this solvent system was applied to the recrystallization of the acetal derivatives, Ia, IIIa and Va were successfully isolated from products containing up to 50% impurity (of aldehyde, mono- and di-esters) in the order of 80% recovery of 95–100% purity. Larger amt of impurity could be tolerated because the lower crystallization temp of the acetals allowed removal of most of the impurities crystallizing at higher temp. The products obtained by this crystallization procedure could be distilled rapidly without careful fractionation.

Partial ozonization of polyunsaturated fatty acids or esters followed by oxidative decomposition of the ozonolysis products is being studied further.

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Alkaline Isomerization of Methyl Crepenynate[1]

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Abstract

Methyl crepenynate (methyl-*cis*-9-octadecen-12-ynoate) is readily isomerized by potassium hydroxide in ethylene glycol to an 8,10,12-octadecatrienoic acid in good yield. A reaction carried out at 120C for 1 hr in 10% base is sufficient to convert the nonconjugated enyne system completely to conjugated triene systems.

Introduction

AN EARLIER REPORT from this laboratory (11) described the isolation and characterization of crepenynic (*cis*-9-octadecen-12-ynoic) acid from seed oil of *Crepis foetida* L. Preliminary data showed that crepenynic acid formed conjugated triene on heating with alkali (11) according to the AOCS method for determining polyunsaturated acids (1) in vegetable oils. These data also indicated that the reaction with crepenynic acid is more rapid and results in a greater yield of conjugated triene than does the comparable alkaline isomerization of linolenic acid.

The rearrangement of simple acetylenes to allenes and isomeric acetylenes by treatment with base has been well established by many workers. They have shown that conjugated enynes (8,10), conjugated diynes (3,7) and nonconjugated diynes (6,15) also undergo rearrangement reactions when treated with base. One of the conjugated enynes, ximenynic (*trans*-11-octadecen-9-ynoic) acid, forms conjugated trienes (8).

The object of this study is to present detailed information on the reaction of crepenynic acid with alkali under variations in time, temp, base concn and sample size. Since crepenynic acid represents a new type of naturally occurring acetylenic fatty acid, it is desirable to identify the alkali-isomerization products. Therefore, results of some experiments designed to characterize these rearrangement products are given here.

Experimental

Preparation of Methyl Crepenynate. Pure methyl crepenynate was obtained by countercurrent distribution of the mixed methyl esters of *Crepis foetida* L. seed oil as described previously (11).

Infrared Analyses. The IR spectra were determined on carbon disulfide solutions of the methyl esters in a 1-mm sodium chloride cell with a Perkin-Elmer Model 137-0001 spectrophotometer.

Alkali Treatment of Methyl Crepenynate. The potassium hydroxide-ethylene glycol solutions were prepared and standardized according to the AOCS method for polyunsaturated acids. The base solution (25.0 ml) was placed in a 50-ml round-bottom flask fitted with a small cold-finger condenser inside a larger air condenser. Nitrogen was admitted through a bubbler, placed so that the tip was well immersed in the solution. Two such apparatus (one for a blank) were then heated in an oil bath at the desired temp and allowed to remain in the bath for 15 min. The sample of ester was then introduced in a glass cup, and the contents of the flask were swirled periodically during the first few min. At predetermined intervals, 1.0-ml aliquots of sample and blank were removed, diluted immediately with absolute ethanol, and analyzed promptly on a Beckman DU spectrophotometer for conjugated triene content (1). No corrections for expansion of the ethylene glycol solutions were made in the calculations.

The preparative-scale isomerization (1.0 g) was carried out in the same manner except that a 500-ml flask containing 200 ml of 10% potassium hydroxide in ethylene glycol at 121C was used.

[1] Presented at AOCS Meeting in New Orleans, 1964.
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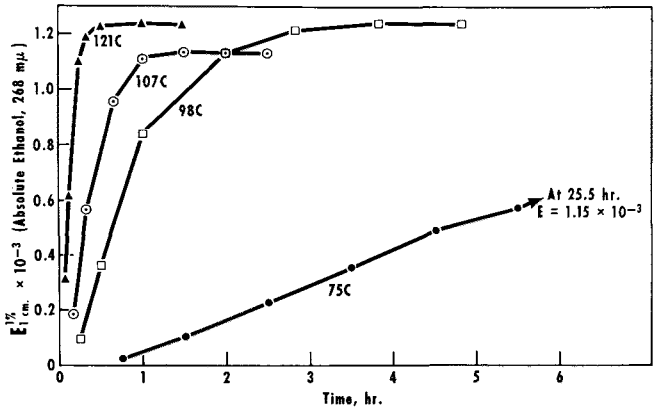

FIG. 1. Effect of temp on rate of isomerization of methyl crepenynate (10% potassium hydroxide).

The alkali-isomerization products were recovered by diluting the alkaline solution with water, acidifying with 6 *N* hydrochloric acid (solution held between 10 and 20C), and extracting with diethyl ether. The extract was washed thoroughly with distilled water and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* at room temp or below.

