The Spectrographic Determination of Linoleic, Linolenic and Elaeostearic Acids

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Saturated aliphatic substances are transparent, except in the far ultra-violet region amenable to study only with a vacuum spectrograph. The presence of an ethylenic linkage results in a displacement of the selective absorption in the direction of longer wavelengths, but the maximum remains below 215$m\mu$ in most cases. Two *conjugated* double bonds bring about a shift of the maximum to 230–235 $m\mu$, and three conjugated double bonds to 265–275 $m\mu$. If the absorbing molecule contains two or more unconjugated double bonds, λ max. will be unaffected but ϵ max. will increase approximately linearly with the number of ethylenic linkages. If a carboxyl group is isolated by CH_2 from the conjugated system its chromophoric rôle is negligible; thus, *e.g.*, (*cf.* Smakula[1]).

	λ max.
$CH_3CH_2(CH=CH)_2CH_2.COOH$	228 $m\mu$
$CH_3CH_2CH_2(CH=CH)_2.COOH$	260 ,,
$CH_3CH_2(CH=CH)_3.CH_2.COOH$	265 ,,

A high degree of unsaturation in a fat is not normally accompanied by selective absorption in the region 200–400 $m\mu$. Thus, cod-liver oil with a high proportion of polyethylenic acids only displays selective absorption due to a small amount of vitamin A with its conjugated double bonds. Heilbron, Morton *et al.*[2] observed (1931) that the mixed acids recovered from cod-liver oil after thorough alkaline saponification are more strongly absorbing and exhibit a large number of well-defined narrow bands, and also found that the appearance of these bands is not confined to cod-liver oil, but may also occur during similar treatment of unsaturated seed oils. These workers considered that the absorbing acids were produced during the saponification process, but believed that they were not ordinary fatty acids, whereas it is now known that the specific absorption arises from molecular rearrangements of the latter which take place in an alkali medium at elevated temperatures, whereby unconjugated acids are in part isomerised to conjugated acids. Dann and Moore[3] (1933) were the first to observe that the development of selective absorption depends upon the prolongation of alkaline hydrolysis in boiling alcohol, and Moore[3] (1937) found that linoleic and linolenic acids are respectively isomerised to conjugated dienoic, and to a mixture of conjugated dienoic and trienoic acids by prolonged boiling with alcoholic potash. Kass, Miller and Burr[4] in 1939 showed that this change was effected much more rapidly at higher temperatures (*e.g.*, by use of a solution of potassium hydroxide in ethylene glycol at 180° C.), and could be used to determine the proportion of linoleic acid in a mixture of the latter with saturated acids and oleic acid. Later, Mitchell, Kraybill and Zscheile[5] proposed a method whereby the proportions of saturated, oleic, linoleic and linolenic acids in a mixture can be at once determined from the iodine value of the mixed acids together with the extinction coefficients for ultra-violet absorption at 234 $m\mu$ and 268 $m\mu$ after isomerisation under standardised conditions (180° C. for 25 min.) with potassium hydroxide in solution in ethylene glycol.

Whilst this method leads to the approximate determination of linoleic and/or linolenic acids in a single analytical operation with probably greater precision and facility than thiocyanometric analysis, and should thus be of great value in the rapid technical evaluation of drying oils, we have formed the opinion that it is not possible to obtain values of the greatest accuracy for the proportions of both linolenic and linoleic acids by a single isomerisation carried out at a given temperature for a given time, for reasons which are briefly as follows. Linolenic acid is rapidly isomerised in presence of alkali from about 160° C. upwards, and at 180° C. the max. amount of di- and tri-ethenoid conjugation is already reached in 10 min ; thereafter the amount of both conjugated forms declines, evidently owing to progressive

thermal polymerisation. This polymerisation is fairly rapid at 180° C. and at 170° C., as will be seen from the following values of $E^{1\%}_{1\,cm}$ for linolenic acid (*cf.* Fig. 2):—

Time of isomerisation (min.)	$E^{1\%}_{1\,cm}$ 268 $m\mu$		$E^{1\%}_{1\,cm}$ 234 $m\mu$	
	15	60	15	60
170° C.	532	490	622	594
180° C.	512	482	610	569

On the other hand, linoleic acid is converted to conjugated diene forms relatively slowly at 170° C., and at 180° C., in our experience, the maximum value of $E^{1\%}_{1\,cm}$ at 234 $m\mu$ is not reached until heating has continued for about 60 min.; thereafter a slow decline due to polymerisation sets in (Fig. 2).

