Unsaturated Aldehyde Oils by the Partial Ozonization of Soybean Oil[1]

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Abstract

Aldehyde oils containing an average of one or two aldehyde groups per glyceride molecule were prepared by partial ozonization of soybean oil. Ozonization was carried out in ethyl acetate-methanol solvent, and reduction of the ozonolysis products was done catalytically with palladium on charcoal in the presence of pyridine. Under these conditions, maximum conversion of unsaturated to carbonyl functionality was achieved without saturation of the unozonized double bonds. The products were oils having three different types of functionality: aldehydic, olefinic, and glyceride ester.

Introduction

A PREVIOUS PAPER described aldehyde oils prepared by complete ozonization of soybean oil (3). These aldehyde oils are viscous liquids having 2.4–2.6 aldehyde groups per glyceride molecule; they are highly reactive toward urea, polyols, and phenolic compounds. The products from these various reactions are soft, flexible, but infusible and insoluble gels, indicating a high degree of crosslinking. The aldehyde functionality in the completely ozonized oil may be higher than necessary or desirable for many reactions. For this reason, we investigated the partial ozonization of soybean oil to obtain products having a lesser degree of aldehyde functionality and to prepare products having some residual unsaturation, which could act as possible sites for further reactions. Three different types of functionality would then be present in an unsaturated aldehyde oil: aldehydic, olefinic, and the glyceride ester group. Such products might have value as modifying agents for many applications.

To preserve residual unsaturation, the ozonolysis products could be reduced either chemically, for example, with zinc and acetic acid (3), or catalytically with hydrogen over palladium on charcoal in the presence of pyridine (4). When the latter method was employed for the reduction of methyl oleate ozonolysis products, pyridine not only prevented hydrogenation of residual unsaturation, but also improved yields of pelargonaldehyde and methyl azelaaldehydate (4). The improved yields resulted from decreased byproduct methyl pelargonate and dimethyl azelate formation. The methanol-pyridine solvent system worked well for methyl oleate, but did not give a homogeneous solution with soybean oil. The effect of pyridine on the hydrogenation of partially ozonized soybean oil in an ethyl acetate-methanol solvent system (3) is described in this paper.

Experimental

The general procedure for ozonization has been previously described in detail (3). Discussion of two of the experiments summarized in Table I will suffice to illustrate catalytic reduction with and without pyridine present.

Reduction Without Pyridine (Run A). Soybean oil having 1.538 moles unsaturation/mole fatty acid (40.0 g, 0.207 mole of unsaturation) was dissolved in 200 ml of ethyl acetate and 50 ml of absolute methanol. Oxygen containing 1.01 moles of ozone/liter of oxygen was passed through the solution (chilled to 12C) at a rate of about 2.06 l./min for 40 min. The amount of unreacted ozone passing through the reactor was less than 0.3% of theory. The amount of ozone absorbed by the reaction was 39.7% of theory. The solution and reaction flask were flushed with nitrogen, and 0.1 g 10% palladium-on-charcoal catalyst was added. Hydrogenation was carried out at room temperature and atmospheric pressure for 330 min, during which time the catalyst became slowly coated with stearins that eventually prevented completion of the hydrogenation. The solution was warmed to 40C to release the catalyst, and hydrogenation continued for another 180 min until the peroxide test was negative. The solution was filtered, and 200 ml of CH_2Cl_2 were added to the filtrate. The solution was then washed three times with water to remove any malonaldehyde, and each aqueous extraction was backwashed with a small amount of CH_2Cl_2. The solution was dried, filtered, and distilled *in vacuo* to a maximum pot temperature of 120C, using a nitrogen capillary ebullator. The yield of unsaturated aldehyde oil was 34.55 g with a carbonyl conversion of 35.1% (equivalent to 0.976 aldehyde groups/molecule or 0.325 mole/mole of fatty acid). Unreacted olefinic unsaturation was 0.043 mole or .342 mole/mole of fatty acid.

