NEW PRODUCTS FROM FISH OILS

Part III - Preparation and Separation of Fatty Alcohols Made from Fish Oils--A Preliminary Study

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ABSTRACT

Sodium reduction of methyl palmitate, methyl stearate, methyl oleate, and of the natural glycerides of menhaden and pilchard oils was carried out in order to prepare the corresponding long-chain fatty alcohols. The resulting prepared fatty alcohols were then fractionated into saturated and unsaturated alcohols by acetone crystallization at low temperature. A complex mixture of unsaturated alcohols prepared from pilchard oil was used in studies of the efficiency of various separation methods. The methods studied were fractional distillation, urea countercurrent distribution, and column chromatography. Ultraviolet spectrophotometry was used to measure qualitatively the degree of unsaturation in the various fatty-alcohol fractions.

INTRODUCTION

A project has been undertaken at the Seattle Technological Laboratory to prepare long-chain unsaturated fatty alcohols from fish oils and to study the corresponding physical properties of these alcohols, with the ultimate objective of providing information that will stimulate the development of new fish-oil products (Stansby 1956).

Why study fatty alcohols? The fatty alcohols are extremely valuable in organic research because of the wide variety of other substances that can be prepared from them. They are among the most important of the synthetic fatty derivatives (Ralston 1948) that can be prepared from fatty acids and esters.

Some of the reactions of primary aliphatic alcohols are esterfication, oxidation or catalytic dehydrogenation to aldehydes, dehydration to unsaturated hydrocarbons, and halogenation to alkyl halides. The alkyl halides thus formed from fatty alcohols also act as important intermediates in the synthesis of other compounds. The formation of various esters,[1] aldehydes, and alkyl halides offers the greatest potentialities for fatty alcohols with respect to chemical synthesis of new products or the modification of older established products.

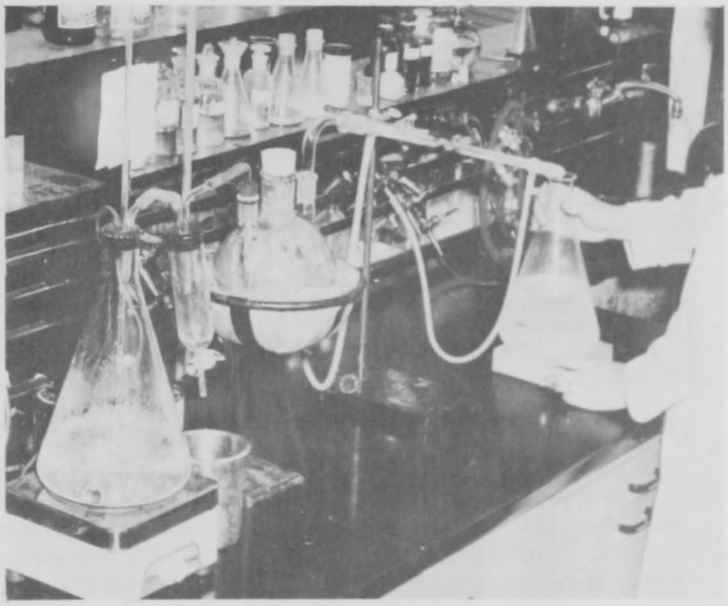

FIG. 1 - HYDROLYSING A SODIUM-REDUCTION-MIXTURE OF FISH OILS IN THE PREPARATION OF LONG-CHAIN FATTY ALCOHOLS.

Owing to the fact that unsaturated alcohols of high molecular weight are difficult to prepare in a state of high purity, few reliable constants for such compounds have been reported in the literature (Ralston 1948). The initial work at the Seattle laboratory therefore has been to obtain information regarding a satisfactory method of isolating pure polyunsaturated fatty alcohols and to report in the scientific literature

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[1] FOR EXAMPLE $R'COOR''$, WHERE R'' IS THE PORTION DERIVED FROM THE LONG-CHAIN FATTY ALCOHOL.

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reliable constants for these alcohols. The techniques commonly employed for separating fatty acids and their derivatives are as follows: (1) fractional distillation (Weitkamp 1955), (2) fractional solvent crystallization (Brown 1955), (3) countercurrent distribution (Dutton 1955), (4) chromatography (Allen 1955), and (5) fractionation by the formation of inclusion compounds (Schlenk 1954). The research work here has employed all of these techniques.

EXPERIMENTAL RESULTS AND DISCUSSION

<u>PREPARATION OF FATTY ALCOHOLS</u>: To prepare long-chain fatty alcohols from fish oil, we used a sodium reduction process (Hansley 1947; Pryde 1951). The method is as follows: To a rapidly-stirred suspension of 92 grams (4.00 moles) of finely divided sodium, in 100 milliliters of refluxing xylene, is slowly added a mixture of 300 grams of fatty acid glycerides, 264 milliliters (214 grams, 2.10 moles) of 4-methyl-2-pentanol (the reducing alcohol), and 600 milliliters of xylene. After the reaction of fatty-acid glycerides with the sodium and reducing alcohol is complete the resulting mixture is hydrolyzed.