We therefore prefer not to employ one isomerisation at 180° C. for such a time (*e.g.*, 25 min.) that a compromise has to be effected between (i) loss of conjugated isomerisation from linolenic acid due to thermal polymerisation and (ii) failure to reach maximum conjugated diene formation from linoleic acid, but prefer to make two separate determinations:

1. From the value of $E^{1\%}_{1\,cm}$ at 268 $m\mu$ after isomerisation at 170° C. for 15 min. the amount of linolenic acid is determined.

2. From the value of $E^{1\%}_{1\,cm}$ at 234 $m\mu$ after isomerisation at 180° C. for 60 min. the amount of linoleic acid is determined. When both acids are present, the increment of $E^{1\%}_{1\,cm}$ at 234 $m\mu$ due to conjugated dienes produced from linolenic acid must be allowed for; this is based on the observation that pure linolenic acid isomerised at 180° C. for 60 min. shows a value of $E^{1\%}_{1\,cm}$ at 234 $m\mu$ of 569 (*cf.* table above).

The observations on which these proposals rest are discussed below, with other data which confirm the findings of Mitchell *et al.*[5] that mixtures of linoleic and linolenic acids or esters behave additively so far as the isomerisation data are concerned. We have also found that the method can be applied to the determination of linolenic and/or linoleic acid in presence of elaeostearic acid, and we have illustrated the proposed application of the technique to sunflower seed oil, niger seed oil, linseed oil, tung oil, and a mixture of the two last-named oils.

Experimental

PREPARATION OF FATTY ACIDS AND ESTERS—(1) *Methyl linoleate*—Tetrabromostearic acid (m.p. 114–115° C.), prepared from the unsaturated acids of cottonseed oil, was debrominated in methyl alcohol with activated zinc dust and hydrochloric acid (Rollett[6]), and the methyl linoleate obtained was fractionated in a vacuum through an electrically-heated and packed column. The main fraction had iodine value 172·5 (calc. 172·8) and thiocyanogen value 91·8 (*cf.* Hilditch and Murti[7]).

(2) *Methyl linolenate*—Hexabromostearic acid (m.p. 180–181° C.), prepared from the acids of linseed oil, was debrominated in pyridine solution with activated zinc dust (Kaufmann and Mestern[8]). The crude linolenic acid obtained was esterified with methyl alcohol in presence of 0·5% of sulphuric acid, and the methyl ester was distilled as above. The purest fraction of methyl linolenate had iodine value 255·3 (calc. 260·8) and thiocyanogen value 152·2 (Hilditch and Murti,[7] 154·5).

(3) *α-Elaeostearic acid*—Tung oil (50 g) was saponified with alcoholic potash and the mixed fatty acids were liberated and crystallised, first from light petroleum (b.p. 40–60° C.) and thereafter several times from alcohol. The α-elaeostearic acid melted at 46–46·5 C.*

(4) *β-Elaeostearic acid*—Tung oil (30 g) was mixed with flowers of sulphur (10 mg) and allowed to stand in daylight for 4 days. It was then hydrolysed, and the liberated mixed fatty acids were crystallised successively from light petroleum and from alcohol, when β-elaeostearic acid, m.p. 70·6–71·2° C., was obtained.

REAGENTS—(1) *Alkaline glycol solution*—Dissolve 7·5 g of potassium hydroxide A.R. (assaying at least 85% KOH) in 100 ml of ethylene glycol (which has been purified by fractional distillation under reduced pressure); heat the solution at 190° C. for 2 min., cool, and store in a stoppered flask.

(2) *Absolute alcohol*—Heat abs. alcohol (1000 ml) under reflux for 1 hr. with zinc dust (20 g) and potassium hydroxide (20 g) and then distil the purified alcohol.

