heat stability method to expoxidized oils were discussed. It was decided that there is need for development of a standard heat stability test on epoxidized oil. As the first phase of this study, the Chairman will assemble the various heat stability tests used by the members and distribute them to the Subcommittee for comments.

Polymerized Fatty Acids Subcommittee, G. G. Wilson, Chairman

The Polymerized Fatty Acids Subcommittee has approved the use of AOCS methods on sampling, acid value, saponification value, unsaponifiable, Gardner color and percent water for testing of polymerized fatty acids. The Subcommittee is now working on the development of a satisfactory method for determination of unsaturation and, also, a satisfactory gas chromatograph method.

Drying Oils Subcommittee, Don Bolley, Chairman

The Drying Oils Subcommittee is continuing to work on the development of a method for measuring haze or cloud in drying oil samples. Ed Handschumaker of Spencer Kellogg Company reported on work done on measurement of haze using a nepholometer. The results of this work look very promising and the study will continue in the hopes of developing a method suitable for routine laboratory use.

New Subcommittees

Following the 1963 fall meeting it was reported that the Society would be surveyed to determine if there was enough interest to establish Subcommittees on Alkyd Resins, Dibasic Acids and Hydrogenated Oils.

Francis Scofield reported that the methods already published in the Oils and Derivatives Sections of AOCS Official Methods are apparently adequate for the analysis of alkyd resins and there is little interest among Society members for the establishment of an Alkyd Resin Subcommittee. Mr. Scofield was requested to write a Recommended Practices Method for the testing of alkyd resins to be included in the Industrial Oils and Derivatives Section.

Don Roblin reported considerable interest in the establishment of a Dibasic Acid Subcommittee, also, that a similar Committee is being established in ASTM E-15. Mr. Roblin was requested to proceed with the organization of a Dibasic Acid Subcommittee to work as a liaison group with the ASTM D-15 Committee.

Ross Walker reprted that there is sufficient interest in a Subcommittee on Hydrogenated Oils to establish a Subcommittee. Mr. Walker was requested to proceed with the organization of a Hydrogenated Oils Subcommittee and Society members interested in Hydrogenated Oils should contact Mr. Walker.

Hydrogenation of Linolenate. X. Comparison of Products Formed with Platinum and Nickel Catalysts[1]

C. R. SCHOLFIELD, R. O. BUTTERFIELD, V. L. DAVISON and E. P. JONES,
Northern Regional Research Laboratory,[2] Peoria, Illinois

Abstract

One mole of hydrogen/mole of ester was added to methyl linolenate over a platinum catalyst at 20C and atmospheric pressure. The product was separated into trienoic, dienoic and monenoic esters by countercurrent distribution (CCD) with acetonitrile and hexane. Each of these ester fractions was further separated by CCD with methanolic silver nitrate and hexane.

Comparison with hydrogenations, in which a commercial nickel catalyst at 140C and atmospheric pressure was used, shows that with platinum more stearate is formed; i.e., the platinum hydrogenation was less selective. Also, a smaller amt of *trans* esters was formed with platinum, and there was less shift of double bonds from the original 9, 12 and 15 positions.

Introduction

IN A PREVIOUS PAPER (14) a study was described of the products formed by partial hydrogenation of methyl linolenate with a nickel catalyst. The reduced fatty acid methyl esters from three hydrogenations were separated into triene, diene and monoene fractions by CCD between acetonitrile and hexane. Some of these fractions were further separated by CCD with an argentation system previously described (15). To compare these products with those formed when linolenate was reduced with a different catalyst and under different conditions, a hydrogenation was made at 20C with a platinum catalyst, and the product was fractionated by CCD in the same way as had been done with the nickel-catalyzed products. The composition of products obtained with platinum is compared here with those from nickel discussed previously (14).

Experimental

Methyl linolenate (50 g) was reduced at 20C by stirring under hydrogen at atmospheric pressure with a magnetic stirrer and using 0.25% platinum as a 5% platinum-on-carbon catalyst. One mole of hydrogen/mole of sample was added in 4.25 hr. Analytical data on the product are compared with the nickel-catalyzed products in Table I.

