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The chemical constitution of the seed fat and seed pod fat of Acia Giraffae (Kaneeldoorn)

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THE CHEMICAL CONSTITUTION OF THE SEED FAT AND SEED POD FAT OF ACACIA GIRAFFAE (KAMEELDOORN).

A Thesis
presented in the
University of the Witwatersrand
for the degree
of
Doctor of Philosophy
by

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This work was carried out in the Department of Chemistry and Chemical Engineering under the supervision of

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November 1951.

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Thesis

Candidate's Record.

1950. March. Graduated B.Sc.(Eng.)(Rand) in the Branch of Chemical Engineering, with distinction.

1950-1952. Engaged in the research described in this thesis and demonstrated in Chemistry to the extent of six hours per week during the University terms.

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1. SUMMARY.

1. The physical and chemical constants, as well as the fatty acid compositions of the seed and seed pod fats of Acacia giraffae, were determined while their probable glycoride compositions were computed.

2. Both fats were shown to contain $\Delta^{9,12}$ hexadecadienoic acid and the constitution of the latter was proved by destructive oxidation and partition chromatography of the resulting dibasic acids.

3. The chemical constitution of the testa wax was investigated.

4. Hydroxyl value determinations were used to show the presence of mucilage and phosphatides in the fats.

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2. NEW METHODS AND APPARATUS.

1. A method using partition chromatography to identify dicarboxylic acids was modified to include the identification of the lower molecular weight acids ($C_3 - C_6$) as well as those with chains of six or more carbon atoms ($C_6 - C_{13}$).

2. Hawke's method for determining hydroxyl values was modified to overcome difficulties encountered when carrying out the determination on the seed pod fat.

3. An extractor was designed, with C. van der Pol, to handle three to four kilograms of seeds. Although operating on the Soxhlet principle, the solvent is heated in the extractor body.

4. A receiver was designed for use with the "E.H.P." ester fractionation apparatus.

5. For liquid - liquid extraction, a technique was developed employing devices for ensuring efficient mixing of the two liquids.







3. INTRODUCTION.

This investigation was undertaken with a view to increasing the knowledge of fats from indigenous South African plants. Hilditch[1], suggests that the seed fats of the family Leguminosae, and, more especially, its sub-division, Mimosoidae, "may prove a very interesting field for further investigation of the component acids of seed fats".

No workers have previously published data from investigations into the seed fat or pod fat of Acacia giraffae, although the presence of such fats has been reported.

Verdoorn[2] reports the moisture, crude protein, crude fibre, ash, and carbohydrate content of both the seeds and the pod husks, together with a fat-content of 7·95% for the seeds and 0·96% for the pod husks.

Henkel[3] reports the above as percentages on "complete pods", giving a fat content of 1·6%.

These, and other references[4, 5] to the seed pods of Acacia giraffae are concerned chiefly with their use as a cattle food and not with the properties and composition of their fats.

The Kameeldoorn, (Acacia giraffae) is found in the sandy regions of the northern parts of the Cape Province, the sandy basaltic loams around Potgietersrust in the Transvaal, and also in the Bloemhof district, Bechuanaland, and South West Africa[2].

It is a tree from 25 - 40 feet high with a trunk 12 - 20 inches in diameter. The spreading branches form an umbrella-shaped crown and they are covered with stout brown thorns. In early spring, the flowers appear in yellow globose heads, and soon after, the dense spring foliage makes its appearance. The plant bears large, somewhat curved, pods averaging four inches long by one-and-a-half inches broad. These are covered with a dense grey felt, and have thick spongy walls.

The average weight of a seed pod is 15 gm. and there are usually between 18 and 20 seeds in a pod, forming about 35% of the total weight.

The seeds themselves are tough and very hard with an average weight of 270 mgm.

It was found difficult to obtain sound samples of pods as these are often infested with insects. A small weevil attacks the seeds and destroys them, while the larvae of a moth eat out the insides of the spongy walls of the pod husks.

The pods are at present used only as a cattle food. When ground to a fine meal, they are very palatable to cattle and are reported to increase the milk yield.

The heartwood of the Kameeldoorn is alone valuable as a timber and as this only develops when the tree is of considerable age, the young trees are fit only for firewood[2].

4. THE SEED FAT.

The fat, which forms about 3·5% of the total weight of the seeds,, is a bright orange-coloured liquid when first extracted but after standing for one or two months, it tends to darken to a brownish orange. This, according to Columbic[6] is due to the oxidation of tocopherols resulting in the formation of chroman 5,6-quinones.

At room temperature, the seed fat was not a homogeneous liquid but contained suspended solid matter. This was completely dissolved on heating to 40°C. and was at first considered to be due to high-melting glycerides. Later, however, this suspended matter was shown to be due to the presence of phosphatides and a small quantity of wax from the testa. Similar observations have been reported in the case of soya-bean oils[7] which were found to resemble the seed fat in many other respects as well.

Filtration of the fat at room temperature removed the suspended solids temporarily, but, on standing, more were found to separate out. The preliminary extraction of the whole (uncrushed) seeds was found to remove part but not all of the solids. The complete removal was eventually effected by filtration of the fat and subjecting the filtered fat to treatment with a boiling brine solution (see page 81).

4.1 Physical Constants of the Seed Fat.

Those were determined on the freshly extracted fat without further treatment. The temperature of 40° C. was chosen as being the temperature at which the fat was completely homogeneous.

Specific Gravity	$d^{40°}$	=	0·9104
Refractive Index	$[n]_D^{40°}$	=	1·4683
	$[n]_D^{45°}$	=	1·4665
	$[n]_D^{50°}$	=	1·4647
Temperature coefficient of refractive index		=	-0·00036 per °C.
Optical Activity	negligible		
Surface Tension		=	32·91 dynes/cm. at 40° C.
Interfacial Tension with water		=	0·42 dynes/cm. at 40° C.
Viscosity		=	38·55 centistokes at 40° C.
		=	36·14 centipoises at 40° C.

The temperature coefficient of the refractive index is exactly that quoted by Hilditch[3] for fats at temperatures near 40° C.

The surface tension of the fat, 32·91 dynes/cm. is in the normal range for fats, but the excessive lowering of the interfacial tension against water to only 0·42 dynes/cm. is abnormal.

This extremely low interfacial tension against water explains the ease with which the fat formed stable emulsions in all proportions. These emulsions were stable to centrifugation and also to the action of electrolytes. They were, however, broken by the addition of controlled quantities of ethyl alcohol. (See page 81).

This property has been shown to be due to the presence of phosphatides and mucilaginous material in the fat. When these were removed by washing the fat with a boiling brine solution, the fat was no longer emulsified on shaking with water and the interfacial tension was increased to 20·0 dynes/cm. at 40° C.

4.2 Chemical Constants of the Seed Fat.

Acid value	=	6·00 mgm. KOH/gm.
Saponification value	=	184·8 mgm. KOH/gm.
Hydroxyl value	=	19·60 mgm. KOH/gm.
Iodine value (Wijs)	=	112·9 gm. I_2/100 gm.
Iodine value (Bromine vapour)	=	113·0 gm. I_2/100 gm.
Unsaponifiable matter	=	4·48%
Reichert Meissl value	=	0·11
Polenske value	=	0·45
Fatty acid content	=	90·4%
Glycerol content	=	9·76%
Mean Molecular weight of Fatty Acids	=	284·3 (By direct titration).

The very low Reichert Meissl value confirmed the absence of low molecular weight acids, while the small Polenske value may have been due to traces of C_{14} and C_{16} acids being carried over by the steam.

The negligible difference between the Iodine value (Wijs) and that determined by the Bromine vapour method, indicates the absence of conjugated double bonds in the fat.

The mean molecular weight of the fatty acids as determined by direct titration was considerably higher than that calculated from the formula derived by Schoorman[9]:-

$$m = \frac{1{\cdot}683 \times 10^3 (100 - u)}{S} - 38E$$

where m = mean molecular weight of the fatty acids.

u = percentage unsaponifiable matter.

E = the Ester value of the fat.

S = the Saponification value of the fat.

From this formula, the mean molecular weight of the fatty acids was calculated to be 278·1. This discrepancy was explained in the light of further investigations and is discussed on page 36.

The glycerol content is very close to that calculated from the formula $G = 0{\cdot}05467\,E^{\frac{9}{8}}$, which is 9·75%.

4.3 Changes in Constants with time of Gathering the Pods.

Samples of fat were extracted from seeds collected at various times of the season to investigate changes in chemical constants with time of gathering.

Sample A. This sample of seeds had been gathered between July and October 1949 and stored for use as cattle food. The pods and seeds were badly attacked by weevils and had to be hand-sorted. The fat was extracted only from sound pods lest the attack by weevils had changed its constitution.

Sample B. These seeds were freshly gathered in April 1950 and the fat extracted immediately on receipt of the seeds. The pods were comparatively free from insects, though seeds from a few pods which had been stung were discarded.

Sample C. This sample was collected in June 1950, towards the middle of the season, and the pods were already showing signs of attack by weevils. It would appear that the insects attack the seeds, while still on the tree, as soon as they ripen but the damage is appreciable only towards the end of the season, so that early gathering of the seeds is essential for a sound crop.

All seeds from the above samples which had been attacked by weevils were discarded and the fat extracted from whole seeds only.

The following are the characteristics of the different fat samples:-

	Sample A.	Sample B.	Sample C.
Time of gathering	July - October*	April	June
Percentage fat in the seeds	3·35	3·79	3·70
Acid value (mgm.KOH/gm.)	3·70	7·08	6·00
Saponification value (mgm.KOH/gm.)	184·1	185·2	184·9
Hydroxyl value (mgm.KOH/gm.)	20·48	19·15	19·59
Iodine value (Wijs)	112·9	112·8	112·9
Iodine value (Bromine vapour)	113·0	113·0	112·9
Unsaponifiable matter (per cent)	5·80	3·82	4·50

*Stored until following March.

From these results, it may be seen that the percentage of fat in the seeds falls off slightly towards the end of the season giving fats with lower acid values but higher percentage of unsaponifiable matter. The saponification, iodine and hydroxyl values, however, remain substantially constant.

The colour of sample B was darker than that of C which in turn was darker than sample A.

It would appear that more chlorophyll is extracted from the seeds collected at the beginning of the season than at the end since the fatty acids from samples B and C were of a darker green than sample A. This would then account for the difference in colour between the fat samples themselves.

4.4 Ester Fractionation Data.

The esters were prepared from a mixture of the two samples A & B, thus representing the average composition of the fat throughout the season.

160 gm. of fat was saponified and the unsaponifiable matter extracted with ether. The soaps were then split and the fatty acids extracted with ether. This yielded 142·6 gm. of mixed fatty acids which were then dissolved in acetone for preliminary separation by low-temperature crystallisation.

A total of 4,420 ml. of dry acetone was required to dissolve all the fatty acids at room temperature. The solution of fatty acids was cooled to -30°C and allowed to crystallise at this temperature for 6 hours when 229·6 gm. of solvated fatty acids were filtered off. This yielded 32·7 gm. of solid fatty acids (AG/A/30) which were then esterified with absolute anhydrous ethanol and fractionated (page 79).

The filtrate from the crystallisation at -30°C. was crystallised at -60°C. for 6 hours after which, 173·8 gm. of solvated fatty acids were filtered off. This yielded 49·0 gm. of liquid fatty acids (AG/A/60) which were esterified with anhydrous methanol and fractionated.

The filtrate from this latter crystallisation yielded 59·0 gm. of fatty acids (AG/A/60F) which were esterified with anhydrous methanol and fractionally distilled.

The following tables give the results of the ester-fractionations:-

AG/a/30.

Pressure	=	1·3 mm. Mercury
Weight of esters distilled	=	29·529 gm.
Saponification equivalent	=	305·2
Saponification value	=	183·8 mgm. KOH/gm.
Iodine value	=	31·27 gm. I_2/100 gm.
Hydroxyl value	=	2·89 mgm. KOH/gm.

Sample	Weight gm.	Temperature °C. Oil Bath	Column Head	Saponification Equivalent	Saponification value	Iodine value	Hydroxyl value
I	1·694	208 - 209	149 - 150	283·3	197·9	7·30	-
II	1·955	209 - 211	150 - 152	293·2	191·3	25·08	-
III	3·082	211 - 215	152 - 153	294·7	190·3	25·21	-
IV	5·142	215 - 218	153	296·3	189·4	28·27	-
V	2·744	218 - 219	153 - 154	300·0	187·0	36·21	-
VI	1·884	219 - 222	154 - 158	299·7	187·2	37·02	-
VII	1·113	222 - 230	158 - 159·5	293·2	191·4	37·73	-
VIII	1·385	230 - 246	160 - 174	296·2	189·4	41·83	2·01
IX	2·200	246 - 257	174 - 182	304·1	184·5	47·18	-
X	1·791	257 - 284	182 - 192	307·5	182·0	45·88	-
XI	1·993	284 - 292	192 - 220	326·3	172·0	38·35	-
XII	0·739	Residue in Column.		346·2	161·9	19·90	-
XIII	3·253	Residue in Flask.		366·3	154·1	14·48	18·97
Total	28·975						

To check on the Saponification, Iodine and Hydroxyl values of the fractions, the sums of the products of each value and the corresponding sample weight, were divided by the sum of the sample weights. This gave the mean Saponification value (S), Iodine value (I) and Hydroxyl value (H) of the samples.

$$28{\cdot}975 \times S = (197{\cdot}9 \times 1{\cdot}694) + (191{\cdot}3 \times 1{\cdot}955) + (190{\cdot}3 \times 3{\cdot}082) + (189{\cdot}4 \times 5{\cdot}142) + (187{\cdot}0 \times 2{\cdot}744) + (187{\cdot}2 \times 1{\cdot}884) + (191{\cdot}4 \times 1{\cdot}113) + (189{\cdot}4 \times 1{\cdot}385) + (184{\cdot}5 \times 2{\cdot}200) + (182{\cdot}0 \times 1{\cdot}791) + (172{\cdot}0 \times 1{\cdot}993) + (161{\cdot}9 \times 0{\cdot}739) + (154{\cdot}1 \times 3{\cdot}253)$$

$$\therefore S = \frac{5329{\cdot}2}{28{\cdot}975} = 183{\cdot}9$$

$$28{\cdot}975 \times I = (7{\cdot}30 \times 1{\cdot}694) + (25{\cdot}08 \times 1{\cdot}955) + (25{\cdot}21 \times 3{\cdot}082) + (28{\cdot}27 \times 5{\cdot}142) + (36{\cdot}21 \times 2{\cdot}744) + (37{\cdot}02 \times 1{\cdot}884) + (37{\cdot}73 \times 1{\cdot}113) + (41{\cdot}83 \times 1{\cdot}385) + (47{\cdot}18 \times 2{\cdot}200) + (45{\cdot}88 \times 1{\cdot}791) + (38{\cdot}31 \times 1{\cdot}993) + (19{\cdot}90 \times 0{\cdot}739) + (14{\cdot}48 \times 3{\cdot}253)$$

$$\therefore I = \frac{877{\cdot}6}{28{\cdot}975} = 30{\cdot}29$$

$$28{\cdot}975 \times H = (2{\cdot}01 \times 1{\cdot}385) + (18{\cdot}97 \times 3{\cdot}253)$$

$$\therefore H = 2{\cdot}29.$$

It will be noticed that the calculated saponification value is very close to that of the original crystal fraction, as is also the hydroxyl value. The calculated Iodine value is, however, one unit lower than that of the crystal fraction. This may be due to oxidation of the column and flask residues as suggested by Hilditch. This is further substantiated by the abrupt fall in the iodine values of the residues as compared with those of the preceding fractions. Hilditch[10] recommends that the iodine and saponification values of the residues be assumed equal to those of the last distilled fraction, but this gives a degree of unsaturation higher than that of the total crystal fraction.

It was found that when values of iodine value versus weight of distilled esters, and saponification equivalent versus weight of distilled esters were plotted, they fell on smooth curves except for the iodine values of the two residual fractions. If, however, the curves were produced, and values extrapolated for these iodine values, a degree of unsaturation much closer to that of the original crystal fraction was

$$28{\cdot}975 \times S = (197{\cdot}9 \times 1{\cdot}694) + (191{\cdot}3 \times 1{\cdot}955) + (190{\cdot}3 \times 3{\cdot}[illegible]) + (189{\cdot}4 \times 5{\cdot}142)$$
$$+ (187{\cdot}0 \times 2{\cdot}744) + (187{\cdot}2 \times 1{\cdot}884) + (191{\cdot}4 \times 1{\cdot}113) + (189{\cdot}4 \times 1{\cdot}385)$$
$$+ (184{\cdot}5 \times 2{\cdot}200) + (182{\cdot}0 \times 1{\cdot}791) + (172{\cdot}0 \times 1{\cdot}993) + (161{\cdot}9 \times 0{\cdot}739)$$
$$+ (154{\cdot}1 \times 3{\cdot}253)$$

$$\therefore S = \frac{5329{\cdot}2}{28{\cdot}975} = 183{\cdot}9$$

$$28{\cdot}975 \times I = (7{\cdot}30 \times 1{\cdot}694) + (25{\cdot}08 \times 1{\cdot}955) + (25{\cdot}21 \times 3{\cdot}082) + (28{\cdot}27 \times 5{\cdot}142)$$
$$+ (36{\cdot}21 \times 2{\cdot}744) + (37{\cdot}02 \times 1{\cdot}884) + (37{\cdot}73 \times 1{\cdot}113) + (41{\cdot}83 \times 1{\cdot}385)$$
$$+ (47{\cdot}18 \times 2{\cdot}200) + (45{\cdot}88 \times 1{\cdot}791) + (38{\cdot}31 \times 1{\cdot}993) + (19{\cdot}90 \times 0{\cdot}739)$$
$$+ (14{\cdot}46 \times 3{\cdot}253)$$

$$\therefore I = \frac{877{\cdot}6}{28{\cdot}975} = 30{\cdot}29$$

$$28{\cdot}975 \times H = (2{\cdot}01 \times 1{\cdot}385) + (18{\cdot}97 \times 3{\cdot}253)$$

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SAPONIFICATION EQUIVALENT
300
295
290
285
280
IODINE VALUE
160
140
120
100
0
10
20
30
40
50
WEIGHT OF DISTILLED ESTERS GM

FIG. 1.

obtained. Consequently those extrapolated iodine values and, in later determinations, extrapolated saponification equivalents were used in preference to the determined values, or those based on Hilditch's assumption.

Thus the iodine values for fractions XII and XIII were corrected to 32·1 and 22·0 respectively giving a calculated iodine value of 31·34 compared with 31·27 determined on the original sample AG/A/30.

The method of correcting both the saponification values and the iodine values is illustrated in Fig. I, in which are the curves for the iodine values and saponification equivalents of fraction AG/A/60F.

AG/A/60

Pressure	=	1·0 - 1·9 mm. Mercury
Weight of Esters distilled	=	44·840 gm.
Saponification equivalent	=	291·2
Saponification value	=	192·7 mgm. KOH/gm.
Iodine value	=	117·1 gm. I_2/100 gm.
Hydroxyl value	=	3·26 mgm. KOH/gm.
Thiocyanogen value	=	77·8 gm. I_2/100 gm.

Sample	Weight gm	Temperature °C		Pressure mm. Mercury	Saponification Equivalent	Saponification value	Iodine value	Hydroxyl value
		Oil Bath	Column Head					
XIV	7·582	213 - 222	158 - 163	1·0	288·6	194·4	108·2	-
XV	3·930	222 - 224	163 - 165	1·0	292·5	191·8	117·5	-
XVI	2·215	224 - 240	165 - 167·5	1·0	290·3	193·2	112·0	-
XVII	6·515	240 - 250	181 - 185	1·9	291·6	192·4	117·6	-
XVIII	5·096	250 - 259	170 - 181	1·3	289·7	193·6	120·1	-
XIX	10·029	259 - 274	181 - 183·5	1·4	290·3	193·3	118·4	-
XX	4·159	274 - 280	185 - 187	1·7	292·6	191·7	113·9	-
XXI	1·107	Residue in column		-	273·8	204·9	73·9	25·04
XXII	3·868	Residue in flask		-	294·4	190·6	83·8	30·20
Total	44·501							

The saponification, iodine and hydroxyl values of the mixed esters was calculated as for AC/A/30:

$$44{\cdot}501 \times S = (194{\cdot}4 \times 7{\cdot}582) + (191{\cdot}8 \times 3{\cdot}930) + (193{\cdot}2 \times 2{\cdot}215) + (192{\cdot}4 \times 6{\cdot}515) + (193{\cdot}6 \times 5{\cdot}096) + (193{\cdot}3 \times 10{\cdot}029) + (191{\cdot}7 \times 4{\cdot}159) + (204{\cdot}9 \times 1{\cdot}107) + (190{\cdot}6 \times 3{\cdot}868)$$

$$\therefore \quad S = \frac{8597{\cdot}4}{44{\cdot}501} = 193{\cdot}1$$

$$44{\cdot}501 \times I = (108{\cdot}2 \times 7{\cdot}582) + (117{\cdot}5 \times 3{\cdot}930) + (112{\cdot}0 \times 2{\cdot}215) + (117{\cdot}6 \times 6{\cdot}515) + (120{\cdot}1 \times 5{\cdot}096) + (118{\cdot}4 \times 10{\cdot}029) + (113{\cdot}9 \times 4{\cdot}159) + (73{\cdot}9 \times 1{\cdot}107) + (83{\cdot}8 \times 3{\cdot}868).$$

$$\therefore \quad I = \frac{5076{\cdot}1}{44{\cdot}501} = 114{\cdot}1$$

$$44{\cdot}501 \times H = (25{\cdot}04 \times 1{\cdot}107) + (30{\cdot}20 \times 3{\cdot}868)$$

$$\therefore \quad H = \frac{145{\cdot}3}{44{\cdot}501} = 3{\cdot}27.$$

As in the previous fraction, there is a sudden drop in the iodine values of the residues in the column and flask, but in this case it is much greater than in the former due to the greater susceptibility of di-unsaturated esters to oxidation.

