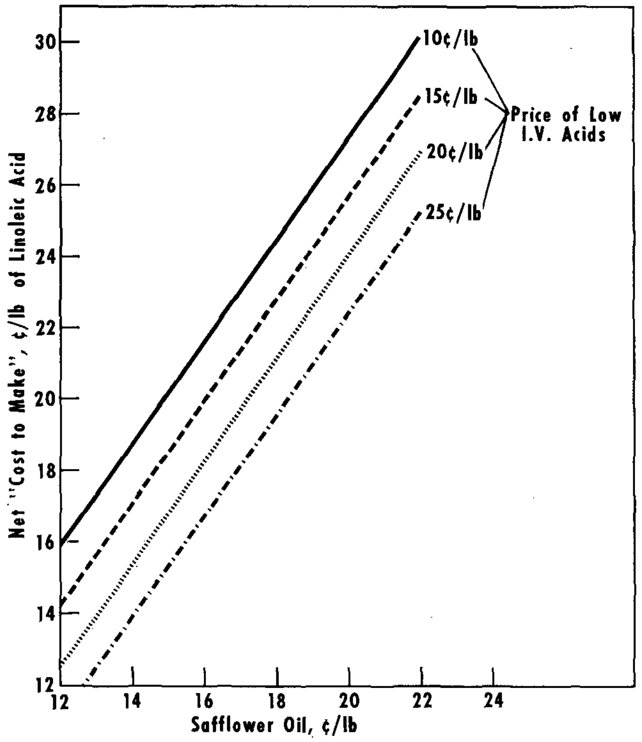

FIG. 3. Effect of changes in price of safflower oil on net "cost to make" of linoleic acid at various prices for low I.V. acids.

be produced in the plant, operations could be adjusted to meet variations in market conditions thereby improving the economics of the individual processes.

Market potential for purified linoleic acid is exceedingly difficult to predict and will be determined to some extent by its selling price. In some applications, such as in plasticizers, substitution of linoleic acid for natural oil may impart improved qualities to a particular product. The use of the acid may possibly be justified under such conditions even though its price may be higher than that of the oil.

For at least one application, an industry source has indicated that linoleic acid at about 40 cents per pound may find a potential market. With a net "cost to make" of 21 cents per pound of 97% linoleic acid, apparently the selling price would be substantially under 40 cents once normal production rates are attained.

Even though linoleic acid must compete in some applications with products now on the market, it would appear that a "cost to make" of 21 cents a pound should create an interest in the product.

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The Synthesis of Tritium Labeled 9, 10-Oleic Acid[1, 2]

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Abstract

Tritium labeled 9,10-oleic acid was prepared from stearolic acid by reduction with tritium gas, in the presence of 5% Palladium on Charcoal catalyst, at room temperature and under partial vacuum. No stearic or elaidic acids were formed. Unreacted stearolic acid was removed by low temperature crystallization from Skellysolve F. The tritium labeled *cis* 9,10-oleic acid was prepared with a specificity of greater than 90% of the activity at the 9 and 10 positions. Specific radioactivity of the oleic acid was 1000 mc of Tritium/g. Tritium labeled stearic acid with a specific activity of 927 mc of Tritium/g was also prepared.

Introduction

EXPOSURE to tritium gas can cause non-specific labeling. The Wilzbach technique (1) is an application of this fact for producing non-specifically labeled compounds. Recently, Dutton and Nystrom (2,3) produced labeled saturated fatty acid esters of high specific activity by their exposure to tritium gas. On the other hand, unsaturated fatty acids were found to preferentially hydrogenate, rather than substitute in the carbon chain, when exposed to tritium gas (4).

In the present study, a method is reported which gave *cis* 9,10-oleic acid, labeled with a high degree of specificity at the double bond. The oleic acid obtained was free from radiochemical impurities, and was obtained in good yield, as well as high specific activity.

Experimental

Methylation. Diazomethane (5) was prepared from "DIAZALD".[4] Esterification was carried on at room temperature for 10 min. A solution of 0.4 g of potassium hydroxide in 10 ml 96% ethanol was poured into a round-bottom flask and 2.0 g "DIAZALD" in 30 ml ether added. If cloudy, more ethanol was added to the mixture. Boiling chips were added, a condenser attached, and the solution warmed at about 50C on a steam bath. The ethereal diazomethane solution was collected in a round-bottom flask that was cooled below −10C in a dry ice—acetone bath. A calcium chloride tube was attached to keep out moisture. The reaction was terminated when the solution lost its yellow color. The solution of diazomethane was transferred to a graduated cylinder fitted with a ground glass stopper and methanol was added to make a 10% methanol in ether solution. Schlenk and Gellerman (6) reported that esterification was slow or incomplete in pure ether solution. Also following the procedure of Schlenk and Gellerman, esterification was

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[2] Presented in part at the Federation of American Societies meeting for Experimental Biology, 1961.