The nickel-catalyzed hydrogenations are numbered in the same way as in the previous paper (14). Although they were all performed at 140C, at atmos-

TABLE I

Analysis of Reduced Methyl Linolenates

Run	Time of reaction, hr	Wijs IV	*trans*,[a] %	Diene conjugation $a_{231-234}$	Composition by CCD			
					Triene, %	Diene, %	Monoene, %	Stearate, %
1 (Ni)	0.5	153.5	37.2	0.5	21.1	40.8	37.2	0.9
2 (Ni)	13	.....	61.8	1.1	39.2	38.7	20.4	1.7
3 (Ni)	11	138.2	62.7	0.4	6.9	59.9	32.8	0.4
Pt	4	170.9	16.2	3.7	43.4	27.2	22.5	6.6

[a] Methyl elaidate standard.

[1] Presented at AOCS Meeting, Atlanta, 1963.
[2] A laboratory of the No. Utiliz. Res. & Dev. Div., ARS, USDA.

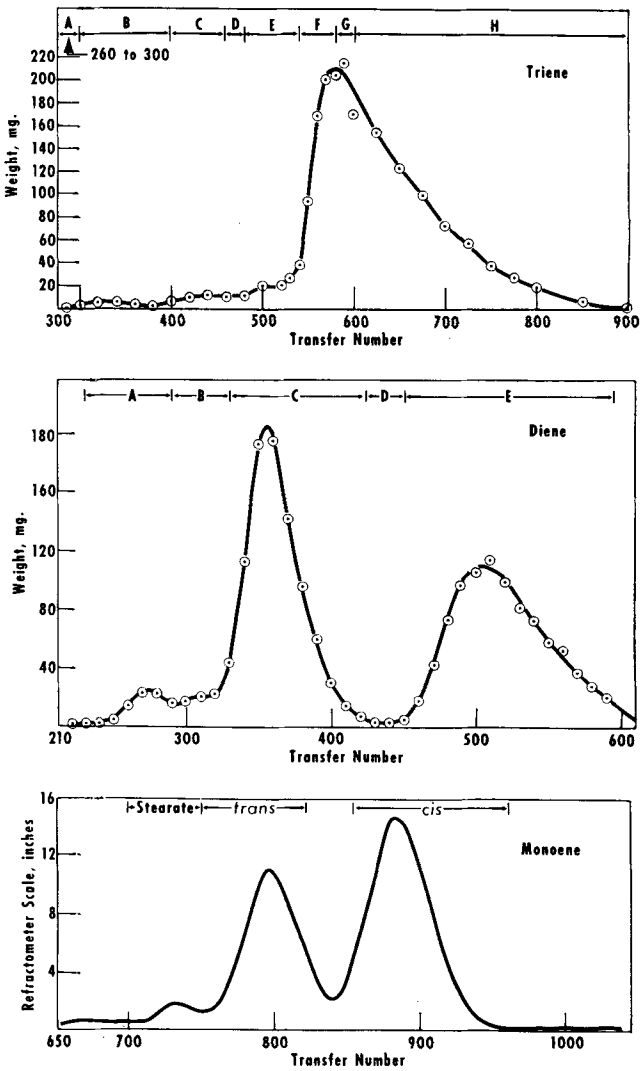


FIG. 1. CCD of fractions from hydrogenated methyl linolenate between 0.2N $AgNo_3$ in 90% methanol and hexane. In triene and diene curves, transfer number is plotted against fraction wt; in monoene curve transfer number is plotted against scale reading of a recording differential refractometer.

pheric pressure, and with 0.5% nickel that was the same type of commercial nickel-on-kieselguhr catalyst, there were great differences in rates of reaction. These are believed to be caused by differences in catalyst activity, agitation and catalyst poisons in the linolenate. Nickel run 1, the last hydrogenation conducted and the one described in detail in the preceding paper was done with fresh catalyst, efficient agitation, and linolenate that had been treated with dilute acid and washed with water until neutral to remove possible traces of mercury which might have been introduced during its preparation.

The fractionation of the platinum-catalyzed product was similar to that described before for nickel-catalyzed materials. It was first separated into triene, diene and monoene fractions by CCD with acetonitrile and hexane. Values for *trans* double bonds in these fractions were monoene 33.0%, diene 19.3% and triene 0%. Methyl elaidate was used as a standard. A small amt of conjugated diene was present in the diene and triene fractions as shown by values of $a_{232} = 2.7$ and $a_{234} = 9.4$, respectively. Each of these fractions was further fractionated by CCD with 0.2N $AgNO_3$ in 90% methanol as the lower phase and hexane as the upper phase.