The saponification equivalent of the column residue also drops suddenly. This, too, is probably due to oxidation, since this fraction contained free fatty acids, giving it an acid value of 15·74 mgm. KOH/gm. The true saponification and iodine values, however, could not be calculated from the acidity since some double bonds were apparently oxidised only as far as the diketone or aldehyde stages, while others were oxidised completely to the acids.

An explanation for the greater oxidative effect on the residue in the column than on that in the flask, is that, when the vacuum was broken at the completion of the distillation, the esters in the column presented a greater surface area than those in the flask and were therefore more extensively oxidised.

Oxidation of a double bond to a diketo compound or to two aldehydes, considerably decreases the iodine value without affecting the saponification value to any great extent. When, however, oxidation results in the formation of carboxylic acids, the saponification value is increased considerably and the iodine value lowered.

The effects of the two stages of oxidation are shown by the following calculation.

Consider a fraction of methyl esters with a saponification value of 190 mgm. KOH/gm. and an iodine value of 130 gm. I_2/100 gm.

(i) Let all the double bonds in methyl linoleate (forming 5% of this fraction) be oxidised to aldehydes and diketones, i.e. two atoms of oxygen are added to each double bond.
The equivalent weight of the oxidised methyl linoleate then becomes:- $294 \cdot 5 + 64 = 358 \cdot 5$

$\therefore$ Total weight of oxidised esters from 1 gm. of unoxidised esters $= 0 \cdot 95 + \frac{358 \cdot 5}{294 \cdot 5} \times 0 \cdot 05$

$= 1 \cdot 0108$ gm.

The saponification value of the unoxidised portion of the esters $= \frac{190 \times 1 - 0 \cdot 05 \times 190 \cdot 5}{0 \cdot 95}$

$= 189 \cdot 9$ mgm. KOH/gm.

and the iodine value of the unoxidised portion of the esters $= \frac{130 \times 1 - 0 \cdot 05 \times 172 \cdot 40}{0 \cdot 95}$

$= 127 \cdot 9$ gm. I_2/100 gm.

Let S = saponification value of the total oxidised fraction, then $(189 \cdot 9 \times 0 \cdot 95) + (\frac{56 \cdot 100}{358 \cdot 5} \times 0 \cdot 0608) = S \times 1 \cdot 0108$

$\therefore$ S = 187·6

Let I = iodine value of the total oxidised fraction,
then $(127{\cdot}9 \times 0{\cdot}95) + (0{\cdot}0608 \times 0) = I \times 1{\cdot}0108$

$$\therefore \quad I = 120{\cdot}1$$

(ii) Now let the double bonds in the same 5% of methyl linoleate be completely oxidised to acids, i.e. 4 atoms of oxygen are added on to each double bond.

∴ The saponification equivalent of the oxidised methyl linoleate

$$= \frac{294{\cdot}5 + 128}{5} = 84{\cdot}5 \text{ mgm. KOH/gm.}$$

and the total weight of oxidised esters from 1 gm. of unoxidised esters $= 0{\cdot}95 + \frac{422{\cdot}5}{294{\cdot}5} \times 0{\cdot}05$

$= 1{\cdot}0216$ gm.

The saponification and iodine values of the unoxidised portion remains the same as before.

Let S^1 = saponification value of the total oxidised fraction,
then $(189{\cdot}9 \times 0{\cdot}95) + \frac{(56{,}100 \times 0{\cdot}0716)}{84{\cdot}5} = S^1 \times 1{\cdot}0216$

$$\therefore \quad S^1 = 222{\cdot}4 \text{ mgm. KOH/gm.}$$

Let I^1 = iodine value of the total oxidised fraction,
then $(127{\cdot}9 \times 0{\cdot}95) + (0{\cdot}0716 \times 0) = I^1 \times 1{\cdot}0216$

$$\therefore \quad I^1 = 118{\cdot}7 \text{ gm. } I_{2/100 \text{ gm.}}$$

For the acid value, the equivalent weight of the oxidised methyl linoleate is 105·6
$\frac{1}{4}$ (molecular weight of methyl linoleate + 8 x atomic weight of oxygen)
$= \frac{1}{4}(294{\cdot}5 + 128) = 105{\cdot}6$
Thus the acid value of the oxidised fraction

$$= \frac{0{\cdot}05 \times \frac{56{,}100}{105{\cdot}6}}{1{\cdot}0216}$$

$$= 25{\cdot}96 \text{ mgm. KOH/gm.}$$

From these calculations, it can be seen how the saponification and iodine values of ester fractions can be altered quite considerably by a relatively small amount of oxidation. It is also evident that to attempt to calculate the true constants of an oxidised ester fraction from experimental data is virtually impossible.

Thus, the method used for obtaining values for use in calculating the fatty acid constitution of the two residue samples XXI and XXII was the same as for AG/A/30. Curves of iodine values and saponification equivalents versus weights of esters distilled were plotted, and these for the two residues extrapolated so as to give calculated values for the total crystal fraction closer to the determined values.

The iodine values of XXI and XXII were corrected to 113·6 and 112·2 respectively giving a calculated iodine value for the crystal fraction AG/A/60 of 116·8 as compared with a determined value of 117·1.

The saponification equivalent of XXI was corrected to 293·5. The saponification value thus became 191·2, giving a calculated saponification value for AG/A/60 of 192·8 compared with the determined value of 192·7.

After calculation of the composition of the ester fraction AG/A/60, the theoretical thiocyanogen value of the fractions was computed and compared with that determined on the mixed esters (see page 34).

Pressure	=	0·8 - 1·2 mm. Mercury
Weight of esters distilled	=	53·181 gm.
Saponification equivalent	=	295·2
Saponification value	=	190·0 mgm. KOH/gm.
Iodine value	=	152·4 gm. I_2/100 gm.
Thiocyanogen value	=	96·92 gm. I_2/100 gm.
Hydroxyl value	=	27·49 mgm. KOH/gm.

Sample	Weight gm	Temperature °C		Saponification Equivalent	Saponification value	Iodine value	Thiocyanogen value	Hydroxyl value
		Oil Bath	Column Head					
XXIII	4·256	223	144 - 159·5	279·8	200·5	143·9	93·03	8·28
XXIV	9·609	223-224	160	292·7	191·7	159·1	90·72	3·47
XXV	7·421	224-226	160 - 160·5	294·7	190·4	158·8	92·32	3·88
XXVI	11·288	226-230	160·5 - 161	294·8	190·3	159·1	94·77	3·41
XXVII	8·539	230-250	161 - 163	296·8	189·1	156·0	110·50	4·82
XXVIII	2·511	Residue	in column	291·8	192·3	112·3	101·20	45·90
XXIX	9·415	Residue	in flask	300·4	186·7	100·6	97·31	59·93
Total	53·039							

The saponification, iodine, and thiocyanogen values of the mixed esters were calculated as above.

$$53·039 \times S = (200·5 \times 4·256) + (191·7 \times 9·609) + (190·4 \times 7·421) + (190·3 \times 11·288) + (189·1 \times 8·539) + (192·3 \times 2·511) + (186·7 \times 9·415).$$

$$\therefore \text{ Saponification value} = \frac{10105}{53·039} = 190·3 \text{ mgm. KOH/gm.}$$

$$53·039 \times I = (143·9 \times 4·256) + (159·1 \times 9·609) + (158·8 \times 7·421) + (159·1 \times 11·288) + (156·0 \times 8·539) + (112·3 \times 2·511) + (100·6 \times 9·415)$$

$$\therefore \text{ Iodine value} = \frac{7676·0}{53·039} = 144·2 \text{ gm. } I_2/100 \text{ gm.}$$

$$53·039 \times C = (93·03 \times 4·256) + (90·72 \times 9·609) + (92·32 \times 7·421) + (94·77 \times 11·288) + (110·50 \times 8·539) + (101·20 \times 2·511) + (97·31 \times 9·415)$$

$$\therefore \text{ Thiocyanogen value} = \frac{5130·9}{53·039} = 96·7$$

As before, oxidation was observed to have taken place in the two residues and the iodine, saponification and thiocyanogen values were accordingly corrected as above.

The iodine values of XXVIII and XXIX were corrected to 146·9 and 136·0 respectively giving a calculated iodine value of 152·6 while the value determined on the crystal fraction AG/A/60F was 152·4.

The thiocyanogen values of the same two fractions were corrected to 105·8 and 101·2 respectively giving a calculated value of 97·1 for AG/A/60F compared with the experimentally determined value of 96·92.

The saponification equivalent of XXVIII was corrected to 299·4 to bring the saponification value to 187·4 and the calculated saponification value of AG/A/60F to 190·1 whereas the value as determined was 190·0.

4.5 The Hydroxyl Value of the Separated Fatty Acids.

After the presence of hydroxylated compounds in the fatty acids and ester fractions was proved by means of hydroxyl values, these were calculated in terms of hydroxy fatty acids.

It was then proposed to attempt to isolate and identify these hydroxy acids. To this end, a fresh sample of fat was extracted, the unsaponifiable matter removed, and the fatty acids separated.

These were then dissolved in dry acetone at a concentration of 1: 25 which was the highest concentration at which all the fatty acids were completely soluble at room temperature. This solution was then crystallised at the lowest temperature obtainable using alcohol and dry ice (-78°C) for six hours, and filtered.

The hydroxy acids, together with linolenic and a small quantity of linoleic acids were expected in the filtrate. When the solvent was stripped off at room temperature using the technique of Hawke[11], it was observed that solid particles began to separate out. After the solution had been concentrated to about 1: 3, it was filtered and 0·7 gm. of solids obtained. The remaining solvent was then stripped from the filtrate.

The solid had an acid value of only 16·3 mgm. KOH/gm. but the hydroxyl value was found to be 368·1 mgm. KOH/gm., and its Iodine value (Wijs) was 103·7 gm. $I_2/100$ gm.

The acid value was calculated to linolenic acid, and the iodine and hydroxyl values corrected accordingly, giving the "non-saponifiable"* matter an iodine value of 89·1 gm. $I_2/100$ gm. and an hydroxyl value of 389·7 mgm. KOH/gm.

That there was still some hydroxy body remaining in the crystallised fraction (at -78°C) and the filtrate left after the removal of the solid material, was shown by hydroxyl values of 11·0 and 23·1 respectively.

Since this hydroxylated substance appeared to be appreciably soluble in acetone even at low temperatures, it was decided to attempt complete

*In this thesis, the term "non-saponifiable" matter is used to refer solely to material which is not saponifiable but which is not removed with the unsaponifiable matter as determined by the standard methods.

separation by exhaustive extraction of the soap solution with ether. Accordingly, a sample of fatty acids, from which the unsaponifiable had been extracted, was treated with boiling alcoholic potassium hydroxide solution for one hour. After dilution with water, the soaps were extracted eight times with ether in a separating funnel. The free fatty acids were then liberated and the hydroxyl and acid values of the fatty acid samples determined before and after treatment.

The acid value was observed to increase from 194·8 to 197·2 and the hydroxyl value to decrease from 32·1 to 15·6. The procedure was repeated but th acid and hydroxyl values both remained substantially unchanged.

The soaps of the fatty acids were again prepared and extracted with ether for 100 hours in the continuous liquid-liquid extractor (page 78). This brought about a reduction of only 2·2 units (to 13·4) in the hydroxyl value of the fatty acids and an increase in the acid value to 198·3. It was obvious that extraction of the soaps with ether would not remove the hydroxy compounds and work along these lines was discontinued.

In an attempt to utilise the higher melting point of the hydroxylated compounds as a means of separating it, the re-extracted fatty acids were heated to 60° C and filtered at this temperature. This left some solid of hydroxyl value 109·0 and acid value 169.6; but the filtrate still contained hydroxyl groups as indicated by a hydroxyl value of 9·9, while its acid value was 198·6. As this method appeared to offer no means of separation of the hydroxylated compounds, it too was discontinued.

A fresh sample of fatty acids was dissolved in three parts of acetic anhydride and warmed under reflux for 30 minutes on a boiling water bath. The solution was then cooled and allowed to stand overnight, when white crystals separated out. These, however, still had an acid value of 158·4 mgm. KOH/gm. and a hydroxyl value of only 169·4 mgm. KOH/gm. The saponification value was the same as the acid value, showing that no acetylation had taken place.

The filtrate was boiled several times with water to remove the acetic anhydride, and the remaining fatty acids saponified. The soaps

were split and the fatty acids extracted. These were found to have an acid value of 188·7 and an hydroxyl value of 11·5 so crystallisation from acetic anhydride was abandoned as a possible method of separation.

Various forms of absorption and partition chromatography were attempted but found to be of no use in separating the fatty acids and the hydroxylated compounds, as both were eluted, or absorbed simultaneously.

Since all these attempts at separation failed, it was decided to re-calculate the fatty acid composition from the data already obtained, assuming all the hydroxyl value to be due to "non-saponifiable" matter with an hydroxyl value of 389·7 and an iodine value of 89·1.

These values were arrived at from the constants determined on the solid which separated out from the filtrate, when crystallising the fatty acids from acetone at -78°C (page 20), by calculating the acid value to linoleic acid. The hydroxyl and iodine values were then corrected accordingly.

The substance gave a positive result from nitrogen in the Lassaigne test for the elements by fusion with sodium.

It was found to be insoluble in ether, acetone, alcohol and water, except in the presence of large quantities of fatty acids, when the solubility in ether and acetone was enhanced considerably.

Owing to the small quantity of the substance available, no further tests were carried out.

Sphingosin, obtained from the hydrolysis of sphingomyelins, has the formula $CH_3(CH_2)_{12}CH = CHCHCHCH_2NH_2$ (OH CH) and hydroxyl and iodine values of 387·1 mgm.KOH/gm. and 84·9 gm. I_2/100 gm. respectively.

According to Boilstein[12], it is insoluble in ether, acetone, alcohol and water. In this respect it is very similar to the solids associated with the fatty acids of the seed fat. Its hydroxyl and iodine values, too, are very close to those calculated for the "non-saponifiable" matter above. These values were, of necessity, determined on very small samples of material and were accordingly accurate to only within ± 5 units.

The presence of 0·85 mgm. phosphorus per gm. of seed fat is indicative of the presence of phosphatides which usually include lecithins, cephalins and sphingomyelins.

The removal of these by treating the fat with a boiling salt solution, also resulted in the removal of hydroxy bodies from the fatty acids, which then had zero hydroxyl value.

From this evidence, the likelihood that the hydroxy body, associated with the fatty acids of the seed fat, is sphingosin, is very strong. It also shows conclusively, the absence of hydroxy acids in the seed fat.

4.6 The Mucilaginous Material in the Fat.

On washing the seed fat with a sodium chloride brine, (page 81), phosphatides and mucilaginous material, to the extent of 4% of the total weight of fat, were removed.

This material, on hydrolysis with dilute sulphuric acid according to the method of Anderson[13], yielded mannose and galactose after neutralisation with barium carbonate.

These sugars were identified by their phenyl osazones. After five minutes treatment with phenyl hydrazine in a boiling water bath, pale creamy-white crystals were observed to separate out which were identified as the osazone of mannose. These were filtered from the solution which was returned to the water bath. After a further thirty minutes, orange-yellow crystals separated out which were identified as galactosazone.

Hence, the mucilaginous material in the seed fat probably consists of galacto-mannans, since these are reported to occur to the exclusion of other polysaccharides in mucilaginous material from seeds[15].

That this mucilaginous material together with the phosphatides, was responsible for the emulsifying properties of the fat was shown by the increase in the interfacial tension between the fat and water caused by their removal.

The interfacial tension was observed to increase from 0·42 dynes/cm. to 20·01 dynes/cm. at 40°C. after treatment of the fat with sodium chloride brine.

The hydroxyl value, too, was found to be affected by this treatment and dropped from 20·5 mgm. KOH/gm. to 8·0 mgm. KOH/gm. which was completely accounted for by the 5·6% unsaponifiable matter with an hydroxyl value of 140·1 mgm. KOH/gm.

4.7 Calculation of Fatty Acid Composition.

4.71 Thio-cyanometrically.

The fatty acid composition was calculated according to the principles put forward by Hilditch[14]. Depending on the values of the constants of the fraction, one of four methods was used.

In the case of fractions found to have an hydroxyl value, this was calculated to the "non-saponifiable" substance of iodine value 89·1 and hydroxyl value 389·7, and the modified constants of the esters in the fraction computed. These were then used in the calculations.

The following are examples of the four basic types of calculation used.

A. This method was used for calculating fractions with low iodine values and was based on the assumption that the saturated and unsaturated esters in the fraction have the same mean molecular weights: viz. the saponification equivalent of the fraction. The compositions of fractions I - XIII were calculated according to this method, and the following is the detailed calculation for fraction II.

Saponification equivalent	=	293·2
Iodine value	=	25·08
Weight	=	1·955 gm.

Let P = Ethyl palmitate	- saponification equivalent	= 284·47
S = Ethyl stearate	- saponification equivalent	= 312·52
O = Ethyl oleate	- saponification equivalent	= 310·50
	Iodine value	= 81·75
H = Ethyl hexadecenoate	- saponification equivalent	= 282·46
	Iodine value	= 89·87

Let the weight of unsaturated esters = u

$\therefore$ weight of saturated esters = 1·955 - u

& H + O = u

Assume that the unsaturated and saturated esters have the same mean molecular weight.

Then, from the saponification equivalents,

$$\frac{H}{282.46} + \frac{\theta}{310.50} = \frac{H + \theta}{293.2}$$

$$\therefore 3.5403H + 3.2206\,\theta = 3.4107H + 3.4107\,\theta$$

$$\therefore 0.1296H = 0.1901\,\theta$$

$$\& \quad H = 1.4668\,\theta$$

From the iodine value:

$$89.87H + 81.75\,\theta = 25.08 \times 1.955$$

$$\therefore 1.4668 \times 89.87\,\theta + 81.75\,\theta = 25.08 \times 1.955$$

$$\therefore \theta = \frac{25.08 \times 1.955}{213.57}$$

$$= 0.2296$$

$$\therefore H = 0.3368$$

$$\therefore \text{Saturated esters} = 1.955 - 0.566$$
$$= 1.389$$

$$\therefore \frac{P}{284.47} + \frac{S}{312.52} = \frac{1.389}{293.2}$$

$$\therefore 3.5155P + 3.1998S = 4.7340$$

but $P = 1.389 - S$

$$\therefore 4.8792 - 3.5155S + 3.1998S = 4.7340$$

$$\therefore S = \frac{0.1452}{0.3157}$$

$$= 0.460$$

$$\therefore P = 0.928$$

∴ Composition is Ethyl hexadecenoate = 0·337
Ethyl palmitate = 0·928
Ethyl oleate = 0·230
Ethyl stearate = 0·460

B. In this type of calculation, the fraction was assumed to consist of three components only. It was used for fractions in which the saponification equivalent was within five units of that of the oleate, while the iodine value was above that of the oleic ester. Hilditch[14] suggests this method rather than the previous one in this case, as the latter tends to give high results for the hexadecenoic acid content. (Fractions XIV - XXII).

The method was also applied where a fourth component might have been present, but had been eliminated by considering the crystallisation conditions. Thus in fractions XXIII - XXV, palmitic acid was eliminated by this reasoning.

The following is the detailed calculation for fraction XV.

Saponification equivalent = 292·5
Iodine value = 117·5
Weight = 3·930 gm.

Let P = Methyl palmitate - Saponification equivalent = 270·45
θ = Methyl palmitate - Saponification equivalent = 296·48
- Iodine value = 85·62
N = Methyl linoleate - Saponification equivalent = 294·47
- Iodine value = 172·40

Then, from saponification equivalents,

$$\frac{P}{270{\cdot}45} + \frac{N}{294{\cdot}47} + \frac{\theta}{296{\cdot}48} = \frac{3{\cdot}930}{292{\cdot}5}$$

$$\therefore\ 3{\cdot}6975P + 3{\cdot}3959N + 3{\cdot}3729\theta = 13{\cdot}436 \text{ - - - - - - - - - (i)}$$

From iodine values,

$$172{\cdot}40N + 85{\cdot}62\theta = 117{\cdot}5 \times 3{\cdot}930$$
$$= 461{\cdot}78 \text{ - - - - - - - - - - - - (ii)}$$

Also $P + N + \theta = 3{\cdot}930$

$\therefore \quad P = 3{\cdot}930 - N - \theta$ - - - - - - - - - - - - - - (iii)

Eliminate P from (i) and (iii)

$14{\cdot}531 - 3{\cdot}6975N - 3{\cdot}6975\theta + 3{\cdot}3959N + 3{\cdot}3729\theta = 13{\cdot}436$

$\therefore \quad 0{\cdot}3016N + 0{\cdot}3246\theta = 1{\cdot}095$

$\therefore \quad \theta = \frac{1{\cdot}095 - 0{\cdot}3016N}{0{\cdot}3246}$ - - - - - - - (iv)

Eliminate θ from (ii) and (iv)

$172{\cdot}40N + \frac{85{\cdot}62}{0{\cdot}3246}(1{\cdot}095 - 0{\cdot}3016N) = 461{\cdot}78$

i.e. $172{\cdot}40N + 288{\cdot}83 - 79{\cdot}55N = 461{\cdot}78$

$\therefore \quad N = \frac{172{\cdot}95}{92{\cdot}35}$

$= 1{\cdot}863$

Substituting in (iv) $\theta = 1{\cdot}643$

$\therefore \quad P = 0{\cdot}424$

Thus the composition is:-

Methyl palmitate	=	0.424 gm.
Methyl oleate	=	1·643 gm.
Methyl linoleate	=	1·863 gm.