* It is essential to avoid the use of rubber corks during the isolation or storage of α-elaeostearic acid, otherwise some isomerisation to the β-acid is liable to occur. The pure α-elaeostearic acid does not keep well, even when stored in evacuated vessels.

METHOD OF ISOMERISATION—This was essentially as described by Mitchell *et al.*,[5] except for variations in the temperature and times of heating. Fatty acids, methyl esters or fats were employed (the necessary correction being made in the latter cases to obtain the equiv. weight of fatty acids present). Difficulty was at times encountered in obtaining accordant results during alkali-glycol treatment at 170° C. for 15 min. when fats were directly employed (especially in cases of high linolenic acid content). It is recommended that, at least for the isomerisations at 170° C. and 15 minutes, the mixed fatty acids should be first prepared from the fat, and used in the determinations. Methyl esters of fatty acids, however, gave no difficulty.

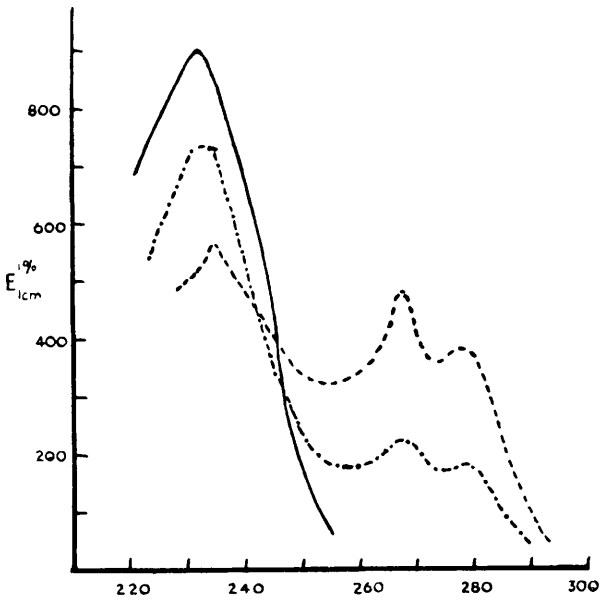

Fig. 1*a*. Linoleate (————), linolenate (--------); mixture of 49% of linoleate with 51% of linolenate (-·-·-·-); all isomerised at 180° C. for 60 min.

Weigh the fatty acids, esters or fat (*ca.* 0·1 g) accurately into a small capsule, and drop the latter into a loosely-stoppered Pyrex test-tube (6 in. × 1 in.), containing the glycol reagent (10 ml), and maintained in an electrically heated oil bath at the desired temperature (±0·3° C.). At the end of the required time, cool the tube quickly, and transfer its contents quantitatively to a 250-ml graduated flask, and make up to 250 ml with abs. alcohol. After standing at 0° C. overnight, filter the soln. and dilute with abs. alcohol to an appropriate concn. for spectrographic examination.

Make a blank determination with the alkaline glycol soln. in exactly similar conditions throughout, and use the final soln., diluted with alcohol to the same degree as the soln. containing the alkali-isomerised product, in the compensator cell of the spectrograph, making duplicate determinations in all cases.

The final values adopted as standards for the different individual acids represent the mean of four or more separate accordant determinations.

The apparatus employed was a Hilger E3 Quartz Spectrograph with sector photometer and an iron-nickel arc. War-time conditions have thus far not permitted us to use a photoelectric method, but we hope soon to obtain a Beckmann spectrophotometer as employed by Mitchell *et al.*,[5] which will simplify, and enhance the accuracy of, determination of the extinction-coefficients. It is estimated that the limits of experimental error in the determination of $E^{1\%}_{1cm}$ with the apparatus used in the present work are ±2%.