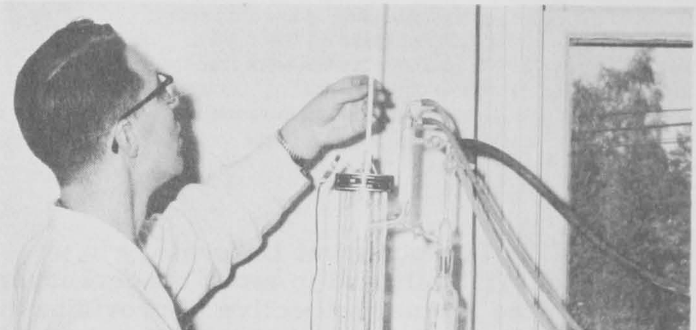

FIG. 2 - FRACTIONAL DISTILLATION APPARATUS WITH PROVISION FOR INTERNAL-INERT ATMOSPHERE THROUGH THE USE OF AN ATTACHED NITROGEN-FILLED BALLOON.

The hydrolysis apparatus used (fig. 1) provides for simultaneous steam distillation of the xylene and the 4-methyl-2-pentanol. At the start of the hydrolysis, 300 milliliters of boiling water is added to a 3-liter, three-neck "hydrolysing flask." Steam, from a steam generator, is passed through the hot water via a gas-inlet tube attached to one of the side arms of the hydrolysing flask. The steam generator is fitted with a water-vapor trap between it and the gas-inlet tube. To the other side arm of the hydrolysing flask is connected a Liebig-type condenser. At the end of the condenser is a flask to receive the condensed xylene and reducing alcohol.

After the steam has passed through the hydrolysing flask for 3 minutes, the sodium-reduction mixture is added through the center neck of the flask, by the use of a large funnel-tube that extends beneath the surface of the hot water. The joint at the center-neck of the flask is tightly sealed to prevent air leakage. The long-chain fatty alcohols, liberated during the hydrolysis, are recovered from the hydrolysis-mixture by an ethyl ether-petroleum ether extraction.

Experience with the details of the sodium-reduction process was gained through the preparation of cetyl alcohol (1-hexadecanol) from methyl palmitate, octadecyl alcohol (1-octadecanol) from methyl stearate, and oleyl alcohol (9-octadecen-1-ol) from methyl oleate. The process was then applied to the natural glycerides of menhaden and pilchard oils. With the latter oils, the crude mixtures of saturated and unsaturated fatty alcohols were obtained as light-brown, mildly viscous liquids in yields of 85 to 90 percent. The saturated and unsaturated fractions were separated by crystallization in acetone at -18° C. It was the unsaturated fraction, obtained in the foregone manner, that became the starting material for further studies of the separation and the identification of unsaturated fatty alcohols.

FRACTIONAL DISTILLATIONS: A sample of mixed unsaturated fatty alcohols (prepared from pilchard oil) was used to study the effects of fractional distillation. The distillation apparatus (fig. 2) included an electrically-heated column jacket for simulating adiabatic conditions, a still-head with internal reflux condenser and adjustable take-off for controlling the rate of distillate take-off for a given reflux ratio (usually about 8 to 1), and a pressure regulator for maintaining a constant pressure during distillations.

The distillations were carried out at a pressure of 0.1 to 10.0 millimeters of mercury. A considerable amount of decomposition and polymerization occurred during the course of a distillation, thereby decreasing the yield of the more highly unsaturated fatty alcohols. The data in table 1 show iodine numbers and refractive indices of the distilled fractions. The yield of total distillate indicated that a large amount of high boiling, polyunsaturated alcohols were not recovered. Unrecovered pot residue amounted to as much as 34.3 percent of the starting material for one of the best distillations.

Table 1 - Analytical Data for Alcohol Fractions Obtained by Fractional Distillation

Fraction[1]	Boiling Point		Refractive Index	Iodine Number	Hydroxyl Equivalent[2]
	Temperature	Pressure			
No.	°C.	Mm. Hg.	n_D^{30}		
1	113.0-126.0	0.50	1.4458	26.6	234.6
3	139.0-141.0	0.50	1.4562	104.0	252.8
9	163.0-165.0	0.51	1.4620	132.5	277.2
11	167.0-170.0	0.63	1.4630	134.2	290.0
12	170.0-172.0	0.50	1.4611	131.4	285.0
17	187.0-189.0	0.60	1.4843	198.2	311.8
18	189.0-191.0	0.65	1.4899	216.4	311.7
21	204.0-208.0	0.65	1.4960	199.8	329.9
22	208.0-210.0	0.65	1.4980	222.9	348.3

[1] THESE ARE THE MAJOR FRACTIONS THAT APPEARED TO CONTAIN NEARLY PURE COMPOUNDS.
[2] THEORETICAL MOLECULAR WEIGHTS OF SATURATED ALCOHOLS ARE AS FOLLOWS: C_{16}, 242; C_{18}, 270; C_{20}, 298; C_{22}, 326; C_{24}, 354.

