are determined directly, and that in calculations of glyceride composition, no theories of acyl group distribution need to be invoked.

The interpretation of chromatograms of oils such as coconut and palm kernel is complicated by the influence of the chain lengths of the esterified acids on the Rf values of the constituent glycerides. For those fats for which we quote data here, the component fatty acids are predominantly palmitic and steric acids or their unsaturated analogues, and no fractionation which could be attributed to differences in chain length was observed. In the case of the nut oils, the differences in fatty acid chain length are much greater, and exert some noticeable influence on the Rf values of the component glycerides.

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Methods for Improving Yields of Cyclic Acid from Linseed Oil[1]

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Abstract

Liquid C-18 saturated monocarboxylic acids, which are termed "cylic acids" because they contain a ring structure, have been prepared by the action of excess sodium hydroxide on linseed oil in ethylene glycol at elevated temperatures, followed by distillation and hydrogenation of the resulting free fatty acid monomers and by separation of the straight-chain components by low-temperature crystallization from acetone. In a survey of other possible catalysts and reaction conditions, cyclic acid yields were improved from the previously reported 32.4 g to 43.5 g of cyclic acid per 100 g of linseed fatty acids by removing water from the starting materials and using the monosodium derivative of ethylene glycol as catalyst. The corresponding amount of polymer formed decreased because of a decrease in hydroxylation and subsequent polyester formation.

Introduction

EARLIER C-18 saturated monocarboxylic acids that fail to crystallize at −70C were reported to have been prepared from linseed oil, linolenic acid, and tung oil (3). The relative proportions of liquid cyclic acids, straight-chain monomeric acids, and polymer formed varied with the substrate and the conditions employed. Under reported conditions, an appreciable loss in total acid equivalents occurred during distillation of crude acids, as indicated by the carboxyl balance. Subsequent investigations showed that the polymers had neutralization equivalents ranging from 450–1,100. Saponification of these polymers reduced neutralization equivalents to about 300, only slightly higher than expected for normal polymeric acids. Esterification and subsequent distillation of the saponified fraction yielded additional cyclic and other monomeric acids, as well as a residue of polymeric acid having a neutralization equivalent slightly above 300.

In this latest work, removing water from the reaction mixture and using the monosodium derivative of ethylene glycol as catalyst increased yields of cyclic acids from a previously reported 32.4 g (3)–43.5 g/100 g of linseed fatty acids, with correspondingly less hydroxylation and subsequent polyester formation.

Experimental

Preparation of Catalyst-Solvent Systems. Except for the nickel catalyst, systems were prepared by dissolving a catalyst in an appropriate amount of solvent to produce a concentration such that a 1:3 (wt/vol) linseed fatty acid to solvent ratio would give a 50% excess of catalyst.

Ethylene glycol-sodium hydroxide: 60 g (1.5 moles) of reagent grade NaOH was dissolved in 834 ml of stock ethylene glycol.

Dry ethylene glycol-sodium hydroxide: made by dissolving 60 g of reagent grade NaOH in 834 ml of ethylene glycol which had been distilled from $\frac{1}{20}$ its weight of Na. A constant boiling fraction was collected at 196C.

Ethylene glycol-monosodium derivative of ethylene glycol: made by dissolving 34.5 g of Na in 870 ml of stock ethylene glycol with vigorous stirring. Tempera-

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[1] Presented at the AOCS meeting, in Chicago, Ill., 1961.

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ture must be kept below 50C during addition of the sodium. If the sodium becomes molten the reaction becomes very difficult to control.

Dry ethylene glycol-monosodium derivative of dry ethylene glycol: prepared by dissolving 34.5 g of Na in 870 ml of dry ethylene glycol.

T-butyl alcohol-K derivative of T-butyl alcohol: made by dissolving 17 g of potassium tertiary butoxide in 175 ml of reagent grade T-butyl alcohol, from MSA Research Corp., Callery, Pa.

All these catalyst-solvent systems are extremely hydroscopic. For this reason all were prepared immediately before use.

Heptane-Ni system: the S-poisoned Ni conjugation catalyst was like that used by Miller et al. (6) for the isomerization of safflower oil.