The distributions obtained with this argentation

TABLE II

Analysis of Triene Fractions from CCD of Methyl Linolenate Reduced with Platinum Catalyst

Sample	Weight % of total triene	Alkali conjugable [a] Ln, %	Alkali conjugable [a] Lo, %	Lipoxidase conjugable, %	*trans* [b] %	Preformed conjugation
A	>0.07	.....	....	.....	.....	$a_{230} = 22.5$ $a_{267} = 16.7$
B	1.6	.....	....	.....	29.8	$a_{240} = 61.5$
C	2.1	192	0	0	31.0	$a_{235} = 73.1$
D	0.7	167.6	0	.....	43.2	$a_{236} = 52.7$
E	4.4	97.6	3.6	72.8	69.3	$a_{233} = 9.8$
F	20.2	98.9	0.4	96.0	0	$a_{232} = 3.4$
G	11.2	99.6	1.8	101.3	0	$a_{233} = 2.8$
H	59.7	94.4	5.0	99.4	0	$a_{234} = 4.9$

[a] Reaction time—45 min.
[b] Methyl elaidate standard.

system are illustrated in Figure 1. Analyses of the combined triene fractions, as shown in Figure 1, are given in Table II, and analyses of the combined diene fractions in Table III.

The monoene fraction was recycled twice through the instrument before collection to give better resolution.

Diene fractions C and E and the *cis* and *trans* monoene fractions were oxidized by the permanganate-periodate procedure, and the dibasic acids obtained were analyzed by gas chromatography of their methyl esters (9). These values show in Figures 2 and 3.

IR analyses for *trans* double bonds were made by measurement of carbon disulfide solutions in a 0.5-mm. cell. Transmission at the 10.4 μ band was measured from a base line drawn tangent to the adsorption curve at approx 9.5 and 10.7 μ. The percentage of *trans* bonds was calculated on the basis of methyl elaidate as 100%. For some fractions the true value will be higher since the adsorption/*trans* double bond for 9,12-mono-*trans* and di-*trans*-linoleates is ca. 85% of that for elaidate (15).

Preformed conjugation was measured in isooctane solution and is reported as absorptivity at the maxima in the diene and triene regions.

Alkali isomerization of diene fractions was at 180C for 4.75 hr to obtain equal conjugation of *cis* and *trans* isomers (7). Triene fractions were isomerized at 180C for 45 min (3). Pure linoleate and linolenate standards were run for comparison.

Lipoxidase isomerizations were by the procedure of MacGee (11).

Separations and characteristics of fractions obtained were also followed by capillary gas chromatograms of individual CCD fractions and combined fractions. The columns used were 200 ft x 0.01 in I.D. and coated with either Apiezon L or diethylene glycol succinate polyester.

Discussion

Comparison of the analyses in Table I shows that the *trans* ester content is higher in the products reduced with nickel and highest in the two preparations where the reduction was slow. Diene conjuga-

TABLE III

Analysis of Diene Fractions from CCD of Methyl Linolenate Reduced with Platinum Catalyst

Sample	Weight % of total diene	Alkali conjugable [a]	Lipoxidase conjugable	*trans* [b] %	$a_{232-234}$
A	3.7	.....	0	34.6	58.6
B	4.2	89.2	11.5	70.0	4.1
C	43.7	76.1	63.3	29.8	0
D	0.3	.....	.....	51.9	.....
E	48.1	0.2	0	12.2	

[a] Isomerization time—4.75 hr.
[b] Methyl elaidate standard.

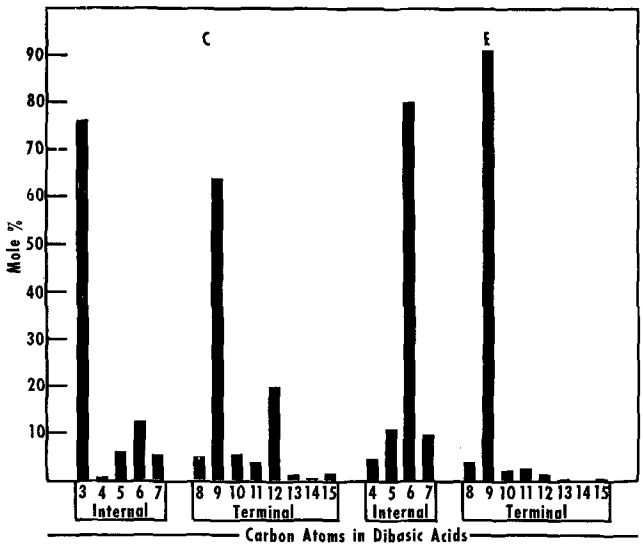


FIG. 2. Dibasic acids from diene fractions obtained in distribution shown in Figure 1.

