenia caffra* Sond., *Ximenia caffra* var. *natalensis* Sond. and *Ximenia americana* var. *microphylla* Welw. commonly known as "Sour Plum".

(b) *Sclerocarya caffra* Sond., commonly known as "Maroola".

(c) Three members of the Strychnos family viz. *Strychnos innocua*, *Strychnos dysophylla* and *Strychnos coccoloides*, commonly known as the "Klapper".

The oil content of the seeds of the *Ximenia* genus varied between 42-51 per cent, the oil content of the "Maroola" seeds was 7.5 per cent, while the oil content of the *Strychnos* fruit was below 4 per cent. In view of the fact that the oil content of the *Strychnos* fruit is so low, the composition of these oils was not determined and only the oil content of the fruit pulp and seeds, and the characteristics of one of the oils have been reported.

The /...

The Ximenia and Maroola fruit are the most popular wild fruit of the sub-tropical region of South Africa. The fruit of the Ximenia ripens in December - January, while the fruit of the Maroola ripens in March - April. The kernel of the Ximenia is protected by a soft hull, while the Maroola kernels (three separate kernels in each seed) are covered with a hard, fibrous hull.

The seeds (and kernels) of Ximenia yielded on extraction with acetone a light yellow oil which, on standing, became turbid with the deposition of a white crystalline compound; this compound gave the oil a viscous thixotropic nature. It is insoluble in light petroleum and could be separated from the oil by diluting it with 3-4 volumes of light petroleum. Filtration of this solution followed by evaporation of the light petroleum yielded a light yellow oil which retained its stability for a long period. Extraction of the seeds with petroleum yielded a light yellow, somewhat cloudy, viscous oil. On dilution of this oil with acetone, a rubbery material was precipitated which appeared to be poly-isoprene.

The absorption spectrum of the acetone extracted and petroleum treated oil (as determined in a Beckman spectrophotometer) revealed the presence, in a fairly large percentage, of a constituent having two unsaturated centres in conjugation and a small proportion of a constituent having three unsaturated centres in conjugation -- further investigation showed that these constituents were present in the fatty acid portion of the oil and the research was directed towards the isolation and characterisation of the fatty acid with two unsaturated centres.

By combination of low temperature crystallisation and distillation, a new conjugated enyne acid, for which the

name /...

name Ximenynic acid was proposed, was isolated from the Ximenia oils. Ximenynic acid is a C_{18} acid, having a double and triple bond in conjugation and from the split products on oxidation of the acid with potassium permanganate and the split products isolated on ozonolysis (followed by reduction of the ozonide with zinc and acetic acid) the following structure was assigned for ximenynic acid:

$$CH_3(CH_2)_5CH{=}CH{\cdot}C{\equiv}C.(CH_2)_7.COOH$$

To calculate the composition of the oils, the total fatty acids were crystallised from acetone (1 g. of acid per 10 ml. acetone) at -25°; the soluble and insoluble acids so obtained were converted into the methyl esters which were fractionally distilled into fractions of 6-8 g. The saponification equivalents, iodine values and absorption intensities [E(1%, 1 cm.)] of all these fractions were determined and from these constants the composition of the fractions (and of the total fatty acids in the oil) could be calculated.

The saponification equivalents and iodine values of the later fractions of the solid acids revealed the presence, in appreciable amounts, of C_{26} saturated and C_{26} unsaturated and C_{30} unsaturated fatty acids, and crystallisation of the fatty acids of the appropriate fractions yielded these acids of 95-97% purity.

The composition of the total fatty acids of the three Ximenia oils have been summarised in the thesis. An attempt was also made to analyse the unsaponifiable portion of the oil, but owing to the lack of raw material only 44% of the total constituents could be accounted for.

The /...

The residual seed meal was also analysed; these meals are relatively rich in protein and could be used as a fodder.

The oil from the Maroola seeds was saponified and the isolated fatty acids were separated into three fractions by crystallisation from ether at -40° and crystallisation of the soluble fatty acids from acetone at -60°. These fractions were converted into the methyl esters which were fractionally distilled into smaller fractions of 6-8 g.; these were analysed and their composition calculated. The unsaponifiable fraction of the oil was also investigated, but only 47% of the constituents could be accounted for.

Owing to the fact that one of the previous investigators (Lehman, The Star, 1948) of the fruit of one of the Strychnos species reported that the fruit contained over 40% of oil, the determination of the oil content of the three members of the genus was undertaken. As the oil content was found to be below 4 per cent., further investigation was limited to the determination of the characteristics of one of the oils.

(e) Fractional Distillation of the Methyl Esters.

The fractionations of the different fractions were carried out in a two foot electrically heated column, packed with single and multiple turn glass helices. The column is a modification of the one described by Longenecker[85]. During the distillation any flooding of the column was avoided, and a temperature difference of about 100° was maintained between the bath temperature and that of the head of the column. An attempt was made to keep the initial fractions relatively small (4-5 g.), and the subsequent fractions were cut at about 5-7 g. The fractionation was continued until about 6-8 g. were left behind in the distilling flask; the distillation was then stopped and the apparatus cooled. After cooling, the column, side-arm and Perkin triangle were rinsed with small volumes of acetone. The acetone washes were combined with the residue in the flask and the solvent removed by distillation. The last traces of solvent were removed under vacuum and the flask cooled and weighed. This fraction was then resaponified according to the standard procedure and the non-sap. extracted. The free fatty acids were then extracted and analysed as such. The details of the fractional distillation (b.p's., pressures, etc.) have been summarised in Tables XLIII - XLV.

(f) Analyses of the Fractions.

In order to be able to compute the composition of each fraction, the saponification equivalent and iodine value are required. Additional information of each fraction was obtained on alkali isomerisation at 180° especially those fractions having an iodine value higher than about 100. From measurements of the absorption intensities at 234 mμ and 268 mμ the percentages of linoleic and linolenic acids were calculated (see Appendix K).

Iodine /...

Iodine Values of all the fractions were determined by using the Wijs reagent (see Appendix A).

Saponification Equivalents. The saponification equivalents of the fractions were determined by refluxing the methyl ester (approx. 1.5 g.) or acid with N/2 alcoholic potash in alkali resistant Erlenmeyers for 20 minutes, and back-titrating the excess alkali with standardised N/3 hydrochloric acid, using phenol phthalein as indicator.

All the iodine values and saponification equivalents were carried out in duplicate and where these values were not within 0.5 - 1.0% agreement, a third determination was carried out. The mean of the two values was taken as the iodine value and saponification equivalent for the calculation of the composition of each fraction.

(g) Villavechia Test for Sesame Oil[17].

The modified Villavechia test was carried out according to the method outlined in the standard A.O.C.S. methods; an abstract of this method has been given in Appendix M.

(vi) Determination of Total Tocopherols in the Kernel Oil.[112]

Reagents:

(1) Purified absolute alcohol (see Appendix A).

(2) αα'-Dipyridyl reagent. Dissolve 0.25 g. of α-α'-dipyridyl in 50 ml. of purified absolute alcohol, and store in a dark bottle away from light.

(3) Ferrichloride hexahydrate. Dissolve 0.10 g. of material in 50 ml. of absolute alcohol. Store in a dark bottle away from direct light.

(4) Palladinised calcium carbonate. This was prepared as follows: Suspend 50 g. of well-

washed /...

washed $CaCO_3$, which has been precipitated while hot, in about 200 ml. of water. Mix the suspension by shaking and gentle warming with a solution of palladium chloride. When the liquid over the calcium carbonate is completely decolorised, pour off and wash the solid a few times with distilled water and collect the precipitate on a Buchner filter. Wash the precipitate until the wash water is free from chloride ions and dry in a vacuum desiccator and store in a well stoppered bottle.

Procedure.

About 0.9 to 1.0 g. of fat or oil was accurately weighed out in a sublimer, 2" wide and 3-4" deep, which was then connected to a high vacuum and mercury pump, capable of giving 10^{-5} to 10^{-6} mm. of pressure. The sample was degassed by closing the sublimer with a flat rubber stopper and applying vacuum to 10^{-5} to 10^{-6} mm. at room temperature for a short while. (This minimised splashing of the sample in subsequent distillation.) The pressure was restored to atmospheric pressure, and without unnecessary delay the condenser was inserted and the pressure again reduced to 10^{-5} to 10^{-6} mm. Dry ice was then placed in the condenser and a small volume of acetone added to improve the efficiency of the condenser. The sublimer was placed in an oil bath and the depth of immersion so arranged that the bottom of the condenser was level with the surface of the oil bath. The oil bath was heated rapidly to 220° and the temperature kept at 215° to 220°C for 30 minutes. The oil bath was then removed and the sublimer allowed to cool to room temperature under vacuum. The distillate was washed off into a pressure flask with pure chloroform, the chloroform then removed by distillation under reduced pressure and the dried residue dissolved in 10 ml. purified anhydrous alcohol. The solution was reduced with gaseous hydrogen under a pressure of 30 lbs/sq. inch, using palladium calcium carbonate catalyst for 15-20 minutes. The catalyst was removed in the centrifuge and the precipitate washed a few times with alcohol, and the total alcohol solution made up to a standard volume in a measuring flask.