C. In the calculation by this method, the fraction was assumed to contain palmitic and hexadecenoic esters together with unsaturated C_{18} esters, as suggested by Hilditch[14]. The unsaturated C_{18} esters were taken to have iodine and saponification values equal to those of the C_{18} esters in the next fraction, since this has been shown to be very nearly true[14].

The only fraction calculated by this method was No. XIV since its saponification equivalent was more than five units below that of the oleic ester.

The details of the calculation of this fraction are given below.

Saponification equivalent = 288·6

Iodine value = 108·2

Weight = 7·582 gm.

Let P = Methyl palmitate - saponification equivalent = 270·45

H = Methyl hexadeconoate - saponification equivalent = 268·42

iodine value = 94·57

C = Methyl esters of C_{18} acids - saponification equivalent = 295·4 } Calculated from XV.

iodine value = 131·7 }

From saponification equivalents,

$$\frac{P}{270\cdot45} + \frac{H}{268\cdot42} + \frac{C}{295\cdot41} = \frac{7\cdot582}{288\cdot6}$$

$$\therefore\ 3\cdot6975P + 3\cdot3851C + 3\cdot7255H = 26\cdot271 \text{ - - - - - - - - (i)}$$

From iodine values,

$$131\cdot7C + 94\cdot57H = 7\cdot582 \times 108\cdot2$$

$$= 820\cdot37 \text{ - - - - - - - - - (ii)}$$

Also $P = 7\cdot582 - C - H$ - - - - - - - - - - - - - - - - - - - (iii)

Substitute for P in (i)

$$28\cdot036 - 3\cdot6975C - 3\cdot6975H + 3\cdot3851C + 3\cdot7255H = 26\cdot271$$

i.e. $0\cdot3124C - 0\cdot0280H = 1\cdot7615$

$$\therefore\ C = \frac{1\cdot765 + 0\cdot0280H}{0\cdot3124} \text{ - - - - - - - - - - - - - (iv)}$$

Substitute in (ii)

$$\frac{131\cdot71}{0\cdot3124}(1\cdot765 + 0\cdot0280H) + 94\cdot57H = 820\cdot37$$

$$\therefore\ H = \frac{76\cdot23}{106\cdot38}$$

$$= 0\cdot717$$

$$\therefore\ C = \frac{1\cdot7851}{0\cdot3124}$$

$$= 5\cdot714$$

$\therefore$ P = 1·151

$\therefore$ Composition is:

Methyl hexadeconoate	=	0·717 gm.
Methyl palmitate	=	1·151 gm.
Methyl oleate	=	2·678 gm.
Methyl linoleate	=	3·036 gm.

D. Calculation by this method, involved the use of the thiocyanogen value as well as the iodine value. The saponification equivalent was used only when the determined value differed appreciably from that of the corresponding linoleate, otherwise, the fraction was calculated to a mixture of unsaturated C_{18} esters using only the iodine and thiocyanogen values. This was the case in fractions XXVII - XXIX but fraction XXVI, with a saponification equivalent of 292·0 (after correcting for "non-saponifiable" matter) was calculated to include methyl palmitate as well.

The method of calculation is as follows:-

Saponification equivalent	=	294·8
Iodine value	=	159·1
Thiocyanogen value	=	94·77
Hydroxyl value	=	3·41
Weight	=	11·288 gm.

Let X = weight of "non-saponifiable" matter

Hydroxyl value	=	390
Iodine value	=	90

$\therefore$ $X = \frac{3·41}{390} \times 11·288 = 0·099$ gm.

$\therefore$ Esters = 11·189 gm.

$\therefore$ $\frac{11·189}{\text{S.E. of esters}} = \frac{11·288}{294·8}$

$\therefore$ Saponification equivalent of esters = 292·0

Iodine value of esters = $\frac{(11·288 \times 159·1) - (0·099 \times 90)}{11·189}$

= 159·6

$$\text{Thiocyanogen value of esters} = \frac{(11.288 \times 94.77) - (0.099 \times 90)}{11.189}$$

$$= 94.16$$

	Saponification Equivalent	Iodine Value	Thiocyanogen Value.
Let H = Methyl hexadecenoate	268.42	94.57	94.07
O = Methyl oleate	296.48	85.62	85.12
N = Methyl linoleate	294.47	172.40	92.10
L = Methyl linolenate	292.45	260.38	159.0

Calculate for 1 gm. esters to simplify the arithmetic

$$\text{then } H = 1 - N - O - L. \quad \text{---------- (i)}$$

From Saponification equivalents,

$$\frac{H}{268.42} + \frac{O}{296.48} + \frac{N}{294.47} + \frac{L}{292.45} = \frac{1}{292.0}$$

$$\therefore\ 3.7255H + 3.3729O + 3.3959N + 3.4191L = 3.4227 \quad \text{---------- (ii)}$$

From Iodine values,

$$94.57H + 85.62O + 172.40N + 260.38L = 159.6 \quad \text{---------- (iii)}$$

From Thiocyanogen values,

$$94.07H + 85.12O + 92.10N + 159.0L = 94.16 \quad \text{---------- (iv)}$$

Eliminate H from (i) and (ii)

$$0.3286O + 0.3296N + 0.3061L = 0.3028 \quad \text{---------- (v)}$$

Eliminate H from (i) and (iii)

$$-8.95O + 77.83N + 165.81L = 64.43 \quad \text{---------- (vi)}$$

Eliminate H from (i) and (iv)

$$+ 8.95O + 1.97N - 64.93L = -0.09 \quad \text{---------- (vii)}$$

(vi + (vii)

$$79.80N + 100.88L = 64.32 \quad \text{---------- (viii)}$$

$(V) \times \frac{8.95}{0.3526}$ + (vi)

$$86.18N + 173.58L = 72.12 \quad \text{---------- (ix)}$$

(ix)— (viii) $\frac{86.18}{79.30}$

$$64.68\,L = 2.64$$

$$\therefore L = 0.410$$

Substitute in (ix)

$$N = \frac{72.12 - 7.08}{86.18}$$

$$= 0.756$$

Substitute in (v)

$$\theta = \frac{0.3028 - 0.2491 - 0.0126}{0.3526}$$

$$= 0.119$$

$$\therefore H = 0.084$$

$\therefore$ Composition is

Methyl hexadecenoate	=	0.940 gm.
Methyl oleate	=	1.332 gm.
Methyl linoleate	=	8.456 gm.
Methyl linolenate	=	0.459 gm.

Using the above methods of calculation, the following are the compositions of the distilled fractions:

AG/A/30.

Fraction	Weight gms.	Ethyl Esters of Saturated Acids					Ethyl Esters of Mono-ethonoid Acids				"Non-Saponifiable Matter
		C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{14}	C_{16}	C_{18}	C_{20}	
I	1.694	0.058	1.499				0.005	0.132			
II	1.955		0.928	0.460				0.337	0.230		
III	3.082		1.338	0.842				0.487	0.415		
IV	5.142		1.915	1.530				0.820	0.877		
V	2.744		0.665	0.905				0.414	0.760		
VI	1.884		0.480	0.626				0.335	0.443		
VII	1.113		0.523	0.259				0.134	0.197		
VIII	1.385		0.396	0.313				0.329	0.347		
IX	2.200		0.293	0.668				0.290	0.944		0.005
X	1.791		0.132	0.663				0.098	0.898		
XI	1.993			0.492	0.519				0.408	0.574	
XII	0.739				0.233	0.190				0.316	
XIII	3.253				1.318	0.938				0.835	0.162
Total	28.975	0.058	8.159	6.758	2.070	1.128	0.005	3.376	5.519	1.725	0.167

AG/A/60

Fraction	Weight gm.	Methyl Esters of Saturated Acids		Methyl Esters of Mono-ethonoid Acids		Methyl Esters of Di-ethonoid Acids	Non-Saponifiable Matter
		C_{16}	C_{18}	C_{16}	C_{18}	C_{18}	
XIV	7.582	1.151		0.717	2.678	3.036	
XV	3.930	0.424			1.643	1.863	
XVI	2.215	0.413			0.721	1.081	
XVII	6.515	0.925			1.357	4.233	
XVIII	5.096	1.024			1.037	3.035	
XIX	10.029	1.824			2.618	5.587	
XX	4.159	0.446			1.918	1.795	
XXI	1.107	0.300	0.054			0.688	0.065
XXII	3.868	1.240				2.320	0.308
Total	44.501	7.747	0.054	0.717	11.972	23.638	0.373

The calculated thiocyanogen value of this fraction is 77·1 which agrees well with the value of 77·8 determined experimentally

AG/A/60F

Fraction	Weight gms.	Methyl esters of Mono-Ethenoid Acids		Methyl esters of Di-ethenoid Acids		Methyl esters of Triethenoid Acids	Non-Saponifiable Matter
		C_{16}	C_{18}	C_{16}	C_{18}	C_{18}	
XXIII	4·256	1·808		1.333	1.022		0·093
XXIV	9·609	1·447	0·086		7·988		0·088
XXV	7·422	0·756	0·435		6·155		0·076
XXVI	11·288	0·940	1·332		8·456	0·459	0·101
XXVII	8·539		4·459		1·197	2·775	0·108
XXVIII	2·511		1·085		0·565	0·558	0·303
XXIX	5 .5		4·213		2·373	1·345	1·484
TOTAL	53·040	4·951	11·610	1·333	27·756	5·137	2·253

Those calculations give the weights of the various esters in the total distilled samples, but allowance must be made for slight losses during crystallisation, etc. and also samples removed for analysis.

To obtain an accurate fatty acid composition, therefore, the weights of distilled esters were corrected to weights equivalent to the fatty acids in the original fractions from the low-temperature crystallisations, from the following data.

	AG/A/30	AG/A/60	AG/A/60F
Fatty acids as crystallised (gm.)	32·7	49·0	59·0
Fatty acids corrected for losses (gm.)	33·1	49·7	59·8
Saponification equivalent of esters	305·2	291·2	295·2
∴ Calculated Saponification equivalent of acids.	277·1	277·2	281·2
Weights of esters extracted (gm.)	32·0	49·0	58·3
Weights of Unesterified acids (gm.)	0·8	0·1	0·7
Weights of esters calculated from fatty acids corrected for losses (gm.)	36·44	52·22	62·83
Weights of esters actually distilled (gm.)	28·98	44·50	53·04
Correction factor to obtain the esters equivalent to the original fatty acids	1·258	1.173	1·184

	Corrected Ester Fractions			Fatty Acids				Per-centage
	AG/A/30	AG/A/60	AG/A/60F	AG/A/30	AG/A/60	AG/A/60F	Total	
Myristic	0.073			0.065			0.065	0.046
Palmitic	10.280	9.084		9.262	8.612		17.874	12.80
Stearic	8.502	0.063		7.739	0.060		7.799	5.59
Arachidic	2.604			2.390			2.390	1.71
Behenic	1.419			1.311			1.311	0.94
Tetradecenoic	0.006			0.006			0.006	0.004
Hexadecenoic	4.247	0.841	5.882	3.824	0.799	5.556	10.179	7.28
Oleic	6.944	14.040	13.780	6.316	13.380	13.100	32.796	23.49
Eicosenoic	2.170			1.990			1.990	1.43
Hexadecadienoic			1.589			1.496	1.496	1.07
Linoleic		27.725	32.895		26.396	31.298	57.694	41.45
Linolenic			6.092			5.789	5.789	4.15
"Unsaponifiable"	0.197	0.473	2.563	0.197	0.473	2.563	3.203	-
TOTAL	36.442	52.226	62.810	33.100	49.720	59.802	142.622	100

Thus the fatty acid composition of the seed fat, when corrected to the nearest 0.1% is as follows:-

	Weight Percent.		Molar Percent	
Myristic	trace		trace	
Palmitic	12.8		13.8	
Stearic	5.6		5.5	
Arachidic	1.7		1.5	
Behenic	0.9		0.7	
Saturated		21.0		21.5
Tetradecenoic	trace		trace	
Hexadecenoic	7.3		7.9	
Oleic	23.5		23.1	
Eicosenoic	1.4		1.3	
Monoethenoid		32.2		32.3
Hexadecadienoic	1.1		1.2	
Linoleic	41.5		40.8	
Linolenic	4.2		4.2	
Di-and Tri-ethenoid		46.8		46.2

The neutralisation equivalent of this mixture of fatty acids was calculated to be 278·1.

This was exactly the same as the mean molecular weight of the fatty acids calculated from the acid and saponification values, and the percentage of unsaponifiable matter of the fat itself. (page 7).

There was, however, a considerable difference between these calculated values and that determined by direct titration of the fatty acids; this was found to be 284·3. This value was determined on fatty acids containing the hydroxy body which was shown to be "non-saponifiable" and thus would have had the effect of increasing the neutralisation equivalent of the fatty acids.

The determined neutralisation equivalent was then corrected as follows, allowing for the "non-saponifiable" matter present.

Associated with 100 gm. of fatty acids, there were 2·298 gm. of "non-saponifiable" matter.

$\therefore$ If the neutralisation equivalent of fatty acids alone = N

then $\frac{100}{N} = \frac{102 \cdot 298}{284 \cdot 3}$

$\therefore$ N = 278·0

This is extremely close to the two calculated values, and well within the limits of experimental error.

The calculated iodine value of this fatty acid mixture is 119·9 gm. $I_2/100$ gm. which agrees well with the value of 120·1 gm. $I_2/100$ gm. determined on the mixed fatty acids.

Also, computing the hydroxyl value of the "fatty acids" as extracted, from the above data on the assumption that the hydroxy body had an hydroxyl value of 389·7, close agreement was obtained with the determined value of 9·10 mg. KOH/gm.

The calculated value was 8·97 mgm. KOH/gm.

4.72. Spectrophotometrically.

The fatty acid composition of another sample of fatty acids was later determined using the alkali isomerisation and spectrophotometric method of Hilditch, Morton and Riley[15].

$E_{1\,cm.}^{1\%}$ 268 mμ = 33·00 (after isomerisation at 170°C for 15 minutes)

$E_{1\,cm.}^{1\%}$ 234 mμ = 426·5 (after isomerisation at 180°C for 60 minutes)

Iodine value = 121·9 gm. $I_2/100$ gm.

∴ Percentage linolenic acid $= \frac{33·00}{532} \times 100 = 6·2\%$

Percentage di-ethenoid acids (as linoleic) $= \left[\frac{426·5}{532} - 33·00 \times \frac{569}{532}\right] \frac{100}{906}$

$= 43·2\%$

Percentage mono-ethenoid acids (as oleic) $= \frac{100 \times 121·9 - (6·2 \times 273·5 + 43·2 \times 181·0)}{89·9}$

$= 30·0\%$

Percentage saturated fatty acids (by difference) $= 20·6\%$

The agreement between these values and those obtained by the ester fractionation and thiocyanometric procedure is within the limits of accuracy claimed for this latter determination: viz. ± 2%

4.8 The Probable Glyceride Composition of the Seed Fat.

According to Hilditch and Moara[16], the amounts of mixed glycerides, present in natural vegetable fats, can be computed with "some approach to exactitude" from the proportions of their component fatty acids by applying the "rule of even distribution".

From these methods of calculation, a glyceride-composition of the seed fat has been computed as indicated below.

For the purposes of calculation of glyceride-composition, minor component acids were considered either collectively or included with the most suitable major component.

Thus the arachidic and behenic acids were grouped together as "arachidic", hexadecadienoic acid was included under "linoleic" and eicosenoic acid under "oleic".

The molar percentages of fatty acids were then as follows:-

"Linoleic"	=	42·0
"Oleic"	=	24·4
Palmitoleic	=	7·9
Linolenic	=	4·2
Palmitic	=	13·8
Stearic	=	5·5
"Arachidic"	=	2·2
		100·0

From these figures, there had to be at least one molecule of "linoleic" acid in each glyceride molecule, and not more than one of any other acid, if the rule of even distribution was to hold.

"Oleic" acid would probably form "oleo" - "linoleins" with a molecule of one of the remaining acids, and the excess of "linoleic" acid could possibly have been present as di-"linoleins".

For this to be possible, the following equation would have to hold:

percent. "linoleic" = percent. "oleic" + 2(percent. fatty acids other than oleic" and lin.leic" — percent. "oleic").

The percent. "linoleic" acid = 42·0

The right hand side of the equation = 24·4 + 2(33·6 - 24·4)

= 42·8

Thus the above equation is very nearly true, and the glyceride composition based on this assumption was calculated. This was corrected to the nearest whole number and found to be as follows:-

	Molar percentage.	
"Oleo"-"linoleins"		74
Palmitoleo-"oleo"-"linolein"	18	
"Oleo"-"linoleo"-linolenin	9	
Palmito-"oleo"-"linolein"	30	
Stearo-"oleo"-"linolein"	12	
"Arachido"-"oleo"-"linolein"	5	
Di-"linoleins"		26
Palmitoleo-di-"linolein"	6	
Di-"linoleo"-linolenin	3	
Palmito-di-"linolein"	11	
Stearo-di-"linolein"	4	
"Arachido"-di-"linolein"	2	
		100

4.9 The Unsaponifiable Matter in the Seed Fat.

The unsaponifiable matter was found to have the following composition when analysed according to the methods of Karnovsky et. al.[17]

	Weight Percentage
Saturated hydrocarbons	15·6
Unsaturated hydrocarbons (as Squalene)	2·0
Sterols (as Sitosterol)	48·5
α-Glycoryl ethers (as Solachyl alcohol)	3·2
	69·3

After separation of the sterols as their digitonides, these were split according to the method of Dam[18] and the free sterols extracted. These crystallised in tufts of short needles and had a melting point of 139-139·2° C. With the Liebermann-Burchard reaction, a greenish violet-colour was produced. From this data, it was concluded that sitosterol was present almost to the exclusion of other phytosterols.

The hydroxyl value of the unsaponifiable matter was 140·0 mgm.KOH/gm., and from this was calculated the hydroxyl value of the 30.7% unaccounted for in the above analysis.

Sitosterol has the hydroxyl value of 170 mgm.KOH/gm.
and Solachyl alcohol one of 330 mgm.KOH/gm.

If x = hydroxyl value of remainder of the unsaponifiable matter, then:-

$$30{\cdot}7x + (170 \times 48{\cdot}5) + (3{\cdot}2 \times 330) = 140 \times 100$$

$$\therefore \quad x = \frac{14000 - 8.245 - 1056}{30{\cdot}7}$$

$$= 153{\cdot}0 \text{ mgm.KOH/gm.}$$

This hydroxyl value is probably due to the higher-aliphatic alcohols from the testa wax and also to choline from the hydrolysis of the phosphatides.

The carotenoid content was determined by finding the extinction of a 1% solution of the unsaponifiable matter in ether at 450 mμ.[19]

The value of $E_{1\,cm.}^{1\%}$ 450 mμ = 0·08

From this the percentage of carotenoids in the unsaponifiable matter, as β carotene $= \frac{\cdot 08 \times 100}{2,500}$

$= \underline{\cdot 0032\%}$ which is negligible.

5. THE SEED POD FAT.

The fat from the seed pods is a very dark green solid fat with a not unpleasant herb-like odour. On standing at room temperature for some time, a white substance was found to separate out on the surface of the green fat.

This was probably mucilaginous material, since the separation no longer occurred after removal of the mucilaginous material with sodium chloride brine.

When the pods were extracted immediately after removal of the seeds, and without further grinding, the petroleum ether extract was found to be only 0·45% by weight of the material; but when the pods were finely ground, in a laboratory hammer mill, the fat was found to form 1·2% of the husks. These fats were found to have widely differing chemical constants, but, due to insufficient of the former material being available, the physical constants were determined only on the more completely extracted fat.