Reduction with Pyridine (Run B). After ozonization in a similar experiment, the solution and reaction flask were flushed with nitrogen, and 26.0 g of pyridine (10% by wt of solution) and 0.1 g of 10% palladium-on-charcoal catalyst were added. Hydrogen was bubbled through the solution at room temperature and atmospheric pressure for 195 min until the peroxide test was negative. The solution was filtered, and 200 ml of methylene chloride were added to the filtrate. The solution was washed with dilute hydro-

TABLE I

Ozonization of Soybean Oil in Ethyl Acetate-Methanol Solvent (1.538 Moles unsaturation/mole fatty acid in original oil)

Run	Reaction variables: Ozone consumed,[a] % of theory	Reaction variables: Pyridine present during: Ozonization	Reaction variables: Pyridine present during: Hydrogenation	Product analysis, moles/mole of fatty acid: Unsaturation	Aldehyde	Methyl ester[b]	Total (estimated)
A	40	—	—	0.342	0.325	0.057	0.724
B	35	—	+	0.681	0.329	0.029	1.039
C	37	+	+	0.728	0.264	0.082	1.074
D	77	—	—	0.140	0.473	0.119	0.732
E	82	—	+	0.375	0.610	0.059	1.044
F	123	—	—	0	0.612	0.167	0.779
G	109	—	+	0	0.714	0.079	0.793

[a] Ozone was consumed by solvent to a small extent as well as by unsaturation.
[b] Methyl ester in weight fraction from GLC analysis.

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TABLE II
Gas Liquid Chromatography of Aldehyde Oils
(As methyl esters)

Run	Composition, area %							
	Methyl azellaaldehydate dimethyl acetal	Dimethyl azelate	Methyl palmitate	Methyl stearate	Methyl oleate	Methyl linoleate	Methyl linolenate	Unknown
Original oil[a]	0	0	10.9	3.4	26.0	53.6	6.2	—
A	21.1	5.7	15.1	21.0	34.4	1.5	0.6	0.5
B	15.1	2.9	18.5	3.9	25.4	33.1	0.6	1.4
C	18.0	8.7	16.5	2.2	20.6	30.0	1.4	2.6
D	36.9	12.5	16.7	10.8	10.8	2.1	0	10.2
E	49.9	5.9	18.8	5.4	11.7	6.7	0	1.4
F	59.8	16.8	17.1	5.2	0	0	0	1.1
G	69.1	7.9	16.8	5.4	0	0	0	0.8

[a] Original soybean oil before ozonization.

chloric acid and then with water until neutral. Each water wash was backwashed with a small amount of CH_2Cl_2. The aldehyde oil was isolated by removal of the volatile aldehydes (principally caproaldehyde and pelargonaldehyde) *in vacuo* as before. The yield of unsaturated aldehyde oil was 34.78 g with a carbonyl conversion in the aldehyde oil of 39.5% (equivalent to 0.991 aldehyde groups/molecule or 0.329 mole/mole of fatty acid). Unreacted olefinic unsaturation in the aldehyde oil was 0.096 mole or 0.681 mole/mole of fatty acid.

Analytical Methods

Hydroxylamine-Hydrochloride Method of Determining Carbonyl. The procedure followed was that described by Siggia (5), except aqueous alcoholic solutions were used. A sample of aldehyde (containing 20–25 milliequivalents) was weighed into a 125-ml glass-stoppered Erlenmeyer flask, 25 ml of 1*N* $NH_2OH \cdot HCl$ in 75% alcohol was added, and the mixture was refluxed for 2 hr. After reflux, the sample was cooled and titrated on a Beckman automatic titrimeter with 0.1*N* NaOH to the pH of a blank $NH_2OH \cdot HCl$ solution (approximately 3.2).

Microhydrogenation of Aldehyde Oil to Determine Total Unsaturation. A modified hydrogenation procedure described by Ogg and Cooper (2) was employed. Four milliliters of *n*-butyl propionate, 1 ml of glacial acetic acid, and about 20 mg of H_2-saturated platinum catalyst were used. Other solvents such as isopropanol, butanol, triacetin, glacial acetic acid, and *n*-hexane (commercial) were unsatisfactory because of insolubility of the sample in the solvents or because of excessive hydrogen uptake by the solvents.

The platinum used for the catalyst reduced both the aldehyde and olefinic unsaturation. Olefinic unsaturation was then found by subtracting the value for the carbonyl unsaturation, as determined above, from the total.