A convenient volume (10 ml. if possible) of the standard solution was pipetted into the Evelyn photometer tube, and the total tocopherols measured with the 520 filter (referred to as L_{520}) according to the method of Quaife and Harris[112]. The tube with the 10 ml. ethanol plus 1 ml. of dipyridyl reagent was placed in the photometer and the galvanometer set at 100. The tube was removed, ferric chloride added and the tube shaken for at least 5 seconds and the reading taken after 50 seconds. The colour due to tocopherol reaction was L_{520} of unknown minus L_{520} of blank. The tocopherol content of the sample in the tube was found from a calibration curve prepared with samples of pure natural tocopherol in purified alcohol, with amounts of 25-100 mg. of tocopherol in 10 ml. of alcohol.

Quaife and Harris found a value of 1.423×10^{-1} for K in the formula

$$c = K \times L,$$

where c = mg. of tocopherol in 10 ml. aliquot,

and $L = 2 - \log G$.

Calibration Curve.

0.0705 g. of pure tocopherol was dissolved in 10 ml. of absolute alcohol and 3 ml. of this standard solution diluted to 200 ml.

Thus /...

Thus concentration of tocopherols = 10.575 mg. per ml. To draw the curve various aliquots of this solution were taken and diluted to standard volume (10 ml.) and the readings taken in the photometer (L_{520}), together with the blank.

The mean value for K was found as 1.81 (± 0.04) x 10^{-1}

Only galvanometer readings between 0.3 and 0.8 were taken for the unknowns; if the readings were outside these values, the concentration of the solution was changed accordingly. The results are given in Table XXVIII and the curve given in Fig. 5.

TABLE XXVIII.

(1) Total Tocopherol Content of Decolorised "Maroola" Oil.

No. of sample:	1	2
Weight of Oil:	1.2602	1.0011 g.

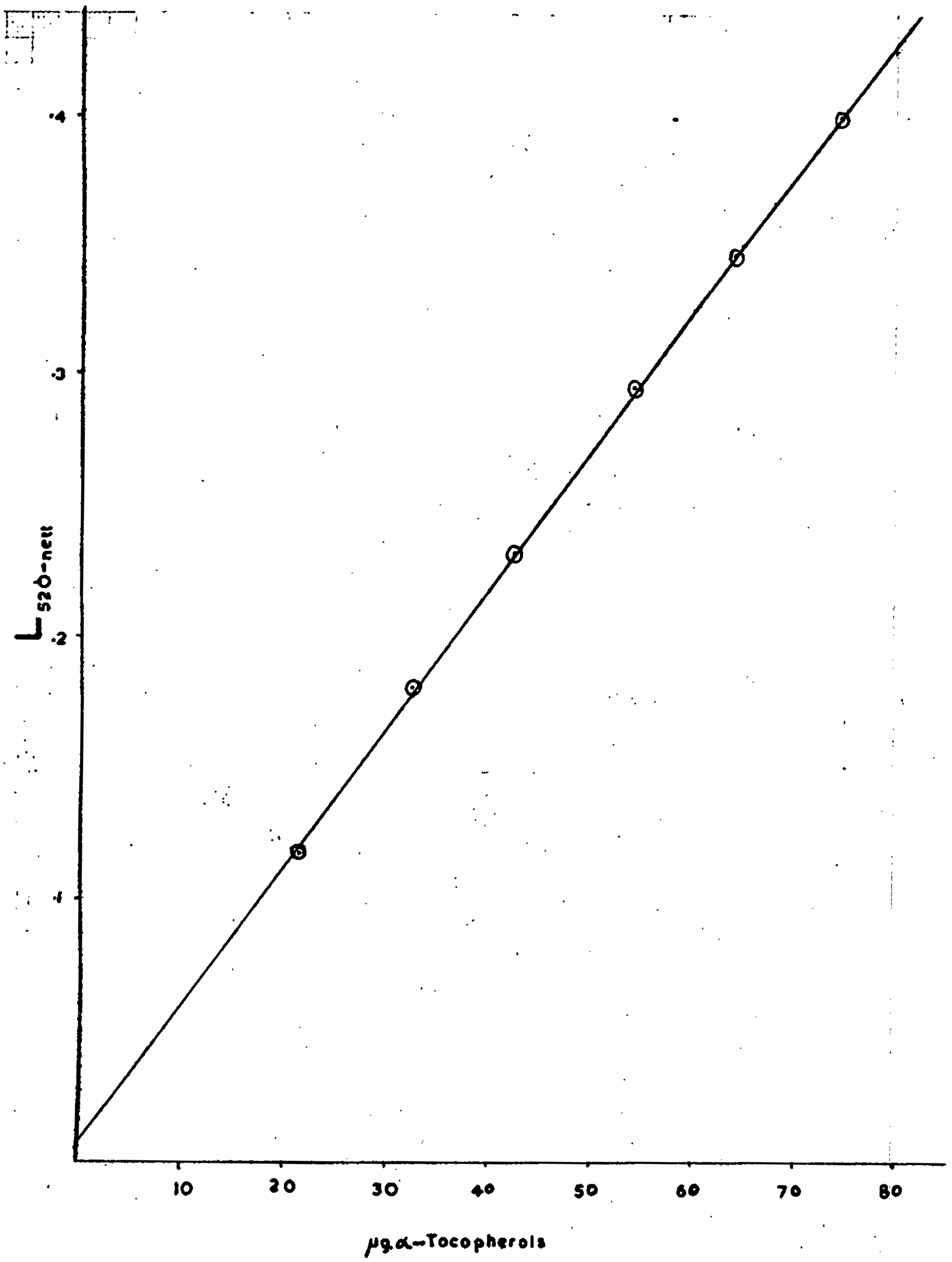
After distillation and hydrogenation the solution was made up to 50 ml. Readings were taken in the photometer, without dilution.

No.	G_{520} (50 secs.)	Mean G_{520}	L_{520}	L_{520} Nett	Total Tocopherols mg./g. oil
Control	80.0 80.5 80.25	80.25	0.0955	-	-
1 10 ml. soln.	27.2 27.5 27.0	27.2	0.5670	0.4715	0.338
1 5 ml. of soln. 5 ml. of alcohol	46.2 47.0	46.6	0.331	0.2355	0.338
Control	80.1 79.5 79.8	79.8	0.0980	-	-
2 10 ml. of soln.	33.2 33.1	33.1	0.481	0.385	0.346

(2) /..

Fig. 5.

Calibration Curve for the Determination of Tocopherols.



(2) Total Tocopherol Content of Impure "Maroola" Oil.

No. of sample:	1	2	3
Weight of oil:	0.9108	0.9038	0.9800 g.

Sample 1 was not hydrogenated, and after distillation the sample was made up to 50 ml. and the total tocopherol content determined in the usual way. Samples 2 and 3 were distilled, hydrogenated and made up to 50 ml. Two ml. of this stock solution were diluted to 50 ml. and the readings taken in the photometer.

No.	G_{520} (50 sec.)	Mean G_{520}	L_{520}	L_{520} Nett	Total Tocopherols mg./g. oil.
Control	80.1 79.5 79.8	79.8	0.0980	-	-
1 10 ml. soln. (2 ml. → 50 ml.)	36.6 36.8	36.7	0.436	0.338	8.34
2 10 ml. soln. 2 ml. → 50 ml.	40.0 40.2 40.00 40.2	40.1	0.396	0.298	7.46
3 10 ml. soln. 2 ml. → 50 ml.	38.9 38.2 38.8	38.6	0.413	0.315	7.39

(vii) Investigation and Analysis of the Unsaponifiable Material of the Kernel Oil.

The unsaponifiable fraction of the oil was extracted and the excess acids removed according to the S.P.A. method[24].

The determination of sterols, α-glyceryl ethers, saturated and unsaturated hydrocarbons was carried out according to the method of Rapson *et al.*[70]

The hydrocarbon content of the non-sap. was determined by separating it from the hydroxylic components by chromato-

graphy /...

graphy on an alumina column. The details are as follows:

1-1.5 g. of non-sap. was dissolved in 25 ml. of light petroleum (b.p. 40°-60°) and chromatographed on a column of alumina, 10 cm. in length and 2 cm. in diameter. The alumina was activated by heating overnight at 140°. The chromatogram was developed with a further 150 ml. of light petroleum (b.p. 40°-60°), and the whole petroleum fraction collected in one flask and analysed for the hydrocarbon content of the non-sap. (Fraction 1).

The chromatogram was further developed with 250 ml. of dry ether, which was collected in a separate flask and analysed for "sterols". The chromatogram was then developed with 100 ml. absolute alcohol containing 5% acetic acid (fraction 3), this last eluent should remove the unsaponifiable material completely from the column.

Analysis of Fraction 1.

The petroleum was removed by distillation through a 25 ml. tared distilling flask and the residual material dried under vacuum on the water bath and weighed. The iodine value was determined by the Rosenmund-Kuhnhenn method and the unsaturated material expressed as the "squalene content" of the non-sap.; the difference between this and the total weight of fraction 1 was designated as the saturated hydrocarbons.

Analysis of Fraction 2.

The ether was removed by distillation through a tared distilling flask and the residual material dried under vacuum. The fraction was analysed for the sterol content according to the colorimetric method of Pijoan and Walter[71] (used for the determination of cholesterol).

Analysis of Fraction 3.