Linseed fatty acids: nonbreak linseed oil was steam hydrolyzed. The fatty acids were distilled under vacuum to give a light-colored product containing 53.2% linolenic acid (GLC) and having a neutralization equivalent of 278.

Isomerization and Cyclization of Linseed Fatty Acids. Again, except with the Ni catalyst, all isomerizations were carried out in a 2,000-ml stainless-steel, medium pressure, stirred autoclave. After the autoclave was flushed with dry nitrogen, the reactants (linseed fatty acids and catalyst-solvent solution) were siphoned in to eliminate atmospheric moisture. The reaction mixture was heated rapidly with steam to 100C, then the autoclave was placed in an electric heating jacket and brought to the desired temperature: about 30 min at 235C, 45 min at 260C, and 1 hr at 295C. The temperature was automatically controlled within ± 3C. Samples were taken at regular intervals for analysis; after the reaction was terminated, the autoclave and its contents were quenched in cold water.

Samples of reaction products were diluted with 10 vol of distilled water in a separatory funnel. First, 5 vol of n-heptane was added and then an excess of dilute HCl. The liberated free fatty acids were extracted in the heptane phase by shaking the funnel. The layers were separated and the heptane layer was washed three times with distilled water. The heptane solution was then dried over anhydrous sodium sulfate.

After stripping off the heptane, the crude acids were distilled under a pressure of 25 μ through an 8-in. Vigreaux column to a pot temperature of 250C. The distillate was collected in two fractions, that boiling below 150C (forerun) and the main one at 150–180C.

Hydrogenation of the main fraction was carried out as follows: 3-g samples of the main fraction were hydrogenated under a pressure of 2,000 psi at 150C for 3 hr with 6 mg of 10% Pd on powdered charcoal as catalyst. The hydrogenation bomb was allowed to cool to room temperature before opening to prevent darkening of the product. The oily, semisolid product was dissolved in acetone and the catalyst filtered off. The acetone was then stripped off to yield the hydrogenated acids (I.V. < 1).

Separation of straight chain saturates from cyclic acids was done by redissolving samples in 10 vol of acetone and cooling to −40C with constant stirring. The straight chain materials crystallized and were filtered. The solid acids were recrystallized twice from acetone to remove the last traces of cyclic acids. The apparatus employed for this procedure has been described in a recent publication. (2).

Results of the isomerizations are shown in Table I.

Discussion

By using a 50% excess of catalyst and eliminating water from the starting materials, the yield of cyclic acids obtained from linseed fatty acids (53.2% linolenic) was increased from about 30 g to 43.5 g per 100 g.

Figure 1 shows the effect of reaction time on the yield of cyclic acids at three different temperatures when the ethylene glycol-monosodium derivative of ethylene glycol solvent-catalyst system was used. At 295C (Table I, No. 9–14) the cyclic acid yield reaches a maximum in 15 min and then decreases rapidly with increased reaction time. At 235C (Table I, No. 1–3) the cyclization is comparatively slow and fails to reach a maximum in 7⅔ hr. At 260C (Table I, No. 4–8) the yield of cyclic acid reached a maximum in 3 hr and then decreased slowly. Since this temperature gave the highest yield, it served as a basis for comparison of the various solvent-catalyst systems.

Figure 2 shows the effect of reaction time on the

TABLE 1
Isomerization and Cyclization of Linseed Fatty Acids in 50% Excess Alkali[a]