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[4] "DIAZALD" is the trade name for N-methyl-N-nitroso-P-toluenesulfonamide, obtained from Aldrich Chemical Co., Inc.

carried on at room temperature for 10 min. Removal of ether and diazomethane under vacuum increased the stability of the esters which otherwise became colored yellow in a few days, even in the refrigerator under nitrogen. A further precaution to prevent yellowing involved the storage of esters in solution.

Radioassay. Radioactivity was determined with an automatic Packard Tri-Carb Liquid Scintillation Spectrometer. Wheaton 5-Dram Liquid Scintillation sample vials were used. The photomultiplier high voltage was set at Tap 6 and discriminator settings of 10–50 V and 50–100 V employed. Samples were taken up in 15 ml of a solution containing 3 g PPO [6] and 50 mg POPOP [7]/liter of redistilled toluene.

Gas Chromatography. An Aerograph Gas Chromatograph was used. Carrier gas was helium; the column was diethylene glycol succinate on firebrick (DEGS) (10 ft); maintained at 220C.

Samples were taken for radioactivity determination every 30 sec by insertion into the exit of a tapered glass tube, stuffed with cotton, and soaked with methanol. The used tubes were placed in scintillation vials until completion of the run. The cotton was removed by insertion of a sturdy wire through the narrow end of the tube. The cotton and tube were replaced in the scintillation vial until the methanol evaporated from the cotton as methanol would cause quenching. When free from methanol, 15 ml of scintillation solution was added and the glass tube and cotton soaked for several hours. After removal of the glass tube and the cotton by hooking with a wire, radioactivity was determined by scintillation counting. The efficiency of this technique of sample collection was tested with a pure sample of tritium labeled methyl oleate. The sum of all fractions amounted to 233,300 counts/min, while the control sample gave 220,290 counts/min. The deviation above 100% recovery was within the experimental error of counting.

Preparation of Stearolic Acid. Stearolic acid was prepared by a modification of the alcoholic-potassium hydroxide method of dehydrodehalogenation that has been reported in various forms by numerous authors (7,8,9,10,11,12). Yields were comparable with those obtainable with alcohol solvents (30–40%), but not as high as that reported by Khan, Deatherage, and Brown (13) who dehydrodehalogenated with sodamide in liquid ammonia. The method reported here, however, was rapid, and the stearolic acid was recovered in a relatively pure state.

Two hundred ninety-six and five tenths g of methyl oleate was slowly brominated with 160 g of bromine while chilled in an ice-water bath. The excess bromine was removed and the reaction mixture transferred to a beaker. Separately, in a large beaker, 336 g potassium hydroxide was dissolved in 216 ml ethylene glycol by heating on a hot plate, while bubbling nitrogen slowly through the solution (to prevent darkening), complete solution occurred overnight. The warm solution of ethylene glycol-potassium hydroxide was slowly poured into the beaker containing dibromostearic acid. An instantaneous reaction occurred, causing foaming. The mixture was covered with a watch glass and heated to a solution temperature of 140–170C for five hr. The mixture was cooled in ice, acidified with concentrated hydrochloric acid to phenolphthalein, and to methyl orange. The stearolic acid was extracted with two liters of Skellysolve F, washed with water and cooled to 1C overnight. The crude stearolic acid which precipitated was removed on a Buchner funnel. The crude material had a mp of 41–44C. The mother liquor contained an appreciable quantity of stearolic acid and was concentrated and recrystallized. Purification was accomplished by repeated crystallization from 100% acetone and from 84% acetone (aqueous). A constant mp of 46.2–46.6C was obtained. Recrystallization from ethyl ether gave large crystals but recovery was not high. The final mp was 46.4–46.6C, taken on a temperature gradient of 1C/min, until near the melting point, and then the temperature was increased at the rate of 0.2C/min. Gas chromatography of the methyl ester on a DEGS column, 220C, showed that the material was of high purity. The stearolic acid was stable and showed no signs of deterioration after storage for one year at room temperature under an atmosphere of nitrogen, in a brown bottle.