The alcohol was distilled through a tared distilling flask and the residue dried under vacuum and weighed. This fraction was analysed for the sterol content by the above-mentioned method and also for the α-glyceryl ether content. The glyceryl ethers was determined as follows: The fraction was dissolved in ethyl acetate and oxidised with a periodic acid reagent according to the method proposed by Rapson *et al.*[102]. The formaldehyde formed in the oxidation was determined with dimedone. The results of the analysis have been given in Table XXVII.

INVESTIGATION OF THE FRUIT OF THE STRYCHOS SPECIES.

The genus Strychnos is a member of the Loganiaceae or Strychnine family, and is found abundantly in the Bushveld of the Transvaal, Natal, Mozambique and Madagascar[113]. The fruits of the species are rather unusual in structure; they are giant berries with a hard woody rind (or shell). The seeds of some of these species have been reported to be poisonous, but the pulp and juice in most fruit are harmless and pleasant to eat. The species are known under the common names of "Botter Klapper", 'Klapper", "Monkey Apple", while "Mkwakwa" is the common native name for it.

Hilditch makes no reference to any oil from these species, and it is apparent that very little has been done on the determination of the oil content of related species of this family.

In a popular article on the possible sources of vegetable oil in South Africa, Lehman[114] stated that the kernel covering pulp of the "Mkwakwa" (Strychnos innocua) contained 40% of oil, and that 400 tons of oil could be obtained annually from about 100,000 trees.

Articles on the possible cultivation of the trees and production of the oil appeared in "The Farmers Weekly"[115].

The Division of Chemical Services analysed Strychnos innocua fruit from Zululand for Lehman[116] and found the following composition for the fruit and oil content of the pulp:

Table /...

TABLE XXX.

Composition and Analysis of *Strychnos innocua*.

Fruit:

Weight whole fruit	253 g.	
Weight of shell	74 g.	= 29%
Weight of pulp and kernels	179 g.	= 71%

Fruit (dry weight):

Weight of shell	44 g.	= 33.6%
Weight of kernel	37 g.	= 29.2%
Weight of pulp	50 g.	= 38.2%
Moisture content of pulp and kernel		46.1%
Nitrogen content of kernel		1.205% = 7.7% protein
Nitrogen content of pulp		0.74% = 4.62% protein

Oil Content:

Yield of oil from pulp as received	11.8%
Yield of oil from pulp finely cut and dried	13.0%
Yield of oil from stamped pulp as received	27.3%
Yield of oil from stamped pulp dried	29.3% *
Yield of oil (calculated on whole dried fruit)	11.2%
Yield of oil (calculated on total fruit)	5.79%

(* Author's Note: There is obviously a mistake in the above report. If the pulp contains 46% of moisture, and the wet pulp contains 27.3% oil, the dried pulp should contain at least 52% of oil.)

During this investigation the oil content of the fruits of the following three *Strychnos* species have been determined:

Strychnos innocua, Del., collected in Pondoland.

Strychnos coccoloides, Bak, collected in the Waterberg district of the Transvaal.

Strychnos dysophylla, collected in the Middelburg - Groblersdal district of the Transvaal.

Strychnos dysophylla is known as the "Poisonous Klapper", and part of the flesh and kernels were fed to rats to test their toxic nature. No poison, however, could be detected in either the flesh or the kernels.

Owing to the low oil content of the kernels and flesh, no large scale investigation of the oils was undertaken and only the composition of the fruit and the constants of the oil are given.

TABLE XXXI.

Composition of the Fruit of *Strychnos* species.

	Strychnos innocua	*Strychnos coccoloides*	*Strychnos dysophylla*
Weight of fruit (g.)	67 - 462	73 - 380.5	94 - 480
Average weight (g.)	168	166.7	194.5
% Shells in fruit	-	-	20.3 - 37.1
% Kernel in fruit	-	-	8.1 - 17.7
Oil content of pulp (%)	3.80	0.85	2.11
Oil content of kernels (%)	-	-	0.5
Moisture content of total pulp (%)	66.9	71.0	78.2

Table XXXII. /....

TABLE XXXII.

Characteristics of *Strychnos* Oils.

	Strychnos innocua	*Strychnos coccoloides*	*Strychnos dysophylla*
Colour	Reddish colour	Reddish colour	Reddish colour
Specific gravity (25°/25°)	-	-	0.9208
Refractive index at 25°C	-	-	1.4673
Acid value of oil	-	-	10.5
Iodine value	80.5	119.9	87.3
Saponification value	192.1	168.6	188.4
Acetyl value	5.5	9.15	9.7
Reichert-Meissel value	0.24	1.64	1.58
Polenske value	0.64	0.51	0.38
% Unsaponifiable material	3.27	19.9	4.50
Results of Alkali Isomerisation of Total Acids:			
% Linoleic acid	1.9	19.2	12.3
% Linolenic acid	7.23	5.2	2.4

A compound with a slightly bitter taste was extracted with the oil from the fruit pulp of *Strychnos dysophylla*. Several attempts to purify this compound by crystallisation were unsuccessful. As only a small quantity of material was available further investigation was not undertaken.

Experimental. /....

EXPERIMENTAL.

The fruits of the different species were collected in the above-mentioned areas.

After picking, the fruit was packed in wooden cases and dispatched to our department and analysed with delay. The range of size of the fruit, determined by selecting the smallest and largest fruit in the consignment, is shown in Table XXXI. The average weight of the fruit was determined by weighing about 100 fruit which were more or less representative of the consignment. This fruit was broken up into shell, pulp and kernels, which were independently weighed to determine the composition of the total fruit (not determined in the case of Strychnos innocua and Strychnos coccoloides).

The total kernels and pulp (or separately in the case of Strychnos dysophylla) were minced and extracted with acetone in a Soxhlet extracter. After 10-12 hours of extraction the acetone was replaced with fresh acetone and the extraction continued for another 10-12 hours. The total volumes of acetone were combined and the acetone removed by distillation through a tared distilling flask and the residual oil dried under vacuum.

The oil from Strychnos dysophylla was a semisolid and the heterogeneous character indicated that it contained a solid compound. Tests with the different solvents showed that a solid compound could be separated on addition of ether, light petroleum (b.p. 40°-60°) and benzene.

The chemical and physical constants of the oils were determined according to the standard A.O.C.S. methods (outlined in Appendices B - J) and these values are listed in Table XXXII.

The linoleic and linolenic acids were determined on the total free fatty acids according to the alkali isomerisation method of Kraybill, Mitchell and Zschiele[49], the results of which are also given in Table XXXII.

In the bulk extraction of the total minced pulp and kernel, acetone was used as solvent and replaced with fresh acetone after 10-12 hours extraction. The total acetone was combined and the acetone removed by distillation and the residue dried. The total extract was diluted with light petroleum (b.p. 40°-60°, 2-3 volumes per volume of oil) and the insoluble material collected on a Buchner filter.

The light petroleum was removed by distillation and the oil dried under vacuum and stored under CO_2 in a refrigerator. The residue was freed of any adhering oil and dried under vacuum. In the small scale extraction of the oil, it was not possible to determine the percentage of solid material (the material tends to adhere to the inside of the flask). In the large scale extraction, approximately 35 Kg. of wet pulp and kernels yielded 24 g. of solid material. This material was insoluble in water, light petroleum, benzene and slightly soluble in ether. The material was readily soluble in acetone, chloroform, alcohol, ethyl acetate and acetic acid, also soluble in a 5% sodium hydroxide solution, but insoluble in 5% hydrochloric and sulphuric acid solutions. Tests for nitrogen and sulphur were negative. The compound may be a glycoside.

Appendix A. /...

APPENDIX A.

Determination of Unsaturation, i.e., Iodine Value.

The following iodine value reagents have been used in the course of this investigation:

(a) Wijs' reagent[51].

Iodine trichloride (9 g.) was dissolved in 1 litre glacial acetic acid, followed by 10 g. of iodine. The flask was stoppered with a loosely fitting cork and heated on a water bath until the colour of the solution changed to a deep reddish brown. The solution was cooled and stored in a brown bottle.

Determination:

The material under examination (0.1 - 0.3 g., depending on the I.V.) was accurately weighed off and dissolved in 10 ml. of dry carbon tetrachloride, and the reagent added. A contact time of 60 minutes was allowed, after which period the excess halogen was back-titrated with standard thiosulphate after addition of 10 ml. of 20% KI and 80 ml. of water. At the same time blanks were carried out. For successful determination of the iodine values, not less than 100% of excess reagent must be present and the iodine value of the duplicate determinations must not vary by more than 1%.

(b) Woburn Reagent for Conjugated Unsaturated Compounds.[52]

Iodine (20.5 g.) was dissolved in 925 ml. of acetic acid with moderate heating and the solution cooled to room temperature; 25 ml. of this solution was measured out and titrated with 0.17 N sodium thiosulphate solution. From the titration the number of additional mls. Y required to dilute the solution to 0.32 N were calculated as follows:

a = ml. of thiosulphate used

b = g. of iodine equivalent to 1 ml. of thiosulphate solution

then $Y = 1773 \times a \times b - 900$

The weight of bromine X necessary to double the halogen equivalent was calculated as follows:

$$X = 22.7 \times a \times b$$

The solution was made up to volume and stored in a dark bottle.

Determination:

The sample under consideration was dissolved in 10 ml. carbon tetrachloride and 400-600% excess of reagent added, and the solution allowed to stand for one hour and back-titrated with 0.17 N thiosulphate according to the method described under (a).

