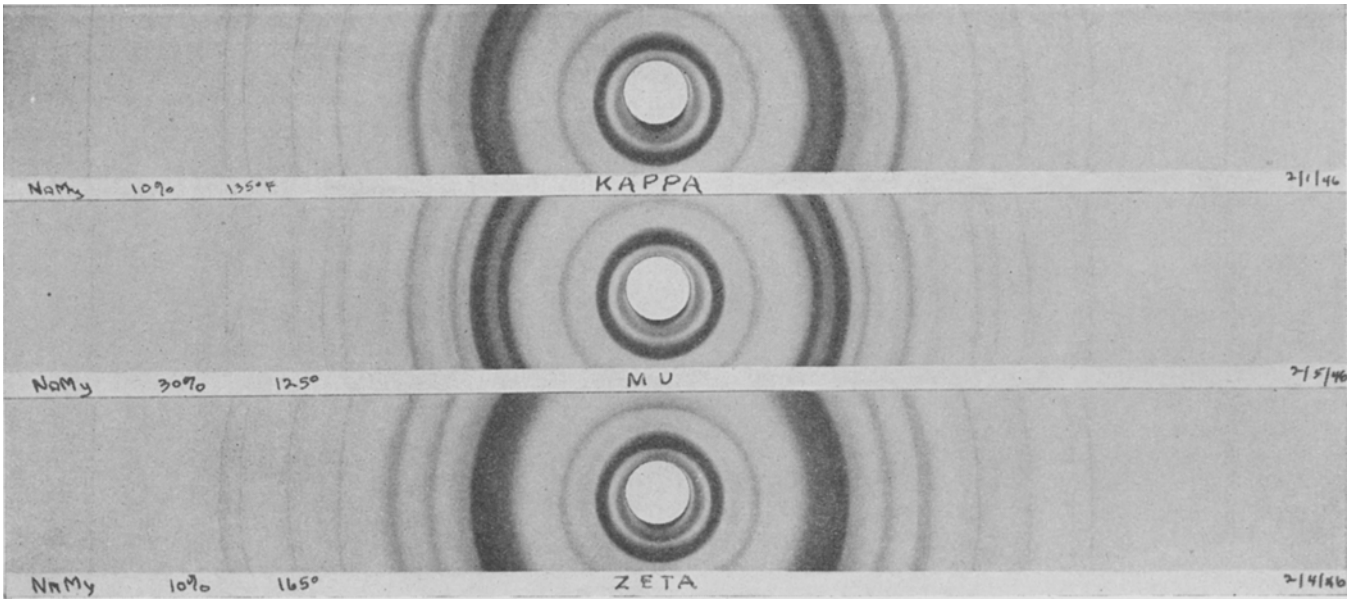

FIG. 3.

down to higher moisture values. This boundary corresponds with McBain's T_c, the temperature of final melting of the curd soap. It is apparent that the phase κ is not an equilibrium phase in this second field. Evidently the equilibrium phase is liquid crystal and κ is a descendant phase.

This T_c boundary is composed of two segments. At moistures higher than about 35%, the boundary is substantially a straight line. At lower moistures the boundary curves sharply upward. The straight part of the boundary has below it a phase field containing the phase ζ plus solution while below the curved boundary lies a two-phase field containing ζ plus κ. If the latter field represents an equilibrium collection of phases, it can contain no solution phase in addition since three-phase fields cannot occur in a two-component system. The question, therefore, arises whether the lower boundary of the ζ plus κ field represents the composition of the ζ crystals.

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The Non-Uniformity of B-Linoleic Acid[1]

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THE linoleic acid isolated from natural oils (1, 2) and the α-linoleic acid[3] obtained on the debromination of crystalline tetrabromostearic acid (3) are believed to be identical (4, 5, 6). However, the identity of the β-linoleic acid obtained on the debromination of liquid tetrabromostearic acid is still subject to speculation. This acid is believed to be either an isomer of α-linoleic acid (6, 7, 8, 9) or α-linoleic acid contaminated with conjugated acids (10) or products of the brominating reaction (5).

In the characterization of new vegetable oils the linoleic acid content has usually been reported as α-linoleic acid. In the case of sorghum grain oil, however, the presence of β-linoleic acid has also been reported (11). Biological assays have indicated that α-linoleic acid was more effective than β-linoleic acid in the prevention of dermatitis in rats (12). Interest in the composition and nutritive value of sorghum grain oil (13, 14) prompted a reinvestigation of the identity of β-linoleic acid.