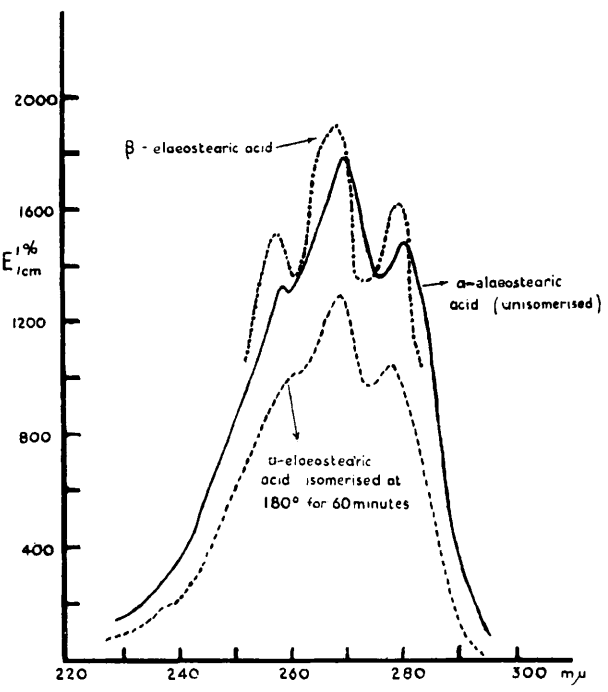

Fig. 1*b*.

The extinction-coefficients ($E^{1\%}_{1cm}$) were determined for the bands with heads at 234 $m\mu$ (conjugated diene) and 268 $m\mu$ (conjugated triene). A second band characteristic of conjugated triene systems in long-chain aliphatic compounds occurs at 278 $m\mu$, but this is less well-defined in character and it has not been necessary to use it in the present work.

Typical absorption curves for some of the individual acids are shown in Fig. 1*a* (linoleic and linolenic acids, and a mixture of these acids, isomerised at 180° C. for 60 min.) and Fig. 1*b* (α- and β-elaeostearic acids).

INFLUENCE OF TEMPERATURE ON ISOMERISATION—*Linolenic Acid*—Methyl linolenate was isomerised, and the standard conditions described were followed at 160° C., 165° C., 170° C. and 180° C. for various periods. The mean results for each time and temp., expressed as $E^{1\%}_{1cm}$ for the linolenic acid present after hydrolysis, are given in Table I.

Linoleic Acid—Methyl linoleate was similarly isomerised at 170° C. and 180° C., with the mean results given in Table II.

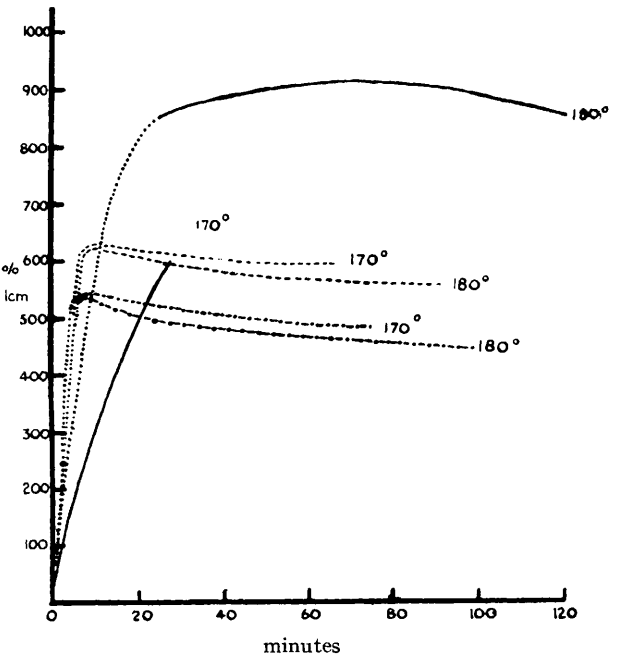

Fig. 2. Linoleate at 234 $m\mu$ (————); linolenate at 234 $m\mu$ (--------); linolenate at 268 $m\mu$ (· · · · · ·).