Reduction with Palladium on Charcoal Catalyst (8)

Model Experiment. Oleic acid was prepared from stearolic acid by hydrogenation in ethyl acetate solution containing 20% pyridine, in the presence of 5% Palladium on Charcoal (Pd/C) catalyst. The procedure was the same as that described for preparation of tritium labeled oleic acid, and was derived from the procedure of Hofmann and Sax (14) who prepared *cis*-11-octadecenoic acid.

The non-radioactive oleic acid, thus prepared, was light yellow in color, with n_D^{28} of 1.45605 and contained no *trans*-isomers (infrared) or triple bond (ultraviolet). Due to traces of solvent the iodine number was 87.1 (theory 89.9). When converted to the methyl ester and analyzed gas chromatographically, using a 10 ft Craig Polyester succinate on firebrick column at 220C, it was shown that no stearic or stearolic acids were present. Only methyl oleate appeared.

Preparation of Tritium Labeled Oleic Acid. One-tenth g of 5% Pd/C catalyst and 7 ml of ethyl acetate containing 20% pyridine (dried over potassium hydroxide), was allowed to saturate with hydrogen for two hr, at room temperature, with stirring. One-half g of stearolic acid in 4 ml of ethyl acetate (20% pyridine) was then injected through the constriction by means of a syringe and a long needle. The syringe was washed twice with 2 ml of the ethyl acetate (20% pyridine) solution and the washings injected into the flask. The flask was chilled with liquid nitrogen, a vacuum drawn (0.001 mm Hg), and the constricted neck closed off. The Toepler pump was connected, and one cubic centimeter of tritium gas was forced in through the break-seal joint adaption. Forty cubic centimeters of hydrogen were then added, by the same means. The solution was stirred for 2 hr, at room temperature. It was freed of catalyst by filtering through a glass sintered filter, and the solvent evaporated. An aqueous methanolic solution of hydrochloric acid was added to remove pyridine and the solution extracted with Skellysolve F. The extract was washed with water and dried with anhydrous sodium sulfate. Both the aqueous and the Skellysolve F extract were assayed for radioactivity. The Skellysolve F solution contained 500 mc of tritium radioactivity, whereas the aqueous extract contained only 0.68 mc. The Skellysolve F solution was concentrated to a volume of about 12 ml and then cooled to −63C in a dry ice-acetone mixture. The solution was allowed to warm, over an 8 hr period, to −37C and the precipitate removed by filtration through a Buchner funnel

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[5] Wheaton Glass Co., Millville, N. J.

[6] PPO-2,5-Diphenyloxazole, Pilot Chemicals, Inc., Waltham, Mass.

[7] POPOP-p-Bis-2-(5-Phenyloxazolyl)-Benzene.

packed in dry ice. About 9 mg of stearolic acid was removed in this manner. A sample of the mother liquor which contained the tritium labeled oleic acid was methylated with diazomethane. The tritium labeled methyl oleate was diluted with a standard mixture of methyl esters and analyzed by gas chromatography on a 10 ft DEGS column, at 220C, with simultaneous collection of samples at 30 sec intervals, as timed with a stop watch. No radioactivity appeared at the stearate fraction (Fig. 1). The solvent was removed from the oleic acid and replaced with reagent grade benzene (50 ml) for storage.

Preparation of Tritium Labeled Stearic Acid. Tritium labeled stearic acid was prepared in the same manner as described for the preparation of tritium labeled oleic acid, except that after the initial uptake of hydrogen and tritium an additional 100 ml of hydrogen were added, and the reaction was allowed to proceed for an additional 2 hr. Stearic acid was separated from the oleic acid, and purified, by repeated crystallization from acetone. Gas chromatographic analysis of the methyl ester and a melting point determination of the free acid showed the stearic acid to be quite pure chemically. Radioactivity of the stearic acid was 927 mc/g.

The oleic acid which remained in the acetone was purified by the addition of non-radioactive stearic acid and by recrystallization of the diluted tritium labeled stearic acid from acetone solution. When gas chromatographically pure, the oleic acid had an activity of 25.5 mc/g. The difference in activity between the stearic and oleic acids possibly indicates that the hydrogen on the catalyst was first used (in reducing stearolic acid to oleic acid), and then the hydrogen-tritium gas mixture was utilized through the catalyst to form the tritium labeled stearic acid.