Interesterification of Aldehyde Oils with BF_3-MeOH Reagent for GLC Analyses. The procedure used by Metcalf and Schmitz (1) for the esterification by fatty acids with BF_3-methanol reagent was modified somewhat to obtain simultaneous methanolysis and acetalization of the aldehyde oil. Two grams of aldehyde oil were refluxed in the presence of 10 ml of 10% boron trifluoride in methanol for 1 hr. The solution was cooled and neutralized with a dilute sodium bicarbonate solution to the methyl orange end point. The mixture was then extracted three times with a small amount of CH_2Cl_2, and the methylene chloride fractions were combined and washed with H_2O until neutral. Two washes were required. The solution was dried, filtered, and evaporated under nitrogen on a steam bath to remove solvent. Gas-liquid chromatographic (GLC) analysis was run on the residue, which consisted of methyl esters of the fatty acids, the dimethyl acetal of methyl azelaaldehydate, and dimethyl azelate.

TABLE III
Some Characteristics of Aldehyde Oils

Run	Molecular weight[a]		Aldehyde groups/mole glyceride	Refractive index n_D^{30}	Gardner viscosity at 25C	Gardner color
	Calculated	Observed				
A	778	820	0.98	1.4540	Semisolid	10
B	778	743	0.99	1.4720	I	9
C	778	776	0.79	1.4713	G	15
D	652	679	1.42	1.4630	J (Contains solids)	8
E	652	690	1.83	1.4680	G	13
F	600	658	1.84	1.4620	E	10
G	600	633	2.14	1.4635	G	12

[a] Molecular weights determined by the freezing point depression method in cyclohexane.

Gas-Liquid Chromatography. A Pye-Argon chromatograph with a Radium D ionization detector and an integrator was used. The column was 4 ft by ¼ in. glass tubing packed with 10% Craig polyester succinate on Chromosorb. The temperature of the column was 160C; pressure, 470 mm; gas flow rate, 50 cc/min.

Results and Discussion

In the preparation of either a monoaldehyde oil (Runs A and B, Table I) or a dialdehyde oil (Runs D and E), twice as much unsaturation remained and one-half or less byproduct azelate ester was formed when pyridine was present during hydrogenation than when absent. Similarly, less than half of the azelate ester was formed in completely ozonized soybean oil (Runs F and G). Further, in the absence of pyridine, the monoaldehyde oil obtained was a solid at room temperature although the unsaturation had not been completely hydrogenated; dialdehyde oil was partially solid at room temperature.

Although the presence of pyridine in the ethyl acetate-methanol solvent system during hydrogenation gave improved results, its presence during ozonization led to a number of side reactions. This effect is in contrast to the experiments with methyl oleate, wherein ethyl acetate was absent and pyridine was present during both ozonization and hydrogenation steps. Perhaps ethyl acetate prevented hydrogen bonding between methanol and pyridine with the result that pyridine oxide formation became a competitive reaction. This result is brought out in Run C, Table I, in which for the same ozone consumption, less unsaturation was attacked by the ozone, less aldehyde formed, and more methyl ester formed than when pyridine was present only during hydrogenation (Run B). Accordingly, optimum results were obtained when pyridine was present during hydrogenation only.

Analyses of the aldehyde oil products were carried out by several different means. Microhydrogenation gave the total amount of olefinic unsaturation and carbonyl. Analysis by hydroxylamine hydrochloride reagent then gave the carbonyl content alone, and the difference between the two determinations gave the amount of olefinic unsaturation alone. The aldehyde oils were converted to volatile products for GLC analysis by treatment with BF_3-methanol. Simultaneous methanolysis of the glyceride and acetalization of the aldehyde groups then occurred. The GLC analyses (Table II) confirmed the results obtained by chemical analysis and illustrated very well the improved results obtained by the use of pyridine. Thus, in the prepara-

tion of monoaldehyde oil (Runs B and C) 21.0% of stearate was formed when hydrogenation was conducted in the absence of pyridine. When pyridine was present, the stearate content increased only slightly over that of the original oil (3.4%).