(c) Wijs-Kemp Reagent.[101]

Made up according to reagent (a), but using carbon-tetrachloride as solvent instead of glacial acetic acid.

Determination:

This reagent was used in the determination of the unsaturation of compounds which are insoluble in glacial acetic acid (e.g., raw rubber, polymers, etc.

The finely divided rubber sample (15-20 mesh) of 0.1 - 0.06 g. was placed in a 500 ml. Pyrex glass-stoppered bottle with 50 g. of pure p-dichlorobenzene. The flask, with contents, was placed on a hot-plate at a temperature of 175°-185° and the contents swirled gently from time to time to facilitate solution. Time of solution varied from about one to two hours. The flask with contents was removed and allowed to cool to room temperature. Before the p-dichlorobenzene was completely crystallised, 50 ml. of dry chloroform

were /...

were added, followed by the reagent and the flask allowed to stand in the dark for one hour. The excess reagent was then back-titrated with standard thiosulphate.

(d) Rosenmund-Kuhnhenn Reagent.[53]

Glacial acetic acid (40 ml.) was placed in each of three Pyrex Erlenmeyers. To the first, 28.5 g. of dry freshly distilled pyridine were added with cooling; to the second, 35.5 g. of concentrated sulphuric acid were added slowly, cooled, and when cool the contents of the second flask were added to the contents of the first flask with cooling. Bromine (28.4 g.) was added to the third flask (cooling). This solution was added to the mixture of the first two solutions and made up to 1 litre with glacial acetic acid followed by another $2^1/_2$ litres of glacial acetic acid. This solution was approximately 0.1 N with respect to bromine.

A 2.5% solution of mercuric acetate in glacial acetic acid was also made up.

Determination:

To the sample dissolved in 5 ml. of carbon tetrachloride was added 50 ml. of reagent, followed immediately by 10 ml. of 2.5% of mercuric acetate solution. The sample was allowed to stand for one hour in the dark and then back-titrated with standard thiosulphate according to the standard procedure.

APPENDIX B.

Moisture and Volatile Matter.[17]

(A.O.C.S. Method Aa 3-38.)

Duplicate samples of 5-10 g. each were accurately weighed into tared 2-4 inch Pyrex petri dishes, provided with a cover.

The petri dish and cover were placed in an oven and

dried /...

dried at 105°-110° for 12-16 hours (overnight). The dishes were removed from the oven, immediately covered and cooled in a desiccator to room temperature and weighed. The dishes were placed back in the oven and the drying continued until the weight remained constant.

APPENDIX C.

The Society of Public Analysts' Method for the Determination of Unsaponifiable Matter in Oils.[24]

Reagents:

(i) N/2 Alcoholic KOH, using purified alcohol.

The alcohol was purified as follows:[72] $2^1/_2$ litres of 96% alcohol were refluxed with 24 g. of potassium hydroxide and 12 g. of aluminium powder for one hour. The alcohol was then distilled and 2 litres collected after the initial 200 ml. were discarded.

(ii) Ethyl ether.

Saponification:

2.0 - 2.5 g. of oil, accurately weighed, were boiled for one hour under reflux with 25 ml. of the N/2 alcoholic KOH solution.

Extraction:

The soap solution, which was slightly cooled, was poured into a total of 50 ml. of water (including washes, etc.) contained in a separating funnel and successively extracted with 50 ml. portions of ether (3 times). During the extraction, the separating funnel and contents were mildly swirled to avoid emulsion formation. The ether solutions were combined in one separating funnel and washed once with 20 ml. of water. The ethereal solution was then washed twice with 20 ml. portions of N/2 aqueous KOH, followed by 2-3 more water washes to remove excess alkali. In all cases utmost care was taken to avoid the formation of emulsions.

The ether was then removed by distillation through a tared distilling flask and the residue dried on a water bath under vacuum of a water pump.

Extraction of Acids:

For the quantitative determination of the fatty acids, the first water-wash and the two alkali washes were added to the aqueous solution and this total solution just acidified with concentrated hydrochloric acid and the fatty acids extracted with three volumes of ether (50 ml. each). The ether extracts were combined, washed free of excess mineral acid, the ether removed by distillation through a tared distilling flask and the residual acids dried on a water bath under vacuum.

APPENDIX D.

Specific Gravity at 25°/25°C.[17]

For the determination of the specific gravity of oils, ordinary specific gravity bottles (25 ml.) were used and for the determination of the specific gravity of smaller samples (e.g., methyl esters) pycnometers (2 ml.) were used. The bottles (or pycnometers) were standardised as follows:
The bottle was cleaned, dried and weighed. The bottle was then filled with distilled water at 20° and carefully swirled to remove any air bubbles. The stopper was inserted and the bottle immersed in a water bath at 25° ±0.2°C for 30 minutes. The bottle was then carefully removed, dried and the last traces of moisture removed on the tip with a piece of filter

paper. /...

paper. The bottle and contents were weighed. The difference between this weight and the weight of the empty bottle represented the weight of water at 25°C.

The bottle was dried and filled with oil at 22°-23°C and kept at 25° for 30 minutes, redried and weighed.

$$\text{S.G. of oil } (25°/25°) = \frac{\text{Weight of oil}}{\text{Weight of water at } 25°\text{C}}$$

APPENDIX E.

Refractive Index.[17]

The refractive indices of the oils were taken with an Abbé refractometer at the required temperatures (25° or 40°C).

APPENDIX F.

Free Fatty Acids (Acid Value).[17]

A solvent was made consisting of 50 parts 96% alcohol and 50 parts benzene and any free acids in this solvent (carbon dioxide) neutralised with a few drops of 0.1 N aqueous NaOH until permanent pink to phenol phthalein as indicator.

10-20 g. of oil (accurately weighed) were placed in a 200 ml. Erlenmeyer flask, 50 ml. of neutralised solvent added and the contents heated on a waterbath for a short period. The contents were slightly cooled and titrated with 0.1 N NaOH until the pink colour persisted for 30 seconds.

The free fatty acids were calculated as percentage oleic acid, with the formula:

$$\frac{\text{ml. Alkali} \times N \times 28.2}{\text{Weight of sample}}$$

Appendix G. /...

APPENDIX G.

Saponification Value (or Equivalent).[17]

A 0.3 N alcoholic potash solution was made up (purified alcohol, see Appendix A) and freshly filtered before use. A weighed quantity (1.4 - 1.6 g.) oil (or methyl ester) was placed in a 200 ml. alkali resistant Erlenmeyer (ex Schott and Gen. Jena); 20 ml. of 0.3 N alcoholic potash added and the solution refluxed for 20 minutes. After this period the excess alkali was back-titrated with standard N/3 aqueous hydrochloric acid solution, using phenol phthalein as indicator.

A control of 20 ml. alcoholic potash was also carried out at the same time.

Saponification Value:

$$\frac{56.1 \times N \times (\text{Titre of Control} - \text{Titre of Sample})}{\text{Weight of Sample.}}$$

Saponification Equivalent:

$$\frac{\text{Weight of Sample}}{(\text{Titre of Control} - \text{Titre of Sample}) \times N}$$

APPENDIX H.

Acetyl Values.[17]

A mixture of ca. 50 ml. of oil and 50 ml. of acetic anhydride was refluxed for two hours in a convenient g.g. flask. The mixture was then poured into a beaker containing 500 ml. distilled water and boiled for 15 minutes under carbon dioxide. It was cooled, the water siphoned off and the acetylated fat boiled twice more with 500 ml. of water. The acetylated fat was transferred to a 250 ml. separating funnel and washed twice more with warm water (60°-70°). The water was drained off as completely as possible, the acetylated oil dissolved in a small volume of ether and the solution dried

over /...

over anhydrous sulphate.

The solution was filtered, the ether removed by distillation and the residue dried under vacuum at 80°-100° and cooled. The saponification values of the oil and acetylated product were determined as in Appendix G, and the acetyl value calculated with the formula:

$$\text{Acetyl value} = \frac{S' - S}{1.000 - 0.00075\,S}$$

where S = Saponification value before acetylation.

S' - Saponification value after acetylation.

APPENDIX J.

Reichert-Meissel and Polenske Values.[17]

Five g. of oil were accurately weighed into a 300 ml. distilling flask, 20 ml. of a glycerol-sodium hydroxide solution (20 ml. of a 50% sodium hydroxide solution to 180 ml. 'Analar' glycerol) were added and the flask heated until the solution became clear. Water (135 ml.) was then added, followed by 5 ml. of sulphuric acid (200 ml. conc. H_2SO_4 diluted to 1 litre with distilled water) and the 110 ml. of water distilled off through a standard apparatus (see A.O.C.S. Methods, Cd. 5-40).

This distillate was cooled to 15°C and the solid acids collected on a filter paper; 100 ml. of the filtrate were titrated with 0.1 N NaOH solution, using phenol phthalein as indicator.

A blank was carried out under exactly the same conditions.

Reichert-Meissel Value:

1.1 x (Titre of Sample - Titre of Blank)

Polenske /....

Polenske Values:

The solid fatty acids (on the filter paper) were washed three times with 15 ml. of water each, and then dissolved in 45 ml. of neutralised 95% alcohol. The alcohol solution was titrated with 0.1 N NaOH solution, using phenol phthalein as indicator.

Polenske Value: ml. of 0.1 N NaOH - Solution.

APPENDIX K.