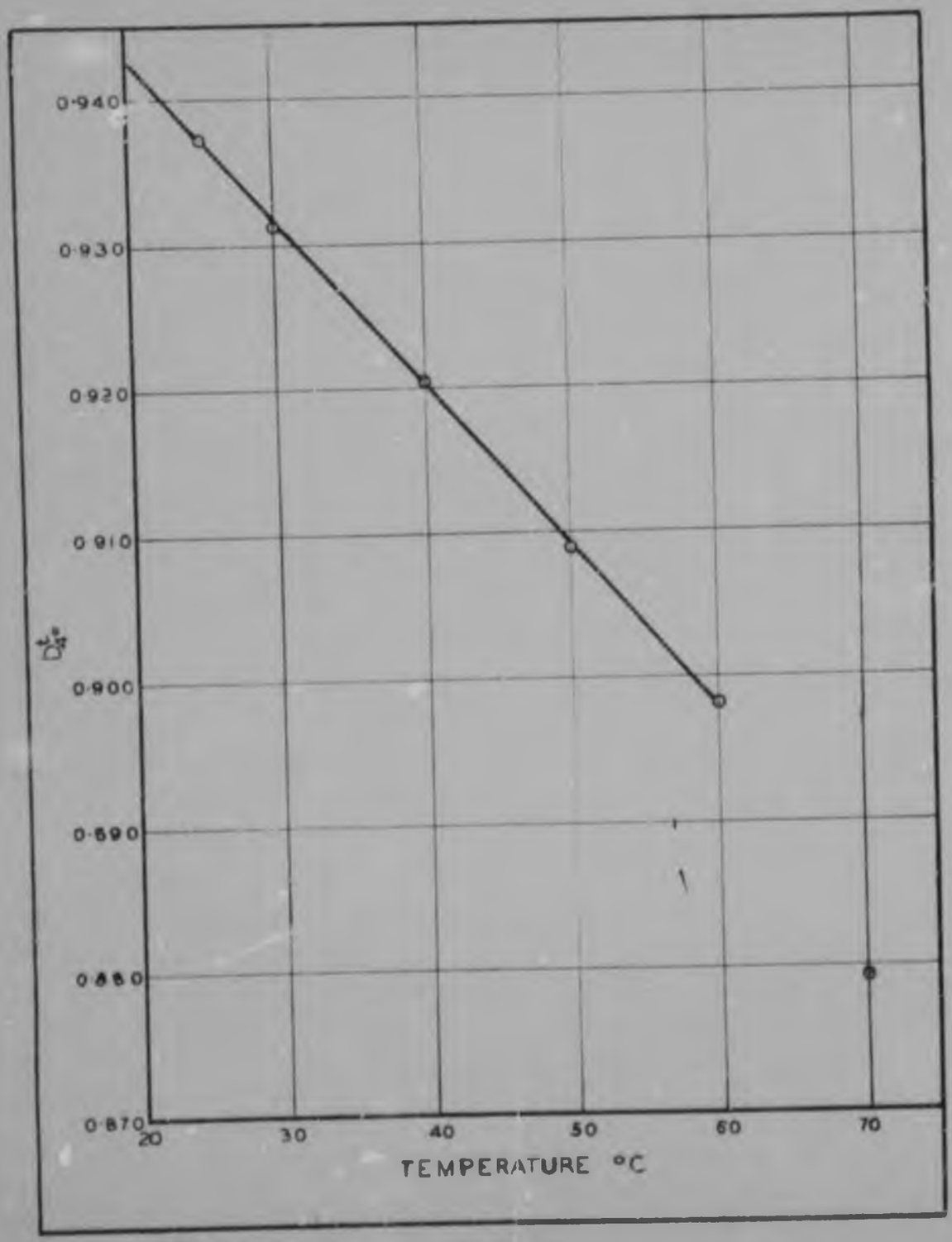

0·940
0·930
0·920
0·910
0·900
0·890
0·880
0·870
20
30
40
50
60
70
TEMPERATURE °C

FIG 2.

5.1 Physical Constants of the Seed Pod Fat.

Melting range = 63·0 - 63·8° C

Specific gravity.

	Temperature °C	$d_4^{t°}$ =
Solid Fat	25	0·9373
	30	0·9310
	40	0·9203
	50	0·9088
	60	0·8981
Liquid fat	70	0·8792

Refractive Index $[n]_D^{65°}$ = 1·4613

$[n]_D^{70°}$ = 1·4599

$[n]_D^{75°}$ = 1·4582

Temperature coefficient of refractive index = −0·00036 per °C

Optical activity negligible

Surface tension = 35·10 dynes/cm. at 70° C

Interfacial tension with water = 8·91 dynes/cm. at 70° C

The Melting Range of the Fat.

Although the fat was found to have a very short melting range, changing from solid to completely molten over a range of 0·8° C, it was found to have a very large softening range. At room temperature and up to about 35° C, the fat was quite hard, but above 40° C it started softening and between 55° C and its melting point, it was found to be plastic.

The Specific Gravity.

The softening of the fat above 40° C and its becoming plastic at higher temperatures, did not affect the linearity of the Specific Gravity - Temperature curve for the solid fat (Fig. 2). Within the limits of experimental error, the specific gravity was found to vary linearly with

the temperature between 25° and 60° C according to the equation:-

$$d^1 = d - .00112 (t^1 - t)$$

Where d^1 = specific gravity at temperature t^1

and d = specific gravity at temperature t

At 70° C, however, when the fat was molten, the specific gravity dropped suddenly and no longer fell on the curve. This is to be expected in a change of state when changes in rate of expansion almost invariably take place.

The Refractive Index.

The temperature coefficient of the refractive index was observed to be slightly lower than normal which is given as -0.00038 per °C.[8] at temperatures near 70° C. This is, however, not of much significance.

Interfacial Tension with Water.

As with the seed fat, there was a considerable and abnormal lowering of the interfacial tension with water. This was however not quite as great as in the former case, which explains why the emulsions formed with water separated into two layers on standing without the addition of ethyl alcohol.

This property was again shown to be due to mucilaginous material and phosphatides and their removal resulted in an increase of the interfacial tension to 24·2 dynes/cm. at 70° C.

Phosphatides were present to the extent of 0·30 mgm. phosphorus per gram of fat and together with the mucilage, they formed 6·7% of the fat.

5.2 The Chemical Constants of the Seed Pod Fat.

The chemical constants of the fat from finely-ground pods, and that extracted from the pods broken for the removal of the seeds, and without further grinding, are tabulated below.

	Sample I. Fat from finely ground pods.	Sample II. Fat from broken pods.
Percentage fat	1·2%	0·45%
Acid value (mgm.KOH/gm.)	57·3	58·7
Saponification value (mgm.KOH/gm.)	143·2	103·5
Hydroxyl value (mgm.KOH/gm.)	30·2	78·0
Iodine value (Wijs) ($gm.I_2/100$ gm.)	69·0	55·0
Unsaponifiable matter (%)	26·0	32·7
Fatty acids (%)	70·8	62·4
Mean molecular weight of Fatty Acids (by direct titration)	305·3	385·0
Reichert Meissl value	0·38	-
Polenske value	0·28	

The percentage of fat extracted in both cases remained substantially constant, varying only between 1·17 and 1·3% and 0·40 and 0·55% respectively. This would seem to indicate that the petroleum ether soluble matter was present in two distinct layers in the pod husk; one on or near the surface and quite readily dissolved by the solvent; the other protected by outer layers of plant tissue through which diffusion of extracted solution was difficult.

Since the saponification and iodine values were observed to be greater in Sample I than in Sample II, while the hydroxyl value was correspondingly lower, it would appear that the more completely extracted fat sample contained a larger quantity of glycerides. That the proportion of free fatty acids extracted was more or less constant was shown by a decrease

of only 1.4 units for sample I compared with sample II.

The calculated mean molecular weight of the fatty acids (276.3) was again observed to be much lower than that determined by direct titration, but this discrepancy was satisfactorily explained in the light of further investigations (page 59).

The negligible Reichert Meissl and Polenske values indicate the absence of low molecular weight fatty acids. The values may be ascribed to small quantities of myristic and palmitic and the corresponding unsaturated acids which are unavoidably carried over by the steam.

5.3 Ester Fractionation Data.

The esters were prepared from the fatty acids derived from 150 gm. of fat after preliminary separation by low temperature crystallisation from acetone.

After saponification of the fat and extraction of the unsaponifiable matter, the soaps were split and the fatty acids extracted with ether. During the extraction of the unsaponifiable matter, a highly solvated solid separated out between the ether and aqueous layers. This was filtered off and set aside for further investigation.

This procedure yielded 106.3 gm. of fatty acids of which 101.0 gm. were dissolved in one litre of dry redistilled acetone and heated to boiling under reflux until all the fatty acids had dissolved. The solution was cooled to room temperature (25°C) when it was crystallised for six hours with constant stirring and then allowed to stand for a further 12 hours before filtering. This yielded 100.0 gm. of solvated product from which 16.6 gm. of fatty acids were obtained (PAG/A/+25).

The filtrate, together with 100 ml. of dry acetone used to wash the precipitate, was then crystallised at 0°C for 18 hours and filtered. The crystals weighed 32 gm. when solvated, but on removal of solvent, 11.7 gm. of fatty acids were obtained (PAG/A/0).

The filtrate from this crystallisation was then cooled slowly to -35°C with constant stirring, when a considerable quantity of crystals separated out. On attempting to cool to -40°C, however, the whole solution together with separated solids set into a firm jelly-like mass. Consequently the crystallisation was carried out at -35°C for 6 hours.

This yielded 130 gm. of solvated product from which 33.4 gm. of fatty acids were obtained (PAG/A/-35).

From the filtrate, 38.9 gms. of fatty acids (PAG/A/-35F) were recovered.

The neutralisation and iodine values of the fatty acid fractions were determined before conversion into the methyl esters. All the fractions were esterified with anhydrous methanol since the difference in melting points between the ethyl and corresponding methyl esters was found to be inappreciable in the fractional distillation of the esters of the fatty acids

from the seed fat.

The following are the neutralisation and iodine values of the fatty acid fractions:

Fraction	PAG/A/+25	PAG/A/0	PAG/A/-35	PAG/A/-35F
Neutralisation value	122·8	169·8	195.2	189·7
Iodine value	43·57	25·09	39·93	128·0

The apparent anomaly in the magnitudes of the iodine values is explained in the light investigations into the constitution of the solids separating out at various stages in the resolution of the fatty acids. (This is discussed on page 65).

After esterification of both PAG/A/+25 and PAG/A/0, large quantities of a fluffy precipitate settled out on cooling; this was filtered off before dilution with water and extraction of the esters with ether. During this latter procedure, more solid had to be filtered from these fractions but similar trouble was not experienced with the other two. The solids were extracted with ether to remove all traces of esters and the ether solutions added to the corresponding bulk extractions of the esters.

After removal of unesterified fatty acids, and distilling off the ether, the esters were fractionated through an E.H.P. column.

The following tables give the results of the ester-fractionations:

PAG/A/-35F.

Pressure	=	0·9 - 1.0 mm. Mercury
Weight of esters distilled	=	36·31 gm.
Saponification equivalent	=	297·3
Saponification value	=	188·5 mgm. KOH/gm.
Iodine value	=	120·9 gm. I_2/100 gm.

Fraction	Weight gm.	Temperature °C.			Saponification Equivalent	Saponification Value	Saponification Value
		Oil Bath	Column	Column Head			
I	4·201	200 - 206	130 - 145	140 - 150	267·6	209·6	107·6
II	3·234	206 - 208	145 - 150	150 - 155	286·9	195·5	125·7
III	4·422	208 - 210	150 - 155	155 - 160	289·0	194·1	128·1
IV	6·727	210 - 211	155	160	291·9	192·2	129·8
V	3·418	211 - 214	155 - 158	160 - 163	292·2	192·0	127·5
VI	2·008	214 - 220	158 - 160	163	295·2	190·0	124·0
VII	2·114	220 - 230	160 - 165	163 - 165	293·0	191·4	123·7
VIII	2·371	230	165	166	291·0	192·8	121·7
IX	2·493	Residue in column			287·6	195·0	97·83
X	5·075	Residue in flask			364·4	153·7*	86·9
Total	36·063						

*0·1347 gms. of solid separated out per gm. of esters during the saponification value determination.

This was arrived at as follows:- The solids separated after titrating the saponification mixture with hydrochloric acid, were filtered off on a tared sintered glass crucible. The crucible was then washed three times alternately with 0·5 N Hydrochloric acid and ether to ensure the removal of any fatty acids present as their potassium soaps, and finally with 25 ml. of ether. The crucible and contents were then dried in a vacuum desiccator and weighed.

The theoretical saponification and iodine values of the mixed esters were calculated, as described on page 11.

$$36{\cdot}063 \times \text{Sap. value} = (4{\cdot}201 \times 209{\cdot}6) + (3{\cdot}234 \times 195{\cdot}5) + (4{\cdot}422 \times 194{\cdot}1) + (6{\cdot}727 \times 192{\cdot}2) + (3{\cdot}418 \times 192{\cdot}0) + (2{\cdot}008 \times 190{\cdot}0) + (2\,114 \times 191{\cdot}4) + (2{\cdot}371 \times 192{\cdot}8) + (2{\cdot}493 \times 195{\cdot}0) + (5{\cdot}075 \times 153{\cdot}7)$$

$\therefore$ Saponification value = 189·2

$$36{\cdot}063 \times \text{Iodine value} = (4{\cdot}201 \times 107{\cdot}6) + (3{\cdot}234 \times 125{\cdot}7) + (4{\cdot}422 \times 128{\cdot}1) + (6{\cdot}727 \times 129{\cdot}8) + (3{\cdot}418 \times 127{\cdot}5) + (2{\cdot}008 \times 124{\cdot}0) + (2{\cdot}114 \times 123{\cdot}7) + (2{\cdot}371 \times 121{\cdot}7) + (2{\cdot}493 \times 97{\cdot}83) + (5{\cdot}075 \times 86{\cdot}96)$$

$\therefore$ Iodine value = 117·5

From the discrepancy in the calculated values, and also the sudden drop in iodine values of two residual fractions, oxidation must have taken place. The saponification and iodine values of those two fractions were accordingly adjusted as described previously (Page 12).

The saponification values were corrected to 190·0 and 150·8 respectively giving a calculated saponification value of 188·6 for the mixed ester fractions as compared with the determined value 188·5.

The saponification value of fraction X was lowered to 150·8 so that, when correction was made for the "non-saponifiable" matter which separated out, the value became 173·8 which lay on the curve of saponification value versus weight of esters distilled.

The corrected iodine values of 121·0 and 103·3 for fractions IX and X respectively gave a calculated value of 120·8 compared with the determined value of 120·9.

The iodine value of fraction X was further increased to 119·3 by allowing for the separated "non-saponifiable" matter which had zero iodine value.

PAG/L/-35.

Pressure	=	0·6 - 0·8 mm. Mercury
Weight of esters distilled	=	30·61 gm.
Saponification equivalent	=	308·8
Saponification value	=	192·6 mgm. KOH/gm.
Iodine value	=	36·13 gm. I_2/100 gm.

Fraction	Weight gm.	Temperature °C			Saponification Equivalent	Saponification Value	Iodine Value
		Oil Bath	Column	Column Head			
XI	5.085	206 - 208	130 - 132	130-132.5	267.9	209.4	9.92
XII	4.874	208 - 212	132 - 135	132.5-135	273.3	205.3	14.00
XIII	2.330	212 - 216	135	135 - 140	277.2	202.4	17.23
XIV	3.068	216 - 218	135 - 140	140 - 146	281.1	199.5	42.37
XV	3.312	218 - 225	140 - 144	146-150.5	290.5	193.1	72.49
XVI	3.658	223 - 240	145	150.5-155.5	295.5	189.9	77.07
XVII	3.009	Residue in column			308.7	181.8	37.47
XVIII	5.015	Residue in flask			310.1	163.5*	27.64
Total	30.351						

*0.0936 gm. of solid separated out per gm. of esters during the saponification value determination.

The theoretical saponification and iodine values for the mixed esters were calculated as before.

Sap. Value x 30.351 = (5.085 x 209.4) + (4.874 x 205.3) + (2.330 x 202.4)
+ (3.068 x 199.5) + (3.312 x 193.1) + (3.658 x 189.9)
+ (3.009 x 181.8) + (5.015 x 163.5)

∴ Saponification value = 192.8

Iodine value x 30.351 = (5.085 x 9.92) + (4.874 x 14.00) + (2.330 x 17.23)
+ (3.068 x 42.37) + (3.312 x 72.49) + (3.658 x 77.07)
+ (3.009 x 37.47) + (5.015 x 27.64)

∴ Iodine value = 35.2

In this case, due to the absence of diethenoid acids, oxidation was not very severe in that the calculated and determined values are in reasonably good agreement, but a slight correction was still necessary for the iodine values. The iodine values of fractions XVII and XVIII thus became 42.0 and 31.7 giving a calculated value of 36.1 while the value determined on the mixed esters was 36.13.

After making allowance for the "non-saponifiable" matter as under PAG/4/-35F, the saponification and iodine values of fraction XVIII became 181·1 and 35·0 respectively.

PAG/4/0

Pressure	=	0·7 mm. Mercury
Weight of esters distilled	=	8·64 gm.
Saponification equivalent	=	308·2
Saponification value	=	172·9 mgm. KOH/gm.
Iodine value	=	20·6 gm. I_2/100 gm.

Fraction	Weight gm.	Temperature °C			Saponification Equivalent	Saponification Value	Iodine Value
		Oil Bath	Column	Column Head			
XIX	1·328	210 - 220	155-165	144 - 157	283·0	198·3	29·90
XX	1·919	220 - 228	165-180	157 - 160	304·3	184·4	41·02
XXI	1·157	228 - 230	180-190	160 - 164	310·8	180·5	22·17
XXII	1·705	Residue in column			313·9	178·6	3·49
XXIII	2·528	Residue in flask			326·8	141·9*	10·48
Total	8·637						

*0·1728 gm. "non-saponifiable" matter separated out per gm. of esters during saponification value determination.

The calculated saponification and iodine values of the mixed esters were again computed.

Sap. value x 8·637 = (1·328 x 198·3) + (1·919 x 184·4) + (1·157 x 180·5) + (1·705 x 178·6) + (2·528 x 141·9)

∴ Saponification value = 172·7 mgm. KOH/gm.

Iodine value x 8·637 = (1·328 x 29·90) + (1·919 x 41·02) + (1·157 x 22·17) + (1·705 x 3·49) + (2·528 x 10·48)

∴ Iodine value = 20·5 gm. I_2/100 gm.

As would have been expected in a fraction of low iodine value, very little oxidation appeared to have taken place and no corrections were necessary.

On allowing for the "non-saponifiable" matter in fraction XXIII, the saponification and iodine values became 171·4 and 12·81 respectively.

P.G/A/+25.

Pressure	=	0·7 mm. Mercury
Weight of esters distilled	=	8·68 gm.
Saponification equivalent	=	321·2
Saponification value	=	174·5 mgm.KOH/gm.
Iodine value	=	59·28 gm. I_2/100 gm.

Fraction	Weight gm.	Temperature °C			Saponification Equivalent	Saponification Value	Iodine Value
		Oil Bath	Column	Column Head			
XXIV	1·336	208 - 230	140 - 155	139·5-144·5	275·6	203·5	55·81
XXV	2·145	230 - 240	155 - 160	144·5-145	286·3	196·0	82·69
XXVI	0·673	240 - 246	160 - 170	145-152·5	289·7	193·6	85·35
XXVII	1·339	Residue in Column			315·7	197·0	46·39
XXVIII	3·154	Residue in flask			395·6	141·8*	27·99
Total	8·647						

*0·2210 gm. solid separated out per gm. of esters during saponification value determination.

The saponification and iodine values calculated for the mixed esters were as follows:-

Sap. value x 8·647 = (1·336 x 203·5) + (2·145 x 196·0) + (0·673 x 193·6) + (1·339 x 197·0) + (3·154 x 141·8)

∴ Saponification value = 177·2 mgm.KOH/gm.

Iodine value x 8·647 = (1·336 x 55·81) + (2·145 x 82·69) + (0·673 x 85·35)
+ (1·339 x 46·39) + (3·154 x 27·99)

∴ Iodine value = 53·26 gm. I_2/100 gm.

Once again, oxidation was observed to have taken place, and the saponification and iodine values of the two residual fractions were corrected as before.

The saponification values were corrected to 192·0 and 136·1 respectively, giving a calculated value of 174·5 for the mixed esters, which was the same as that determined on PAG/4/+25.

The iodine values became 70·0 and 34·7 respectively, bringing the calculated iodine value to 59·3 as compared with the determined value of 59·28 for the mixed esters.

On allowing for the "non-saponifiable" matter in fraction XXVIII, the saponification and iodine values were calculated as 175·2 and 44·5 respectively.

5.4 Calculation of the Fatty Acid Composition.

5.41 From Ester Fractionation Data.

The methods of computing the fatty acid composition from the ester fractionation data were the same as those employed for the seed fat. From these data, the following compositions of the ester fractions were calculated.

Methyl Esters of PAG/4/-35F.

Fraction	Weight gm.	Saturated Acids		Mono-ethenoid Acids			Di-ethenoid Acids		"Non-saponifiable" matter
		C_{14}	C_{16}	C_{16}	C_{18}	C_{20}	C_{16}	C_{18}	
I	4·201	0·256		3·307			0.638		
II	3·234		0.068	0·679	0·980		0·132	1·375	
III	4·422		0·049	0·694	1·481		0·133	2.065	
IV	6·727		0·800		1.707			4·220	
V	3·418		0·376		1.019			2.023	
VI	2.008		0.022		1.074			0.912	
VII	2·114		0.197		0·838			1·079	
VIII	2·371		0·370		C·650			1·351	
IX	2·493				1·477			1.016	
X	5.075					2·475		1·916	0·684
Total	36.063	0.256	1.882	4·680	9·226	2·475	0.903	15·957	0·684

Methyl Esters of PAG/A/-35

Fraction	Weight gm.	Saturated Acids				Mono-ethenoid acids			"Non-Saponifiable" Matter
		C_{14}	C_{16}	C_{18}	C_{20}	C_{14}	C_{16}	C_{18}	
XI	5.085	0.407	4.618			0.009	0.051		
XII	4.874		3.685	0.453			0.595	0.141	
XIII	2.330		1.404	0.487			0.291	0.148	
XIV	3.068		0.963	0.663			0.748	0.694	
XV	3.312		0.149	0.414			0.539	2.210	
XVI	3.658		0.165	0.201				3.292	
XVII	3.009			1.138	0.394			1.477	
XVIII	5.015			1.436	1.255			1.854	0.470
Total	30.351	0.407	10.984	4.792	1.649	0.009	2.224	9.816	0.470

Methyl Esters of PAG/A/0.

