Resolution Into Various Acid Fractions

The oil was saponified with 0.5*N* alcoholic KOH solution under reflux for 4.5 hrs. The alcohol was then distilled under reduced pressure from the soap solution. The residual soap was diluted with water and extracted with diethyl ether to remove the nonsaponifiable matter. Fatty acids were obtained from the soap by the addition of dilute sulphuric acid.

The fatty acids were separated into ''solid'' and ''liquid'' fractions by Twitchell's lead salt-alcohol method as adapted by Hilditch (5), as shown in Table II. The acids were characterized through bromo-derivatives of the liquid acids and methyl esters of the ''solid'' acids.

TABLE II

Lead Salt Acid Separation

Values	Total acids	"Solid" acids	"Liquid" acids
Percentage	......	10.1	89.9
Saponification value	219	213.5	202.3
Iodine value	148	1.1	153.6

Liquid Acids

Bromo-derivatives of the liquid acids were prepared according to Jamieson and Baughman (6). From the derivatives the amounts of C_{18} unsaturated acids were calculated.

Solid Acids

The solid acids were converted to their methyl esters according to Hilditch (7) and fractionated by distillation under reduced pressure.

By combining results of these analyses and calculations, the composition of the oil is indicated in Table III.

The nonsaponifiable matter in diethyl ether was concentrated under reduced pressure. The residue was crystallized from 95% alcohol. The m.p. of the crystals was noted to be 129°C. (uncorrected). Acetyl, benzoyl, bromo-, and digitonide derivatives of the sterol had 119°, 119.5°, 105–109°, and 234–237°C. (decomp.) as their melting points, respectively. From these tests the crystals above were inferred to be of fucosterol. The alcoholic mother liquor contained a yellow substance which was not identified.

TABLE III

Composition of Oil from *Asphodelus fistulosus*

Acid	% as Glyceride
Myristic	= 0.5
Palmitic	= 5.7
Stearic	= 3.6
Oleic	= 33.1
Linoleic	= 54.9
Nonsaponifiable matter	= 1.8
Total	= 99.6

Discussion

The bromo-derivatives indicated that oleic and linoleic acids were present in the liquid acids. Further, that the liquid acids were of the same carbon content was concluded from their saponification equivalents and the amounts of the crystallizable bromo-derivatives. Where the acids have the same carbon content, esterification and fractionation of the esters are nonconclusive. From the saponification values given in Table II, only C_{18} acids constitutue the liquid acids. Since a hexabromide derivative from the liquid acids was not obtained, it was inferred that linolenic acid was absent. Tetrabromide derivative (m.p. 112–113° C.) established the presence of linoleic acid. The rest of the liquid acids was taken as oleic acid.

REFERENCES

1. Muhammad, S., P. Jr. Sci., I, 35 (1949).
2. *Idem, ibid., 2,* 29 (1950).
3. Official Methods of Analysis of the A.O.A.C., 8th ed., pp. 462–467 (1955).
4. Williams, K.A., "Oils, Fats, and Fatty Foods," 3rd ed., J. and A. Churchill Ltd., London, pp. 76–111 (1950).
5. Hilditch, T.P., "Chemical Constituents of Natural Fats," 3rd ed., Chapman and Hall Ltd., London, p. 574 (1956).
6. Baughman, W.F., and Jamieson, G.S., J. Am. Chem. Soc., pp. 154 and 1199 (1920).
7. Hilditch, T.P., "Chemical Constituents of Natural Fats," 3rd ed., Chapman and Hall Ltd., London, p. 581 (1956).

[Received July 5, 1960]

• *Letter to the Editor*

The Preparation of Pure Methyl Linoleate

THE UREA ADDUCT METHOD (1) has been used for the preparation of large quantities of methyl linoleate from safflower seed oil. The yield was 21–24%, and the final product was contaminated with 1% of an impurity assumed to be methyl oleate.

In this laboratory the same method has been closely followed. Whereas Keppler *et al.* (1) used an alkali-refined safflower seed oil, we have used a ''non break'' sample containing 1.25% FFA. To neutralize these free fatty acids an equivalent amount of metallic sodium has been added in addition to the amount used in the previous method. Separation of methyl linoleate from 2,886 g. of methyl esters, isolated from 3,000 g. of safflower seed oil, was followed by gas liquid chromatographic analysis, using an eight-foot column packed with 20% diethylene glycol adipate polyester on Celite at 207°C.