tion is low in all products but higher with platinum than with nickel. CCD with hexane and acetonitrile shows that with platinum catalyst more stearate is formed. These differences, which are in agreement with previous results, are caused both by differences in the catalyst used and by differences in the temp of hydrogenation. Richardson and Snoddy (12) in 1926 showed that platinum was less selective than nickel for hydrogenation of cottonseed oil. They found that isooleic acid formed at 40C was quite low, but that it increased with increasing temp. More recently, Cousins (4) found that under identical operating conditions platinum produced more *trans* isomers from methyl linoleate than did nickel. In hydrogenating an equimixture of methyl linolenate and linoleate, Johnston (8) obtained lower *trans* values with platinum at 25C than with nickel at 140C.

The *trans* content of the fractions from CCD with acetonitrile and hexane is all lower than that of the corresponding fractions from nickel reductions. With nickel, values of 53–71% *trans* for monoene, 42–76% for diene and 13–70% for triene have been obtained. In the triene fraction from the platinum-catalyzed product, no isolated *trans* bonds were found although Table III shows isolated *trans* was coned in some small triene fractions. A summation of the triene fractions in Table II indicates 4.5% *trans* in the whole triene.

CCD between hexane and 0.2N $AgNO_3$ in 90% methanol of the fractions in Table II produced distribution curves similar in appearance to those obtained from the corresponding fractions of run 1 with nickel. However, differences were found upon analysis of the fractions.

In CCD of the triene shown in Figure 1 and Table II, fractions H, G and F make up 91% of the sample. Analytical data, together with capillary gas chromatography, indicate that these are mostly unchanged linolenate with a small amt of mono-*trans* isomer shown by GLC in F. E is also shown to contain mono-*trans* isomers as well as unchanged linolenate.

The smaller fractions A, B, C and D give exceedingly complex capillary gas chromatograms with many unidentified components. Here isolated *trans* double bonds and diene conjugation are present. Fraction A is the only one containing triene conjugation.

High values for alkali-conjugable linolenate, like those of C and D, have been reported before for

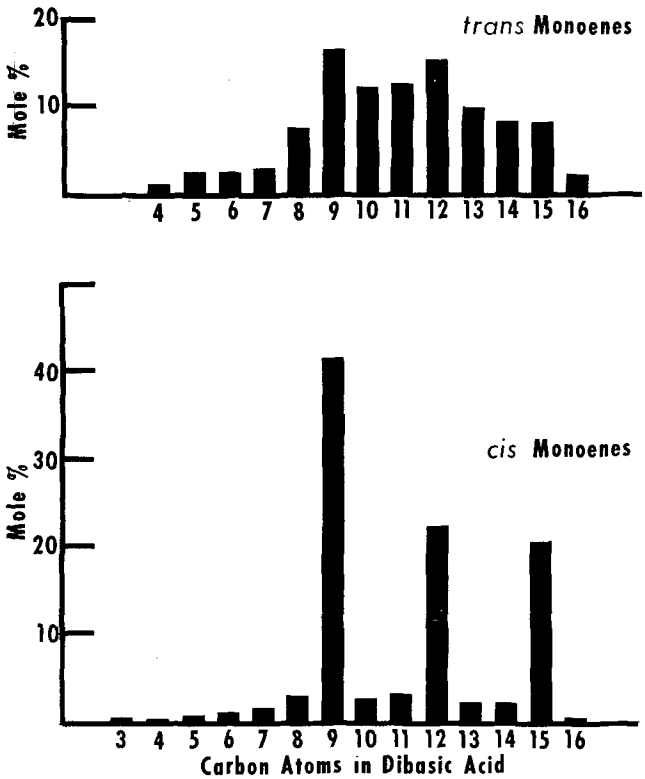


FIG. 3. Dibasic acids from monoene fractions obtained in distribution shown in Figure 1.

fractions from nickel-catalyzed reductions (14). These results show that we are applying the analytical alkali isomerization procedure to samples for which the standard constants are not suitable. Absorptivity at 267 μ for C and D are 83 and 94, whereas it is 49 for linolenate under the same conditions. Absorption in the diene region, which was originally high, was destroyed by alkali and no diene conjugation remained. The nature of the esters having this unusual behavior with alkali is not known.