Fraction	Weight gm.	Saturated Acids				Mono-Ethenoid Acids			"Non-Saponifiable" Matter
		C_{16}	C_{18}	C_{20}	C_{22}	C_{16}	C_{18}	C_{20}	
XIX	1.328	0.470	0.416			0.203	0.239		
XX	1.919		0.511	0.489			0.919		
XXI	1.157		0.298	0.561			0.298		
XXII	1.705		0.721	0.977			0.007		
XXIII	2.528			1.669	0.080			0.342	0.427
Total	8.637	0.470	1.946	3.696	0.080	0.203	1.463	0.342	0.427

Methyl Estors of PAG/A/+25.

Fraction	Weight gm.	Saturated Acids			Mono-ethenoid Acids			"Non-Saponifiable" Matter
		C_{16}	C_{18}	C_{20}	C_{16}	C_{18}	C_{20}	
XXIV	1·336	0·422	0·106		0·584	0·224		
XXV	2·145	0·060	0·086		0·687	1·312		
XXVI	0·673	0·005	0·012		0·145	0·511		
XXVII	1·339	0·194	0·051			1·094		
XXVIII	3·154		0·233	0·947		0·167	1·111	0·696
Total	8·647	0·681	0·488	0·947	1·416	3·308	1·111	0·696

Those calculations give the weights of the various estors in the tota distilled samples but allowance must be made for slight losses during crystallisation, et cetera and also for samples removed for analysis.

To obtain an accurate fatty acid com osition, therefore, the weights of distilled estors were corrected to those weights equivalent to the fatty acids in the original fractions from the low temperature crystallisations, using the foll ing data:-

	PAG/A/+25	PAG/A/0	PAG/A/-35	PAG/A/-35R.
Fatty acids as crystallised (gm.)	16·60	11·72	33·49	38·97
Fatty acids corrected for losses (gm.)	16·64	11·74	33·57	39·06
Fatty acids estorified (gm.)	15·008	10·332	31·402	37·167
Solids separated out during estorification	5·30	0·91	-	-
Saponification equivalent of Estors	321·2	324·0	291·3	297·4
∴ Calculated saponification equivalent of acids	307·2	310·0	277·3	283·4
Weights of estors extracted (gm.)	9·247	9·369	31·488	37·187
Weights of unestorified acids (gm.)	0·20	0·15	0·42	0·51
Weights of estors calculated from the fatty acids corrected for losses (gm.)	11·218	11·211	35·238	41·033
Weights of estors actually distilled (gm.)	8·647	8·637	30·351	36·063
Correction factor to obtain the estors equivalent to the original fatty acids.	1·301	1·297	1·162	1·136

	Methyl Esters					Fatty Acids	
	PAG/A/+25	PAG/A/0	PAG/A/-35	PAG/A/-35F	Total	Total	Weight Percent.
Myristic			0·473	0·291	0·764	0·72	0·8
Palmitic	0·884	0·610	12·750	2·141	16·385	15·53	17·0
Stearic	0·633	2·525	5·563		8·721	8·31	9·1
Arachidic	1·228	4·797	1·914		7·939	7·60	8·3
Behenic		0·104			0·104	0·10	0·1
Tetradecenoic			0·010		0·010	0·01	0·01
Palmitoleic	1·837	0·264	2·582	5·322	10·005	9·48	10·4
Oleic	4·291	1·900	11·400	10·490	28·081	6·75	29·3
Eicosenoic	1·442	0·444		2·815	4·701	4·50	4·9
Hexadecadienoic				1·036	1·036	0·98	1·1
Linoleic				18·160	18·160	17·30	19·0
"Non-Saponifiable"	0·903	0·567	0·546	0·778	2·794	2·79	-
Total	11·218	11·211	35·238	41·033	98·700	94·07	100·0

The fatty acid composition, rounded off to one decimal place is then as follows:-

	Weight Percent.	Molar Percent.
Myristic	0·8	1·0
Palmitic	17·0	18·3
Stearic	9·1	8·8
Arachidic	8·3	7·3
Behenic	0·1	0·1
Saturated acids	35·3	35·5
Tetradecenoic	Trace	Trace
Palmitoleic	10·4	11·3
Oleic	29·3	28·8
Eicosenoic	4·9	4·5
Mono-ethenoid acids	44·6	44·6
Hexadecadienoic	1·1	1·2
Linoleic	19·0	18·7
Di-ethenoid acids	20·1	19·9
	100·0	100·0

The neutralisation equivalent of this mixture of fatty acids was calculated to be 276·4. This agrees very well with the mean molecular weight of the fatty acids calculated from the acid and saponification values and the percentage unsaponifiable matter of the fat itself. This value was 276·3 (See page 46).

There is, however, a considerable difference between this value and that determined by direct titration of the mixed fatty acids which was found to be 305·3.

This was determined on fatty acids containing large quantities of "non-saponifiable" matter which was later separated during the esterification process and the determination of saponification values of ester fractions.

When corrected to account for this "non-saponifiable" matter, the value approaches very closely to the two calculated above.

Associated with 91·28 gms. fatty acids, there were 2·79 gm. "non-saponifiable" matter removed during saponification value determinations and also 6·93 gm. of "non-saponifiable" matter removed during esterification, giving a total of 101 gm. "fatty acids" crystallised.

Hence, allowing for these, the neutralisation equivalent of the fatty acids becomes

$$305{\cdot}3 \times \frac{91{\cdot}28}{101}$$

$$= 276{\cdot}0$$

The calculated iodine value for the mixed fatty acids is 79·4 gm.I_2/100 gm., while the value determined on the mixed "fatty acids" was 71·9 gm.I_2/100 gm. When this latter is corrected for the "non-saponifiable" matter as above (Iodine value less than 5 gm.I_2/100 gm.) it becomes 79·7 gm.I_2/100 gm. which is in good agreement with the calculated value.

5.42 Spectrophotometrically.

The fatty acid composition of another sample of fatty acids from the pod fat was later determined using the alkali isomerisation and spectrophotometric method of Hilditch, Morton and Riley[15].

$E^{1\%}_{1\ cm.}$ 268 mμ = 2·73 (After isomerisation at 170°C for 15 minutes)

$E^{1\%}_{1\ cm.}$ 234 mμ = 205·8 (After isomerisation at 180°C for 60 minutes)

Iodine value = 79·2 gm.I_2/100 gm.

Percent. linolenic acid $= \frac{2\cdot73 \times 100}{532} = 0\cdot5\%$

Percent di-ethenoid as linoleic acid $= \left[205\cdot8 - 2\cdot73 \times \frac{569}{532}\right] \frac{100}{906} = 22\cdot3\%$

Percent mono-ethenoid as oleic acid $= \frac{79\cdot2 \times 100 - (0\cdot5 \times 273\cdot5 + 22\cdot3 \times 181\cdot0)}{89\cdot9}$

$= 41\cdot6\%$

Percent saturated acids (by difference) $= \underline{35\cdot6\%}$

$100\cdot0$

This fatty acid composition agrees well with that determined by ester fractionation.

5.5 The Probable Glyceride Composition of the Pod Fat.

Using the method of Hilditch and Meara[16], it was possible to calculate a glyceride composition for the pod fat from the fatty acid composition.

The fatty acids contained

35·5% (molar) Saturated acids
44·6% Mono-ethenoid acids
and 19·9% Di-ethenoid acids.

Hence, there would have to be at least one saturated and one mono-ethenoid acid in each glyceride molecule, and not more than one di-unsaturated acid in any molecule.

Therefore, for 100 molecules of fatty acids, there were three main groups of glycerides.

A. 11·3 molecules containing 1 saturated and 2 mono-ethenoid acids.
B. 2·2 molecules containing 2 saturated and 1 mono-ethenoid acid.
C. 19·9 molecules containing one each of saturated, mono-ethenoid and di-ethenoid acids.

The saturated acids were further divided into

19·3 mols. "Palmitic" (Including 1·0 mol. of myristic)
8·8 mols. Stearic
7·4 mols. "Arachidic" (Including 0·1 mol. of Behenic)

and the mono-ethenoid acids into

33·3 mols. "Oleic" (Including 4·9 mols. Eicosenoic)
11·3 mols. Palmitoleic

Hexadecadienoic and Linoleic acids were grouped together as "Linoleic" acid - 19·9 mols.

The glycerides in group A were then calculated to comprise

6·1 mols. "Palmito" - palmitoleo - "olein"
2·8 mols. Stearo - palmitoleo - "olein"
and 2·4 mols. "Arachido" - palmitoleo - "olein"

Those in group B were further sub-divided into

1·2 mols. "Palmito" - stearo - "olein"
and 1·0 mols. "Palmito" - "arachido" - olein.

The composition of the third group of glycerides was computed to be

10·8 mols. "Palmito" - "oleo" - "linolein"
4·9 mols. Stearo - "oleo" - "linolein"
and 4·2 mols. "Arachido" - "oleo" - "linolein".

The molar percentage composition of the glycerides, rounded off to the nearest whole number, was then found to be as follows:-

Palmitoleo - "oleins"		33
"Palmito" - palmitoleo - "olein"	18	
Stearo - palmitoleo - "olein"	8	
"Arachido" - palmitoleo - "olein"	7	
"Palmito" - "oleins"		7
"Palmito" - stearo-"olein"	4	
"Palmito" - "arachido" - "olein"	3	
"Oleo" - "linoleins"		60
"Palmito" - "oleo" - "linolein"	33	
Stearo - "oleo" - "linolein"	15	
Arachido" - "oleo" - "linolein"	12	
		100

5.6 Analysis of Unsaponifiable Matter.

The unsaponifiable matter was found to have the following composition:-

	Weight percent.
Saturated hydrocarbons	9·1
Unsaturated hydrocarbons (as squalene)	0·3
Sterols (as sitosterol)	15·8
Glycoryl ethers (as solachyl alcohol)	1·7
	26·9

As described previously (page 40) the free sterols were separated as their digitonides and isolated by the method of Dam[18].

The sterols crystallised in tufts of short needles melting at 139 - 139·6°C. and gave a greenish violet colour with the Liebermann-Burchard reaction. From this it was deduced that the sterols consisted almost entirely of sitosterol, although the greater melting range in this case than in that of the sterols from the seed fat would seem to indicate the presence of a slightly larger amount of other phytosterols.

The hydroxyl value of the total unsaponifiable matter was found to be 93·0 and from this, the hydroxyl value of the 73·1% unaccounted for in the above analysis was calculated.

If X = hydroxyl value of the remainder of the unsaponifiable matter,
then $73{\cdot}1 \times X + (170 \times 150{\cdot}8) + (330 \times 1{\cdot}7) = 9300$

$$\therefore \quad X = \frac{9300 - 2686 - 561}{73{\cdot}1}$$

$$= 82{\cdot}9 \text{ mgm.KOH/gm.}$$

This hydroxyl value, according to Karnovsky et al.[17] is indicative of the presence of fatty alcohols in the unsaponifiable matter: the low value, however, indicates the presence of other non-hydroxylated substances.

The carotenoid content was determined from the extinction of light at 450 m/ [19].

$$E^{1\%}_{1\text{ cm.}} = 0{\cdot}463$$

$$\therefore \text{ Percentage carotenoids as carotene} = \frac{0{\cdot}463}{2{,}500} \times 100$$

$$= {\cdot}0185\% \text{ which is negligible.}$$

5.7 The "Non-Saponifiable" Matter in the Pod Fat.

(i) The solids which separated out during the extraction of the unsaponifiable matter from the soap solution (page 47) were acidified with dilute hydrochloric acid, and thoroughly extracted with ether to remove any fatty acids. After this treatment, they were dried and weighed.

From 150 gm. of fat, 13.1 gm. of solids were recovered, and these were extracted in a soxhlet apparatus with ether for 24 hours.

The residue in the extraction thimble (4.4 gm.) was found to consist of polysaccharides since, on hydrolysis by boiling with dilute sulphuric acid, a positive reaction for carbohydrates was obtained, using Molisch's test.

The ether extract, after similar treatment, gave no positive reaction for carbohydrates. The extract was crystallised from alcohol, giving a precipitate of 4·5 gm. and leaving 4·0 gm. dissolved in the filtrate. The hydroxyl values and melting points were determined on these two fractions and also the melting points of their acetyl derivatives.

Fraction	Alcohol soluble	Alcohol insoluble
Weight of fraction (gm.)	4·0	4·5
Hydroxyl value (mgm.KOH/gm.)	134·4	126·1
Melting point of fraction	82 - 83°C	85·0 - 85·8°C
Melting point of Acetyl derivatives	64·9 - 66·0°C	68·3 - 69·1°C
Iodine value (gm.I_2/100 gm.)	3·2	1·7

These melting points were similar to those reported by Piper et al.[2] for mixtures of long chain fatty alcohols.

A mixture of 20% C_{26} + 40% C_{28} + 40% C_{30} alcohols was reported to have a melting point of 82·3°C and the acetates of the mixture, a melting point of 65·5°C. The hydroxyl value corresponding to this mixture of alcohols is 135·7 mgm.KOH/gm., hence it would appear that the alcohol soluble fraction contained about 0·8 gm. C_{26}, 1·6 gm. C_{28} and 1·6 gm. C_{30} saturated fatty alcohols. The low iodine value

indicated the absence of appreciable unsaturation in the alcohols.

The alcohol insoluble fraction was found to have characteristics very similar to those of a synthetic mixture of 10% C_{28} + 80% C_{30} + 10% C_{32} fatty alcohols. This mixture was reported to have a melting point of 85·6°C. and the acetyl derivatives prepared from the mixture, a melting point of 68·9°C. The calculated hydroxyl value is 128·2 mgm.KOH/gm. which agrees with the value of 126·1 obtained above within the limits of experimental error of a semi-micro hydroxyl value determination.

Thus the fraction was calculated to contain 0·5 gm. C_{28}, 3·5 gm.C_{30} and 0·5 gm.C_{32} long-chain alcohols.

The composition of the total ether soluble fraction was then calculated to be as follows:-

	Weight	Weight percent.
Hexacosanol	0·8 gm.	9
Octacosanol	2·1 gm.	25
Triacontanol	5·1 gm.	60
Di-triacontanol	0·5 gm.	6
		100

This method of determining the proportions of the alcohols was used owing to lack of enough material for fractional distillation of the acetates.

(ii) The solids separating out from the fatty acid fractions on esterification (page 48) and during the determination of the saponification values of the residues in the distillation flasks after ester fractionation (page 49) were hydrolysed by boiling with dilute sulphuric acid. After this treatment, all the above gave a positive reaction for carbohydrates with Molisch's test, indicating the possibility of their being polysaccharides.

The presence of polysaccharides and other carbohydrates in the fatty acids probably explains the high degree of unsaturation in the least soluble fatty acids in fraction PAG/A/+25. This fraction would

have been expected to contain only the most saturated of the fatty acids.

Rowall[21] indicates that carbyhydrates are associated with fatty acids and phosphatides in a loose combination but without the formation of any stable compound. The acids most commonly associated with the carbohydrates are the unsaturated $C_{16} - C_{20}$ acids, which were present to a considerable extent in PAG/A/+25. This fraction was also found to contain nearly 30% by weight of "non-saponifiable" matter. Thus it would appear that the carbohydrates remained associated with the fatty acids and decreased their solubility in acetone - hence the separation of abnormal quantities of unsaturated acids during the crystallisation of the acetone solution of the "fatty acids" at room temperature.

6. THE TESTA WAX.

When the uncrushed seeds were extracted with Petroleum Benzine, 0·20% of the material was found to be soluble.

The extract, after removal of the solvent, was found to consist of two phases: a solid and a liquid. These were separated by crystallisation from acetone (concentration 1 : 5) at 0°C. for 12 hours, when the bulk of the extract remained in solution. From 25·5 gms. of extract from the seeds, 3·0 gm. was filtered off as insoluble in acetone and 22·1 gm. was recovered from the filtrate.

The material recovered from the filtrate was an orange-coloured liquid similar in appearance to the seed fat which was shown to form the major part of it.

The following were the chemical constants of the material from the filtrate. (Values for the seed fat are shown in brackets).

Saponification value	= 179·5 mgm.KOH/gm.	(184·5)
Iodine value	= 103·5 gm.$I_{2/100}$ gm.	(112·8)
Acid value	= 8·0 mgm.KOH/gm.	(3·7 - 7·1)
Hydroxyl value	= 33·1 mgm.KOH/gm.	(20·0)

The solid material, insoluble in acetone had the following constants:-

Saponification value (after 30 minutes' saponification)	= 18·8
Saponification value (after 2 hours' saponification)	= 30·7
Iodine value	= 6·59
Hydroxyl value	= 89·4

From these data, it would appear that the greater part of the extract of the whole seeds, consisted of fat extracted from the kernel itself through the holes caused by insect attack. The values of the chemical constants determined on the liquid portion of the extract are very similar to those of the seed fat itself. The higher hydroxyl value and correspondingly lower saponification and iodine values were probably the result of incomplete separation of the two phases due to slight solubility of the solid material in acetone at 0°C.

From the saponification values of the small quantity of solids crystallised from acetone, these would appear to be of a wax-like nature, since waxes are never completely saponified in the standard time of 30 minutes with 0.5 N alcoholic KOH, but usually require from 1½ to 2 hours for saponification under these conditions[22].

On the assumption that this material consisted of a mixture of higher aliphatic alcohols, and their esters with the corresponding fatty acids, separation of the alcohols from the acids after saponification for two hours was effected by the method of Chibnall et al.[23].

The neutralisation equivalent of the fatty acids liberated was found to be 134·8 mgm.KOH/gm., giving a mean molecular weight of 416. This corresponds to a mixture of hexacosanoic, octacosanoic and docosanoic acids. From 1·5 gms. of wax, only 0·183 gm. of fatty acids was obtained so that further determinations were not carried out.

The alcohols were crystallised from alcohol (95%) giving 1·163 gm. of alcohol soluble fraction and 0·207 gm. of alcohol insoluble. The hydroxyl values of the fractions were determined together with their melting points and those of their acetyl derivatives.

	Alcohol soluble Fraction	Alcohol Insoluble Fraction
Weight	1·163 gm.	0·207 gm.
Hydroxyl value mgm.KOH/gm.	140·9	119·2
Melting point °C	80·0 - 80·8	86·9 - 87·5
Melting point of acetyl derivatives °C	63·5 - 64·0	72·2 - 72·8

The alcohol soluble fraction corresponds to a mixture of 40% C_{26} + 40% C_{28} + 20% C_{30} alcohols which has been reported[20] to have a melting point of 80·4°C. with acetates melting at 63·8. The calculated hydroxyl value for this mixture is 139·5 mgm.KOH/gm.

The alcohol insoluble fraction corresponds to an equimolar mixture of C_{30}, C_{32} and C_{34} alcohols which is reported to have a melting point of 87·1°C. and the acetates of which have a melting point of 72·5°C. The hydroxyl value of this mixture was calculated to be 120·0 mgm.KOH/gm.

The composition of the alcohols from the tosta wax was then computed to be as follows:-

	Weight (gm.)	Weight Percent.
Hexacosanol	0.465	34
Octacosanol	0.465	34
Triacontanol	0.302	22
Dotriacontanol	0.069	5
Totratriacontanol	0.069	5
	1.370	100

7. $\Delta^{9,12}$ HEXADECADIENOIC ACID.

7.1 Presence in the Seed and Seed Pod Fats.

The presence of a hexadecadienoic acid was first suspected in the ester fraction number XXIII of the seed fat.

Its chemical constants were as follows:-

Saponification equivalent	=	279·8 mgm.KOH/gm.
Iodine value	=	143·9 gm.I_2/100 gm.
Thiocyanogen value	=	93·03 gm.I_2/100 gm.
Hydroxyl value	=	8·28 mgm.KOH/gm.

From the saponification equivalent, it was decided that there would be a large proportion of unsaturated C_{16} acids in the fraction.

The iodine value indicated the presence of di-, and, possibly, tri-ethenoid esters, but the latter were excluded by considering the compositions of the next two ester fractions. When these were calculated to include linolenic acid, in both cases the percentage was less than 0·1% of the fraction. Hence, it was considered unlikely that linolenic acid would be present in fraction XXIII.

The thiocyanogen value was higher than that of pure methyl linoleate (92·10). To account for this value, there would have had to be present in the fraction the esters of either linolenic acid or some di-ethenoid C_{16} acid. Since the presence of the former had already been shown to be unlikely, the fraction was assumed to contain the methyl ester of a hexadecadienoic acid, this being the only method of explaining the values of the chemical constants satisfactorily.