TABLE I

$E^{1\%}_{1cm}$ FOR LINOLENIC ACID AFTER ALKALI-ISOMERISATION

Time	Wavelength 268 $m\mu$				Wavelength 234 $m\mu$			
(Min.)	160° C.	165° C.	170° C.	180° C.	160° C.	165° C.	170° C.	180° C.
10				529				620
15	308	473	532	512	466	578	622	610
25				492				596
30	362	510	517		523	600	613	590
45	458	505	492	476	575	594	596	573
60	512	496	490	483	599	585	594	569
90				447				545

TABLE II

$E^{1\%}_{1cm}$ FOR LINOLEIC ACID AFTER ALKALI-ISOMERISATION

Time	Wavelength 234 $m\mu$	
(min.)	170° C.	180° C.
15	415	
25	564	852
45		863
60		906
80		923
90		863
100		899
120		859

The variation of $E^{1\%}_{1cm}$ for both wavelengths in the cases of linoleic and linolenic acids isomerised at 170° C. and 180° C. is shown graphically in Fig. 2.

Elaeostearic Acids—The extinction coefficients of these acids were measured for the pure compounds, and also after the latter had been heated with alkaline glycol under the standard conditions respectively at 170° C. for 15 min., and at 180° C. for 60 min. (Table III).

TABLE III

$E^{1\%}_{1\,cm}$ FOR α- AND β-ELAEOSTEARIC ACIDS

	α-Acid		β-Acid
	268 mμ	234 mμ	268 mμ
Pure acid	1780	208	1870
Alkali-treated, 170° C., 15 min.	1690	237	1830
,, 180° C., 60 min.	1290	197	1550

From the above observations it was decided to adopt for the present the values for $E^{1\%}_{1\,cm}$ shown in Table IV for the data for the individual acids after receiving the treatment indicated. Not all of these data are actually required in evaluating the components of a mixture of these acids (those not so required are shown in brackets).

TABLE IV

VALUES OF $E^{1\%}_{1\,cm}$ ADOPTED FOR USE IN SPECTROGRAPHIC ANALYSES

	268 mμ	234 mμ
α-Elaeostearic acid, untreated	1780	(208)
β- ,, ,, ,, ...	(1870)	
α- ,, ,, alkali-treated, 170° C., 15 min.	1690	(237)
α- ,, ,, ,, 180° C., 60 ,, ...	(1290)	197
Linolenic ,, ,, 170° C., 15 ,, ...	532	(622)
,, ,, ,, 180° C., 60 ,, ...	(483)	569
Linoleic ,, ,, 180° C., 60 ,, ...	—	906

For comparison, it may be noted that, in a recent paper, Beadle and Kraybill[5] give, for alkali-treatment at 180° C. for 25 min., $E^{1\%}_{1\,cm}$ linolenic acid 532 at 268 mμ and 609 at 234 mμ, and $E^{1\%}_{1\,cm}$ linoleic acid, 860 at 234 mμ.

MIXTURES OF UNSATURATED ACIDS—(i) *Linoleic and linolenic acids*—Six mixtures of methyl linoleate and methyl linolenate of known composition were made up and their composition was determined by the spectrographic method as described above. The resulting data are shown graphically in Fig. 3, in which the circles round the experimental points indicate the probable max. experimental error of the spectrographic determinations. It is seen that the data approximate to the linear relationships indicated by the straight lines drawn between the respective values for pure linoleic and pure linolenic acid (*firm lines*: isomerisation at 180° C. for 60 min.; *broken lines*: isomerisation at 170° C. for 15 min.).

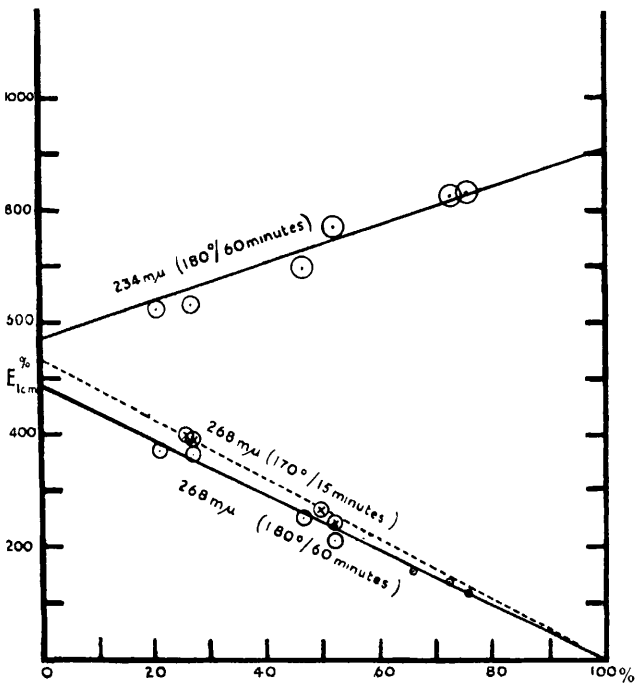

Fig. 3. Isomerisation of linoleate-linolenate mixtures.