Determination of Linoleic and Linolenic Acids.[47]

Reagents: (1) Ethylene Glycol reagent. Ethylene glycol, freshly distilled under reduced pressure over KOH and zinc dust. This ethylene glycol (750 g.) was heated to 190° for 10 min., cooled to 150° and 60 g. of 85% KOH carefully dissolved and the solution again heated at 190°C for 10 min.

(2) Purified 96% Alcohol. (See Appendix A.)

Procedure:

About 0.1 g. of fatty acid was accurately weighed in a glass vial. Ten ml. of the ethylene glycol reagent were pipetted into a 6" x 1" Pyrex test-tube and placed in an oil bath at 180 ±0.5°C. Twenty minutes later the vial, containing the fatty acids, was added and with intermittent shaking kept at 180 ±0.5°C for 25 minutes. At the end of 25 minutes the tube was removed and rapidly cooled under water tap and the contents of the flask quantitatively transferred to a 100 ml. volumetric flask and the solution made up to the mark with 96% alcohol.

A control was likewise treated and made up to 100 ml.

with /....

with 96% alcohol. The solutions were allowed to stand overnight in the refrigerator. They were then allowed to come to room temperature and were filtered through Whatman No. 1 filter paper and the absorption measured in a Beckman spectrophotometer, Model D.U. at 234 and 268 m . The blank solution was placed in the solvent cell and diluted in the same way as the solutions during the determinations.

The percentage linoleic and linolenic acids were calculated from the values given by **Mitchell, Kraybill** and Zscheile.[47]

APPENDIX L.

Preparation of the Methyl Esters of the Acids.[31]

A weight of acid (say 50 g.) was refluxed with 5 times its volume of absolute methanol (250 ml.) in the presence of 1-2% of concentrated sulphuric acid ($^1/_2$ - 1 g.). Refluxing was continued for 4-5 hours; the solution was cooled to room temperature and then poured into double its volume of water (500 ml.). This solution was extracted with three volumes of ether (250 - 500 ml. each, depending on the solubility of the ester in the ether); the ether volumes were combined, washed 2-3 times with water and then extracted 2-3 times with a 5% potassium carbonate solution. The ether solution was then again washed with water; the water removed as completely as possible and the ether removed by distillation. The residue was dried under vacuum at 80°-100°.

The yield of ester should be of the order of 98-100 %.

Appendix M. /...

APPENDIX M.

Modified Villavechia Test for Sesamé Oil.[17]

A sample of the oil (say 10 ml.) was mixed with an equal volume of concentrated HCl in a test-tube. To this mixture was added 0.1 ml. of a 2% furfural (reagent grade) in purified 95% alcohol (v/v). The total solution was well shaken and the emulsion allowed to break. A red or crimson coloration of the lower layer is positive for sesamé oil.

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TABLE XXXIII.

Composition of the Total Fatty Acids
of Oil from *X. caffra* Sond.

Acid	Liquid Acids	Solid Acids	Total (%)
Palmitic Acid	0.36	0.04	0.40
Stearic Acid	1.72	2.34	4.06
Cerotic Acid	-	6.35	6.35
Hexadecenoic Acid	1.49	0.03	1.52
Oleic Acid	31.01	3.75	34.76
Ximenynic Acid	21.55	2.76	24.31
Elaeostearic Acid	0.31	0.06	0.37
C_{20} unsat. Acid	0.45	-	0.45
C_{22} unsat. Acid	1.77	-	1.77
C_{24} unsat. Acid	3.61	-	3.61
Ximenic Acid	1.32	8.99	10.31
Lumeniqueic Acid	1.75	9.51	11.26
C_{34}-2H Acid	-	0.82	0.82
Total (%):-			99.99

TABLE XXXIX.

Composition of the Total Fatty Acids
of oil from *Ximenia americana* var. *microphylla*.

Acid.	Liquid Acids	Solid Acids	Total (%)
Palmitic acid	0.20	0.07	0.27
Stearic acid	0.67	1.34	2.01
Cerotic acid	-	5.46	5.46
Hexadecenoic acid	0.43	0.09	0.52
Oleic acid	33.61	3.48	37.09
Ximenynic acid	19.25	2.69	21.94
Elaeostearic acid	0.14	0.03	0.17
C_{20} unsat. acid	0.57	-	0.57
C_{22} unsat. acid	0.09	-	0.09
C_{24} unsat. acid	0.99	-	0.99
Ximenic acid	5.02	10.88	15.90
Lumeniqueic acid	0.40	13.97	14.37
C_{34-2H} acid	-	0.62	0.62
Total:-			100.00

TABLE XLII.

Composition of the Fatty Acids of *Sclerocarya caffra* Sond. ("Maroole").

Acid.	Fraction A	Fraction B	Fraction C	Total (%)
Palmitic	13.10	3.04	-	16.14
Stearic	4.75	0.30	-	5.05
Arachidic	0.10	-	-	0.10
Behenic	0.98	-	-	0.98
Hexadecenoic	-	-	1.05	1.05
Oleic	10.39	54.62	1.72	66.73
Linoleic	-	2.63	4.64	7.27
Linolenic	-	-	-	trace
C_{20}-2H	-	1.13	1.55	2.68
Erucic	-			
Total (%)				100.00

Linoleic and Linolenic acids in total Fatty Acids:-

	Calculated (%)	Alkali Isomerisation (%)
Linoleic acid	7.27	6.4
Linolenic acid	trace	0.42

TABLE XL

Fractional Distillation of "Liquid" Methyl Esters of *Ximenia americana* var. *Microphylla* Welw.

	Temperature (°C)			Pressure		E(1%, 1 cm.)							Calculated Composition of Methyl Esters:-											
													Saturated			Unsaturated								
Fraction	Head	Middle	Oil Bath	mm's	Weight (a)	268 mμ	229 mμ	I.V.(i)	S.E.(E)	a'	i'	E'	C_{16}	C_{18}	C_{26}	C_{16}	C_{18}	Ximenynic	Elaeostearic	C_{20}	C_{22}	C_{24}	C_{26}	C_{30}
1st drop	134	180	215	0.4																				
I	155	185	215	0.4	5.0097	0.12	8.34	79.8	287.0	4.9333	79.06	286.9	0.4935			1.0428	3.3969	0.0761	0.0003					
II	154	188	217	0.3	5.8368	0.2	9.44	86.8	295.8	5.7379	86.18	295.9					32.2235	0.1003	0.0006					
III	157	191	220	0.45	6.6544	0.3	14.54	87.3	295.8	6.4772	86.27	295.9						0.1762	0.0010					
IV	157	193	220	0.45	6.7944	0.3	16.87	87.9	295.9	6.5848	86.69	296.0						0.2086	0.0010					
V	157	195	220	0.4	7.5494	0.3	25.02	87.5	296.3	7.2045	85.66	296.5						0.3438	0.0011					
VI	157	195	225	0.4	6.7328	0.4	41.8	88.0	296.2	6.2191	84.93	296.3						0.5124	0.0013					
VII	157	197	225	0.4	7.1260	0.5	50.0	87.6	296.5	6.4754	83.94	296.9		1.6010			41.0291	0.6488	0.0018					
VIII	157	197	226	0.35	6.8733	0.5	62.0	87.9	296.4	6.0956	83.22	296.9						0.7759	0.0017					
IX	158	197	227	0.40	7.7787	0.6	70.65	88.4	296.3	6.7764	83.06	296.9						1.0000	0.0023					
X	159	197	227	0.40	7.6078	0.7	87.5	88.8	295.6	6.3928	82.01	296.9						1.2120	0.0027					
XI	159	198	227	0.35	9.7332	1.0	113.0	90.2	296.3	7.7262	81.24	296.9						2.0020	0.0049					
XII	161	202	230	0.4	7.1667	1.3	173.0	93.8	295.3	4.9049	79.66	296.9						2.2570	0.0047					
XIII	164	205	235	0.3	7.6048	1.7	241.2	100.6	294.8	4.2588	81.81	296.9						3.3390	0.0065					
XIV	166	208	236	0.3	7.5806	2.4	355.0	111.4	294.1	2.6716	87.02	297.3					2.5318	4.9000	0.0090	0.1398				
XV	170	211	238	0.3	8.2882	3.2	433.5	121.0	294.1	1.7352	106.0	300.7					1.4107	6.5400	0.0133	0.3245				
XVI	178	217	243	0.25	8.6618	5.1	477.5	125.7	296.5	1.1098	128.5	327.8						7.5300	0.0221	0.9061	0.2037			
XVII	190	237	265	0.1	8.0192	7.4	424.5	122.6	309.3	1.7922	114.3	378.8						6.1970	0.0298		0.0119	1.7805		
XVIII	196	248	269	0.1	6.9193	5.9	384.0	117.9	318.5	2.0613	101.2	403.8						4.8380	0.0204			0.2629	1.7980	
XIX	196	264	272	0.1	3.8652	8.7	149.1	80.5	362.1	2.7992	63.3	405.3						1.0496	0.0168			0.3109	2.4885	
XX		Residue	(as acids)		11.2000	(38.3)	(126.1)	(86.4)	376.6			414.2						2.4757	0.2061				7.5796	0.9386
Total weight of Esters (g.)					147.0033								0.4935	1.6010		1.0428	80.5918	46.1818	0.3474	1.3704	0.2156	2.3543	11.8661	0.9386
As Acids (g.)					140.1958								0.4679	1.5258		0.9883	76.7801	43.9706	0.3076	1.3111	0.2070	2.2675	11.4596	0.9103
% of Total Acids					61.366								0.203	0.668		0.433	33.608	19.247	0.135	0.574	0.091	0.993	5.016	0.398

Degree of unsaturation of C_{20} esters: -2H

.. .. C_{22} esters: 3H

.. .. C_{24} esters: 3.2H

TABLE XLI.