In the case of ester fraction number I of the pod fat, the evidence for the presence of a hexadecadienoic acid was even stronger. The saponification equivalent and iodine value of this fraction were:

Saponification equivalent	=	267·6
Iodine value	=	107·6 gm.I_2/100 gm.

The iodine value was well above that of even methyl tetradecenoate and could only have been due to the methyl ester of a di-ethenoid acid.

The saponification equivalent was 0·8 unit below that of methyl palmitoleate so that presence of methyl linoleate in the fraction was extremely unlikely. Hence, to account for both the low saponification equivalent and also the high iodine value, the presence of methyl hexadecadienoate was again postulated.

1.54 gm. Ester Fraction No. XXIII from Seed Fat.

Oxidation and removal of excess permanganate
Steam distillation (ca. 750 ml. of distillate)

Distillate

Extract with Ether and wash with Na_2CO_3 Aq.

Distil off ether from Neutral Products

0.103 gm.

Saponification equivalent = 262
Probably unoxidised esters

Acidify aqueous layer
Extract with ether
Distil off solvent

0.20 gm.

Neutralisation equivalent = 111.2
Odour of butyric acid

Probably butyric and heptanoic acids

Reacidify, extract, distil off ether

Distil acids at Atmospheric pressure

Distillate
Neutralisation Equivalent = 89
Odour of butyric acid

Residue
Neutralisation equivalent = 118
Probably butyric, capric and heptanoic acids

Flask Residue

Extract with ether

Wash extract with Na_2CO_3 Aq.

Distil off ether from extract

0.20 gm.
Neutral Products

Saponification Equivalent = 283

Probably unoxidised esters

Saponify aqueous layer with KOH, Acidify, Extract with ether, distil off solvent

Triturate with Petroleum ether and wash with solvent

Soluble

Distil off petroleum ether

0.21 gm.

Still odour of butyric acid
Neutralisation equivalent = 119
Probably butyric, capric and heptanoic acids

0.40 gm. Insoluble
Neutralisation Equivalent = 82

Chromatograph

Aqueous layer

Evaporate to near dryness with KOH, acidify with H_2SO_4 and take to dryness

Leach with acetone and distil off solvent

Neutralise with alcoholic KOH, acidify to methyl orange with H_2SO_4, cool and filter

Evaporate to dryness

1.24 gm. Neutralisation Equivalent = 72.8

Chromatograph

FIG. 3.

3·06 gm. Ester Fraction No. I from the pod fat.

Oxidation and removal of excess permanganate
Steam distillation (ca. 750 ml. distillate)

Distillate

Extract with ether and Wash with Na_2CO_3 Aq.

Distil off ether from the Neutral Products

0·30 gm.
Saponification Equivalent = 221

Probably methyl myristate and other unoxidised esters

Acidify aqueous layer Extract with ether Distil off solvent

0·34 gm.
Neutralisation Equivalent = 112·1
Odour of Butyric Acid

Probably butyric and heptanoic acids

Reacidify, extract and distil off ether

Distil acids at atmospheric pressure

Distillate
B.R. 155 - 160°C
Neutralisation Equivalent = 90
Odour of Butyric Acid

Residue
Neutralisation Equivalent = 123
Odour of Butyric acid
Probably butyric and heptanoic acids

Flask Residue

Extract with ether

Wash extract with Na_2CO_3 Aq.

Distil off ether from extract.

0·44 gm.
Neutral products
Saponification Equivalent = 272
Probably unoxidised esters

Saponify aqueous layer with KOH, Acidify, extract with ether Distil off solvent

Triturate with petroleum ether and wash with solvent

Soluble

Distil off Petroleum ether

0·46 gm.
Still odour of butyric acid and probably butyric + heptanoic acids
Neutralisation equivalent = 127

0·40 gm. Insoluble
Neutralisation equivalent
Chromatograph

Aqueous layer

Evaporate to near dryness with KOH acidify with H_2SO_4 and take to dryness.

Leach with acetone and distil off solvent

Neutralise with alcoholic KOH, acidify to methyl orange with H_2SO_4, cool and filter

Evaporate to dryness

1·5 gm. Neutralisation Equivalent = 72·8

Chromatograph

FIG. 4.

7.2. Proof of Constitution.

To confirm the presence of hexadecadienoic acid in the two fats, and to attempt to determine its structure, the two fractions mentioned above (AG/A/60F, No. XXIII and PAG/A/-35F, No. I) were subjected to oxidation by potassium permanganate in acetone[24].

After reducing the excess potassium permanganate with potassium bisulphite, the oxidation products were steam distilled and treated as shown in the flow sheets (Figs. 3 and 4).

In the acid products from the steam distillates of both fractions, the presence of butyric acid was detected by its characteristic odour and further confirmed by means of the neutralisation equivalents as shown.

In the oxidation of fraction Number XXIII (AG/A/60F), butyric acid could be obtained from one of the following esters:-

(i) $CH_3CH_2CH_2CH = CH CH_2CH = CH(CH_2)_7COOCH_3$

(ii) $CH_3CH_2CH_2CH = CH(CH_2)_{10}COOCH_3$

(iii) $CH_3CH_2CH_2CH = CH(CH_2)_3CH = CH(CH_2)_7COOCH_3$

(iv) $CH_3CH_2CH_2CH = CHCH_2CH = CH(CH_2)_9COOCH_3$

(v) $CH_3CH_2CH_2CH = CH(CH_2)_{12}COOCH_3$

Butyric acid could also be derived from fraction Number I (PAG/A/-35F) by the oxidation of one of the following esters:-

(i) $CH_3CH_2CH_2CH = CH CH_2CH = CH(CH_2)_7COOCH_3$

(ii) $CH_3CH_2CH_2CH = CH(CH_2)_{10}COOCH_3$

(iii) $CH_3CH_2CH_2CH = CH CH_2CH = CH(CH_2)_5COOCH_3$

(iv) $CH_3CH_2CH_2CH = CH(CH_2)_8COOCH_3$

Which of the above esters was present in the fractions was indicated by the dicarboxylic acids present in the residues remaining in the steam distillation flasks.

The dicarboxylic acids were determined qualitatively by partition

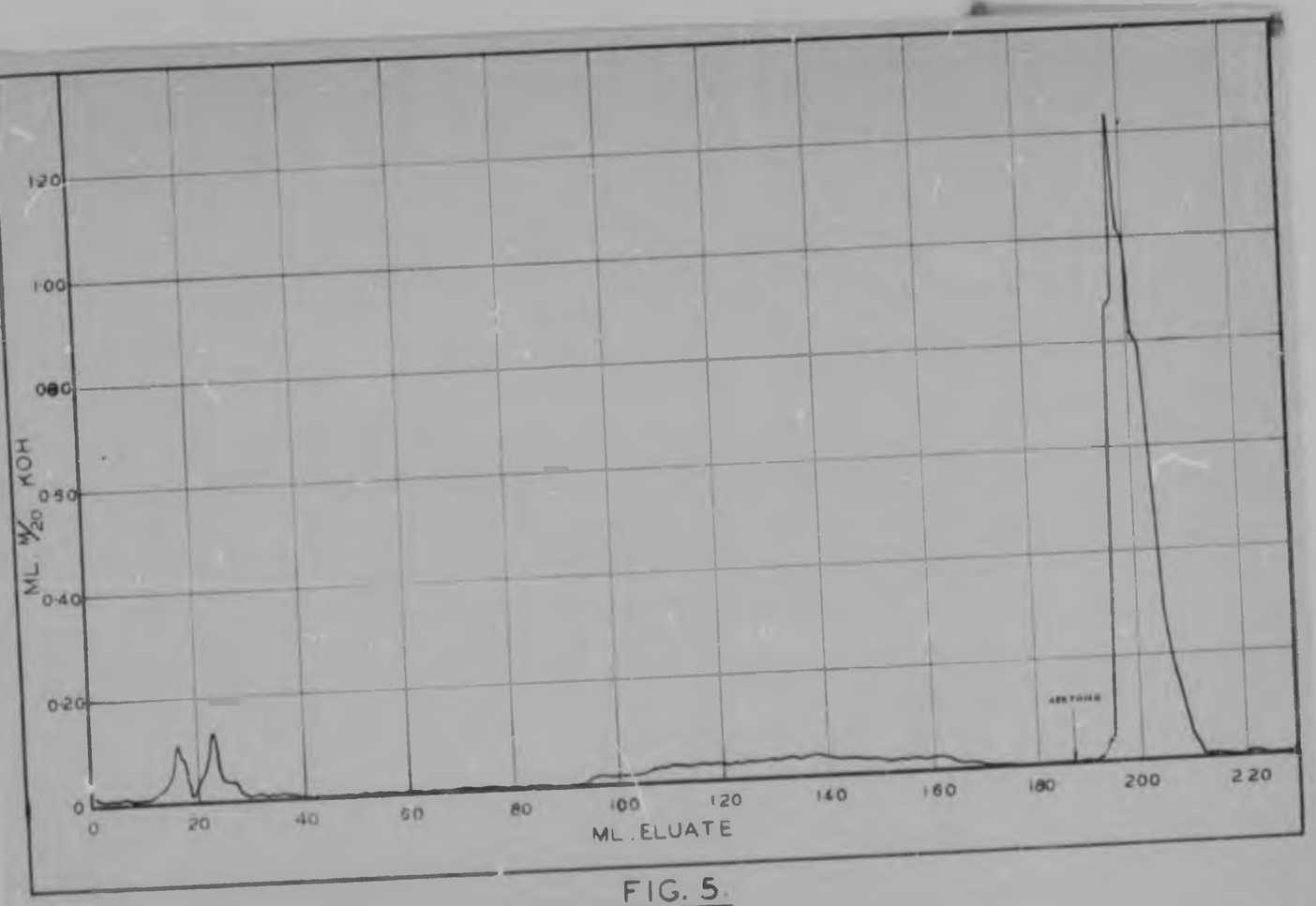

1·20
1·00
0·80
0·60
0·40
0·20
0
ML. N/20 KOH
0
20
40
60
80
100
120
140
160
180
200
220
ML. ELUATE
ACETONE

FIG. 5.

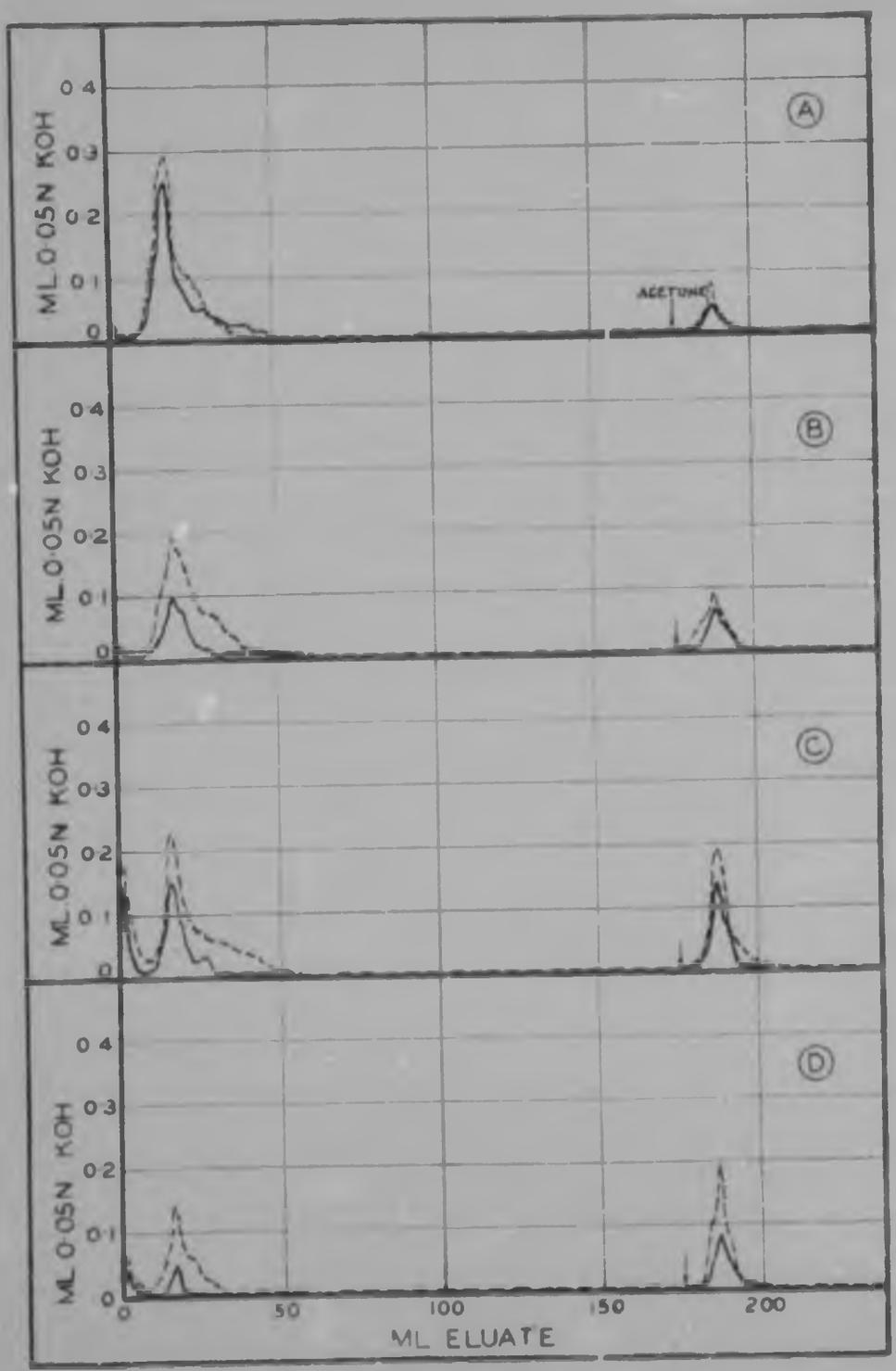


FIG. 6.

chromatography according to the method of Borgemann, Kopplor and Bookonoogon[25].

The method was first tested with a synthetic mixture of one part each of azolaic and adipic acids and five parts each of succinic and malonic acids to determine the applicability of the method to the lower dicarboxylic acids. It was observed that the resolution of azolaic and adipic acids was extremely sharp (Fig. 5) but the succinic acid appeared only after 100 ml. eluate had been collected and then only as a very flat hump in the curve.

It was obvious that malonic acid would have taken even longer to appear and would have had an even flatter hump than succinic acid. Consequently, the method was modified to include the elution of the column with acetone after 170 ml. eluate had been collected. This figure was chosen because, in the above determination, the succinic acid was observed to have been eluted completely by this quantity of benzene eluent.

From the standardisation curve (Fig. 5) the effect of this modification may be seen to be satisfactory and this method was adopted throughout the succeeding determinations.

Fig. 6 shows the four curves obtained from the various samples of dicarboxylic acids. In all cases, the curves drawn with full lines are those of the acids as obtained from the oxidation process. The broken lines are the curves obtained when small quantities of azolaic and malonic acids were added to the unknown acids to identify the peaks.

Fig. 6 A and B are those obtained from the chromatography of the dicarboxylic acids extracted by ether from the residues in the steam distillation flasks.

Figs. 6 C and D are the curves from the determinations on the dicarboxylic acids in the aqueous layers remaining after extraction of the residues in the distillation flasks with ether.

The acids used to obtain Figs. 6 A and C were obtained from the pod fat fraction (Number I) and Figs. 6 B and D from the seed fat fraction (Number XXIII).

Fig. 7 shows the curves of Fig. 6 A. drawn on a larger scale to indicate the variations in titre of successive portions of eluate. From

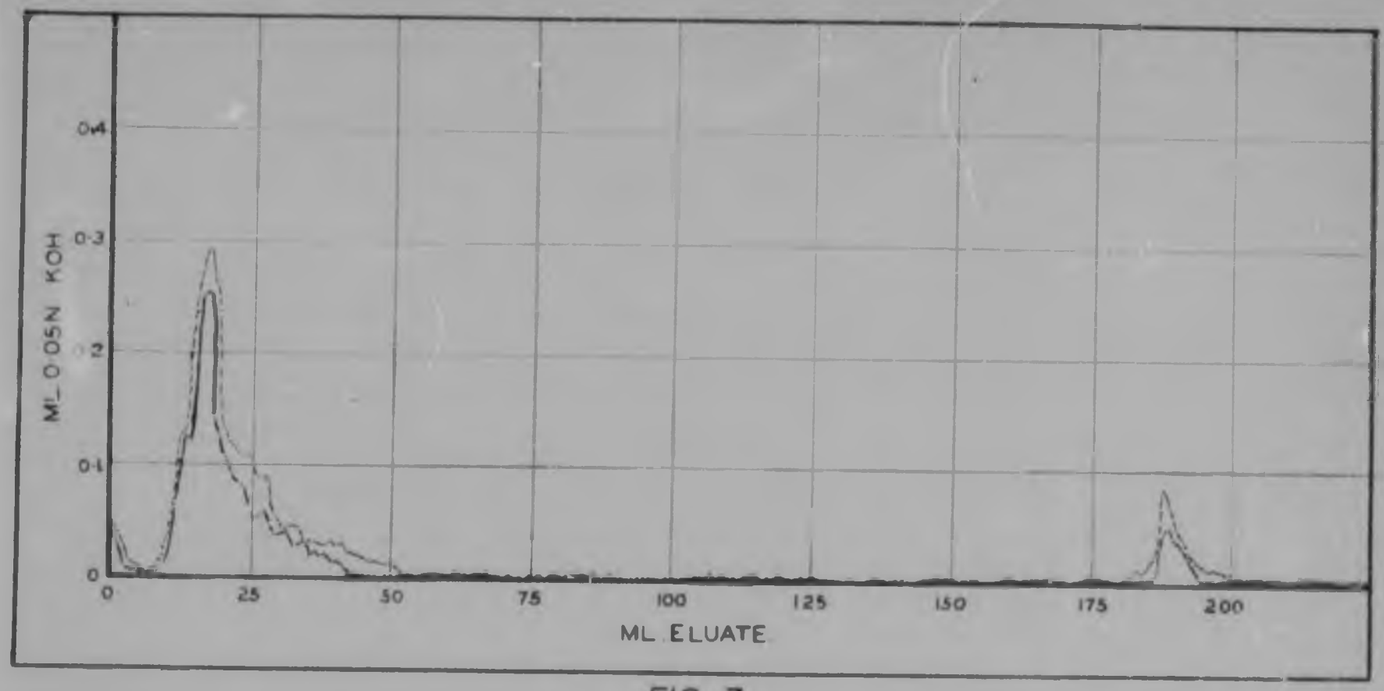


FIG. 7.

this curve, it is obvious that the peaks obtained in the determinations could not possibly be due to experimental error.

In all the curves on dibasic acids from oxidation of the ester fractions, the first three to five ml. of eluate required appreciable quantities of alkali for neutralisation. This was shown to be due to traces of sulphuric acid which had not been washed from the acids. These samples gave precipitates with Barium Chloride solution which were insoluble in boiling concentrated hydrochloric acid.

These curves indicate the presence of azelaic and malonic acids to the exclusion of all other dicarboxylic acids in the oxidation products of the two ester fractions.

The only possible ester from which these two acids could be derived together with butyric acid, is

$\Delta^{9,12}$ Hexadecadienoic Acid.

The evidence offered in the foregoing paragraphs indicates that $\Delta^{9,12}$ hexadecadienoic acid was almost certainly present in the mixed fatty acids from both the seed fat and the seed pod fat.

How this acid, on oxidation with potassium permanganate, yields butyric, malonic and azelaic acids, is shown by the equation below.

$$CH_3CH_2CH_2CH = CHCH_2CH = CH(CH_2)_7COOH + 8O$$

$$= CH_3CH_2CH_2COOH + HOOC\,CH_2COOH + HOOC(CH_2)_7COOH.$$

8. THE TECHNOLOGY OF THE FATS.

(i) The Seed Fat.

The drying time of the seed fat was determined according to the standard method specified by the South African Bureau of Standards[26] and found to be as follows:-

Extracted fat	= 48 hours
Extracted fat, after removal of phosphatides and mucilage	= 30 hours.

The latter drying times is normal for a semi-drying oil and according to Hilditch[27], semi-drying oils of this type are unsuited to use in paint manufacture, since heat treatment has very little effect on their drying properties. Even after fractional crystallisation from suitable solvents, due to the mixed glyceride structure of natural fats, no fraction can be separated which will have a sufficiently high iodine value to render it useful as a drying oil. Hence no further investigation into the drying properties of the oil was made.

The use of the fat as a source of sterols or phosphatides would most probably prove uneconomic due to the small percentage of fat in the seeds. Soya bean oil, with approximately the same proportions of sterols and phosphatides, is much more readily available.

(ii) The Seed Pod Fat.

Being a highly-coloured solid fat containing appreciable amounts of free fatty acids and unsaponifiable matter, the pod fat would appear to have no technological applications.

At present the sole use of both seeds and pods of Acacia giraffae is as a cattle feed and it would appear that this is the only economical way of utilising the fruit of the tree.