Characteristics of these aldehyde oils are listed in Table III. One product (Run A) had considerably higher molecular weight than expected, probably because aldehyde condensation reactions occurred during work-up. The products obtained when pyridine was present during hydrogenation were, generally, slightly more colored and more viscous than those obtained when pyridine was omitted.

Studies on the reactions of these unsaturated aldehyde oils are under way.

Conclusion

The preparation of a potentially useful chemical intermediate containing both unsaturated and aldehydic functionality by partial ozonization of soybean oil has been demonstrated.

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Incorporation of Linolenic-1-C^{14} Acid into Eicosapentaenoic and Docosahexaenoic Acids in Fish

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Abstract

Following intraperitoneal injection of methyl linolenate-1-C^{14} into kelp bass, *Paralablax clathratus,* the highly polyunsaturated fatty acids of their body fats were concentrated by low temperature crystallization from acetone, and eicosapentaenoic and docosahexaenoic acids were isolated from the concentrate by reversed-phase chromatography and hydrogenated. The resulting arachidic and behenic acids were degraded stepwise to margaric acid, and the distribution of activity was determined. The results indicate that the injected linolenic acid was converted to eicosapentaenoic acid and the latter incorporated into docosahexaenoic acid. A probable conversion pathway is linolenic acid ⟶ 6,9,12,15-octadecatetraenoic acid ⟶ 8,11,14,17-eicosatetraenoic acid ⟶ 5,8,11,14,17-eicosapentaenoic acid ⟶ 7,10,13,16,-19-docosapentaenoic acid ⟶ 4,7,10,13,16,19-docosahexaenoic acid.

Introduction

THE EVIDENCE deduced from many studies of the structure of the polyunsaturated fatty acids of fish lipids leaves little doubt that they resemble those found in mammalian lipids in possessing the divinyl methane structure almost exclusively (1–8). Moreover, previous work from this laboratory (9) indicated that the same types of reactions (i.e. chain elongation and dehydrogenation) contribute to the transformation of exogenous linoleic acid to arachidonic acid in a fish *(Tilapia mossambica),* as previously shown to obtain in the rat (10–13). However, the fatty acids generally found in fish on a natural diet are largely of the linolenic type, having the terminal structure $CH_3-CH_2-CH=$ (7) and thus leading to the assumption that they are derived largely from dietary linolenic acid. This view has been strengthened by studies in which it was shown that in young mullet, dietary linolenic acid gave rise to increased deposition of eicosapentaenoic and docosahexaenoic acids, as indicated by gas chromatographic analysis (14).

The present experiments were designed to test the logical supposition that the fed linolenic acid is incorporated *in toto* into the chains of eicosapentaenoic and docosahexanenoic acids. Such a pathway has been indicated in rats by Steinberg et al. (15) and by Klenk and Mohrhauer (17).

Experimental

Synthesis of methyl linolenate-1-C^{14}. Methyl linolenate-1-C^{14} was synthesized following the method of Nevenzel and Howton (16). In the process of synthesis the by-products formed during the Hunsdiecker decarboxylation and debromination of silver 9,10,12,-13,15,16-hexabromostearate were identified (18).

Treatment of fish. Three mature kelp bass *(Paralablax clathratus),*[3] with an average body weight of 167.3 g and average body length of 20.6 cm, were used as experimental animals. The fish were given an intraperitoneal injection of 600 mg methyl linolenate-1-C^{14} (for a total of 0.25 mc) diluted with pure inactive methyl linolenate,[4] and put into a 24 × 7¼ × 7¾ in. salt water glass aquarium at 19C. The aquarium was then placed in a constant temperature laboratory bath[5] set at 9.6C, since previous experience had indicated that a lower temperature might increase the yield of polyunsaturated acids. The temperature in the aquarium reached that of the bath in 110 min. After 5.5 hr of this cold treatment, when two of the fish were becoming weak and only one remained active, they were killed by freezing.

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[2] This paper based partially on work performed under Contract AT(04-1)GEN-12 between the Atomic Energy Commission and the University of California.

[3] Caught by John Prescott, Marineland of the Pacific, Palos Verdes, Calif.
[4] Obtained from the Hormel Institute, Austin, Minn.
[5] American Instrument Co., Inc., Cat. No. 4-8605.