Experimental

Preparation of the methyl esters: The α- and β-methyl linoleate were prepared according to the method of Rollett (3) from corn, cottonseed, and sorghum grain oil. In each case the α-methyl linoleate, iodine value 170.3 (theory 172.4), was prepared from recrystallized tetrabromostearic acid (m.p. 114° C.). The β-methyl linoleate, iodine value 147.0, was prepared from liquid tetrabromostearic acid according to the modification of McCutcheon (4).

The esters were saponified and converted to the free acids when needed. The methyl esters and free

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[2] Contribution No. 323 from the Department of Chemistry.
[3] The term "α" and "β" as such have no proven structural significance. The term "α" refers to the acid obtained from crystalline tetrabromostearic acid and the term "β" refers to the acid obtained from the Skellysolve B soluble residue or liquid tetrabromostearic acid.

acids were not subjected to distillation; the last traces of solvent were simply removed with an oil pump and the colorless product stored under high vacuum.

The treatment of α-methyl linoleate with hydrogen bromide: One hundred g. of α-methyl linoleate (iodine value 170.3) were diluted with 1500 ml. of Skellysolve F and cooled to 0° C. The solution was agitated with an electric stirring motor while anhydrous bromine-free hydrogen bromide, prepared by the action of bromine on red phosphorus in the presence of water, was bubbled through the solution for two hours at a temperature of 0° to 10° C. The gas was passed through an absorption tube filled with red phosphorus and one filled with phosphorus pentoxide in order to remove bromine and moisture respectively.

After standing at —5° C. overnight, the solution was poured into a 3-liter separatory funnel, washed once with 500 ml. of N/10 sodium thiosulphate, three times with 500 ml. of cold water, dried over sodium sulphate, and freed from solvent. The residue was taken up with 200 ml. of absolute methyl alcohol, 100 grams of zinc were added, and the mixture refluxed for two hours. The resulting product (iodine value 145.8) was extracted, purified, and stored under vacuum in the same manner as the α- and β-methyl linoleate.

Chromatographing of the methyl esters: Chromatographing of α- and β-methyl linoleate and of the methyl linoleate treated with hydrogen bromide was carried out according to the method of Swift, Rose, and Jamieson (15). Fifty g. of methyl ester were diluted with 1 l. of Skellysolve F and poured through a column of activated alumina[4] which had been washed with Skellysolve F. This column was 360 mm. long and 45 mm. in diameter. The column was eluted with Skellysolve F until no more soluble material was removed from the column. The column was then eluted with acetone. The eluates were freed of solvent, weighed, and the iodine value of each fraction determined. The percentage of material eluted was plotted against the iodine value (Fig. 1).

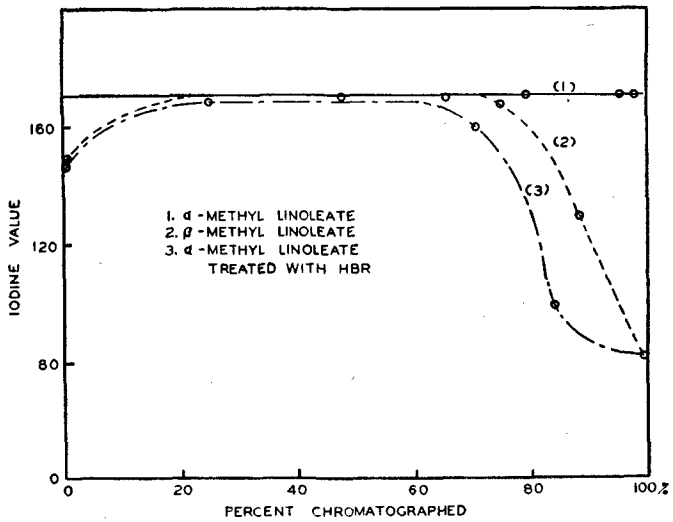

FIG. 1. Relationship of the per cent of methyl ester chromatographed to iodine values of the fractions obtained.

Determination of constants: Iodine values were determined with Wijs solution (1 hr.). Spectrographic analyses were made according to the method of Mitchell, Kraybill, and Zcheile (16) and the various fractions were brominated as described by Hilditch and Jasperson (8). Neutralization equivalents were determined on 0.5 g. samples with the aid of a Fisher Titrimeter; bromine was determined by a semimicro modification of the Carius method (17).