Preparation of Methyl Esters and Low-Temperature Crystallization. Methyl esters were prepared by refluxing the products (under nitrogen) for 45 min with 4% HCl in methanol. The esters were recovered in the usual manner and the IR spectrum was obtained.

A 2.5% solution of the unknown methyl esters in redistilled acetone was cooled to −30C for 30 min and then to −40C for 20 min. The crystals obtained were washed once with cold acetone (−40C) and dried under vacuum. UV and IR analyses were done, the remainder of the sample was saponified, and the resulting acid was recovered.

Maleic Anhydride Adduct (2). The isomerization product methyl esters (0.5 g) and 0.211 g of maleic anhydride in 5 ml of benzene were refluxed under nitrogen for 6 hr. Benzene was removed *in vacuo,* the residue was dissolved in ether, and the ether solution was washed with water until the wash was neutral.

Quantitative Hydrogenation of Alkali-Isomerization Products. The ester sample (0.0143 g) was hydrogenated (at room temp and atmospheric pressure) in 95% ethanol with platinumoxide catalyst. The saturated product was removed by filtering off the catalyst and removing the ethanol *in vacuo.*

Oxidation of Alkali-Isomerization Products. Methyl esters of the alkali-isomerization products were cleaved with permanganate-periodate in *t*-butyl alcohol as described by von Rudloff (14) except the reaction time was 15.5 hr.

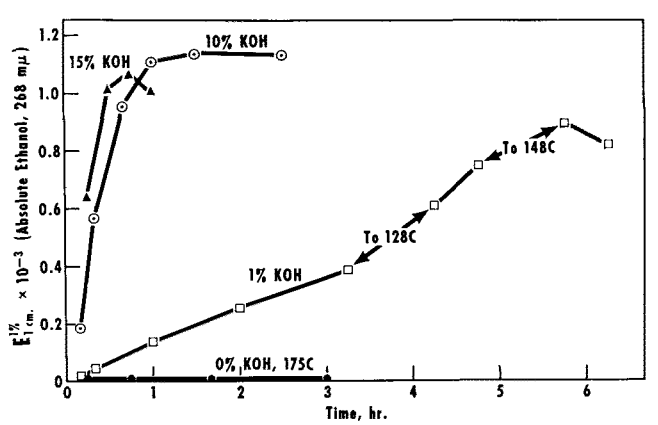

FIG. 2. Effect of base concn on rate of isomerization of methyl crepenynate (107C except where noted).

Results and Discussion

The plots of absorbance versus time in Figures 1 and 2 show graphically the effect of variations in temp and in base concn on the rate of formation of conjugated triene from methyl crepenynate. The formation of triene was determined by measuring the adsorption of the sample at 268 mμ, which was the point of maximum absorption for all samples. All absorption plots gave a 3 maxima curve (λ = 259, 268, 278) very similar to that of β-eleostearic acid. None of the plots showed any conjugated diene adsorption.

To obtain the values for Figure 1, the base concn was held at 10%. The sample sizes varied from 21–26 mg, but the large excess of base allows the sample size to be increased at least threefold (e.g. 21–65 mg) without producing any significant change in the rate of triene formation. The preparative-scale isomerization had a sample-to-base mole ratio of 1:100, which is nearly five times that of the trial runs (1:450), and yet the maximum triene content was obtained in 45 min. This value is very similar to that shown in the plot of the small sample reaction rate.

Figure 1 also shows that the conjugated triene obtained in this reaction is formed under much milder conditions than are necessary to conjugate linolenic acid by the Official AOCS Method (1). Similarly, the conditions used here are milder than those necessary to rearrange ximenynic acid to the maximum amt of conjugated triene (6.6% KOH, 180C for 2 hr) (8). The 75C run took 27 hr to reach a maximum comparable to those of the other 3 runs. The temp was raised to 90C during the 1.5 hr just prior to removal of the 27-hr aliquot. The percentage of conjugated triene [calculated as preformed β-eleostearic acid (1)] ranged from 53–58% for the four plots in Figure 1. These values would range from 69–75% if calculated as α-eleostearic (*cis,trans,trans*) acid and would be near 100% if calculated as the *cis,cis,trans* isomer (5).

Concn of base has a considerable effect on the rate of rearrangement (Fig. 2). To obtain a maximum in a reasonable length of time with 1% of base (mole ratio of sample to base = 1:45), it was necessary to increase the temp during the reaction. In this case and also in the 15% KOH run, the maximum triene was lower than in any of the trials in Figure 1. In addition, the conjugated triene absorption dropped after the maximum had been reached. These phenomena may be due to polymerization or cyclization under the influence of higher temp, in one instance, and to higher base concn in the other.

When methyl crepenynate was heated in ethylene glycol at 175C for 3 hr in the absence of alkali, no increase in absorption at 268 mμ was observed, and no new absorption between 225 and 300 mμ was formed.

An isomerization reaction was stopped just before the maximum conjugated triene was obtained, and the products (46% β-eleostearic acid by UV) were recovered and esterified. Analysis of the esters by GLC (conditions described in Ref. 9) also showed 46% of conjugated triene (13). An unknown component (48%) with an equivalent chain length (12) of 19.9 on an LAC-2-R 446 column was also present. This peak is not unrearranged methyl crepenynate which has an

equivalent chain length of 20.7 in this column.

