Removal of the free fatty acids from the *Chilopsis* oil did not alter the absorbance of the oil at 233 mμ, hence the conjugated acid is considered to be a component of the glycerides. It is also believed that the acid exists in the *trans,trans* form in the oil since no change in configuration would be expected under the mild conditions employed for its isolation.

The *trans*-10-*trans*-12-Octadecadienoic acid was made in 1942 by von Mikusch by dehydrating and isomerizing the ricinoleic acid of castor oil (15). It has also been prepared from alkali-isomerized linoleic acid (16). The ultraviolet spectrum of the methyl ester, determined by Tolberg and Wheeler (17) is similar to the spectrum in Figure 1 and includes the inflection at 241–242 mμ.

A closely-related substance is 9-hydroxy-*trans*-10-*trans*-12-octadecadienoic (dimorphecolic) acid, found by Smith and co-workers in the seed oil of *Dimorphotheca* (Compositae) (18). Although there was no evidence of an hydroxy acid in the *Chilopsis* oil, it is possible that such an acid could be an intermediate in the biosynthesis of the unsubstituted dienoic acid. Occurrence of a reducing enzyme in the seed that would convert dimorphecolic acid to the simple dienoic acid is likely. The conjugated trienoic acid of *Chilopsis* is probably formed by a separate pathway in the plant since its unsaturated grouping is $\Delta^{9,11,13}$.

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Reaction of Acetyl Nitrate with Alcohol Derivatives of Fatty Acids: A Synthesis of Nitrate Esters[1]

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Abstract

Acetyl nitrate was found to react with fatty alcohols, hydroxy esters, and vicinal glycols to give good yields of the corresponding nitrates. The reaction was applied to the synthesis of *n*-octadecyl nitrate, methyl 2-nitrato-octadecanoate, methyl 12-nitrato-octadecanoate, and 18,19-dinitrato-hexatricontane. Alkyl nitrates and nitrato esters were also prepared from alcohols derived from commercially available marine oils. In addition, the long-chain nitrate derivatives were analyzed by infrared spectroscopy and thin-layer chromatography (TLC).

The potential use of nitrate esters for the synthesis of derivatives is discussed, together with their possible effects on human physiology.

Introduction

A NUMBER OF METHODS have been reported for the preparation of short-chain organic nitrates ($R–O–NO_2$), but the instability of many of these compounds and their derivatives has discouraged their use in chemical syntheses. Nevertheless, interest has increased recently in the preparation and properties of organic nitrates, and the chemistry of these compounds has become an important part of the field of organic chemistry (2,7,12,13). The use of nitrate esters for the preparation of a wide variety of derivatives has recently been reviewed by Boschan et al. (2).

[1] Presented at the AOCS meeting in Atlanta, 1963.

It was found in this laboratory that nitrate derivatives of fatty acids are relatively stable, even at elevated temperatures, and are useful intermediates for the preparation of compounds of possible industrial importance. Despite their potential usefulness, however, nitrate derivatives of fatty acids have received little attention in the literature.

A previous communication from this laboratory reported that acetyl nitrate reacts quantitatively with the double bond of methyl oleate to form nitro, acetoxy-nitro, and nitro-nitrate derivatives (9). Further work with this reagent showed that it reacts under similar conditions with fatty alcohols, hydroxy-esters, and vicinal glycols to give good yields of the nitrate derivatives. The present paper summarizes this work and describes a convenient method for the synthesis of the following previously unreported long-chain nitrates: *n*-octadecyl nitrate, methyl 2-nitrato-octadecanoate, methyl 12-nitrato-octadecanoate, and 18,19-dinitrato-hexatricontane. The preparation of alkyl nitrates and nitrato esters from alcohols obtained from commercially available marine oils is also described. In addition, infrared spectroscopy and TLC were found to be desirable complementary methods for the analysis of the long-chain nitrate derivatives reported here.

Experimental

Infrared Spectroscopy and TLC

All materials and products were examined by infrared spectroscopy and TLC. Infrared spectra of thin films of liquids on sodium chloride plates were ob-

tained with a Baird-Atomic infrared spectrophotometer, model NK1. Compounds that were solid at room temperature were examined in the same instrument as Nujol mulls (Table I). Thin layers of Silica Gel G were prepared by previously described methods (10). Spots were indicated by 2′,7′-dichlorofluorescein under ultraviolet light, or by charring with 50% sulfuric acid (11).