Fractional Distillation of "Solid" Methyl Esters of *Ximenia americana* var. *microphylla* Welw.

													Calculated Composition of Methyl Esters:-									
	Temperature of Column (°C)			Pressure		E(1%, 1 cm.)							Saturated			Unsaturated						
Fraction	Head	Middle	Oil Bath	mm's	Weight (a)	268 mμ	229 mμ	I.V.(i)	S.E.(E)	a'	i'	E'	C_{16}	C_{18}	C_{26}	C_{16}	C_{18}	Ximenynic	Elaeostearic	C_{26}	C_{30}	C_{34}
1st drop	120	180	220	0.3	4.5414	0.6																
I	149	186	224	0.7			82.2	71.6	293.3	3.8603	62.17	293.5	0.1602	0.9247		0.2262	2.5492	0.6797	0.0014			
II	160	196	235	0.5	5.2469	0.7	120.4	78.7	299.1	4.0949	65.82	301.0		0.8635	0.0325		3.0062	1.150	0.002	0.1927		
III	162	214	238	0.5	5.6861	1.2	174.2	79.6	310.9	3.8801	58.45	320.3		0.7587	0.2579		2.0757	1.803	0.003	0.7878		
IV	202	230	255	0.2	6.1998	1.3	92.1	53.0	358.9	5.1558	38.42	376.3		0.5363	1.6824		0.6642	1.040	0.004	2.2729		
V	208	239	268	0.3	6.9281	0.4	12.3	44.6	391.9	6.7715	42.69	395.0		0.2388	2.0336		0.4098	0.1556	0.001	4.0893		
VI	210	240	268	0.4	5.5992	0.3	10.1	46.0	403.9	5.4953	44.50	406.8		0.0399	1.5357		0.0481	0.1029	0.001	3.8716		
VII	218	248	268	0.45	6.4990	0.3	10.9	47.3	414.0	6.3691	45.73	417.5						0.1289	0.001			
VIII	220	250	275	0.4	5.8233	0.3	11.2	48.2	418.4	5.7036	46.62	422.2						0.1187	0.001			
IX	220	250	278	0.4	6.9378	0.3	11.7	48.9	427.6	6.7890	47.26	432.0						0.1478	0.001			
X	221	255	281	0.4	6.5806	0.4	11.9	50.0	430.5	6.4370	48.30	435.1			7.7071			0.1426	0.001	15.7430	26.9543	
XI	224	257	283	0.4	6.0764	0.4	12.1	50.8	434.1	5.9415	49.13	438.9						0.1339	0.001			
XII	225	257	285	0.4	7.3578	0.5	13.6	52.1	437.5	7.1736	51.74	443.1						0.1822	0.002			
XIII	228	257	285	0.4	6.3593	0.6	16.50	52.2	440.4	6.1663	49.94	447.5						0.1910	0.002			
XIV	230	263	292	0.4	6.0901	0.65	23.80	53.1	450.3	5.8243	49.84	461.7						0.2638	0.002			
XV	230	270	300	0.05	5.0242	1.6	25.70	53.6	460.3	4.7851	49.97	473.9			0.2784			0.2351	0.004		3.3027	1.2040
XVI	Residue analysed as acids				4.9000		33.6	47.9	450.0	4.5656		468.4						0.2926	0.0417		4.2306	0.3351
Weight of Esters (g.)					95.8500								0.1602	3.3619	13.5276	0.2262	8.7532	6.7678	0.0691	26.9573	34.4876	1.5391
Weight as acids (g.)					92.4725								0.1590	3.2039	13.0664	0.2144	8.3392	6.4437	0.0658	26.0338	33.4486	1.4977
% of Total Acids:-					38.634								0.066	1.339	5.459	0.089	3.484	2.692	0.0275	10.876	13.974	0.626

TABLE XLIII.

Fractional Distillation of the Solid Methyl Esters (Fraction A) of *Sclerocarya caffra* Sond. ("Maroola").

	Temperature of Column (°C)							Calculated Composition of Ester fractions:-						
								Saturated				Unsaturated		
Fraction	Head	Middle	Oil Bath	Pressure mm's	Weight of Fraction (g.)	S.E.	I.V.	C_{16}	C_{18}	C_{20}	C_{22}	C_{16}	C_{18}	C_{20}
1st drop	88	150	216	0.2	6.8188	270.9	6.4	6.3090				Postulated as absent	0.5098	
I	111	155	216	0.2										
II	116	162	214	0.4	6.2080	268.7	4.2	5.9025					0.3055	
III	130	168	220	0.2	6.3568	270.6	7.8	5.7792					0.5776	
IV	131	169	220	0.2	6.6895	276.8	21.8	4.8309	0.1585				1.7001	
V	135	169	220	0.2	5.8081	280.2	25.1	3.4463	0.6649				1.6969	
VI	145	169	220	0.2	5.6576	278.5	27.0	3.7033	0.1734				1.7809	
VII	145	170	223	0.2	6.2278	282.4	33.8	3.1578	0.6149				2.4551	
VIII	145	171	224	0.2	6.2766	286.5	42.1	2.2390	0.9522				3.0834	
IX	145	171	224	0.2	6.6652	289.1	45.6	1.7480	1.3723				3.5448	
X	146	171	224	0.2	6.5341	292.7	47.6	0.9182	1.9894				3.6275	
XI	147	172.5	224	0.2	6.2032	294.5	48.2	0.4845	2.2329				3.4858	
XII	148	175.5	224	0.2	5.7309	294.0	47.5	0.5466	2.0074				3.1769	
XIII	149	180	227	0.2	5.9652	293.5	45.0	0.6784	2.1532				3.1336	
XIV	157	194	240	0.01	5.4596	292.7	36.9	0.8011	2.3063				2.3522	
XV		Residue	-	Pot fraction	3.8716	341.0	12.8			0.3121	2.9797		0.5798	
Total Weight of Methyl Esters (g.)					90.4730			40.5418	14.6264	0.3121	2.9797		32.0099	
Corresponding weight of Fatty Acids (g.)					86.0383			38.4425	13.9393	0.2987	2.8619		30.4959	
% Acids (of total Fatty Acids)					29.321			13.101	4.750	0.102	0.975		10.393	

TABLE XLIV.

Fractional Distillation of the "Intermediate Fraction" (Fraction B) of *Sclerocarya caffra* Sond. ("Maroola").

	Temperature of Column (°C)			Pressure mm's	Weight of Fraction (g.)	S.E.	I.V.	Calculated Composition of Ester fractions:-							
								Saturated			Unsaturated				
Fraction	Head	Middle	Oil Bath					C_{16}	C_{18}	C_{20}	C_{16}	C_{18}	C_{18-4H}	C_{20}	
1st drop	118	171	215	0.5											
I	131	175	216	0.4	5.2342	284.5	60.4	2.1557				2.4958	0.5827		
II	135	175	214	0.2	6.7401	294.1	85.2	0.4260				5.9397	0.3744		
III	138	177	216	0.2	6.2989	293.7	86.4	Fractions combined for calculation							
IV	140	180	220	0.2	6.1969	295.6	86.3								
V	145	184	220	0.2	6.1817	295.2	87.3								
VI	156	186	225	0.4	5.7194	296.2	86.6								
VII	153	188	228	0.2	6.2131	294.6	87.5								
VIII	154	189	228	0.2	6.0433	295.2	87.3								
IX	150	188	228	0.2	6.9952	293.2	87.1								Total Weight
X	150	184	228	0.1	6.1270	295.2	86.9	1.9278				69.0990	2.9213		73.9481
XI	150	184	228	0.1	6.3844	294.9	87.5	S.E. 293.06							
XII	150	184	228	0.1	5.9521	295.4	86.9	I.V. 86.9							
XIII	150	184	228	0.1	5.8773	295.9	86.9								
XIV	150	184	228	0.1	5.9588	296.3	86.4								
XV	Residue in flask				5.0390	304.9	76.9		0.4440			2.9403		1.6547	
Total weight of Methyl Esters (g.)					90.9614			4.5095	0.4440			80.4748	3.8784	1.6547	
Corresponding Weight of Fatty Acids (g.)					86.6418			4.2757	0.4231			76.6686	3.6912	1.5832	
% of Total Fatty Acids					61.722			3.046	0.301			54.618	2.629	1.128	

TABLE XLV.

Fractional Distillation of the "Liquid" Fraction (Fraction C) of *Sclerocarya caffra* Sond. ("Maroola").