CCD of the diene fraction is shown in Figure 1 and Table III. Here the small fraction A is high in diene conjugation. Capillary GLC shows conjugated *cis,trans-*, *trans,cis-*, *cis,cis-*, and *trans,trans*-isomers as well as several unidentified components.

Fractions B and C both contain large amt of alkali-

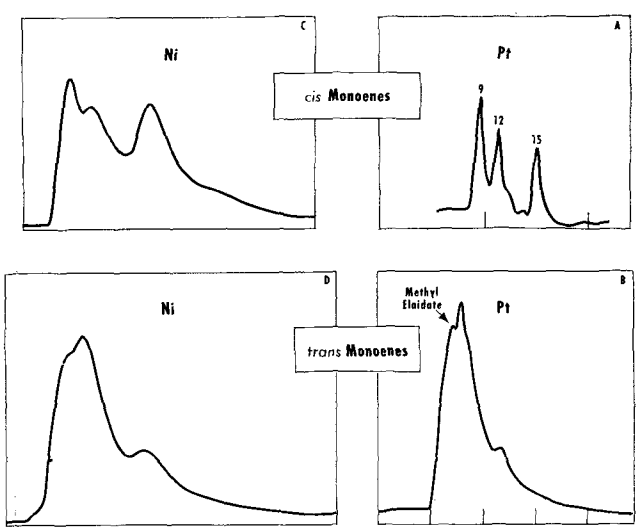


FIG. 4. Capillary gas chromatograms of monoene fractions from methyl linolenate reduced with platinum and with nickel. A 200-ft × 0.01-in. column coated with Apiezon L was operated at 200C.

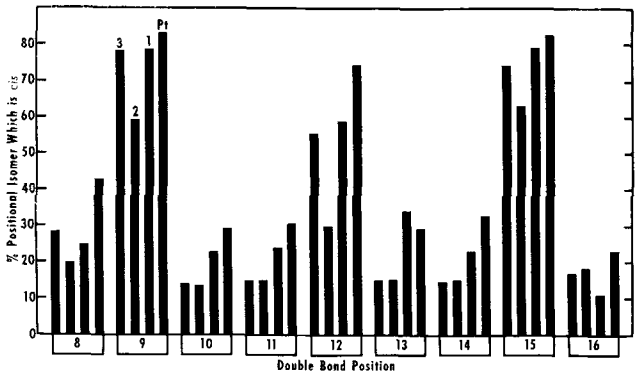


FIG. 5. Percentage of each monoene positional isomer that has a *cis* configuration. The bar graphs corresponding to each hydrogenation are labeled for double bond position 9. They are in the same order for other double bond positions.

conjugable esters, but B is higher in *trans* esters and consequently lower in lipoxidase conjugable.

The analysis of the dibasic acids obtained by oxidation of C shows in Figure 2. As previously (14), we have arbitrarily considered that all dibasic acids of eight or more carbon atoms are terminal—from the carboxyl end—and those with seven or less carbon atoms are internal—from between the double bonds. While this assumption is not strictly true, it is a good approximation and allows classification of the dibasic acids in a way which gives more information than if they were all grouped together. Also, since malonic acid is unstable and its recovery is not quantitative, we have used the value for alkali conjugation as a measure of this acid. Dibasic acids from Fraction C are similar to those from the corresponding fraction from the nickel run 1 reduction but with even more C_9 and C_{12} acids from the terminal portion. These acids from the terminal portion and the high C_3 from the internal portion reflect the high value for alkali-conjugable diene in this fraction.

Fraction E is largely methyl 9-*cis*,15-*cis* linoleate as shown by the dibasic acid analysis, by the absence of conjugable esters and by gas chromatography. Except for its lower *trans* content, it is similar to the corresponding fraction from the nickel run 1 reduction.