Oxidative Cleavage of the Double Bond. The procedure of oxidation was essentially that of Jones and Stolp (15). Two oxidations were carried out, oxidation #1 an 11 hr oxidation, and oxidation #2 a 45 hr oxidation. The procedure, described in detail is that of oxidation #2, however, oxidation #1 was similar.

One hundred fifty-eight and two tenth mg of oleic acid (124 uc/g) was added to a 500 ml ground glass stoppered Erlenmeyer flask which had been freed of oxygen by flushing with purified nitrogen. The addition of 32 ml of 0.5% potassium hydroxide solution formed a soap which dissolved upon addition of 200 ml of distilled water. In a separate container, a solution of 95 ml 1.2% sodium meta-periodate and 1 ml 1.58% potassium permanganate was prepared and titrated to a pH of 5.2 by the addition of 5 ml 0.5% potassium hydroxide solution. The entire solution was added to the potassium oleate solution and made up to a total volume of 500 ml by the addition of water. The reaction was left to proceed for 45 hr, the solution then acidified with 5 ml of 5 N hydrochloric acid, and gaseous sulfur dioxide passed through it until the color of free iodine disappeared. About 1 mg of solid phenolphthalein was added, and the solution made basic with 20 ml of 28% potassium hydroxide solution. The solution was transferred to a beaker and evaporated, under a stream of air, on a hot plate to a volume of about 50 ml. The solution was transferred to a separatory funnel and after acidification, extracted three times with ethyl ether. The ether extracts were combined, washed four times with 10 ml volumes of saturated sodium chloride solution, dried with anhydrous sodium sulfate, and freed from solvent.

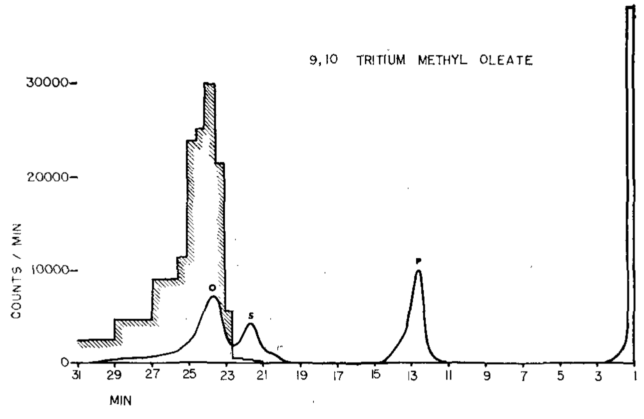

FIG. 1. Data obtained on gas chromatography of tritium labeled methyl oleate diluted with a standard mixture of the methyl esters of oleic (O) palmitic (P) and stearic acids (S) on a 10 ft DEGS column at 220C with simultaneous collections of samples at 30 sec intervals as timed with a stop watch.

A sample of the crude oxidation products was methylated with diazomethane and analyzed by gas chromatography using a 10 ft DEGS column at 220C. The azelate fraction contained no more than 6% of the radioactivity of the original oleic acid while the pelargonate fraction was found to contain no more than 14% of the radioactivity. The azelaic acid from the unesterified mixture was isolated by leaching off the pelargonic acid with Skellysolve F and then taking up the crude azelaic acid in ethyl ether. Preliminary crystallization was made from toluene. The azelaic acid was found to contain 9.54 uc/g (1.8 uc/m mole). Since the starting oleic acid contained 25 uc/m mole, the azelaic acid represented about 7% of the radioactivity in the oleic acid, which checked with the gas chromatographic data.

Dehydrodehalogenation of the tritium labeled oleic acid was performed as an additional check on the specificity of labeling. 4.65 g of methyl oleate was mixed with 0.0178 g (20 λ) of the tritium labeled methyl oleate, to form a new dilution with an activity of 14.85 uc/g. The solution was cooled to 0C and 0.83 ml of bromine added dropwise. Potassium hydroxide in ethylene glycol was added, and the mixture heated at 150C for 6 hr under nitrogen. The stearolic acid was isolated and purified as described in the preparation of stearolic acid. The stearolic acid was found to contain less than 6% of the activity of the oleic acid.

