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SYNTHESIS OF METHYL [18-²H₃], [17-²H₂], [16-²H₂], [14-²H₂] and [12-²H₂] *cis*-9-OCTADECENOATES*

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Methyl $[17^{-2}H_{2}]$ oleate was prepared by stepwise reduction from 17-oxooleate in 24% yield. Methyl $[18^{-2}H_{3}]$, $[16^{-2}H_{2}]$, $[14^{-2}H_{2}]$ and $[12^{-2}H_{2}]$ oleates were synthesized from appropriately deuterated octyl bromides by conversion to deuterated 7-hexadecyn-1-ols and chain extention to deuterated stearolates followed by semihydrogenation; overall yields were about 17%.

I. Introduction

Dideuterated fatty acid esters, with both deuterons on the same carbon, have recently been employed in investigations of NMR spectra. Thus spectra of $[2-{}^{2}H_{2}]$ and $[12-{}^{2}H_{2}]$ oleates were used to assign signals in the ${}^{13}C$ NMR spectrum of methyl oleate [1] and $[12-{}^{2}H_{2}]$ oleate was also used in a ${}^{2}H$ NMR study of the structure of artificial membranes [2]. It was necessary to extend these studies to oleates deuterated at other carbons but, apparently the only syntheses previously reported were those of $[2-{}^{2}H_{2}]$ oleate [3] and of $[11-{}^{2}H_{2}]$ oleate [4]. Syntheses of $[18-{}^{2}H_{3}]$, $[17-{}^{2}H_{2}]$, $[16-{}^{2}H_{2}]$, $[14-{}^{2}H_{2}]$ and $[12-{}^{2}H_{2}]$ oleates have now been carried out and are described here.

II. Materials and Methods

Mass spectra were measured with an MS 12 Mass spectrometer at the Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan. ¹H NMR spectra were obtained in carbon tetrachloride with a Varian HA 100 spectrometer and ¹³C NMR spectra in deuterochloroform with a Varian XL-100-15 spectrometer in the Fourier transform mode at 25.2 M Hz with proton noise decoupling. Products were examined by gas liquid chromatography using a 1 m \times 3 mm column packed with 1.5% Dexsil 300 on 80–100 mesh acid washed and silanized Chromosorb W programmed from 100–300°C (for chain length determination) and using a 6 m \times 3 mm column packed with 15% OV-275 on acid washed and silanized Chromosorb P [18] at 200°C (for determination of saturated and *cis* and *trans* monoenoic and monoynoic esters of the same chain length). Column chromatography was performed on 200–400

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mesh silicic acid (Biosil A, Biorad), activated at 110°C. Separation of *cis* C₁₈ monoenoate from saturated ester and *trans* monoenoate was carried out by column chromatography on silicic acid/30% silver nitrate [19].

Methyl $[17-^{2}H_{2}]$ -cis-9-octadecenoate.

Crude methyl 17-hydroxyoleate, with approx. composition 17-hydroxystearate (5%), 17-hydroxyoleate (70%), 17-hydroxyelaidate (5%), and 18-hydroxyoleate (20%) [5], was used. A solution of chromium trioxide (13 g) in 25% sulphuric acid (55 ml) [20] was added to a solution of methyl 17-hydroxyoleate (50 g) in acetone (1 l) during 45 min with stirring and cooling to 25° C. After 15 min the mixture was poured into ice water (4 l) and excess oxidant reduced with sulphur dioxide, crude methyl 17-oxooleate was extracted with chloroform. The product was refluxed with 5% methanolic HCl and yielded 17-oxooleate (49.6 g) containing approx. 20% dimethyl octadecenedioate (formed by oxidation of 18-hydroxyoleate).

The 17-oxooleate (49.6 g) was reduced in methanol (750 ml) at 20°C with sodium borodeuteride (5 g) for 1 h. Excess reagent was destroyed with acetic acid and, after pouring into water, the product was recovered in chloroform. Product was chromatographed on silicic acid, elution with hexane/ether (96 : 4) gave dimethyl octadecenedioate (11 g) and with hexane/ether (85 : 15) gave methyl [17-²H]-17-hydroxyoleate (29 g).

A solution of the above product (29 g) and triethylamine (14 g) in methylene chloride (650 ml) at -10° C was treated with mesyl chloride (14.9 g.); the mixture was kept at 0° for 24 h, washed three times with ice water and dried. Removal of the solvent gave methyl [17-²H]-17-mesyloxyoleate (33.9 g), which could not be induced to crystallize.

The mesyloxyoleate (33.9 g) was refluxed in ether (1 l) with lithium aluminum deuteride (5.85 g) for 18 h and recovery, as previously described [21], gave the crude tetradeutero alcohol (26 g). Oxidation of the alcohol (26 g) in acetone (1.2 l) with chromium trioxide (17.7 g) in 25% sulphuric acid (70 ml) was carried out as described above. The acid obtained was converted to crude methyl $[17-^2H_2]$ oleate (26.1 g) by reflux with methanolic HCl for 18 h. Chromatography on silicic acid and elution with hexane/ether (98 : 2) gave purified esters (14.8 g).