Results: The characteristics of linoleic acid prepared from sorghum grain oil were found to be identical with those of linoleic acid prepared from cottonseed or corn oil. It had an absorption coefficient of 86.0 at 2340 Å, an iodine value of 180.0, a melting point of —5.5° C., and it yielded 40-45% of crystalline tetrabromostearic acid on bromination. As more cottonseed than sorghum oil was available, further studies on the nature of β-linoleic acid were carried out with α- and β-linoleic acids prepared from cottonseed oil.

The results obtained on chromatographing of the methyl esters indicated that α-linoleic acid was a homogeneous substance while β-linoleic acid was not (Fig. 1). The iodine values of the methyl esters of α- and β-linoleic acid eluted with Skellysolve F did not differ by more than three points. However, the iodine values of the fractions eluted with acetone varied by 91 points. The iodine values of the acetone eluate from α- and β-methyl linoleate were 172.4 and

The acetone eluate of the β-linoleate was a viscous straw-colored liquid which had an absorption coefficient of 9.2 at 2340 Å and an iodine value of 81.4. Qualitative tests revealed the presence of bromine. A portion of the viscous material was again treated with zinc in acidified methyl alcohol, but the resulting product still contained bromine. Apparently, the bromine was present in a combination relatively stable to treatment with zinc. An attempt was made to separate and purify the viscous material. However, it could not be recrystallized from ethyl alcohol, acetone, or Skellysolve F at —50° C. When cooled to —70° C. without solvent it formed a yellow transparent jel. That the viscous material was produced from α-linoleic acid, and not carried over from the sorghum grain or cottonseed oil was shown in the following manner:

Fifty g. of the α-methyl linoleate, which had been proved homogeneous by chromatographing through activated alumina, were converted to linoleic acid, brominated, and the liquid tetrabromostearic acid isolated. This material was debrominated with zinc in acidified methyl alcohol and the resulting β-methyl linoleate chromatographed through activated alumina. The acetone eluate again contained the viscous straw-colored material.

Five g. of this viscous material were diluted with Skellysolve F and rechromatographed through a small column of activated alumina. The column was washed thoroughly with Skellysolve F and eluted with acetone. The acetone eluate had an iodine value of 68.7, a saponification equivalent of 370.5, and a bromine value of 19.8%. If one assumes the addition of hydrogen bromide to one of the double bonds in methyl linoleate, the resulting product would have an iodine value of 67.9, a saponification equivalent of 375.3, and a bromine content of 21.3%. Apparently, therefore, the viscous straw-colored component of β-linoleic acid could be a monobromooleic acid.

An attempt was made to synthesize a similar material as follows: One hundred g. of α-linoleic acid

[4] Activated Alumina, Grade F-20, mesh 80-200, Alumina Ore Co., East St. Louis, Illinois.

TABLE I

Characteristics of the Chromatographed Methyl Esters

Characteristics	α-Linoleate			β-Linoleate			α-Linoleate treated with HBr		
	Before Chromat.	Eluate		Before Chromat.	Eluate		Before Chromat.	Eluate	
		Skelly-solve F	Acetone		Skelly-solve F	Acetone		Skelly-solve F	Acetone
Per Cent Eluted	.....	81.0%	19.0%	.....	78.0%	22.0%	.....	71.0%	29.0%
Iodine Value	170.3	170.6	172.4	147.0	169.6	81.4	145.8	167.2	84.5
Abs. Coef. at 2340 Å	85.8	80.4	81.5	65.8	73.6	9.2	67.4	72.8	24.6
Cryst. Tetrabr. Acid	40.8%	29.4%	29.7%	27.3%	29.8%	8.2%*	30.8%	25.3%	2.4%*

* Liquid, no crystalline tetrabromostearic acid.

were diluted with 1500 ml. of Skellysolve F, cooled to 0° C., and treated with hydrogen bromide instead of bromine. This product was debrominated in acidified methyl alcohol and 50 g. of the methyl ester chromatographed. The characteristics of the fraction eluted with Skellysolve F and the fraction eluted with acetone were similar to the corresponding fractions obtained from β-methyl linoleate (Table 1). The iodine value and absorption coefficient (at 2340 Å) of the fraction eluted with Skellysolve F were 2.4 and 1.6 lower, and for the fraction eluted with acetone 3.1 and 15.4 higher than the iodine value and absorption coefficient of the corresponding fractions obtained from β-methyl linoleate. Bromination of the free fatty acid which was obtained from the acetone eluate yielded no crystalline tetrabromostearic acid. The absorption coefficients were plotted against wave lengths (Fig. 2). The resulting curves also indicated that α-methyl linoleate treated with hydrogen bromide and β-methyl linoleate were identical in character.