Fraction	Temperature of Column (°C) Head	Middle	Oil Bath	Pressure mm's	Weight of Fraction (g.)	S.E.	I.V.	C_{16-2H}	C_{18-2H}	C_{18-4H} (Calculated)	C_{18-6H}	C_{20-2H}
1st drop	91	162	239	0.1								
I	142	180	255	0.1	5.1943	283.2	130.7	2.0884	0.6357	2.4702		
II	142	180	255	0.05	4.7731	289.7	146.8	0.7799	0.7252	3.2680		
III	142	178	255	0.05	4.4732	292.4	145.6	0.2192	1.1976	3.0564		
IV	150	189	286	0.1	4.7790	294.8	143.2		2.0000	2.7790		
Pot fraction	Residue				7.0980	312.8	106.2		0.4860	2.0577		4.5543
Total weight of Methyl Esters (g.)					26.3176			3.0875	5.0445	13.6313		4.5543
Corresponding weight of Fatty Acids (g.)					25.0718			2.9262	4.8059	12.9822		4.3575
% of total Fatty Acids					8.95			1.05	1.717	4.638		1.55

TABLE XXXVIII.

Fractional Distillation of "Solid" Methyl Esters of *Ximenia caffra* var. *natalensis* Sond.

Calculated Composition of Esters:-

	Temperature (°C)			Pressure		E(1%, 1cm.)							Saturated			Unsaturated						
Fraction	Head	Middle	Oil Bath	mm's	Weight (s) (g.)	268 mμ	229 mμ	I.V.(i)	S.E.(E)	a'	i'	E'	C_{16}	C_{18}	C_{26}	C_{16}	C_{18}	Ximenynic	Elaeostearic	C_{26}	C_{30}	C_{34}
1st drop	147	187	220	0.5																		
I	160	194	225	0.5	4.8376	1.22	64.3	58.7	294.7	4.2684	49.83	295.0	0.1761	1.6208		0.0802	2.3913	0.5662	0.003			
II	160	201	235	0.5	5.2478	0.76	79.1	46.3	304.5	4.4900	33.02	306.6		2.4223	0.2766		1.5675	0.7558	0.002	0.2236		
III	164	206	243	0.3	5.8492	0.95	100.0	62.1	313.0	4.7812	48.04	318.0		1.4669	0.4361		2.1561	1.065	0.003	0.7221		
IV	186	222	258	0.3	6.3681	1.27	100.2	52.8	335.0	5.2031	27.29	346.3		2.6055	1.6602		0.9169	1.161	0.004	1.0205		
V	201	233	265	0.3	6.0460	1.01	48.5	42.3	366.4	5.5093	28.88	375.6		0.7794	2.3752		0.5450	0.5337	0.003	1.8097		
VI	204	235	270	0.3	6.1954	0.55	20.6	41.4	382.8	5.9610	38.05	387.5		0.8965	1.4121			0.2324	0.002	3.6524		
VII	207	236	270	0.3	6.4586	0.41	13.3	42.2	388.1	6.3012	40.12	391.3		0.7734	1.4569			0.1564	0.001	4.0709		
VIII	209	240	280	0.3	6.0170	0.36	7.8	42.2	391.0	5.9305	40.95	392.9		0.6598	1.3600			0.0855	0.001	3.9107		
IX	212	242	282	0.3	6.6832	0.44	11.7	43.3	398.5	6.5389	41.42	401.7		0.3309	1.8466			0.1423	0.002	4.3614		
X	216	245	285	0.3	5.9471	0.43	12.3	45.1	405.7	5.8130	43.22	408.3		0.0380	1.7293			0.1331	0.001	4.0457		
XI	220	248	285	0.3	5.8590	0.40	12.6	46.7	417.5	5.7235	44.87	420.9						0.1345	0.001			
XII	226	253	288	0.3	6.4063	0.43	11.9	47.8	428.5	6.2665	46.06	433.0			5.0454			0.1388	0.001	6.9491	15.8310	
XIII	230	255	296	0.3	6.0065	0.35	12.0	48.7	434.3	5.8743	46.97	439.1						0.1312	0.001			
XIV	233	263	296	0.3	5.9549	0.64	15.7	50.4	442.3	5.7827	48.20	449.2						0.1702	0.002			
XV	220	274	296	0.02	4.3818	1.55	25.1	50.8	447.5	4.1785	47.12	459.4						0.2003	0.003			
XVI		Residue			2.9200	(15.6)	(48.00)	(55.2)	441.0	2.6503								0.2477	0.022		2.6503	
Weight of Esters (g.)					91.1785								0.1761	10.5935	17.5984	0.0802	7.5768	5.8541	0.052	30.7661	18.4813	
Total weight as acids (g.)					87.8156								0.1670	10.0958	16.9985	0.0760	7.2184	5.5738	0.0495	29.7122	17.9245	
% of total acids					29.164								0.055	3.353	5.645	0.025	2.397	1.851	0.016	9.867	5.953	

TABLE XXXVII.

Fractional Distillation of "Liquid" Methyl Esters of *Ximenia caffra* var. *natalensis* Sond.

Fraction	Temperature (°C)			Pressure		E(1%, 1cm.)						
	Head	Middle	Oil Bath	mm's	Weight (a)	268 mμ	229 mμ	I.V.(i)	S.E.(E)	a'	i'	E'
1st drop	90	176	220	0.5								
I	155	187	220	0.5	4.0627	4.9	17.15	76.1	280.0	3.9258	74.38	282.6
II	155	190	220	0.5	4.9450	0.8	7.74	87.7	295.6	4.8733	87.16	295.6
III	155	192	220	0.45	5.3345	0.4	8.62	89.1	296.6	5.2497	88.46	296.7
IV	155	190	220	0.45	7.0258	0.3	10.10	89.2	296.8	6.8955	88.50	296.9
V	155	190	223	0.4	5.3974	0.2	13.40	89.1	296.2	5.2657	88.24	296.3
VI	157	192	225	0.5	5.7136	0.2	13.20	89.6	296.7	5.5763	88.73	296.8
VII	157	190	225	0.5	5.9605	0.2	13.32	89.3	297.9	5.8160	88.44	298.0
VIII	157	191	226	0.5	5.7793	0.15	15.67	89.1	296.9	5.6144	88.05	297.0
IX	157	194	230	0.5	6.0194	0.2	19.15	89.3	296.7	5.8096	88.02	296.9
X	158	198	233	0.5	7.0648	0.2	36.1	89.3	296.3	6.6005	86.80	296.6
XI	165	204	240	0.5	6.5862	0.4	138.0	91.9	295.0	4.9302	80.85	296.7
XII	170	207	247	0.5	6.2433	0.8	411.5	118.3	293.8	1.5633	98.70	298.1
XIII	170	207	247	0.5	7.1071	0.9	525.5	126.6	293.6	0.3041	166.01	323.3
XIV	170	209	252	0.5	6.0036	1.1	514.0	125.5	294.4	0.3816	135.191	327.4
XV	181	225	267	0.2	5.8087	4.2	401.0	109.0	301.8	1.5567	65.46	330.9
XVI	192	255	282	0.05	6.1762	5.0	323.0	106.3	321.2	2.5292	79.35	374.4
XVII	196	276	292	0.05	3.5752	11.10	124.4	87.3	357.1	2.7455	75.59	382.7
XVIII		Residue			6.4900	(42.6)	(98.3)	(89.4)	(340.0)	5.2415		
Weight of Esters (g.)					105.2931							
Weight as acids (g.)					100.4177							
% of total acids					70.836							

Fraction	Calculated Composition of Esters:-											
	Saturated			Unsaturated								
	C_{16}	C_{18}	C_{26}	C_{16}	C_{18}	Ximenynic	Elaeostearic	C_{20}	C_{22}	C_{24}	C_{26}	C_{30}
1st drop												
I	0.3499	0.3155		1.4796	1.7809	0.1269	0.0079					
II						0.0697	0.002					
III						0.0837	0.0011					
IV						0.1292	0.001					
V		1.1638			50.5372	0.1317						
VI						0.1378						
VII						0.1445						
VIII						0.1649						
IX						0.2098						
X						0.4643						
XI		0.2920			4.6380	1.655	0.0012					
XII					1.4270	4.677	0.003	0.1363				
XIII						6.800	0.003	0.2888	0.0153			
XIV						5.619	0.002	0.3103	0.0715			
XV						4.240	0.0122	1.1495	0.408			
XVI						3.632	0.0156		0.4756	2.0536		
XVII						0.8099	0.0198			2.4697	0.2758	
XVIII						1.1157	0.1328			1.2906	3.9509	
Weight of Esters (g.)	0.3499	1.7713		1.4796	58.3829	30.2106	0.2041	1.8857	0.9704	5.8139	4.2267	
Weight as acids (g.)	0.3317	1.6881		1.4023	55.6205	28.7641	0.1943	1.8023	0.9318	5.5997	4.0819	
% of total acids	0.234	1.191		0.989	39.236	20.291	0.137	1.271	0.657	3.951	2.879	

Degree of unsaturation of C_{20} methyl esters:- -4.0 H

.. .. C_{22} .. .. :- -3.7 H

.. .. C_{24} .. .. :- -2.0 H

TABLE XXXIV.

Fractional Distillation of "Liquid" Methyl Esters of *Ximenia caffra* Sond.