No.	Temp C	Time at temp, hr	NE crude acids	Forerun, %	Monomeric acids, %	NE monomeric acids	Polymer, %	NE polymer	Cyclic acids, %
Solvent-Catalyst System: Crude glycol and monosodium derivative									
1	235	1	283	....	95.2	280	4.8	348	24.2
2	235	3	282	....	93.2	280	6.8	344	32.6
3	235	7⅔	282	....	89.2	281	10.8	321	38.1
4	260	½	282	....	95.1	281	4.9	334	36.4
5	260	1½	284	....	91.1	278	8.9	347	39.1
6	260	3	288	....	88.6	280	11.4	356	42.5
7	260	4	289	....	90.2	282	9.8	380	40.8
8	260	6	295	....	84.9	287	15.1	379	39.8
9	295	0	284	....	93.4	280	6.6	375	40.2
10	295	¼	290	....	92.7	282	7.3	363	40.9
11	295	½	286	1.5	88.0	281	10.5	381	40.5
12	295	1	301	1.9	81.0	284	17.1	458	36.8
13	295	2	314	2.3	78.0	288	19.7	553	......
14	295	7	356	3.1	53.2	290	43.7	900	24.8
Solvent-Catalyst System: Dry glycol and monosodium derivative									
15	260	1	294	....	93.7	280	6.3	343	38.4
16	260	2	296	....	90.0	283	10.0	349	39.6
17	260	3	293	0.5	88.4	282	11.1	347	43.5
18	295	0	297	0.7	92.2	282	7.1	368	41.0
19	295	½	294	1.7	87.7	283	10.6	391	40.2
20	295	1	312	2.4	78.2	283	19.4	488	35.2
Solvent-Catalyst System: Dry glycol and NaOH									
21	260	3	292	0.6	86.7	282	12.7	331	39.4
Solvent-Catalyst System: Crude glycol and NaOH									
22	260	3	285	0.8	84.8	282	14.4	338	37.3
Solvent-Catalyst System: T-butyl alcohol and K derivative									
23	235	5	293	....	93.7	286	6.3	415	44.0

[a] All percentages are based on recovered crude acids.

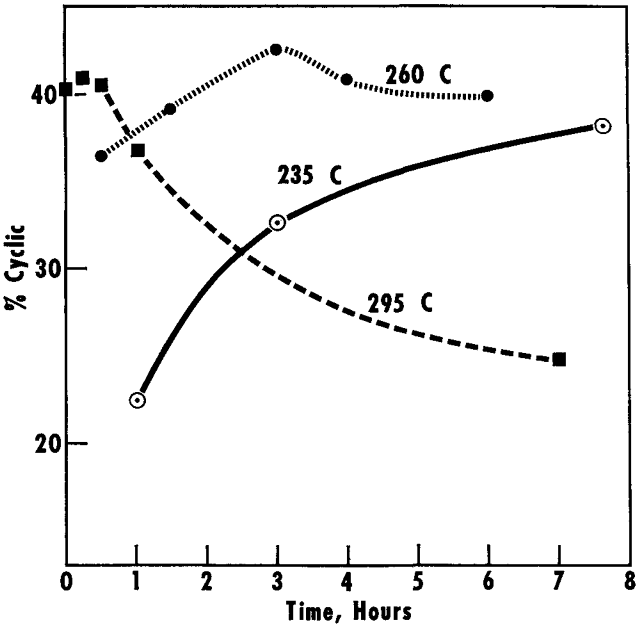

FIG. 1. Yield of cyclic fatty acids at various temperatures using crude ethylene glycol as a solvent and its monosodium derivative as a catalyst (50% excess).

neutralization equivalent of the polymeric residue at three different temperatures when the ethylene glycol-monosodium derivative of ethylene glycol solvent-catalyst system was used. The increase in neutralization equivalent observed at 295C was caused by esterification. The polymer (NE 900, Table I, No. 14) was saponified yielding crude acids (NE 318) which were distilled under vacuum to give additional monomer. Table I (No. 9–14) shows that the amount of polymer as well as its neutralization equivalent, increases rapidly with time at 295C. The slight, but definite, decrease observed in the neutralization equivalent of the polymer formed at 235C is unexplained.