(ii) *Linolenic and α-elaeostearic acids*—Similar determinations were made with mixtures of α-elaeostearic acid and methyl linolenate of known, but varying, composition, with results shown graphically in Fig. 4. In these instances, of course, the extinction-coefficients for 100% α-elaeostearic acid are taken from the observed values after heating with alkaline glycol under the standard conditions, respectively, at 170° C. for 15 min. (1690 at 268 mμ and 237 at 234 mμ) and at 180° for 60 min. (1290 at 268 mμ and 197 at 234 mμ). The relationship is again linear, within the limits of error of the method.

ILLUSTRATIONS OF THE METHOD—(i). *Fats containing saturated, oleic and linoleic acids only*—If linolenic acid is shown to be absent from a fat, determination of the iodine value and of the extinction-coefficient at 234 $m\mu$ after alkali isomerisation at 180° C. for 60 min. gives the proportions of saturated, oleic and linoleic acids. This is illustrated by the analysis of specimens of sunflower seed and niger seed oils (Table V); analyses by ester-fractionation had been made earlier on the same specimen of each oil, and the results of these (quoted in Table V) indicate reasonable accordance between the two methods.

(ii) *Fats containing saturated, oleic, linoleic and linolenic acids*—In this case the proportion of linolenic acid is determined from the value of $E^{1\%}_{1cm}$ at 268 $m\mu$ after alkali treatment at 170° C. for 15 min. The proportion of linoleic acid is then obtained from $E^{1\%}_{1cm}$ at 234 $m\mu$ after alkali treatment at 180° C. for 60 min., after deducting the increment of this $E^{1\%}_{1cm}$ due to the observed proportion of linolenic acid ($E^{1\%}_{1cm}$ at 234 $m\mu$ for 100% linolenic acid after alkali treatment at 180° C. for 60 min. being 569; *cf.* Tables I and IV). The oleic acid is then determined from the iodine value of the mixed fatty acids in the oil, after allowing for that due to the observed proportions of linolenic and linoleic acids. The saturated acids (with any unsaponifiable matter) are obtained by difference.

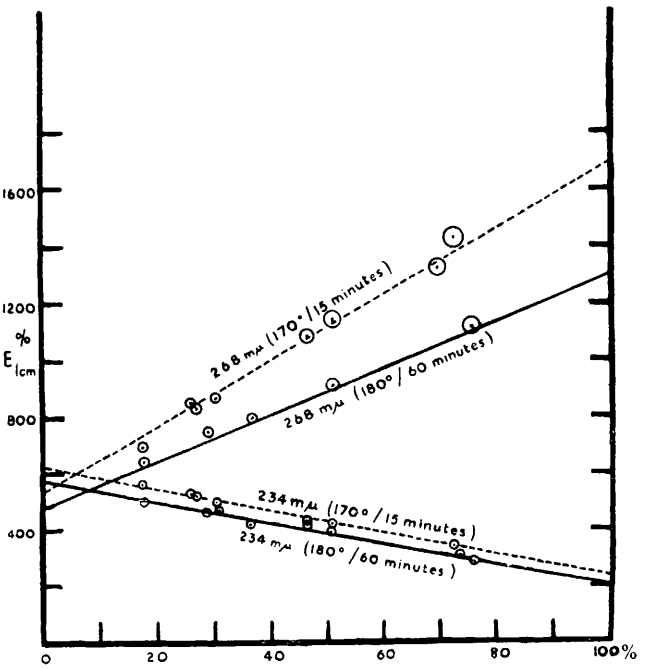

Fig. 4. Isomerisation of linolenate-α-elaeostearic acid mixtures.