Materials

Fatty Alcohols. A sample of *n*-octadecanol was obtained from a commercial source and was found to be 95% pure. In addition, a fraction of saturated fatty alcohols from menhaden oil was prepared from molecularly distilled (4) methyl esters (I.V. 188.4, sap. eq. 298.4) by a lithium aluminum hydride reduction (5) followed by hydrogenation.

Hydroxy-Esters. DL-2-hydroxy-octadecanoic acid and DL-12-hydroxy-octadecanoic acid, from a commercial source, were obtained in 90–95% purity after one crystallization from petroleum ether. These compounds were converted to methyl esters with methanol-HCl and used without further purification. Monohydroxy acids were synthesized by the procedure of Knight et al. (6) from 95 g of monoenoic fatty acids (90%) obtained from dogfish *(Squalus acanthias)* liver oil by previously described methods (8). The mono-hydroxy acids (65.5 g) were obtained after two crystallizations from petroleum ether. The ester derivatives (I.V. 1.7, sap. eq. 337.0, 5.4% OH) were prepared by esterification with methanol-HCl. The only impurity detected in this fraction was 5–10% unsubstituted methyl esters.

Vicinal Glycol. Hexatriconta-18,19-diol, prepared by previously reported methods (5), was obtained in 95% purity after one crystallization from 85% ethanol.

Preparation of Nitrate Derivatives

n-Octadecyl Nitrate. The reaction of acetyl nitrate with *n*-octadecanol, which is representative of the procedure used for the nitration of the other alcohol derivatives, was conducted as follows: 15.0 ml (0.24 mole) of colorless nitric acid was added slowly to 210 ml of acetic anhydride at 15C. The temperature was maintained at 25C by controlling the rate of addition of nitric acid. The *n*-octadecanol (10.9 g, 0.040 mole), dissolved in 75 ml of glacial acetic acid, was added to the acetyl nitrate solution at 25C over a period of 15 min. Nitration was conducted at this temperature for 1 hr. Reaction was terminated by pouring the reaction mixture into ice water. The ice mixture was then stirred until hydrolysis of the excess acetic anhydride was complete. The product was extracted with diethyl ether and the ether solution was washed twice with 10% sodium bicarbonate, and then with distilled water until neutral. The organic phase was dried over anhydrous sodium sulfate, and the crude *n*-octadecyl nitrate (12.6 g)—a pale yellow, highly fluid oil—was recovered after evaporation of solvent.

The unrefined *n*-octadecyl nitrate was found to be 95% pure by TLC. Highly colored constituents and other impurities in the product were easily removed by column chromatography. The unrefined *n*-octadecyl nitrate was purified on a column of silicic acid (Mallinkrodt, chromatographic grade), using a 1:10 ratio of nitrate to silicic acid. Elution of the column with petroleum ether (bp 30–60C) yielded 7.9 g of a colorless liquid ($n^{25}D = 1.4439$).

Analysis: Calculated for $C_{18}H_{37}NO_3$: C, 68.52; H, 11.82; N, 4.44. Found: C, 68.87; H, 11.45; N, 4.32. Estimated purity by TLC: >98%.

Alkyl Nitrates from Menhaden Oil. When 30.0 g (0.082 mole) of hydrogenated alcohols from menhaden oil were used as starting material, mixed alkyl nitrates were obtained in high yield. The product (34.7 g, $n^{25}D = 1.4418$, $d^{30} = 0.902$) was a pale yellow oil.

Analysis: Calculated: N, 4.47. Found: 4.12. Estimated purity by TLC: >95% alkyl nitrates.

Methyl 2-Nitrato-octadecanoate. Methyl 2-hydroxy-octadecanoate (4.9 g, 0.016 mole) was nitrated using the same methods described for the preparation of *n*-octadecyl nitrate. Chromatography of the crude product (5.1 g) on a column of silicic acid, using 80:20 petroleum ether-diethyl ether as the eluent, yielded 4.6 g of purified methyl 2-nitrato-octadecanoate ($n^{25}D = 1.4459$). TLC of this fraction revealed essentially one spot (Fig. 1,7).

Analysis: Calculated for $C_{19}H_{37}NO_5$: N, 3.90. Found: 3.20.