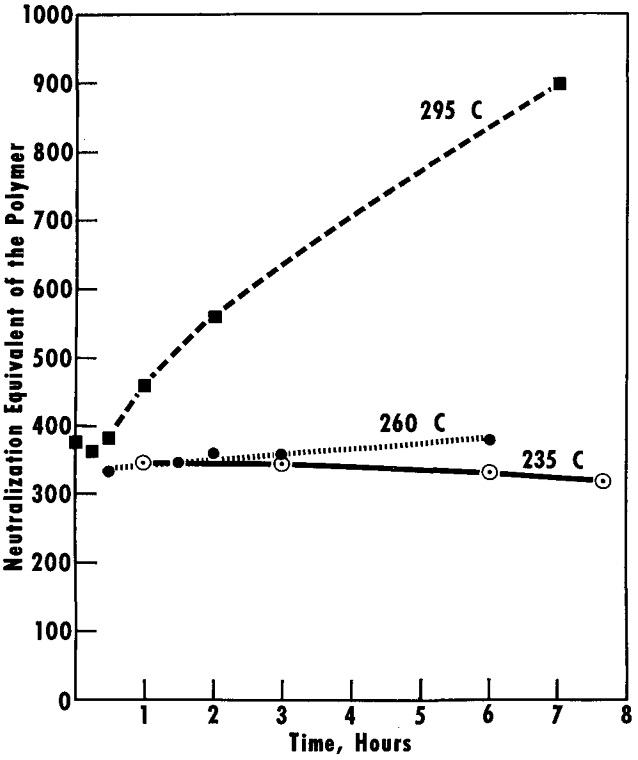

FIG. 2. Change in neutral equivalent of polymer with time and temperature using the same solvent and catalyst as used for Fig. 1.

It can be seen from Table I by comparing No. 22, 21, 6, and 17 that, as greater care was taken to remove water from the reaction media, the yield of cyclic acids increased and the amount of polymer decreased. This increase may result from the presence of a more effective catalyst species, i.e., $^{-}OCH_2CH_2OH$ as compared to OH^-. However, in the presence of water, and particularly at high temperatures, a considerable amount of hydroxylation occurs and subsequent polyesterification occurs during distillation of the crude acids. When large amounts of polyester are formed in the absence of H_2O (Table I, No. 14), the hydroxyl presumably must come from the catalyst or from decomposition of the solvent, provided there are not precursors in the linseed fatty acids. Ethylene glycol dehydrates in the presence of an aluminosilicate catalyst to produce acetaldehyde, acetic acid, and ethylene (7). In the cyclizations carried out at 295C, a flammable gas is produced, but its composition has not been determined.

Beal and coworkers (1) found in the linseed-oil, NaOH, ethylene-glycol system, that if, after the cyclization, the crude acids were esterified thus blocking the possibility of polyester formation, yields of 42.4 g of cyclic acids per 100 g of linseed oil could be obtained. This yield agrees closely with that obtained under essentially anhydrous conditions without esterification. The reduced percentages of polymer found under anhydrous conditions are also equivalent to the percentages obtained by Beal. The polymers formed under anhydrous conditions (Table I) still have slightly high neutralization equivalents and also minor amounts of ester, as indicated by their lower saponification equivalents.

Previously (3) the best conversion of eleostearic acid to a cyclic product by purely thermal means was about 25%, whereas alkali treatment of tung oil (about 80% eleostearic) resulted in about 70% conversion based on the eleostearic acid present. Since neither the alpha nor beta form contains a *cis*-11 bond, the configuration is unfavorable for thermal cyclization.

To further substantiate the necessity of alkali for cyclization, a sulfur-poisoned Ni isomerization catalyst was used. The results were inconclusive. Under the best reaction condition, i.e., 1 part linseed fatty acid, 3 vol heptane, 4.5% catalyst at 180C for 3 hr then 260C for 3 hr, a yield of 14% liquid C-18 saturated acid was obtained after hydrogenation. This material has a different GLC pattern than that obtained by the alkaline treatment. MacDonald (5) also noted that the cyclic material formed by heat treatment of linseed oil showed no absorption above 255 mμ as did the alkaline-treated linseed fatty acids. This absorption is characteristic for cyclohexadienes (8). If these liquid acids produced in the presence of the Ni catalyst are cyclic acids, they must then be formed by a different mechanism from those produced by alkaline isomerization. Although this supposition does not constitute unequivocal proof that alkali catalyzes cyclization as well as conjugation, it certainly lends strong support to this contention. If alkali does catalyze cyclization, a mechanism which would account for the hydroxylation, as well as the cyclization, is shown in Figure 3.