To help identify this new peak, another isomerization was done, but it was allowed to proceed for only 15 min. The reaction was stopped, and the products were recovered and esterified. Analysis of these esters by GLC showed 81% of unrearranged methyl crepenynate, 10% of conjugated triene, and 8% of the unknown component. Preliminary data indicate that this unknown may be formed by thermal rearrangement of the conjugated trienes in the GLC column. Additional research is currently in progress on the characterization of this material and will be reported later.

The IR spectrum of the methyl esters of the isomerization products shows two strong, sharp bands in the 10–11 μ region (Fig. 3A). The first band at 10.1 μ is similar to that observed for materials having a system of *trans,trans* conjugated double bonds such as α- or β-eleostearic acids. The second band at 10.4 μ is very similar to that caused by the isolated *trans* double bond in elaidic acid. In fact the IR spectrum (Fig. 3A) can be obtained from a mixture of tung oil and methyl elaidate in the proper proportions. There is no known single material that gives this absorption pattern. The absence of absorption near 5.1 μ indicates that allenic products or intermediates (3,7,15) were not present.

Low-temp crystallization of the esters was attempted to determine if they could be fractionated. Only one relatively pure fraction (ca. 15% of starting material) was obtained. The IR spectrum of the crystallized material is given in Figure 3B. The intensity of the 10.1 μ band is greatly increased as compared to the intensity of the 10.4 μ band. UV analysis of this material gave a value of 74% conjugated triene as β-eleostearic acid. The liquor from this crystallization showed the 10.4 μ band to be stronger than the 10.1 μ band, but on standing at room temp the 10.1 μ band gradually strengthened until its intensity was again greater than that of the 10.4 μ band. This increase indicates that the isomer causing the 10.4 μ band easily rearranges to the isomer which was obtained from low-temp crystallization.

The crude acid obtained by saponification of the crystallized ester had a mp of 60–71C. After two recrystallizations from 95% ethanol (recovery = 45%) the mp was 73–76C. This melting point is slightly higher than that of β-eleostearic acid (71–72C) (5) and approaches that reported for the all-*trans* 8,10,12-isomer (77–78C) (4). The high melting point indicates that this fraction is probably an all-*trans* acid.

The maleic anhydride adduct of methyl esters of the isomerization products was prepared in another attempt to separate the isomer causing the 10.1 μ band from the one causing the 10.4 μ band. The IR spectrum of the adduct showed no band at 10.1 μ, but did have a strong band at 10.4 μ. The presence of this band is not surprising nor conclusive since the adduct of an all-*trans* triene would show absorption for isolated *trans*. Analysis of the adduct by GLC (after further esterification) indicated ca. 56% of the original material had not reacted. This amt of unreacted material was decreased slightly by an additional 3-hr reflux treatment with maleic anhydride. These results probably mean that the product is a mixture of an adduct-forming material (ca. 50%) having a minimum of two adjacent *trans* bonds and other materials which rearrange slowly to give adduct-forming compounds.

The methyl esters of the isomerization products absorb 3.0 moles of hydrogen/mole of ester to yield only methyl stearate, identified by melting point and GLC characteristics.

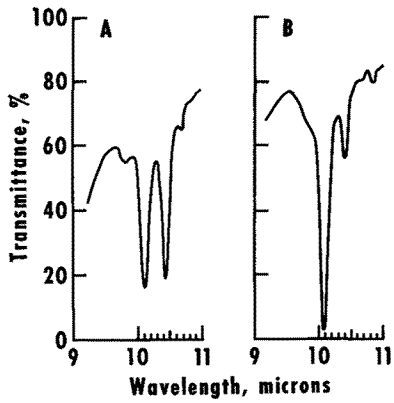

FIG. 3. A. Portion of IR spectrum of methyl esters of isomerization products, 1% in carbon disulfide. B. Portion of IR spectrum of methyl esters crystallized from acetone at −40C, 1% in carbon disulfide.

Analysis by GLC of the monobasic acids obtained by oxidative cleavage of the esters showed 5% butanoic, 14% pentanoic, 69% hexanoic, 9% heptanoic and 3% octanoic acids. Analysis of the dibasic acid methyl esters showed 2% hexanedioate, 8% heptanedioate, 61% octanedioate, 14% nonanedioate, 10% decanedioate and minor amt of unknown components. These results indicate that 60–70% of the rearrangement product is 8,10,12-octadecatrienoic acid and that the remainder is a mixture of other positional isomers. The discrepancy of 8% between the hexanoic acid and octanedioate percentages is not meaningful because it is within the limits of experimental error.

The evidence indicates that the major product from this alkaline rearrangement is 8,10,12-octadecatrienoic acid. Other positional isomers in lesser quantities are also present. The geometric configuration of the 8,10, 12-isomer is being studied, and this work will be reported later along with the results of experiments designed to explain the mechanism of this rearrangement.

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