TABLE V

COMPONENT ACIDS OF SUNFLOWER SEED AND NIGER SEED OILS

	Sunflower seed oil		Niger seed oil	
Saponification equiv.	290·6		293·3	
Iodine value	135·9		138·7	
	Present method	By ester fractionation[9]	Present method	By ester-fractionation[10]
$E^{1\%}_{1cm}$ (after alkali glycol treatment at 180° C. for 60 min.)	615	—	653	
Component acids				
Saturated (+ unsaponifiable)	11·1†	9·5	11·6†	12·7
Oleic	21·0*	24·8	16·3*	16·8
Linoleic	67·9	65·7	72·1	70·5

* Calculated from iodine value, after allowing for observed linoleic acid. † By difference.

A sample of linseed oil (saponification equiv. 290·6, iodine val. 182·2) was hydrolysed, and its mixed fatty acids were isolated (iodine val. 191·3). The mixed acids were submitted to the standard alkali-glycol treatment, and the following values of $E^{1\%}_{1cm}$ were found:

Alkali-treatment at 170° C. for 15 min., $E^{1\%}_{1cm}$ at 268 $m\mu$ 301
,, ,, ,, 180° C. ,, 60 ,, $E^{1\%}_{1cm}$,, 234 $m\mu$ 437

The component acids of the linseed oil, from these data, were calculated to be saturated (+ unsaponifiable) 16·2, oleic 14·4, linoleic 12·8, and linolenic 56·6% (wt.).

(iii) *Fats containing saturated, oleic, linoleic, linolenic and elaeostearic acids*—Here the proportion of α-elaeostearic acid is first determined spectroscopically from the extinction coefficient at 268 $m\mu$ of the mixed fatty acids of the oil (without isomerisation). The

elaeostearic acid found has then to be taken into account in calculating the % of linolenic and linoleic acids from, respectively, $E^{1\%}_{1cm}$ at 268 $m\mu$ after alkali-glycol treatment at 170° C. for 15 min., and $E^{1\%}_{1cm}$ at 234 $m\mu$ after alkali-glycol treatment at 180° C. for 60 min.

The proportions of oleic and saturated acids are given together in these instances in the present communication, since we have been unable to determine the small proportion of oleic acid present by utilising the iodine value of the oil. The calculated iodine values due to the observed elaeostearic, linolenic and linoleic acids were together somewhat in excess of the iodine value found by the method of Toms,[11] whilst, on the other hand, we have been unable to use a "partial iodine value" method such as that described by von Mikusch *et al*,[12] since in our experience the "partial iodine value" of elaeostearic acid varies too widely according to the proportion of elaeostearic acid in the total unsaturated fatty acids, the "partial iodine value" of which is being determined.

We have in progress further investigations which suggest that these difficulties may be partly overcome, and the accuracy of the data for individual acids improved, by suitable preliminary resolution of the mixed fatty acids into a number of groups, in each of which one or other of the various unsaturated types may be concentrated. Our object in the present communication has been to define the details of the alkali-isomerisation and subsequent spectroscopic techniques which we have found most suitable up to the present time.

In the meantime we may record the following analyses of a specimen of tung oil, and of a mixture of equal parts of this tung oil with the linseed oil used in the preceding analysis (Table VI).

TABLE VI

ANALYSIS OF TUNG OIL AND OF 50% TUNG + 50% LINSEED OIL

	Tung oil	Tung-linseed oil	
Mixed fatty acids, iodine value (Toms)	242·0	214·0	
Spectroscopic data	$E^{1\%}_{1cm}$	$E^{1\%}_{1cm}$	
268 $m\mu$ (unisomerised acids)	1370	700	
268 $m\mu$ (alkali, 170° C., 15 min.)	1400	855	
234 $m\mu$ (,, 180° C., 60 ,,)	267	375	
Component acids			Calc.*
Saturated and oleic (+ unsaponifiable) ..	3·4	14·6	17·0
Linoleic	1·0	10·4	6·9
Linolenic	18·6	35·7	37·6
α-Elaeostearic	77·0	39·3	38·5

* *i.e.*, mean of observed values for the tung oil and the linseed oil.

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