A 5-g. sample of the fraction eluted with acetone was oxidized with potassium permanganate in acetone according to the method of Armstrong and Hilditch (18). The oxidized products were freed from the unoxidized methyl ester (14%) and separated by steam distillation as described by Hilditch and Jasperson (19). Characterization of the distillate indicated the presence of caproic acid. The residue had a neutralization equivalent of 307 and contained 18.6% bromine; theoretical for the mono methyl ester of bromo decamethylenedioic acid is 322.9 and 24.8% respectively.

Discussion

Previous workers have reported characteristics for β-linoleic acid which have indicated that it was not an entity. Reimenschneider, Wheeler, and Sando (5) and Hilditch and Jasperson (8) found that fractional distillation of β-methyl linoleate yielded some fractions which had higher saponification equivalents and lower iodine values than α-methyl linoleate. The latter noted that 80% of the distillate had iodine values approximately 40 points higher than the residue, or 156-163 and 119 respectively. The residue yielded no crystalline tetrabromostearic acid on bromination. Furthermore, on oxidation of β-linoleic acid with potassium permanganate, Green and Hilditch (9) could account for only 80% of the total as Δ^9, Δ^{12} octadecadienoic acid. Evidently the remaining 20% was not linoleic acid.

In the present study this 20% of material appeared to be composed mainly of a brominated derivative of linoleic acid from which bromine could not be removed by treatment with zinc. Its formation seemed to be dependent on the conditions which arose when linoleic acid was brominated at 0° to 10° C. Under these conditions a considerable amount of hydrogen bromide seems to form through the action of bromine on either the moisture condensed on the inner walls of the flask or by substitution of the solvent. That hydrogen bromide is actually produced during bromination at 0° to 10° C. can be shown by merely blowing moist air across the mouth of the flask or testing with moist litmus paper. In the present studies it was also shown that hydrogen bromide instead of bromine could add to linoleic acid at 0° C. Furthermore, the data presented by Rollett indicated that liquid tetrabromostearic acid was not brominated completely. He found that crystalline tetrabromostearic acid contained 2.6% more bromine than the liquid acid. On a theoretical basis if one mole of tribromostearic acid is formed for every four moles of liquid tetrabromostearic acid the resulting mixture would contain 50.6% bromine. This value is within 0.03% of the analysis for liquid tetrabromostearic acid as found by Rollett.

The position of the bromine atom in monobromooleic acid was not determined with certainty. The hydrogen bromide could have been added to the double bond between $\Delta^{9,10}$, $\Delta^{12,13}$ or to the active methylene group between these two double bonds. As oxidation of the acetone eluate with potassium permanganate in acetone yielded caproic acid and the mono methyl ester of bromo decamethylenedioic acid, the hydrogen bromide must have been added between $\Delta^{9,10}$ rather than $\Delta^{12,13}$. However, when compared with the theoretical values, the neutralization equivalent and bromine content of the oxidized undistilled residue indicated that the residue was not all the mono methyl ester of bromo decamethylenedioic acid.

The low absorption coefficients of the acetone eluates (Table I) seemed to indicate that the presence of bromine interfered with the conjugation of the double bonds. Maruyama and Suzuki (20) found that potassium hydroxide readily removed the bromine from tetrabromostearic acid. As the bromine could therefore be removed during the isomerization process, it is difficult to explain the lower absorption coefficient of the acetone eluate (Fig. 2) at 2340 Å. Whether the Skellysolve F eluates from α- and β-methyl linoleate were "isomeric" was not determined with certainty. The iodine value indicated that the α-methyl linoleate was chromatographically homogeneous. However, the lower absorption coefficient and lower yield of crystalline tetrabromostearic acid of the Skellysolve F and acetone eluates seemed to indicate that some changes had taken place during chromatographing. On the other hand, α-linoleic acid which had been subjected to molecular distillation (21) yielded the same percentage of crystalline tetrabromostearic acid as the undistilled acid. Further studies on the nature of the isolated linoleic acid are now being carried out in our laboratory.