Calculated Composition of Ester Fractions:-

Fraction	Temperature (°C) Head	Middle	Oil Bath	Pressure mm's	Weight (a) (g.)	E(1%, 1cm.) 268 mμ	229 mμ	I.V.(i)	S.E.(E)	a'	i'	E'	Saturated C_{16}	C_{18}	C_{26}	Unsaturated C_{16}	C_{18}	Ximenynic	Elaeostearic	C_{20}	C_{22}	C_{24}	C_{26}	C_{30}
1st drop	124	180	218	0.4																				
I	158	189	219	0.5	9.5553	0.34	12.12	75.6	284.5	9.3426	74.46	284.3	0.7254	0.8358		3.1742	4.6074	0.2107	0.0018					
II	159	192	221	0.4	7.0101	0.90	9.00	82.2	294.8	6.8920	81.53	294.5	0.0235	0.1822		0.3257	6.3606	0.1149	0.0030					
III	159	194	222	0.45	8.3850	0.50	12.48	83.5	295.6	8.1945	82.55	295.7	0.0177	0.2872			7.8896	0.1905						
IV	159	195	225	0.45	8.3847	0.46	16.32	84.1	295.6	8.1355	82.85	295.7	0.0176	0.2566			7.8613	0.2492						
V	159	196	228	0.45	7.7902	0.44	23.00	83.5	295.6	7.4640	81.69	295.7	0.0256	0.3270			7.1114	0.3262						
VI	159	196	228	0.45	7.1050	0.47	13.50	84.4	295.3	6.9350	82.08	295.4	0.0208	0.2707			6.6390	0.1745						
VII	159	195	229	0.4	7.9353	0.46	28.52	84.3	295.1	7.5225	82.11	295.6	0,0224	0.2961			7.2040	0.4105						
VIII	159	199	230	0.45	8.4753	0.51	34.50	84.9	295.7	7.9421	82.20	296.3						0.5562						
IX	160	199	230	0.45	8.4194	0.93	73.8	85.9	295.3	7.2880	79.89	296.3		1.5553			22.4072	1.1614						
X	164	199	230	0.45	8.4969	2.02	183.0	93.5	294.3	5.6574	77.76	296.3						2.8313	0.0082					
XI	167	202	236	0.40	7.5604	3.22	325.0	108.4	293.4	3.0750	84.12	296.5						4.4701	0.0153					
XII	171	203	237	0.40	9.0415	4.21	442	119.5	293.3	1.7468	95.92	297.1					1.6637	7.2766	0.0181	0.0831				
XIII	172	205	239	0.35	8.6948	5.64	516	129.3	293.2	0.5023	200.4	303.2					0.3492	8.1692	0.0233	0.1531				
XIV	174	207	246	0.4	7.4919	7.60	502.5	130.0	293.0	0.6101	186.5	310.6					0.2418	6.8548	0.0271	0.3682				
XV	182	226	251	0.45	8.0256	20.4	463	122.9	298.4	1.1901	108.4	338.6						6.7575	0.0780	0.4418	0.7483			
XVI	185	235	270	0.15	7.1853	16.77	327.8	115.4	317.8	2.8393	100.1	366.5						4.2889	0.0573		1.2139	1.6254		
XVII	193	250	295	0.05	6.5070	12.35	170.4	94.8	339.0	4.4497	67.57	366.0						2.0189	0.0384		2.1397	2.5100		
XVIII	196	260	300	0.05	8.7744	31.4	115.3	72.9	362.6	6.8011	71.71	389.						1.8421	0.1311			4.3833	2.4179	
XIX	Residue				7.1496	(92.0)	(189.3)	(88.5)	(362.6)	4.6325		456.3						2.4643	0.3288				0.6224	4.0101
Total weight of Esters (g.)					152.2606								0.853	4.0106		3.4999	72.3382	50.3140	0.7304	1.0462	4.1019	8.5187	3.0403	4.0101
Weight as Acids (g.)					145.2597								0.8087	3.8224		3.3170	68.9159	47.9050	0.6954	1.0010	3.9387	8.0122	2.9561	3.8893
% of Total Acids					65.349								0,364	1.720		1.490	31.007	21.554	0.313	0.450	1.772	3.605	1.331	1.75

Degree of unsaturation of C_{20} methyl esters:- -4H

.. .. C_{22} .. .. :- -3H

.. .. C_{24} .. .. :- -2.5H

TABLE XXXV.

Fractional Distillation of "Solid" Methyl Esters of *Ximenia caffra* Sond.

Fraction	Temperature (°C)			Pressure	Weight (a)	E(1%, 1cm.)		I.V.(i)	S.E.(E)	a'	i'	E'	Calculated Composition of Ester fractions:-									
													Saturated			Unsaturated						
	Head	Middle	Oil Bath	mm's	(g.)	268 mμ	229 mμ						C_{16}	C_{18}	C_{26}	C_{16}	C_{18}	Ximenynic	Elaeostearic	C_{26-2H}	C_{30-2H}	C_{34-2H}
1st drop	140	200	220	0.3																		
I	167	195	225	0.4	5.6204	3.73	85.70	70.0	294.5	4.7331	59.64	294.9	0.1470	1.3061		0.1171	3.1629	0.8768	0.0105			
II	168	192	228	0.4	6.3275	5.28	114.4	75.3	298.0	4.9935	61.94	299.6		1.3166	0.0261		3.4920	1.317	0.0167	0.1591		
III	170	200	228	0.4	6.6689	6.85	140.8	75.8	304.0	4.9369	58.37	308.3		1.2613	0.1748		2.9943	1.709	0.0228	0.5067		
IV	185	208	240	0.3	6.6664	9.42	172.3	72.2	314.5	4.5450	47.39	326.0		1.0422	0.4748		2.0175	2.090	0.0314	1.0105		
V	203	220	255	0.3	6.4692	9.42	127.2	55.5	342.5	4.9402	33.79	361.7		1.3355	1.2259		1.1824	1.498	0.0306	1.1964		
VI	208	222	256	0.3	5.9814	4.5	45.77	39.7	369.0	5.4695	29.32	378.7		0.4947	1.7194		1.3570	0.4984	0.0135	1.8984		
VII	216	233	263	0.3	7.1746	1.09	14.03	38.0	387.0	6.9874	36.11	385.4		1.1454	1.7413			0.1833	0.0039	4.1007		
VIII	218	236	266	0.3	6.3943	0.53	8.79	38.0	393.0	6.2903	36.51	399.8		0.6045	1.9876			0.1023	0.0017	3.6982		
IX	219	244	267	0.25	5.8975	0.48	8.34	38.5	397.5	5.8065	37.31	399.8		0.3734	1.9445			0.0896	0.0014	3.4886		
X	222	245	268	0.30	6.2834	0.31	8.33	40.8	410.2	6.1871	39.48	412.8						0.0953	0.001			
XI	230	250	271	0.25	6.1190	0.36	8.92	43.0	414.0	6.0185	41.61	416.9						0.0994	0.0011			
XII	230	250	271	0.25	6.4448	0.4	9.52	44.4	423.8	6.3318	42.99	427.2						0.1117	0.0013			
XIII	235	252	277	0.20	6.5105	0.4	9.80	45.32	426.3	6.3931	43.85	429.9						0.1161	0.0013			
XIV	235	254	274	0.20	6.6642	0.4	10.40	45.6	429.8	6.5367	44.02	433.8			13.7229			0.1262	0.0013	17.5224	30.5698	
XV	235	254	274	0.20	5.9002	0.4	12.32	47.5	433.2	5.7667	45.72	438.1						0.1323	0.0012			
XVI	235	254	276	0.20	6.1372	0.4	13.12	48.1	434.9	5.9898	46.66	440.2						0.1462	0.0012			
XVII	236	254	276	0.20	6.4166	0.4	14.17	49.5	436.4	6.2498	47.51	442.2						0.1655	0.0013			
XVIII	239	254	279	0.25	5.9719	0.6	16.62	50.6	442.4	5.7884	48.24	449.7						0.1807	0.0018			
XIX	244	261	281	0.25	6.8336	1.67	22.0	49.6	449.3	6.5542	46.41	459.8						0.2737	0.0057			
XX	239	270	297	0.03	6.3572	17.5	39.3	52.2	454.9	5.8468	45.43	478.1			0.7248			0.4548	0.0556		2.7122	2.4098
XXI	-- Residue --				2.9500	(16.0)	(39.0)	(52.8)	440.7	2.7242								0.2030	0.0228		2.0917	0.6325
Weight of Esters (g.):-					129.7868								0.1470	8.8798	23.7421	0.1171	14.2061	10.4693	0.2281	33.5810	35.3737	3.0423
Weight as Acids (g.):-					125.0793								0.1393	8.4625	22.9328	0.1110	13.5342	9.9680	0.2172	32.4307	34.3080	2.9605
% of Total Acids:-					34.65								0.039	2.3446	6.354	0.031	3.750	2.761	0.060	8.985	9.504	0.820

TABLE XXXVI

Composition of Total Fatty Acids of Oil from *Ximenia caffra* var. *natalensis* Sond.

	Liquid Acids	Solid Acids	Total (%)
Palmitic acid	0.23	0.06	0.29
Stearic acid	1.19	3.35	4.54
Cerotic acid	-	5.65	5.65
Hexadecenoic acid	0.99	0.03	1.02
Oleic acid	39.24	2.40	41.64
Ximenynic acid	20.29	1.85	22.14
Elaeostearic acid	0.14	0.02	0.16
C_{20} unsat. acid	1.27	-	1.27
C_{22} unsat. acid	0.66	-	0.66
C_{24} unsat. acid	3.95	-	3.95
Ximenic acid	2.88	9.87	12.75
Lumeniqueic acid		5.95	5.95
C_{34}-2H acid			
Total:-			100.02