Reversed-phase partition chromatography on a siliconized silicic acid plate (9) indicated that the low nitrogen content of this fraction was due, in part, to the presence of unsubstituted methyl esters from the starting material. Methyl octadecanoate was not separable from methyl 2-nitrato-octadecanoate by either column chromatography or TLC on silicic acid (Fig. 1,9), and the latter derivative decomposed on attempted distillation. Consequently, because of the difficulties involved, no further purification of the methyl 2-nitrato-octadecanoate was undertaken.

Methyl 12-Nitrato-octadecanoate. Nitration of 14.0 g (0.045 mole) of methyl 12-hydroxy-octadecanoate yielded 14.4 g of crude product. When 11.6 g of this product was chromatographed, using the conditions described for the purification of the 2-nitrato derivative, 10.0 g of methyl 12-nitrato-octadecanoate ($n^{25}D = 1.4471$) was obtained.

Analysis: Calculated for $C_{19}H_{37}NO_5$: C, 63.48; H, 10.37; N, 3.90. Found: C, 63.24; H, 10.03; N, 3.62. Estimated purity by TLC: >95%.

Nitrato Esters from Dogfish Liver Oil. When 10.0 g (0.032 mole) of monohydroxy esters from dogfish liver oil were used as starting material, mixed nitrato esters (10.1 g, $n^{25}D = 1.4470$, $d^{25} = 0.966$) were obtained. The product was a highly fluid yellow oil that was free of unreacted hydroxy esters.

Analysis: Calculated: N, 3.76. Found: 3.64. Composition by TLC: 90% nitrato esters and 5–10% unreacted saturated methyl esters from the starting material.

18,19-Dinitrato-hexatricontane. Hexatriconta-18,19-diol (3.0 g, 0.0056 mole) was dissolved in 60 ml of warm acetic anhydride (40C). The warm solution was then added, over a period of 5 min, to a solution of acetyl nitrate maintained at the same temperature. The nitration reaction was allowed to continue for 30 min. The crude product (3.2 g) was isolated as previously described.

Chromatography of 2.9 g of the crude product on a column of silicic acid, using petroleum ether as the eluent, yielded 1.0 g of 18,19-dinitrato-hexatricontane (mp 40–42C).

Analysis: Calculated for $C_{36}H_{72}N_2O_6$: C, 68.75; H, 11.54; N, 4.45. Found: C, 69.12; H, 11.24; N, 4.24. Estimated purity by TLC: >95%.

Discussion

Nitrations of alcohols with nitric acid in acetic anhydride have been known for many years. Recent work has indicated, however, that nitric acid dissolved in acetic anhydride is, in itself, a relatively

TABLE I

Infrared Bands in Nitrate Derivatives of Fatty Acids

Derivatives	NO_3 Bands (μ)				
	Asym. Str.	NO_2 Sym. Str.	O'N Str.	Out of plane	NO_2[a] bending
n-Octadecyl nitrate	6.14(s)	7.88(s)	11.63(s)	13.1(m)	14.3(m)
Methyl 2-nitrato-octadecanoate	6.08(s)	7.89(s)	11.74(s)	13.2(w)	14.4(w)
Methyl 12-nitrato-octadecanoate	6.16(s)	7.91(s)	11.56(s)	13.3(w)	14.4(w)
18,19-dinitrato-hexatricontane	6.10(s)	7.89(s)	11.75(s)	13.3(w)	14.4–14.5(w)

[a] Probable overtone of C-O-N bending band at 351 cm^{-1} (3).

ineffective nitrating agent and that the formation of acetyl nitrate is essential for successful nitrations (1,13). It has been reported that the addition of nitric acid to acetic anhydride at −10C, e.g., results in the formation of little or no acetyl nitrate (1). In the present work, acetyl nitrate was prepared at room temperature using the conditions recently described by Bordwell and Garbisch (1). This reagent, formed under these conditions, was highly effective for the direct synthesis of a variety of previously unreported nitrate derivatives of fatty acids.

Nitrate derivatives of fatty acids may also be prepared by the reaction of alkyl halides with silver nitrate. This method, however, has few advantages over the reaction described here since alkyl halides are usually prepared by the halogenation of alcohols.

The nitrate derivatives prepared in this work were characterized by a number of well defined bands in the infrared. These bands were similar to those found in a variety of organic nitrates by Brown (3). By virtue of their spectra, nitrate derivatives of fatty acids may be unambiguously identified, even in complex mixtures of polar compounds. Bands that are characteristic of the nitrate derivatives reported in this work are shown in Table I.