Compounds of type I are known to be formed by the alkaline isomerization of linolenic acid (4). Furthermore a compound of type IV is formed if the

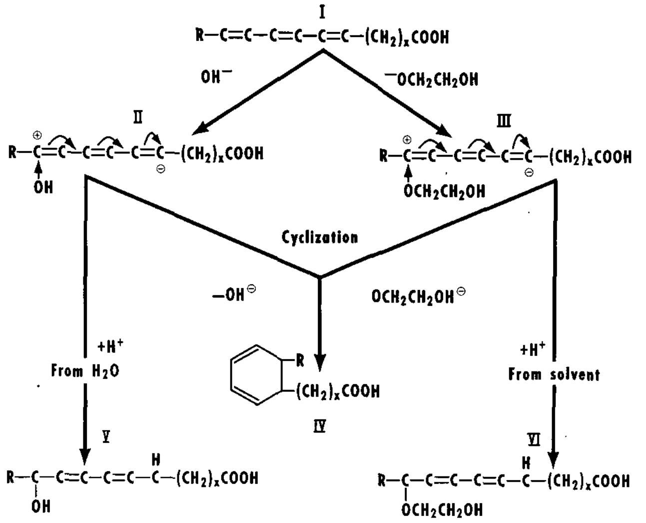

FIG. 3. Possible base catalyzed mechanisms for cyclization.

alkaline isomerization is continued at elevated temperatures (3). One cannot explain the presence of hydroxyl in the reaction products on the basis of a thermal mechanism for cyclization. On the other hand, if a nucleophillic attack by the catalyst on the conjugated system is postulated, intermediates of type II and III could exist which would give rise to structures IV, V, or VI. Although hydroxyl has been found in the reaction products both by spectrophotometry and by acetylation with acetic anhydride, no amount of ether linkage was detectable. Route I → III → VI is therefore improbable. In the presence of water, if the reaction proceeds through an intermediate of type II, the large increase in polymer because of hydroxylation and subsequent polyester formation could be explained. On the other hand in the absence of water, if the reaction proceeds through intermediate III, an increase in IV should result since the alternate route I → III → VI has been ruled out.

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Vernonia anthelmintica (L.) Willd. Enzyme Studies. Conversion of Epoxyoleic Acid to (+)-*threo*-12,13-Dihydroxyoleic Acid[1]

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Abstract

Vernonia anthelmintica (L.) Willd. seed was found to contain enzymes that were active only after the seed was ground. By deactivation of the enzymes, an oil rich in trivernolin (the triglyceride of epoxyoleic acid) was produced, and pure trivernolin was isolated. Acceleration of the enzyme activity altered the composition of the oil as evidenced by changes in free fatty acid content, iodine values, and oxirane oxygen (epoxy) content. Investigation showed that these changes were due, at least partially, to the conversion of epoxyoleic acid to (+)-*threo*-12,13-dihydroxyoleic acid, which was isolated in pure form. Pure (−)-*threo*-12-13-dihydroxyoleic acid was prepared by acetolysis of *V. anthelmintica* seed oil. Neither of these isomers had been obtained previously from mature *V. anthelmintica* seed.

Introduction

AT VARIOUS stages in the maturity of *Vernonia anthelmintica* (L.) Willd. seed there appear to be present four active principles of an enzymatic nature. Miwa et al. (1) reported the presence of two principles in maturing seed; one, an oxidative and, two, a dehydrating principle. A hydrolytic principle in the mature seed, active only after the seed is crushed, was discussed previously (2).

This paper elaborates on the behavior of the hydrolytic principle and on the activity of a fourth principle, a hydrating one, that is active when the mature seed is crushed and stored under a controlled experimental environment. The announcement of the presence of this hydrating principle responsible for the production of (+)-*threo*-12,13-dihydroxyoleic acid was made recently (3). The (+) and (−) isomers of *threo*-12,13-dihydroxyoleic acid were prepared previously by Chisholm and Hopkins (4) by acetolysis of the seed oils of *Malope trifida,* Cav. and *Vernonia colorata,* Drake, respectively.

Since this paper was submitted to the Editor, an article has appeared on the enzymatic production of (+)-*threo*-9,10-dihydroxyoctadecanoic acid in the spores of plant rusts, by A. P. Tulloch, Can. J. Biochem. Physiol., *41,* 1115 (1963).

Experimental

Analytical Methods. The source of seed, analytical methods, Soxhlet, and rapid extraction techniques used in these investigations were described previously (2).

Thin-layer chromatographic procedures were essentially the same as published methods (5,6). The GLC analyses were done on a silicon rubber (SE-30) column using programmed temperatures as described by Herb et al. (7).

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[1] Presented in part at the AOCS meeting in Chicago, Ill., 1961; and Toronto, Canada, 1962.

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