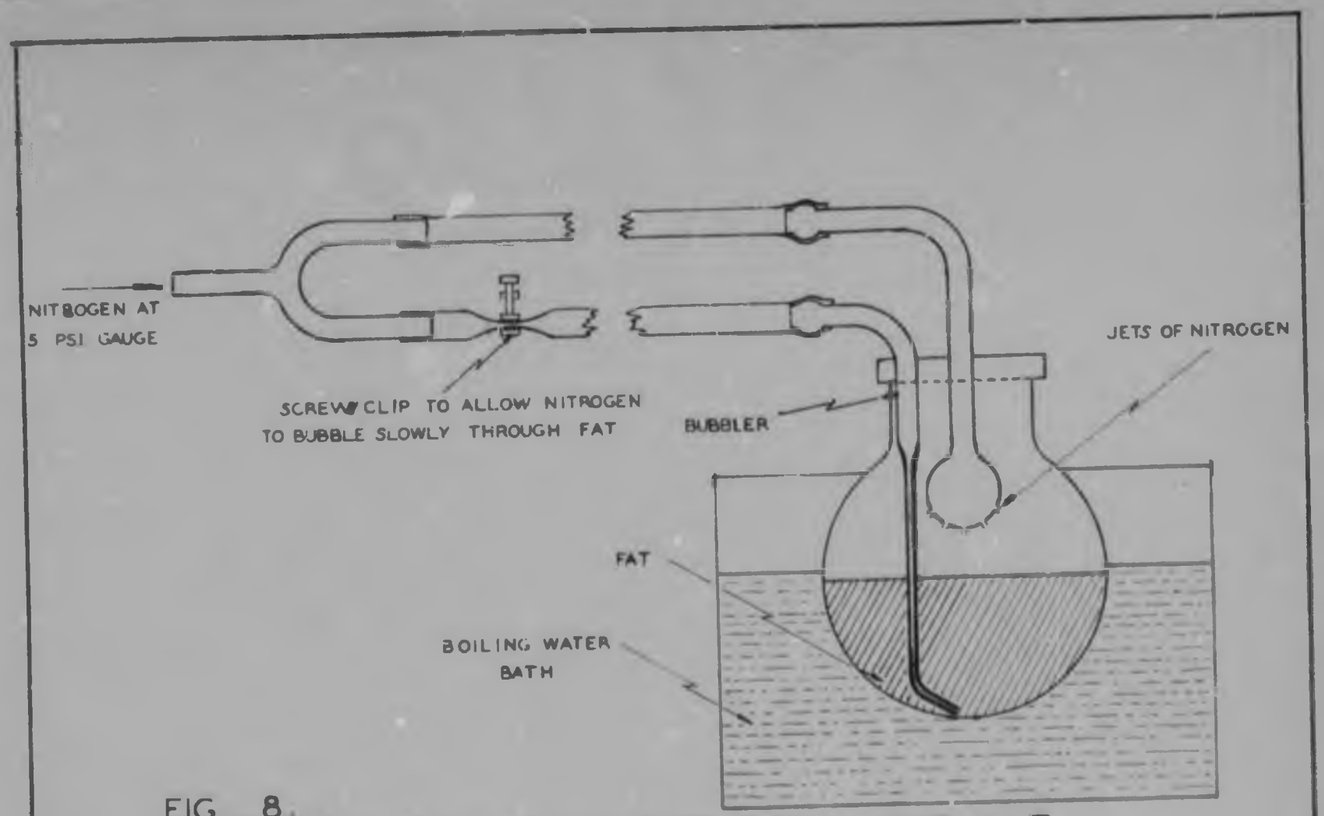


FIG. 8.
APPARATUS FOR REMOVAL OF SOLVENT FROM SEED FAT.

9. EXPERIMENTAL.

9.1 Extraction of Fats.

The seeds were separated from the pod husks by pounding the whole pods and sorting out the seeds by hand. The seeds were then subjected to a preliminary extraction for 24 hours in the extractor described below, using Shell "Special Boiling Petroleum Benzine" (Boiling range 50 - 70°C) as solvent. Petroleum benzine was used, because, while being considerably less expensive than Analar Petroleum Ether, it was found to be in no way inferior (see page 91).

After the "Testa Wax" had been removed, the seeds, owing to the hard tough nature of the testa, had to be passed several times through cracking rolls prior to grinding. The final grinding was carried out either in a large hand-operated coffee mill, or in a laboratory hammer mill.

The finely-ground seeds were then extracted in batches of 3·5 - 4 kgs. for 24 hours at a time. As before, this was carried out in the specially designed extractor, using petroleum benzine.

After drying the solution of fat in benzine over anhydrous sodium sulphate for a period of 12 hours, and filtering, the solvent was distilled off at atmospheric pressure on a water bath. As the solution became more concentrated, suction was applied to the flask but had to be discontinued owing to violent frothing of the fat.

This difficulty was overcome by the adoption of a different technique for the removal of the last traces of solvent. A stream of nitrogen was bubbled through the fat, heated on a boiling water bath, and jets of nitrogen were directed on to the surface to prevent undue frothing. The apparatus is shown in Fig. 8. By this means, the solvent was completely removed in about four hours after the passage of nitrogen was started (Nitrogen could not be replaced by carbon dioxide, from dry ice, for this purpose since a high pressure was required to break the bubbles in the froth).

The pod husks were at first extracted without any further grinding after the removal of the seeds but this was found to give incomplete extraction of the fat, even after 36 hours. Subsequent batches of pods were

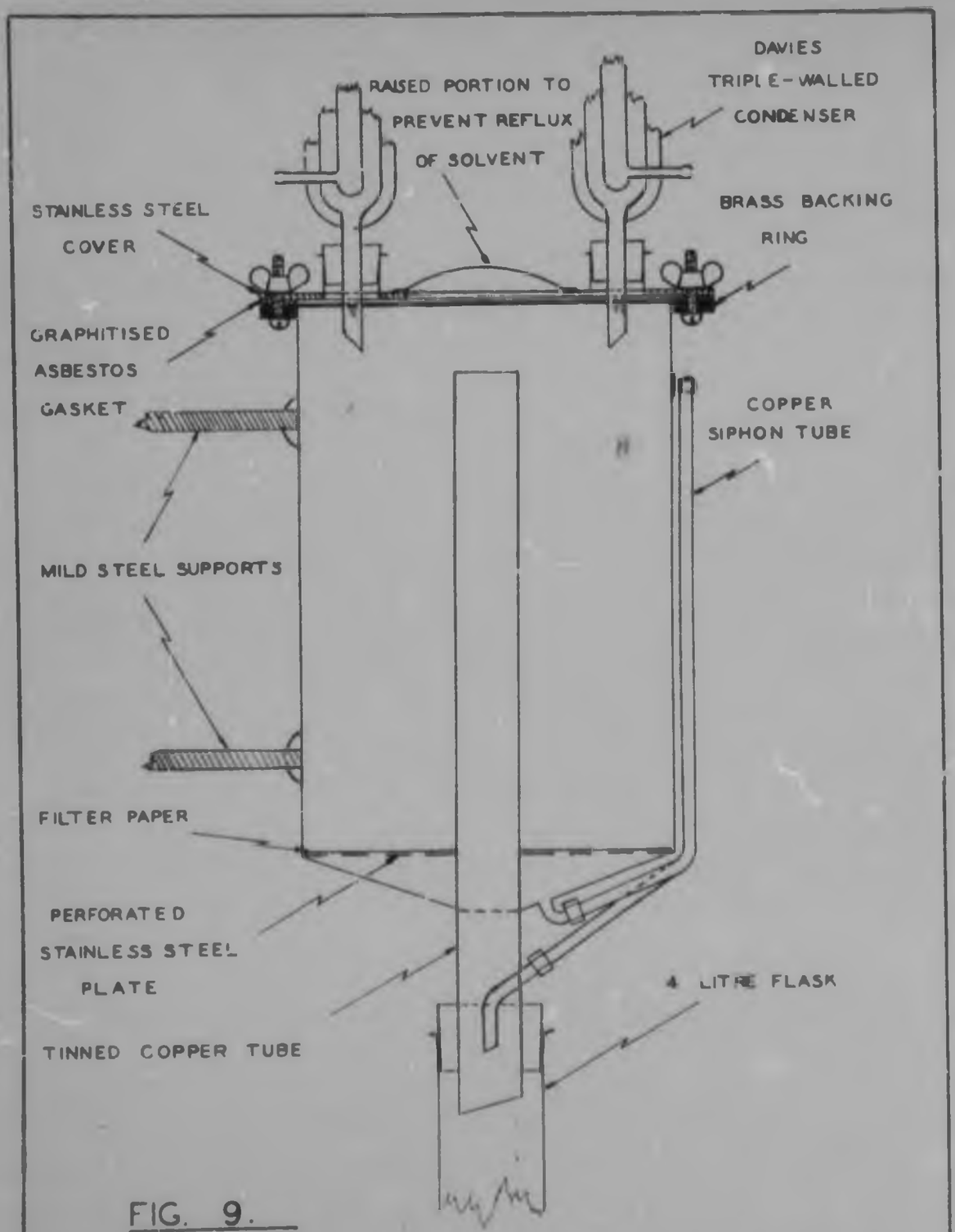


FIG. 9.

STAINLESS STEEL FAT EXTRACTOR

finely ground in a laboratory hammer mill. After this treatment, 24 hours' extraction removed the fat completely.

In the case of the pod fat, the removal of the solvent presented no difficulty and the last traces were removed under reduced pressure. To facilitate this removal, however, carbon dioxide or nitrogen was bubbled through the molten fat.

The Extractor (Fig. 9).

This was designed by the present author together with Mr C. van der Pol.

From the diagram, it can be seen that it operates on the general Soxhlet principle but the vapours pass up through a central tube in the extractor body, thus conferring the added advantage of extraction with hot solvent.

Two condensers were used and the centre portion of the cover raised, to prevent reflux of solvent down the central tube. The condensers as well as the distillation flask were fitted by means of well-rolled, shellac-impregnated, corks previously extracted with petroleum benzine.

Heating was carried out on an electric water bath with Simmerstat control to maintain a temperature of 70-80° C. Under these conditions, the average time taken to fill and drain the extractor was 1½ hours, thus giving at least 15 extractions during a 24-hour run.

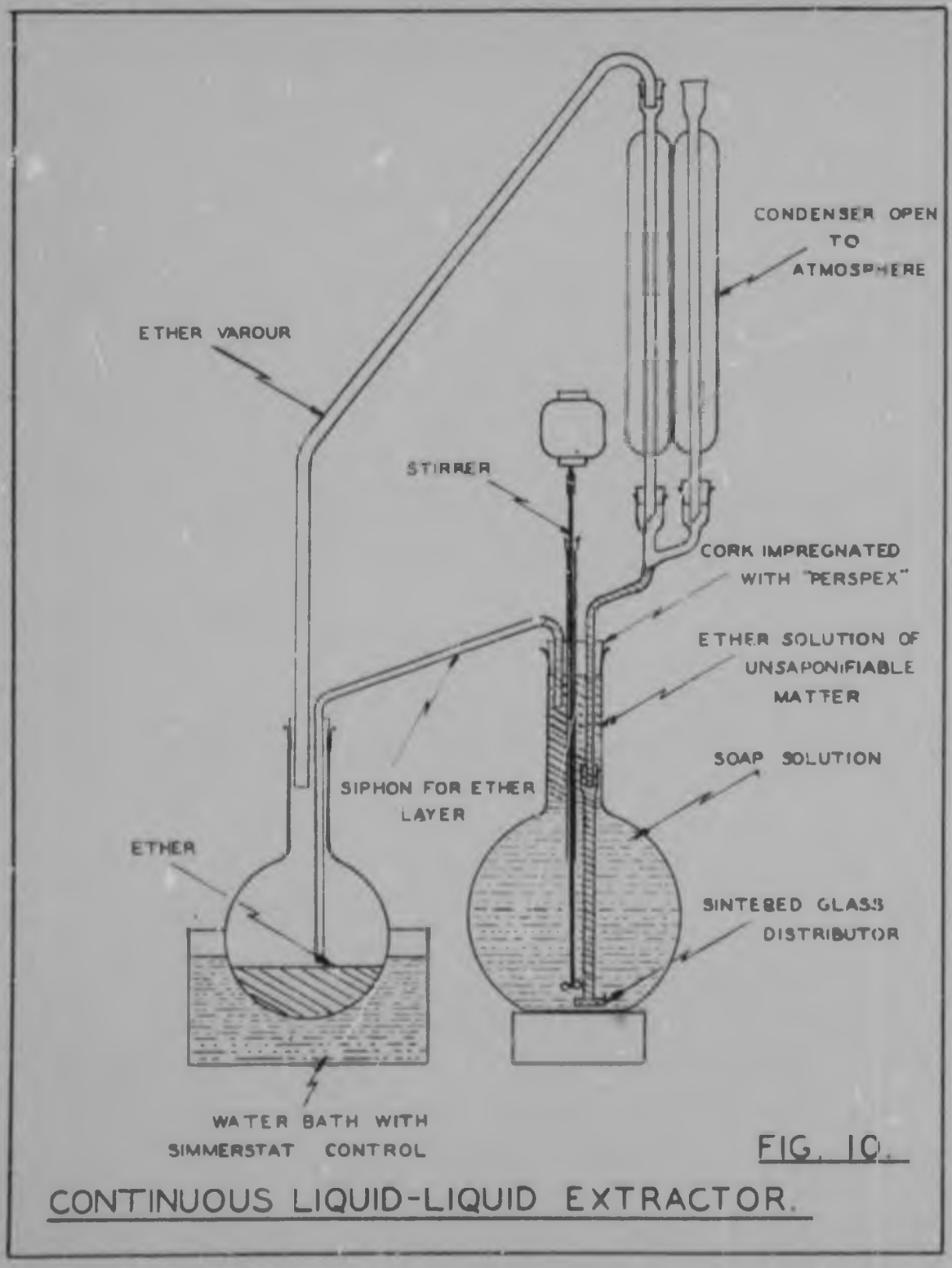


FIG. 10.

CONTINUOUS LIQUID-LIQUID EXTRACTOR.

9.2 Resolution of the Fatty Acids.

9.21 Saponification.

The fats were saponified by boiling for 3 hours under reflux with alcoholic potassium hydroxide solution to ensure complete hydrolysis. For each 100 gm. of fat, 30 gm. of potassium hydroxide in 500 ml. of S.V.R. were used[28].

9.22 Removal of Unsaponifiable Matter.

After completion of the saponification, about three-quarters of the alcohol was distilled off and the solution of soaps made up to twice its volume with distilled water.

The soap solution was then extracted with ether in the continuous liquid-liquid extractor shown in Fig. 10. This is a modification of a similar apparatus suggested by Hilditch[28].

The bottle in this latter apparatus has been replaced by a five litre flat-bottomed flask so as to reduce the volume of the ether layer to a minimum and thus increase the rate of removal of unsaponifiable matter.

A second reflux condenser was added to prevent loss of ether due to incomplete condensation. This was found to be considerable in the Hilditch apparatus, especially during warm weather.

The corks used were impregnated with a solution of "Perspex" in chloroform to obviate diffusion of ether vapours from the flask, as this prevented the efficient operation of the siphon.

Efficient mixing of the ether and soap solution was achieved by the introduction of a sintered glass distributor together with a propeller type stirrer revolving at a low speed to minimise emulsification.

By means of this apparatus, the unsaponifiable matter from 400 gm. of fat could be extracted completely in 8 hours.

The ether solution of unsaponifiable matter was washed three times alternately with 10% potassium hydroxide solution and distilled water and finally with distilled water until the washings were no longer alkaline to phenol phthalein. After drying overnight with anhydrous sodium sulphate, and filtering, the ether was distilled off under reduced pressure.

9.23 Extraction of Fatty Acids.

After removal of the unsaponifiable matter, the soaps were split by warming with dilute sulphuric acid (2N) until definitely acid to methyl-orange. The fatty acids were then extracted with ether, the extraction being continued until the aqueous layer was clear. The ether solution was then dried overnight with anhydrous sodium sulphate, filtered and the ether distilled off under reduced pressure in a stream of nitrogen or carbon dioxide.

9.24 Separation of the Fatty Acids into Fractions by Low-Temperature Crystallisation from Anhydrous Acetone.

Anhydrous acetone was prepared by allowing "chemically pure" acetone to stand over anhydrous potassium carbonate for several days and then distilling through a Vigreaux column. The fraction distilling between 50.5 and 51.0°C (625 mm. Mercury) was collected, stored in glass-stoppered bottles, and used as soon as possible after distillation.

Solutions of the appropriate concentration of fatty acids in anhydrous acetone were crystallised for six hours at the required temperature and the crystals then filtered off[29].

The filtrates were then crystallised at suitably lower temperatures without further treatment and the solvent from the various batches of crystals and the final filtrates removed at room temperature under reduced pressure using a stream of nitrogen or carbon dioxide.

9.25. Esterification of Fatty Acid Fractions.

The solid fatty acid fraction of the seed fat was esterified with absolute ethyl alcohol and the two liquid fractions with anhydrous methyl alcohol.

The ethyl esters were employed at first due to their slightly lower melting points, compared with those of the corresponding methyl esters. Later work showed that no appreciable advantage resulted from this technique and in subsequent work, the methyl esters were used exclusively.

The anhydrous alcohols were prepared by refluxing "chemically pure" material over calcium oxide for two hours followed by distillation through a Vigreaux column. In each case, the distillate was collected over a range of 1°C.

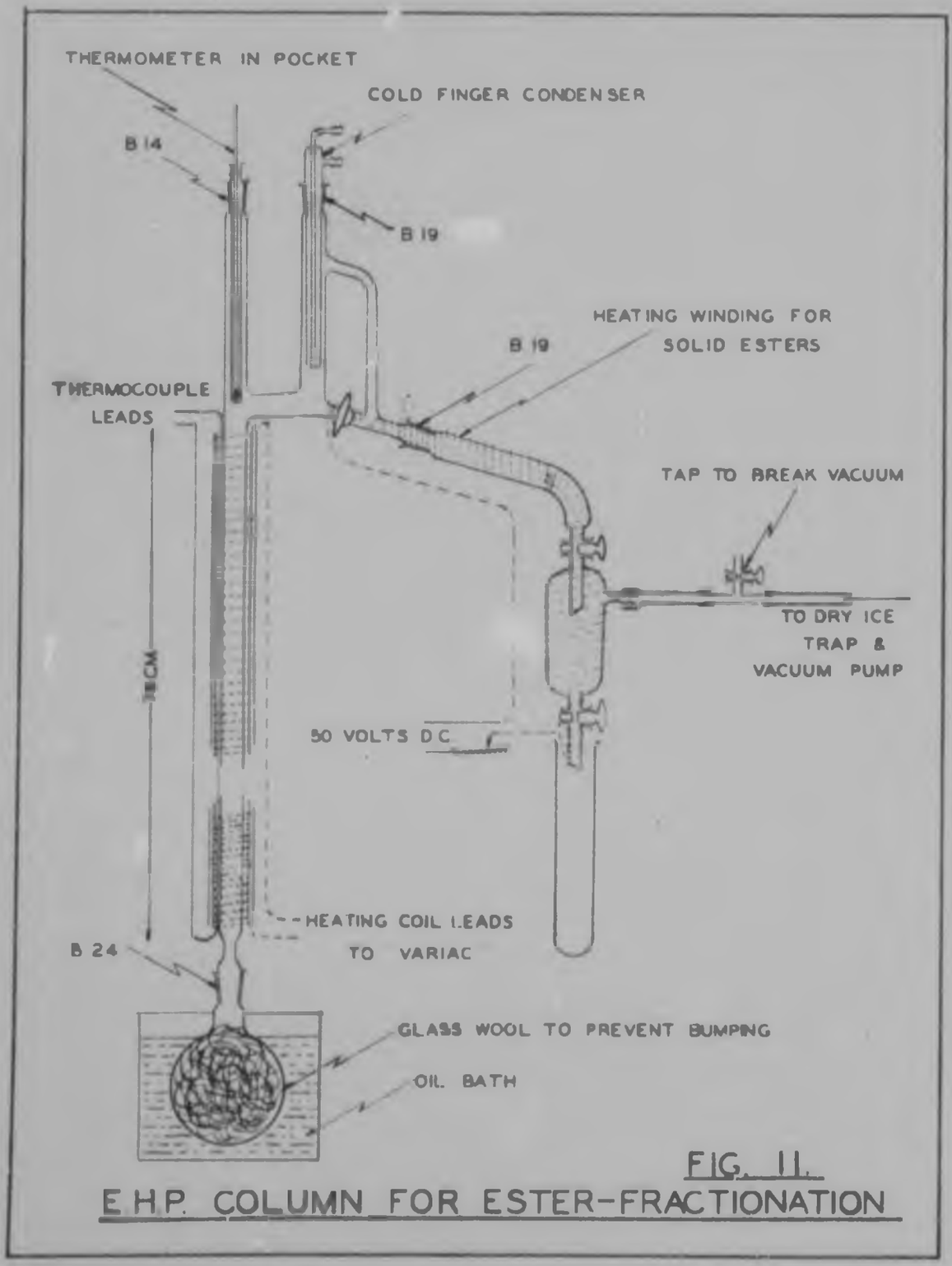


FIG. 11.

E.H.P. COLUMN FOR ESTER-FRACTIONATION

Estorification was carried out by boiling the fatty acids with four times their weight of the particular alcohol in the presence of 1% of concentrated sulphuric acid[30]. This mixture was refluxed for two hours, allowed to stand overnight and refluxed for a further two hours to ensure complete estorification.