Results and Discussion

The results indicated that tritium labeled *cis* 9,10-oleic acid could be prepared with a specificity of greater than 90% of the activity at the 9 and 10 positions. Hoffman and Sax (14) used 5% Pd/C catalyst to prepare *cis*-11-octadecenoic acid. In the present study, the catalyst also proved to be stereospecific in the reduction of stearolic to oleic acid. Under the experimental conditions of this study, the uptake of hydrogen did not cease after the double bond had formed. However, by limiting the quantity of hydrogen and by a short reaction period, the formation of stearic acid was almost completely prevented. A small quantity of stearolic acid remained. Oxidative degradation of the purified oleic acid indicated that greater than 90% of the tritium radioactivity was at the 9 and 10 positions. Bromination of the oleate and subsequent dehydrodehalogenation confirmed the specificity of labeling; the technique of dehydrodehalogenation with potassium hydroxide was made more feasible for analytical, as well as preparatory, work by employing ethylene glycol as the solvent rather than the ordinary alcohols simplifying isolation and purification of the stearolic acid.

ACKNOWLEDGMENT

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Hydrazine-Reduced Linolenic Acids as a Source of C_9, C_{12}, and C_{15} Dibasic Acids[1]

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Abstract

Anticipated reduction in cost of hydrazine, resulting from its increased production as a rocket fuel, suggests the need to reconsider this chemical as an industrial reactant for fats. Hydrazine reduces the individual double bonds of linolenic acid (9,12,15-octadecatrienoic acid) nonselectively and without altering the position of residual unsaturation; thus the monoene fraction from hydrazine-reduced linolenic acid consists of an equimixture of the 9-, 12-, and 15-octadecenoate acids. Equal amounts of the C_9, C_{12}, and C_{15} dibasic acids are derived from this fatty acid mixture by oxidative cleavage along with the corresponding monobasic acids.

Kinetics of reactions, source of raw materials and reactants, and problems of processing and utilization of products are discussed.

Introduction

Linolenic acid by virtue of unsaturation at the 9, 12, and 15 carbon atoms constitutes a potentially unique commercial source of C_{15} dibasic acid. Required processing steps are reduction to the monoene level and oxidative cleavage at the double bond.

Recent studies have demonstrated not only that catalytically hydrogenated methyl linolenate is a complex mixture composed of saturates, monoenes, dienes, and trienes but that these classes of compounds are composed of numerous *cis* and *trans* isomers and positional isomers (1). Within the moneone fraction alone, nine components were demonstrated by capillary gas chromatography. Dibasic acid analysis indicated that the original double bonds at positions 9, 12, and 15 had moved to neighboring carbon atoms in nearly a random fashion. The dibasic acids, resulting from such a complex system, present a mixture difficult to fractionate by current processing equipment. Although catalytic hydrogenation of *alkali-conjugated* linoleic acid soaps (2) produces a mixture of 9, 10, 11, and 12 monoenes, it cannot yield the C_{15} dibasic acid available from linolenic acid.

In contrast with the complex mixture of isomeric monoenes obtained by the heterogeneous catalytic reduction of methyl linolenate, the monoenes from the homogeneous reduction with hydrazine are composed of 9-, 12-, and 15-octadecenoic acids exclusively. They are present in approximately equal amounts (3) and, as Aylward and Rao (4) demonstrated earlier, have no isomers. The monoenes from hydrazine-reduced linolenic acids might thus be expected to produce an approximately equal mixture of the saturated C_9, C_{12}, and C_{15} dibasic acids upon oxidative cleavage.

Anticipated reduction in cost of hydrazine, resulting from its production for a rocket fuel, suggests the need for reconsideration of this chemical as an industrial reactant for fats (5). By 1951 the price for hydrazine hydrate had dropped from a previous high of $5 per pound to $3.50 per pound. Currently it sells for $1.28. Estimates indicate that hydrazine produced by neutron irradiation of ammonia may result in prices as low as 15 to 25 cents per pound, depending upon rate of production. At this price, the chemical cost for reluction of a double bond per pound of linolenic acid is about 2½ cents. Depending upon yields, efficiency of the reaction, and value of the products, hydrazine reduction may become an economically feasible reaction. The present paper reviews the kinetic information on the reaction, considers certain schemes for processing, and presents certain experimental verifications of these concepts.

Mechanism of Hydrazine Reduction. A startling observation was made by Aylward that oxygen must be present if hydrazine reduction was to take place (4). This phenomenon was early verified in our experiments, but the complete mechanism was not known. Our experiments indicated that, based upon measurements of oxygen taken up, of nitrogen evolved, and of hydrazine reduced, the absorption of one-half mole of oxygen resulted in the liberation of

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