Fraction E, which is formed by reduction of the 12,13 double bond, makes up 48% of the diene fraction rather than only one-third as would be expected if the double bonds were reduced at an equal rate. In the nickel run 1 reduction the corresponding fractions made up 49% of the diene.

These 9,15 esters make up part of the "isolinoleates" detected by Vander Veen (16) by ozonization of hydrogenated methyl linolenate and by Lemon (10) by alkali isomerization of hydrogenated linseed oil and its fractions.

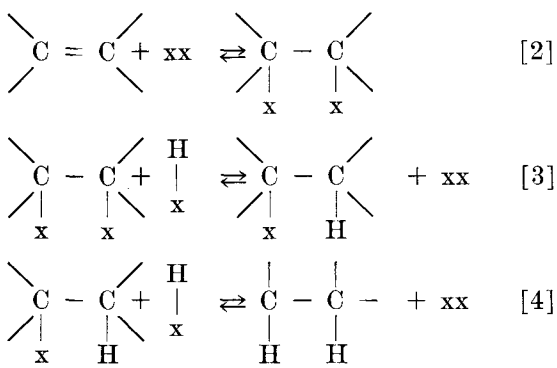
With the monoene fraction, a good separation of *cis* and *trans* isomers was obtained (Fig. 1). Oxidation of these fractions followed by dibasic acid analysis gave the data for Figure 3. As with nickel, but to a greater degree, in the *cis* fraction the double bonds are in the original 9, 12 and 15 positions. However, with platinum the *cis* monoenes decrease in the order C_9, C_{12} and C_{15}; whereas with nickel there is less C_{12} than C_9 or C_{15}. In the *trans* fractions, the double bonds are widely scattered, but more double bonds are in the original 9, 12 and 15 positions than in adjacent positions. With nickel, the greatest amounts are always in the 10, 11, 13 and 14 positions.

The differences between the monoene fractions are well illustrated by the capillary gas chromatograms of Figure 4. With platinum the *cis* fraction shows the 9, 12 and 15 monoenes as three well-separated peaks; with nickel the separations are less distinct because larger amt of other esters with double bonds in intermediate positions are present. The *trans* fractions from both platinum and nickel contain many positional isomers and these give broad bands with incomplete separation of the isomers.

The double bond distributions in the monoenes also may be compared by considering what percentage of each positional isomer is *cis* (Fig. 5). The data in this figure must be interpreted with caution. Many of these percentages represent a quotient of two small numbers of limited accuracy. Nevertheless, certain patterns are evident for the whole figure. The percentages of *cis* isomers are greatest for esters with double bonds in the original 9, 12 and 15 positions. It is greater for the 9 and 15 positions than for 12. It is greater for platinum than for nickel and greater for nickel 1 with a rapid reduction than for nickel 2 and 3.

Catalytic hydrogenation of olefinic double bonds is usually considered to proceed through a half-hydrogenation mechanism as proposed by Horiuti and Polanyi (5).

$$H_2 + xx \rightleftarrows 2\ \overset{H}{\underset{x}{|}}\quad (x = \text{catalyst}) \qquad [1]$$



With fatty acid esters under ordinary conditions step 4 is practically irreversible. Allen (1) suggested that such a mechanism would explain the shifting of double bond configuration and position which occurs during hydrogenation.

The smaller amt of isomerization with platinum at 20C suggests that in step 3 the equilibrium is more to the right than it is with nickel at 140C. That is, the probability that the half-hydrogenated ester in step 3 will be completely hydrogenated in step 4, rather than dehydrogenating to a diadsorbed species, is greater for platinum at 20C than nickel at 140C.

This mechanism does not seem sufficient to account for all of our results. With platinum it does not account for more *trans*-monoenoic esters with double bonds in the original positions than in adjacent positions. Also, it does not explain the combination of a larger amt of conjugation with a smaller amt of double bond shif in the platinum-catalyzed reduction. Diene-conjugated esters formed by the half hydrogenation-dehydrogenation mechanism would be expected to reduce more rapidly than unconjugated esters and to produce a larger amt of double bond shift. The failure of this shift to occur may indicate a greater tendency for conjugated esters to be desorbed from the catalyst at step 2, or it may indicate a smaller difference in the relative reduction rates of conjugated and unconjugated esters.