Keppler *et al.* (1) rejected the mother liquor remaining after the fourth crystallization (Fraction IV, Table I), but we have found that these contained a further 500 g. of linoleate, essentially free of other fatty acid esters. Schlenk (2) has reported that the yield of the urea linoleate adduct can be increased by reducing the volume of the solvent. Therefore the 20 liters of mother liquor were concentrated at reduced pressure under nitrogen to 10 liters. Gas liquid chromatography and iodine value (3) determinations showed that no change in the methyl linoleate had occurred during the concentration. A further 1,500 g. of urea was added to the concentrated mother liquor, the mass was heated and allowed to stand over-night at room temperature. The urea adduct was filtered and washed with 2 liters of methanol.

The fourth crystallization produced 99.4% of linoleate contaminated with 0.6% oleate in 24.3% yield (Table I), fully confirming the results of Keppler *et al.* (1) and emphasizing the great value of the method for obtaining reasonable quantities of methyl linoleate in a comparatively simple manner.

TABLE I

Isolation of Pure Methyl Linoleate from 2,886 g. of Safflower Seed Oil Methyl Esters

Fraction No.	Methylated safflower seed oil	I	II	III	IV	V
G. urea added	1,924	1,588	866	2,405	1,500	......
Fatty acid	Moles percentage of methyl esters obtained by GLC					
Laurate	tr.	tr.	......	......	......	......
Myristate	tr.	0.4	......	......	......	......
Palmitate	6.2	47.3	1.3	......	......	......
Palmitoleate	tr.	tr.	0.7	......	......	......
Stearate	3.1	15.5	......	......	......	......
Oleate	10.9	17.1	40.0	9.5	0.6	......
Linoleate	79.8	17.5	58.0	90.5	99.4	100
Arachidate	tr.	1.8	......	......	......	......
$C_{20:1}$?	tr.	0.4	......	......	......	......
Iodine value	142.3	56.5	135.3	155.9	169.4	172.4
Yield of urea adduct g.	......	1,700	1,820	1,030	2.746	1,580
Wt. % ester in urea adduct	......	26.5	25.5	24.5	25.5	25.4
Yield expressed as % of original methyl esters	......	......	......	......	24.3	13.9

However the formation of the urea adduct from the mother liquor remaining after the fourth crystallization not only increased the yield of methyl linoleate by 13.9%, but, in addition, the product was completely free of other fatty acid methyl esters.

If Fractions IV and V are combined, the additional step which we have introduced provides an increased yield of methyl linoleate and also makes possible the preparation of a product, albeit in smaller yield, of greater purity than has hitherto been possible.

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REFERENCES

1. Keppler, J.G., Sparreboom, S., Stroink, J.B.A., and von Mikusch, J.D., J. Am. Oil Chemists' Soc., *36*, 308 (1959).
2. Schlenk, H., "Progress in the Chemistry of Fats and Other Lipids," Vol. 2, p. 243, Pergamon Press, London, 1954.
3. Official and Tentative Methods of the American Oil Chemists' Society, 1957 Edition, Ka 9-51.

[Received February 9, 1961]

ABSTRACTS . . . . R.A. REINERS, Editor

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• Fats and Oils

NEW SOLVENT SYSTEM FOR SEPARATION OF FATTY ACIDS C_{10}–C_{18} BY COUNTERCURRENT DISTRIBUTION. F. Will, III (Alcoa Res. Labs., Aluminum Co. of Am., New Kensington, Pa.). *Anal. Chem.*. 33, 647–48 (1961). Fatty acids C_{10} to C_{18} have been separated from each other by the countercurrent distribution technique in the new solvent system using petroleum ether as an upper layer and a 9 to 1 ratio of dimethyl sulfoxide to 1-octanol as a lower layer.

OXIDATIVE RANCIDITY IN COOKED MULLET. Marelynn Zipser and Betty Watts (Dept. of Food and Nutrition, Fla. State Univ., Tallahassee). *Food Tech.* 15, 318–321 (1961). The muscle lipids of mullet begin to oxidize very rapidly after cooking, as shown by increases in TBA number and rancid odors. Intensity of the reaction appears to be greater in tissues containing large quantities of lipids and heme pigments than in tissues containing lesser amounts. The oxidative reaction is retarded by limited oxygen supply and by low (freezer) temperatures, but was not completely inhibited under the condition studied. Inhibition was more complete from the antioxidant mixture of sodium tripolyphosphate and sodium ascorbate, either alone or in combination with curing salts.