Most crude nitrate derivatives of fatty acids, owing to their weak polarity, were easily separated by TLC and column chromatography from more polar starting materials and side products. Fatty acid derivatives that contained only nitrate substituents were found to be slightly more polar than hydrocarbons. Consequently, *n*-octadecyl nitrate and 18,19-dinitrato-hexatricontane were easily purified on silicic acid using pure petroleum ether as the eluent. The nitrato esters, having greater polarity due to the ester group, were purified by using a more polar solvent (Fig. 1).

It should be noted that methyl 2-nitrato-octadecanoate was inseparable from methyl octadecanoate by TLC (Fig. 1,7). This finding suggests that some fatty acid derivatives that have weakly polar substituents on the α-carbon atom may not be separable from the unsubstituted compounds by this method. In contrast, methyl 2-nitrato-octadecanoate was completely separable from methyl 12-nitrato-octadecanoate. Fractionation would be expected to occur therefore in the TLC of some mixtures of structural isomers.

A number of workers have studied the thermal decomposition of short-chain nitrates, and the principal products were found to be nitrites (2). The nitrate derivatives prepared in this work were stable at room temperature. Flash distillation (120–150C/0.1 mm) of *n*-octadecyl nitrate and methyl 12-nitrato-octadecanoate resulted in very little decomposition. Some decomposition occurred, however, on attempted distillation of methyl 2-nitrato-octadecanoate, and a slight yellow color was apparent when the 18,19-dinitrato-hexatricontane was heated at temperatures above 100C.

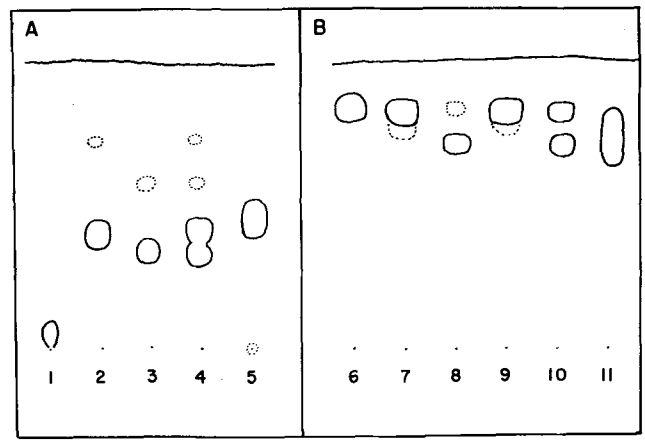

FIG. 1. TLC of nitrate derivatives on silica gel g. Indicator: 50% sulfuric acid. Amount of spot: 100 γ. Eluents: A, petroleum ether (bp 30–60C); B, 90:10:1 petroleum ether-diethyl ether-acetic acid. (1) Methyl octadecanoate, (2) *n*-octadecyl nitrate, (3) 18,19-dinitrato-hexatricontane, (4) mixture of 2 and 3, (5) alkyl nitrates from menhaden oil, (6) methyl octadecanoate, (7) methyl 2-nitrato-octadecanoate, (8) methyl 12-nitrato-octadecanoate, (9) mixture of 6 and 7, (10) mixture of 7 and 8, (11) nitrato esters from dogfish liver oil.

The physiological effects of short-chain nitrate esters have been extensively investigated (2). These compounds are known to oxidize hemoglobin to methemoglobin and depress the muscles in the vascular walls causing dilation. Such changes are accompanied by decreased systolic blood pressure and increased pulse and respiration rates. By virtue of these properties, amyl nitrate, glycerol dinitrate, and other short-chain nitrates have been used widely for the control of high blood pressure. Despite the abundance of data on short-chain nitrate esters, little or no information is available on the physiological properties or metabolic fate of the long-chain nitrates described here. Consequently, the possible biological significance of these compounds may be worthy of investigation.

Organic nitrates are known to undergo a wide variety of reactions. The preparation of important derivatives of nitrate esters via N-nitration, alkylation, and replacement reactions is described in current papers (2,12). It was shown recently that nitrate esters undergo cleavage reactions with sodium salts to form halide, nitrile, thiocyanate, and thiouronium derivatives (12). Nitrate esters, therefore, provide an alternative to the use of sulfonates and alkyl halides for the synthesis of these classes of compounds.

Hydroxy acids may be obtained from various natural oils, such as castor oil, and may be readily prepared from unsaturated oils. Nitrates prepared from these hydroxy acid derivatives may have useful properties. They would also be expected to undergo reactions, such as those described to yield potentially useful compounds.

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