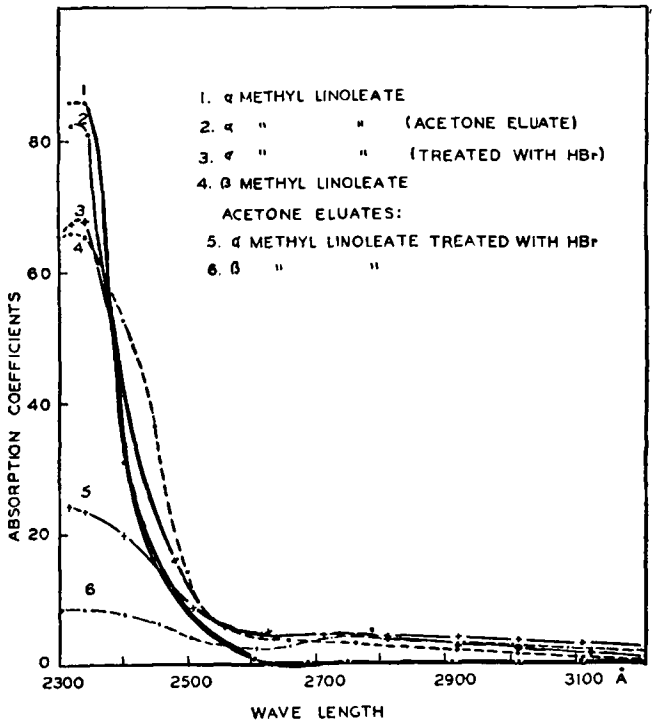

FIG. 2. Relationship between wave lengths and absorption coefficients of the α- and β-methyl esters.

Summary

Methyl esters of α- and β-linoleic acid were chromatographed on activated alumina and separated into fractions eluted with Skellysolve F and acetone, respectively. Methyl α-linoleate was found to be chromatographically homogeneous in character while methyl β-linoleate was not. The latter contained a small amount of yellow viscous material which appeared to be monobromooleic acid; this bromine could not be removed by treatment with zinc in absolute alcohol. A similar substance could be isolated from methyl α-linoleate through which bromine-free, hydrogen bromide had been bubbled for two hours at 0° C. The presence of this bromine containing substance was shown to be partly responsible for the differences in the characteristics of α- and β-linoleic acid.

Acknowledgment

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A Study of Lipids in Commercial Feeds

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Introduction

THE present paper is an investigation of the total extract, phospholipid, unsaponifiable matter, and total fatty acids of cottonseed meal, soybean meal, meat and bone scraps, wheat gray shorts with screenings, alfalfa leaf meal, whole barley, and whole oats in the anhydrous ether and 3 + 1 alcohol-ether extracts. It also includes a study of the phosphorus in the alcohol-ether extract, and a method is proposed for the determination of phospholipids in plant material. The samples were selected at random from those collected by the Feed Control Service of the Texas Agricultural Experiment Station for routine analyses.

It has long been recognized that ether alone does not completely extract lipid matter, especially phospholipid, from biological matter (1) but that alcohol and ether remove them more completely (2, 3). A number of methods of extraction have been recommended, most of which involve the use of hot alcohol plus some fat solvent. Mixtures of alcohol with ether (4), chloroform (5), or benzene (6) have been used. The function of the alcohol is two-fold. It has a higher penetrating power than a solvent not miscible with water, and it "breaks" insoluble lipid-protein complexes unaffected by unmodified fat solvents. The addition of ether, chloroform, or benzene gives added fat-solvent power to the mixture.

A 3 + 1 alcohol-ether mixture is the most commonly used solvent for the extraction of lipids from animal tissues, especially for micromethods (7), and a similar mixture was found by Guerrant (4) to be most effective for the extraction of lipid phosphorus from seeds. Bornmann (5) found that Hertwig's (8) alcohol-ether method for the extraction of "lipoids" from cereal products and eggs was incomplete. It should be pointed out, however, that in the latter method a 10-gm. sample was heated with only 30 ml. of 70% alcohol, then 55 ml. of unheated 95% alcohol, and finally 85 ml. of ether added. Thus the ratio of