Excess alcohol was distilled off on a boiling water bath under atmospheric pressure, the esters cooled and diluted to twice their volume with distilled water. The resulting mixture was extracted with ether until clear and the united ether extracts washed with 50 ml. aliquots of a 1% potassium carbonate solution. Finally, the ether solutions were washed with distilled water until no longer alkaline to phenol phthalin, and dried overnight with anhydrous sodium sulphate. After filtering, the ether was distilled off under reduced pressure in a stream of nitrogen or carbon dioxide.

The potassium carbonate washings were united in each case, acidified with hydrochloric acid and extracted with ether. The extracts were then washed with distilled water, dried over anhydrous sodium sulphate and, after filtering, the ether was distilled off. The conversion to esters exceeded 95% in all cases and hence re-estorification of the ether extract of the potassium carbonate washings was not necessary[30].

9.26 Fractional Distillation of the Esters.

This was carried out in an E.H.P. column similar to that described by Hilditch[31], but fitted with standard ground glass joints throughout. Operating pressures varied between 0·5 and 1·5 mm. Mercury, and were maintained by including a "dry-ice" cooled trap in the system to collect condensible matter.

A modified receiver, Fig. 11, was designed and used. It eliminates the inclusion of a vacuum-tight joint between the receiver and the tubes used for collecting the samples. The time between breaking the vacuum to collect a sample, and the re-attainment of the original pressure, was less than one minute which is comparable with the time used for changing the receiver on a Perkin Triangle. It has, however, the considerable advantage of a very closely reproducible vacuum.

Provision was also made for heating the receiver to prevent solidification of saturated esters while distilling. The heating winding was 28 S.W.G. Nichrome Resistance wire and maintained a temperature of 50°C in the receiver with a current of 1 amp. at 50 volts.

9.3 The Removal of Mucilaginous Material from the Fats.

About 100 gm. of seed fat was boiled with 200 ml. of a 2% sodium chloride solution, and stirred vigorously with a propeller stirrer. The boiling was continued for one hour and the fat and water allowed to stand until cool.

No separation into layers was obtained on standing and even centrifuging failed to break the emulsion, suggesting the presence of powerful emulsifying agents. The mixture was then stirred again without heating and S.V.R. added slowly until the fat showed a tendency to form globules. After centrifuging at 1500 x G for five minutes, the clear fatty layer was separated from the layers of brine and emulsion.

The procedure was repeated and the fat washed several times by boiling with distilled water to remove the last traces of salt. To remove the water, the fat was dried over anhydrous sodium sulphate at 90°C for 2 hours and filtered hot. The fat was then a clear golden yellow colour but still contained traces of alcohol. This had to be completely removed to determine the true hydroxyl value of the fat, since ethyl alcohol has an hydroxyl value of 1,219. This was effected under vacuum on a boiling water bath while bubbling a stream of carbon dioxide through the fat. The acid and hydroxyl values were then determined.

The pod fat was treated similarly, except that the addition of alcohol to break the emulsion was unnecessary and the mixture separated into two well-defined layers on cooling. Thus the final treatment with carbon dioxide was not required.

After separating the fats, the brine extracts from the above procedures were, in both cases, treated with twice their volume of S.V.R. to precipitate the mucilaginous material which was then separated by centrifugation. The solid separating out was dried and then hydrolysed by heating on a boiling water bath for six hours with 35 times its weight of 2% sulphuric acid[13].

After cooling, the hydrolysate was filtered and the acid in the filtrate neutralised with solid barium carbonate. The barium sulphate formed, and excess barium carbonate were then filtered off and washed. The filtrate and washings were united and evaporated to dryness under vacuum on a boiling water bath.

The crystalline product thus obtained was treated with phenyl hydrazine and acetic acid to prepare the osazones of the sugars. After heating for five minutes on a boiling water bath, the separated crystals were examined microscopically and filtered off. After a further 30 minutes' heating, a further crop of crystals had formed and these too were examined.

The osazones from the hydrolysis of the mucilages from both the seed fat and the seed pod fat were compared and found to be similar.

A sample of mucilage was extracted from the seeds themselves according to the method of Anderson[13], hydrolysed, and the osazones of the resulting sugars prepared.

These were examined microscopically and found to be similar to those from both the seed and the seed pod fats.

3.4 The Oxidation and Treatment of the Oxidation Products of Ester Fractions Containing $\Delta^{9,12}$ Hexadecadienoic Acid.

The ester fractions from the seed and seed pod fats which were postulated to contain reasonably large proportions of hexadecadienoic acid were selected: Number XXIII from the seed fat and Number I from the pod fat.

These were separately subjected to oxidation with potassium permanganate in acetone according to the method of Armstrong and Hilditch[24].

The oxidation mixture, after the reduction of excess permanganate with sodium bisulphite and dilute sulphuric acid, was steam distilled. The steam distillate and the residue in the flask were extracted with ether and the aqueous layer of the steam distillate discarded, but that of the flask residue was reserved. It was thought that this latter layer would contain malonic acid, as it is about ten times as soluble in water as in ether, while its partition coefficient between ether and water is 12:1 in favour of water[31].

9.41 The Ether Extract of the Steam Distillate.

This was first washed with 10% aqueous sodium carbonate solution to remove the acid products of oxidation. The neutral residue was left in solution in the ether which was washed with water, the washings being added to the sodium carbonate washings. After distilling off the ether, the saponification equivalent of these neutral products was determined.

The sodium carbonate washings were split and repeatedly extracted with ether. It was considered unnecessary to saponify them, as the semi-esters of the dicarboxylic acids are non-volatile and would therefore have been absent.

The ether extract was dried with large quantities of anhydrous sodium sulphate to remove all water. If any water were left in the ether, it would on distillation, have carried over some of the steam volatile fatty acids with it.

The distillation of the ether was carried out very gently at atmospheric pressure on a water bath at temperatures not exceeding 60°C.

The neutralisation equivalent of these fatty acids was determined and the solution of soaps in alcohol retained. These, after splitting, extracting with ether and stripping off the ether as above, were distilled at room temperature. The weight and neutralisation equivalent of the residue were determined and hence an approximate neutralisation equivalent of the distillate calculated (see Figs. 3 & 4 facing p.72).

9.42 The Ether Extract of the Residue in the Steam Distillation Flask.

This was washed with sodium carbonate solution and the ether layer treated as above.

The sodium carbonate extract was, however, boiled with 10% of sodium hydroxide pellets for one hour to saponify the semi-esters of dicarboxylic acids which were present. The resulting solution of sodium salts was then acidified with sulphuric acid (2N) and repeatedly extracted with ether.

After drying the ether extracts and distilling off the ether, the acids were triturated with 100 mls. "Analar" petroleum ether (Boiling range 40° - 60°C) and allowed to stand overnight to dissolve out the mono-carboxylic acids. After two more washings with petroleum ether, the solid residue was dried at 100°C.

This residue was then chromatographed according to the method of Borgomann, Kepplar and Bookenoogen[25]. Experiments with synthetic mixtures showed this method could not be used for the detection of malonic acid, but its presence could be demonstrated by the following modification.

An equilibrium mixture of 10 parts benzene, 3 parts water and 3 parts ethanol, was prepared. The aqueous phase was used to wet the column of silica gel and the benzene phase for elution of the column until 170 ml. of eluate had been collected. This was determined to be past the point where succinic acid was completely eluted (See Fig. 5).

The benzene phase was then replaced by acetone which eluted malonic acid. The efficacy of this method was proved by chromatographing a synthetic mixture of azelaic, adipic, succinic and malonic acids, when complete resolution was obtained (See Fig. 5).

9.43 The Aqueous Layer Remaining after the Ether Extraction of the Residue in the Distilling Flask.

This was boiled with 10% of sodium hydroxide pellets for one hour and then concentrated by heating in an evaporating basin on an asbestos pad. When solids began to separate out, the residue was acidified to methyl orange with 2N sulphuric acid and evaporated just to dryness. The solids left in the evaporating basin were extracted with hot S.V.R. several times and the alcohol evaporated.

A tarry mass remained which was extracted with cold alcohol three or four times and the alcohol distilled off. The acids remaining were then triturated with petroleum ether and extracted as above. The acids insoluble in petroleum ether were then chromatographed as before.

9.44 Identification of the "Peaks" Obtained in the Chromatographic Treatment of the Di-carboxylic Acids.

Each sample, on which a chromatographic determination had been carried out, was mixed with a known quantity of azelaic and malonic acids and the

chromatographic analysis repeated. By super-imposing the relevant curves, the identity of the known carboxylic acids with those in the fractions was easily demonstrated.

9.5 Methods of Analysis.

9.501 Density[32].

The densities of the seed and seed pod fats were determined using specific gravity bottles of approximately 10 ccs. capacity.

All results were reported as $d_{4^\circ}^{t^\circ}$ and consequently an air-free sample of distilled water had to be used as a reference in each case. To achieve this, double distilled water was boiled for a short while and then allowed to cool under vacuum. This process was repeated immediately before each determination so that errors due to dissolved gases were eliminated. The specific gravity bottles were calibrated at all temperatures at which they were used, maintaining the temperature of the bath to ± ·05° at 60°C and ± .02° at 30°C.

The determinations on the fats when liquid were carried out exactly as for water, but in the case of the seed pod fat below 65°C, special precautions had to be taken.

For each determination, about 5 gm. of molten fat was weighed into each specific gravity bottle and kept at a temperature of 110°C for 30 minutes. The bottles were then removed and allowed to cool in a vacuum desiccator. This was repeated twice more to ensure the removal of all air bubbles from the fat samples. The bottles were then filled with air-free water, replaced in the vacuum desiccator and again evacuated. After this treatment, the bottles were heated to the temperature of the determination for half an hour and then again evacuated in the vacuum desiccator. After this, they were held at the required temperature in the thermostat for one hour, cooled, dried and weighed.

From these determinations, the values of $d_{4^\circ}^{t^\circ}$ were calculated and corrected for buoyancy of the air[33].

9.502 Refractive Index[34].

The refractive indices of the fat samples were determined using a Zeiss Abbé Refractometer and a Zeiss sodium vapour lamp. During a determination, the temperature was maintained constant within ± 0·1°C.

Ample time was allowed after introducing the fat sample for it

to reach the constant temperature before taking a reading. Readings were taken every minute until constant for three minutes. The temperature was then raised 10°C. and a further set of readings taken to determine the temperature coefficient.

9.503 Optical Activity[35].

The optical rotations of the fats were determined on chloroform solutions at a temperature of 20°C ± 0·1°C., using a Winkel-Zeiss precision polarimeter fitted with a jacketed two decimetre tube. A Zeiss sodium discharge lamp was used as a light source.

9.504 Surface Tension and Interfacial Tension with Water.

The surface tension against air was determined using a Cenco-du Nouy precision tensiometer. All glassware used was cleaned with hot bichromate cleaning mixture, washed with distilled water, and dried with absorbent paper. The ring was cleaned in boiling acetone, heated to redness and allowed to cool slowly, this procedure being repeated after each determination.

The determinations were carried out in a squat moisture dish jacketed with a container through which water was circulated to maintain a constant temperature. At least ten readings were taken at each temperature and the mean used in calculating the surface tension.

The interfacial tension was determined by gently floating a layer of molten fat on the surface of distilled water at the temperature of the determination. After allowing the whole to reach thermal equilibrium, the tension at the interface between the water and the fat was determined.

9.505 Viscosity.

The viscosity of the seed fat was determined using a standard No. 3 U-tube viscometer as described in B.S. 188, 1937.

9.506 Saponification Value.

This was determined on 1 to 2 gm. samples of fat using 0·5 N alcoholic potassium hydroxide solution according to the British standard method[36].

9.507 Iodine Value.

The iodine values of all samples of fats, fatty acids and esters were determined according to the method of Wijs[37].

The iodine value of the seed fat was checked using the bromine vapour method of Atmore and Hawke[38] to confirm the absence of conjugated unsaturation.

9.508 Thiocyanogen Value[39].

This was determined on ester fractions in the absence of a spectrophotometer to find the percentages of linoleic and linolenic acids present. The British standard method was used[39] and the lead thiocyanate prepared according to the procedure of Lambou and Dollear[40].

When an ultraviolet spectrophotometer became available, the fatty acid compositions were checked using the alkali isomerisation technique of Hilditch, Morton and Riley[15].

9.509 Hydroxyl Value.

The method used was that of Hawke[41].

It was necessary to modify the method in the case of the pod fat, since, on cooling after hydrolysis, the fat separated as a hard layer on top of the acetic acid pyridine-water mixture, and was found to be almost insoluble in butanol.

The modification consisted of adding 25 ml. portions of chloroform to each of the flasks on completion of the hydrolysis of the excess acetic anhydride and before cooling and adding the neutralised butanol. By this means, an homogeneous system was maintained throughout the determination.

For determining the hydroxyl values of small samples, the method was modified as suggested by Schoorman[42]. A sample of between 0·1 and 0·5 gm. was weighed out and 1 ml. of acetylating reagent added from a graduated pipette or micro burette. Acetylation was carried out for one hour when the excess acetic anhydride was hydrolysed for 15 minutes with 4 ml. of distilled water.

After cooling, 20 ml. of neutralised butanol was added down the condenser and the neck of the flask washed with a further 10 ml. Titration

of the acetic acid formed was carried out using 0·12 N alcoholic potassium hydroxide solution. This normality was chosen to give a blank titration of between 40 and 50 ml., thus making the fullest use of a 50 ml. burette.

Using this method, the maximum difference between two blanks was observed to be 0·04 ml. This on a sample weight of 0·2 gm. with hydroxyl value of 200 mgm. KOH/gm. corresponds to a difference in hydroxyl value of 1·3. Thus the hydroxyl values so obtained are accurate to only 2 units or 1% on a sample weight of 0·2 gm. On a sample weight of 0·5 gm., however, the results are accurate to 0·5 units or 0·25%.

9.510 Acid Value.

This was determined using butanol and pyridine as a solvent mixture and titrating the free acidity with 0·53 N alcoholic potassium hydroxide against a mixed indicator of cresol red and thymol blue in alcohol[41].

For semi-micro determinations, the sample weights were reduced to between 1 and 0·5 gm. and 30 ml. of butanol added instead of 75 ml. The free acids were then titrated with the 0·12 N alcoholic alkali used in the semi-micro determination of hydroxyl values. A blank titration was always carried out on the reagents and found to be between 0·15 and 0·20 mls.

9.511 Unsaponifiable Matter.

The percentage of unsaponifiable matter in the fats was determined using the method of the Society of Public Analysts[43].

9.512 Volatile Acids.

The method for determining the Reichert-Meissl and Polenske values was that adopted as a British standard[44].

9.513 Fatty Acid Content.

The soaps remaining after the extraction of the unsaponifiable matter (see under 8.511) were split with 1 : 1 hydrochloric acid and the fatty acids extracted with ether until the aqueous layer was clear. The united extracts were washed with water until the washings were no longer acid to methyl orange. The ethereal solution was dried overnight with anhydrous sodium sulphate, filtered, and the ether distilled off under reduced pressure in a tared flask.

Both in the case of the seed fat and also in the case of the pod fat, the percentage fatty acids obtained by this method had to be corrected for "non-saponifiable" matter which was not extracted by ether from the soap solution but remained associated with the fatty acids.

9.514 Glycerol Content.

This was determined according to the method of Bertrand and Rutgers[45] on the aqueous layer remaining after the extraction of the fatty acids with ether.

9.515 Analysis of Unsaponifiable Matter.

The total hydrocarbon fraction of the unsaponifiable matter was separated from the hydroxylic components by the chromatographic method of Karnovsky et al.[17]

The eluate from this was analysed according to the method of Fitelson[46,47] to determine the percentage of unsaturated hydrocarbons and the results expressed as squalene.

The α -glyceryl ethers in the unsaponifiable fractions were determined by the method of Karnovsky and Rapson[48] using ethyl acetate as solvent and oxidising with alcoholic periodic acid.

The sterols were precipitated as their digitonides with a 1% alcoholic solution of digitonin in 100% excess. The unsaponifiable matter was dissolved in chloroform for this purpose. This is the method of Dam[18] as modified by Karnovsky et al.[17] The sterols were identified by taking up their digitonides in warm pyridine and treating as described by Dam[18].

9.6 Specification of Special Boiling Petroleum Benzine.

The Petroleum Benzine was tested according to the I.P.T. standard methods for petroleum spirit[49].

In the distillation test, the first fraction distilled over at 44.5°C (625 mm. Mercury) and the final fraction at 63.2°C. These temperatures, when corrected for barometric pressure according to the specification become 50.2°C and 69.0°C respectively.

The residue on evaporation was found to be 0.005%. Thus the petroleum benzine conforms closely with the specification for 50/70 "Analar" Petroleum Ether[50]:-

Non Volatile matter = 0.003%
80% to boil between the limits 50°C to 70°C.

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11. . REFERENCES.

1. Hilditch, "The Chemical Constitution of Natural Fats", Chapman and Hall, London, 2nd Ed., 1949, p.186.

2. Vordoorn, J. Dept. Agr. Union S.Afr., 1924, 8, 415.

3. Honkol, Rhodesian Agr. Journ., 1931, 28, 71.

4. Stoyn, Farming S. Afr., 1943, 28, 71.

5. v.d. Walt and Stoyn, J. Vet. Sci. and Animal Ind., 1939, 2, 335.

6. Columbic, J. Amer. Chem. Soc., 1924, 64, 2337.

7. Bailey, "Industrial Oil and Fat Products", Interscience Publishers, New York, 1945, p.194.

8. Hilditch, "Industrial Fats and Waxes", Bailliére, Tindall and Cox, London, 3rd. Ed., 1949, p.82.

9. Schooman, M.Sc. Thesis, University of the Witwatersrand, 1946, p.26.

10. Hilditch, "The Chemical Constitution of Natural Fats", Chapman and Hall, London, 2nd. Ed., 1949, p.509.

11. Hawke, Ph.D. Thesis, University of the Witwatersrand, 1949, p.53.

12. Beilstein, "Handbuch der Organischen Chemie", Berlin, 4th Ed., 2nd Supplement, 1942, Vol. 4, p.757.

13. Anderson, Ind. and Eng. Chem., 1949, 41, 2887.

14. Hilditch, "The Chemical Constitution of Natural Fats", Chapman and Hall, London, 2nd Ed., 1949, p.505.

15. Hilditch, Morton and Riley, Analyst, 1945, 70, 68.

16. Hilditch and Moara, J. Soc. Chem. Ind., 1942, 61, 117.

17. Karnovsky, Rapson, et al., J. Soc. Chem. Ind., 1948, 67, 106.

18. Dam, Biochem. Z., 1928, 194, 177.

19. Black, Harris and Schwartz, J. S. Afr. Chem. Inst., New series, 1949, 2, 118.

20. Piper et al., Biochem. J. 1934, 28, 2186.

21. Rowald, Biochem. Z. 1929, 211, 199.

22. Jamieson, "Vegetable Fats and Oils", The Chemical Catalog. Co., New York, 1932, p.340.

23. Chibna[illegible] et al., Biochem. J., 1934, 28, 2193.

24. Armstrong and Hilditch, J. Soc., Chem. Ind., 1925, 44, 44 T.

25. Bergemann, Keppler, and Boekenoogen, Rec. Trav. Chim., 1950, 69, 439.

26. S.A.B.S., 87, 1949.

27. Hilditch, "Industrial Fats and Waxes", Bailliére, Tindall and Cox, London, 3rd Ed., 1949, p.305.

28. Hilditch, "The Chemical Constitution of Natural Fats", Chapman and Hall, London, 2nd. Ed., 1949, p.465.

29. Hawke, op. cit., p.48.

30. Hilditch, "The Chemical Constitution of Natural Fats, Chapman and Hall, London, 2nd. Ed., 1949, p.474.

31. Hilditch, op. cit., p. [illegible].

31A. International Critical Tables, Vol. 4, pp. 250 and 396.

32. B.S. 684, 1950, p.75.

33. B.S. 188, 1937, p.32.

34. B.S. 684, 1950, p.77.

35. Jamieson, op cit., p.378.

36. B.S. 684, 1950, p.27.

37. Hilditch, "Industrial Fats and Waxes", Bailliére, Tindall and Cox, London, 3rd. Ed., 1949, p.48.

38. Atmore and Hawke, J.S. Afr. Chem. Inst., New Series, 1950, 3, 23.

39. B.S. 684, 1950, p.38.

40. Lambou and Dollear, Oil and Soap, 1946, 23, 97.

41. Hawke, J.S. Afr. Chem. Inst., New Series, 1948, 1, 85.

42. Schooman, private communication.

43. B.S. 684, 1950, p.28.

44. B.S. 684, 1950, p.46.

45. Bortram and Rutgors, Roc., Trav. Chim., 1938, 57, 681.

46. Fitolson, J. Assoc. Off. Agr. Chem., 1943, 26, 499.

47. Karnovsky and Rapson, J. Soc. Chem. Ind., 1947, 66, 124.

48. Karnovsky and Rapson, J. Soc. Chem. Ind., 1946, 65, 138.

49. "Standard Methods for Testing Petroleum and its Products", The Institute of Petroleum, London, 6th. Ed., 1949, p.506.

50. "Analar" Standards of Laboratory Chemicals, The British Drug Houses Ltd. and Hopkin and Williams Ltd., London, 4th. Ed., 1949, p.174.

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