IRRADIATION FLUOROMETRIC METHOD FOR ESTIMATION OF DIETHYLSTILBESTROL IN BEEF LIVER TISSUE. J.M. Goodyear and N.R. Jenkinson (Control Div., Eli Lilly & Co., Indianapolis, Ind.). *Anal. Chem.* 33, 853–56 (1961). Additional information is given on the application of an irradiation fluorometric method which has been recently developed for the specific measurement of diethylstilbestrol in biological samples.

EFFECTS OF VARYING THE CHEMICAL COMPOSITION OF STATIONARY PHASE ON THE SEPARATION OF CERTAIN C_{19} AND C_{27} STEROIDS BY GAS CHROMATOGRAPHY. S.R. Lipsky and R.A. Landowne (Dept. of Internal Medicine, Yale Univ. School of Med., New Haven, Conn.). *Anal. Chem.* 33, 818–28 (1961). Gas chromatographic analyses of mixtures of C_{19}, C_{21}, and C_{27} steroids were performed. The chemical composition of these substrates was correlated to differences in separation factors for closely related steroids. Relationships were derived which describe the effect of structural changes in the steroid molecule upon their order of elution from the chromatographic column.

AN IMPROVED SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF TOCOPHEROLS USING 4,7-DIPHENYL-1,10-PHENANTHROLINE. C.C. Tsen (Grain Res. Lab., Board of Grain Commissioners for Canada, Winnipeg 2, Man., Canada). *Anal. Chem.* 33, 849–51 (1961). An improved method for the determination of tocopherols, based on the method of Emmerie and Engel, is proposed.

DETECTION OF OLEFINS BY EPOXIDATION AND HYDROXAMATION AND CHARACTERIZATION BY REARRANGEMENT OF EPOXIDES TO CARBONYL COMPOUNDS. J.G. Sharefkin and H.E. Shwerz (Dept. of Chem., Brooklyn College, Brooklyn, N.Y.). *Anal. Chem.* 33, 635–39 (1961). Two most general tests for the olefin bond, decolorization of bromine and permanganate, give positive signs of reaction with many nonolefinic reducing reagents. Such false positive tests are avoided by devising tests in which the sign of a positive reaction depends on a chemical change in the substrate rather than the reagent. Commercial 40% peracetic acid transforms olefins to their glycol monoacetates which are treated with hydroxylamine and the hydroxamic acids are detected as the wine red ferric salt. Olefins are also characterized by rearranging the epoxides with boron trifluoride to carbonyl compounds that are then converted to solid dinitrophenylhydrazone. Reactions may be carried out on a semimicro scale and permit identification of olefins in the presence of acetylenes and other reductants.

DETECTION AND CHARACTERIZATION OF ACETYLENES BY HYDRATION TO CARBONYL COMPOUNDS AND FORMATION OF 2,4-DINITROPHENYLHYDRAZONES. J.G. Sharefkin and E.M. Boghosian (Dept. of Chem., Brooklyn College, Brooklyn, N.Y.). *Anal. Chem.* 33, 640–44 (1961). A method has been developed for differentiating the acetylene from the olefin functional group, both of which decolorize both bromine and permanganate, by hydrating with a boron trifluoride, mercuric oxide, and trichloroacetic acid catalyst mixture. The carbonyl compounds formed are treated with 2,4-dinitrophenylhydrazine and a phenylhydrazone is formed by addition of methanolic potassium hydroxide to produce a wine red color.

NATURALLY OCCURRING EPOXY ACIDS. I. DETECTION AND EVALUATION OF EPOXY FATTY ACIDS BY PAPER, THIN-LAYER, AND GAS-LIQUID CHROMATOGRAPHY. J. Morris, R.T. Holman, and K. Fontell (Hormel Inst. and Dept. of Physiol. Chem., Univ. of Minnesota, Austin, Minn.). *J. Lipid Res.* 2, 68–76 (1961). Paper, thin-layer, and gas-liquid chromatographic (GLC) methods were developed for detection and measurement of epoxy and other oxygenated fatty acids in naturally-occurring oils. Techniques were tested both on synthetic acids and their mixtures