Oxygen-free nitrogen was bubbled through the pot charge while the distillation proceeded, in order to lessen the loss due to polymerization. This bubbling was done by the use of a nitrogen-filled balloon attached to a capillary tube (fig. 2) inserted into the distilling flask containing the charge of unsaturated fatty alcohols. Additional work along these lines is postponed until distillations can be studied on a spinning-band type of distillation column such as the type described by Murray (1951).

UREA COUNTERCURRENT DISTRIBUTION: In another phase of the research, a study was made of the utility of fractionation by the use of urea inclusion compounds incorporated into a countercurrent distribution system (Domart, Miyauchi, and Sumerwell 1955). It was shown that the urea-countercurrent-distribution technique makes possible the separation of highly unsaturated alcohols. To what extent this separation is complete has not yet been determined for mixtures occurring naturally. The experiments performed thus far have shown an abnormal weight-distribution curve for urea countercurrent distribution of the unsaturated alcohols prepared from pilchard oil. Nevertheless, refractive indices of the fractions obtained have indicated that a separation was made. The fractions separated in this manner are currently being studied as to the degree of unsaturation and the chain-length of the respective alcohols.

CHROMATOGRAPHIC SEPARATIONS: The alcohols obtained from fractional distillation have appeared colorless, whereas those obtained from the urea countercurrent distribution have appeared yellowish. Studies on column chromatography were started to determine to what extent the various fractions separated by these other methods could be further purified by the removal of polymerized materials. The chromatography has been carried out principally with fractions from the countercurrent distribution.

Preliminary work was undertaken to find an adsorbent that would remove the impurities and still permit good yields of the purified fractions. A mixture of Fuller's earth, powdered silica, and powdered calcium oxide was found to give fractions much lighter in color. Inasmuch as the fractions going into or coming out of the column were not protected by an inert atmosphere, it is believed that this adsorbent will be more satisfactory when used with nitrogen.

DETECTION OF UNSATURATION: Ultraviolet spectrophotometry has been used to determine the extent of conjugated and nonconjugated double bonds in the polyunsaturated alcohols being studied. The ultraviolet spectra is being used to determine the success in isolating the more highly unsaturated fatty alcohols by the various separation techniques. Alkali isomerization of nonconjugated unsaturation is employed with ultraviolet absorption when the number of double bonds in the alcohols of the particular fractions is being measured.[2]

A certain amount of conjugation has taken place during the sodium-reduction process, as the ultraviolet spectra of the crude alcohol mixtures have indicated. This was expected, since the method used to isomerize and measure the nonconjugated double bonds involves treatment of the unsaturated alcohols with strong alkali [2] at a high temperature (180° C.) and since the unsaturated portions of the prepared alcohols are in contact with a strongly alkaline mixture at a temperature close to the boiling point (138° C.) of the xylene solvent during the sodium reduction process. The amount of isomerization and possible decomposition that occurs during this treatment can be minimized by working rapidly and by utilizing an inert atmosphere over the reaction mixture. To obtain the alcohols that correspond to the naturally-occurring fatty acid glycerides, we will have to develop a method for the removal of the conjugated unsaturated alcohols that occur via the sodium-reduction process and/or other reactions.

Abu-Nasr and Holman (1954) have pointed out that conjugation of unsaturated-fatty-acid esters occurs during fractional distillation. They therefore suggested that fractional distillation be avoided as a final step in purifying polyunsaturated esters. This suggestion would apply also to the polyunsaturated alcohols. For this reason, the detection of conjugated unsaturation in fractions of distilled unsaturated alcohols is not surprising. It is also expected that such analytical data as iodine numbers will be erroneously low [3] when quantities of conjugated unsaturated alcohols are present in the various fractions.

It has been indicated here that double bonds are conjugated by an alkaline treatment during sodium reduction and by fractional distillation. Thus, it can be seen why difficulties may be encountered in isolating pure fractions of nonconjugated polyunsaturated fatty alcohols. Displacement chromatography is one method of isolating pure compounds of this type (Abu-Nasr and Holman 1954). Another method that has

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[2] AMERICAN OIL CHEMISTS' SOCIETY TENTATIVE METHOD CD 7-48.

[3] IODINE IS VERY SLOW TO REACT WITH CONJUGATED DOUBLE BONDS; THEREFORE EXPERIMENTALLY-DETERMINED IODINE NUMBERS ARE LOW WHEN THE CONVENTIONAL ANALYTICAL METHODS ARE USED.

proven successful for the separation of fatty materials is the formation of urea inclusion compounds (Schlenk 1954); (Schlenk and Holman 1950); (Abu-Nasr, Potts, and Holman 1954). Both of these techniques are to be given additional study at this laboratory.

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