The amt of 9,15-type diene (Diene Fraction E) and its corresponding fraction from nickel 1, which are larger than expected by chance, may indicate that the 12,13 double bond in the triene is reduced more rapidly than the 9,10 or 15,16 double bonds. However, this larger amt of 9,15 diene is also caused by the slower reduction of 9,15 dienes (isolinoleate type) compared with 9,12 linoleate. Previous workers have reported such differences in reduction rates for 9,15 and 9,12 linoleates (2,13) and it is reasonable to assume that similar differences occur with the *trans*-containing isomers which are also present in the hydrogenating mixture. In the monoene from a nickel catalyzed linolenate, there are less 12 monoenes than 9 or 15. From the platinum monoenes, however, one would predict that the double bonds farthest from the carboxyl are reduced more rapidly, as believed by Inoue and Suzuki (6). The difference in monoene composition may be caused by differences in the relative rates of hydrogenation of isolinoleic acid-type dienes and other dienes.

The reaction pathways by which this complex mixture of diene and monoene isomers is formed are still not clear. Certainly the isomerized trienes and dienes that are present in small amt participate in the reaction, and some of them—especially conjugated esters—may be quite reactive intermediates. Interpretation of data, therefore, must be tentative, and more information on the identity and relative reaction rates of the intermediates is needed before the reduction is well understood.

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Phospholipids of the South African Pilchard *(Sardina ocellata Jenyns)*

M. H. SILK [1] and A. J. DE KONING, Fishing Industry Research Institute, University of Cape Town, Rondebosch, Cape Town, South Africa

Abstract

Chromatographic resolution of phospholipids from the flesh of the South African pilchard (*Sardina ocellata Jenyns*) revealed the presence of cardiolipins, cephalins, inositol phosphatides, cerebrosides, sphingomyelins, lecithins, lysolecithins and plasmalogens.

Column and paper chromatographic techniques were used to identify ethanolamine, serine, choline, sphinogsine and inositol in pilchard phospholipid hydrolysates.

The fatty acids of pilchard phospholipids comprised large amt of C_{22} hexaenoic, C_{20} pentaenoic and C_{16} saturated acids, with smaller amt of C_{18} dienoic, C_{18} monoenoic and C_{18} saturated acids.

Introduction

THE PILCHARD (*Sardina ocellata Jenyns*) is abundant in South African waters, and is of considerable economic importance in the Republic of South Africa.

The triglyceride composition of pilchard oil has been investigated in considerable detail (30,33), but the phospholipids have not been examined previously.

The phospholipids of cod and haddock have been described in a series of papers by Lovern and Olley (19,20), Olley and Lovern (25,26), Garcia et al. (8) and Lovern et al. (21), while tuna phospholipids have been examined by Igarashi et al. (10,11,12), Zama and Igarashi (13,15) and Katada (14).

These investigations, together with reports concerning the phospholipid composition of other fish, have been reviewed by Lovern (22).

[1] Present address: South African Poliomyelitis Research Foundation, Johannesburg, South Africa.

Pilchard phospholipids were isolated according to a modified procedure which was based on the method of Lovern et al. (21) for codfish.

Experimental

Isolation of Pilchard Phospholipids

Method: Operations were carried out under an atmosphere of carbon dioxide produced by adding dry ice to all extracts and containers. Phosphorus contents were determined according to the method of Bartlett (2).

1) *Solvent Extraction of Mixed Triglycerides and Phospholipids.* Fresh pilchards, caught during the previous 24 hr, were received in the laboratory packed in ice at 0C. The fish were decapitated, degutted, deboned and descaled. After coarse mincing of the flesh with adhering skin, a weighed portion (750 g) was extracted by thorough stirring with 375 ml acetone. The slurry was filtered on a Buchner funnel and the tissue was further drained by subjecting it to a pressure of 2000 lb/sq. in. in the canvas bag of a laboratory Carver press. The extraction process was repeated with a further 375 ml acetone and both acetone extracts were discarded.

The presscake from the second acetone extraction was macerated for 10 min in a Waring blender with approx 700 ml $CHCl_3$:MeOH (2:1, v/v). The suspension was filtered on a Buchner funnel and the disintegrated tissue again blended with 700 ml $CHCl_3$:MeOH (2:1, v/v). After filtering, the residue was suspended in a further 700 ml $CHCl_3$:MeOH (2:1, v/v) and allowed to stand overnight at −20C before removal of the solvent.

The combined $CHCl_3$:MeOH extracts were dis-