The product contained methyl $[17\ {}^{2}H_{2}]$ stearate (5%, derived from 17-hydroxystearate in the starting material) and methyl $[17\ {}^{2}H_{2}]$ elaidate (15%). Chromatography on silicic acid/silver nitrate gave saturated and *trans* esters (eluted with hexane containing 20% benzene) and pure methyl $[17\ {}^{2}H_{2}]$ oleate (8.1 g, eluted with hexane containing 30% benzene). The overall yield based on hydroxyoleate content of the starting material was 24.2%. Mass spectrometry showed that the ester was 96% dideuterated. Methyl $[12\ {}^{2}H_{2}]$ -cis-9-octadecenoate (from castor oil).

Crude methyl 12-oxooleate (34 g, prepared from castor oil methyl esters [7]) was reduced in methyl (200 ml) with sodium borodeuteride (2 g) as described above. The product (29.5 g) was chromatographed on silicic acid; elution with hexane/ether (99:1) gave C_{16} and C_{18} nonoxygenated acids (4.2 g) and with hexane/ether (90:10) gave methyl [12-²H]-12-hydroxyoleate (22.3 g). Methyl [12-²H]-12-mesyloxyoleate

(27 g) was prepared by mesylation of the hydroxy ester (22.3 g) as described above. The mesylate (27 g) was stirred in ether (900 ml) with lithium aluminum deuteride (7 g) at -30° C for 3 h and at 20°C for 18 h. Excess reagent was destroyed by addition of 10% sodium hydroxide solution and product (16 g) was recovered. Both TLC and GLC indicated that the product contained a major amount of a polar, presumably oxygenated, by-product. All product was chromatographed on silicic acid and elution with hexane/ether (85:15) gave a fraction (6.1 g) which resembled oleyl alcohol on TLC and GLC. The alcohol fraction (6.1 g) was oxidized in acetic acid (250 ml) with chromium trioxide (4 g, 25% excess) in 90% acetic acid (44 ml) at 25°C for 30 min. The solution was poured into 4 N sulphuric acid (800 ml) giving a dark green solution indicating that all the oxidant had been consumed. The product (6.9 g) was extracted with chloroform but a portion treated with diazomethane appeared to contain very little methyl oleate (GLC). The product was reoxidized as before with chromium trioxide (3 g) and excess oxidant remained at the end of the reaction. The second oxidation product (6.7 g) was treated with methanolic HCl for 18 h and gave crude esters (6.1 g). Chromatography on silicic acid gave a mixture of methyl $[12-{}^{2}H_{2}]$ oleate and methyl pelargonate (1.4 g, eluted with hexane/ether (99 : 1)). Methyl $[12-{}^{2}H_{2}]$ oleate (0.85 g) was obtained by fractional distillation. The above reactions were repeated and the $[12-^{2}H]-12$ -mesyloxyoleate was reduced with lithium triethylborodeuteride in tetrahydrofuran but there was no improvement in yield obtained at this stage. Methyl $[12-^{2}H_{2}]$ oleate (0.7 g) was also prepared from castor oil esters (20 g)

by this route but using methyl $[12-{}^{2}H]-12$ -tosyloxyoleate as intermediate instead of the mesylate. The ${}^{13}C$ NMR spectrum indicated that the product contained 20% of *trans* isomer.

1. Methyl [16-²H₂]-cis-9-octadecenoate

Butyric acid (22 g) was converted to $[4 \cdot {}^{2}H_{2}]$ hexanoic acid as previously described [3]. Using reaction conditions described before [3] the acid was reduced to the alcohol, converted to the mesylate and by malonate extension [22] followed by reduction converted to $[6 \cdot {}^{2}H_{2}]$ -1-octanol. Bromination with triphenylphosphine dibromide in dimethyl formamide [11] gave $[6 \cdot {}^{2}H_{2}]$ -1-bromooctane (19 g), b.p./20 mm 88–91°C. The tetrahydropyranyl ether of 7-octyn-1-ol [15] (21.2 g) was added to a suspension of lithium amide (2.5 g) in ethylene glycol dimethyl ether (200 ml) and the mixture refluxed for 5 h (W.F. Steck, pers. comm.). The deuterated octyl bromide (19 g) was added and reflux continued for 18 h, the mixture was then poured into ice water and the product extracted with hexane and dried. Removal of hexane gave the tetrahydropyranyl ether of [14 - ${}^{2}H_{2}$]-7-hexadecyn-1-ol (28.5 g). After hydrolysis of the ether by refluxing in methanol/2% HCl for 4 h, the crude alcohol was distilled giving [14 - ${}^{2}H_{2}$]-7-hexadecyn-1-ol (17.1, b.p./0.1 mm 135–140°C).

The methanesulphonate of the above alcohol was prepared as described earlier and, after crystallization from methanol at -12° C, the yield was 17.7 g and the m.p. 17°C. Reaction of the mesylate with diethyl sodiomalonate [22] gave [14-²H₂]-7-hexade-cynylmalonic acid. Crystallization from benzene gave the acid (15.6 g) with m.p.

 $81-82^{\circ}$ C. The malonic acid was heated at 160° for 2 h and distilled giving [16-²H₂]stearolic acid (12.65 g) with b.p./0.1 mm 164°C. The acid was converted to methyl ester and semihydrogenated over Lindlar catalyst (Fluka AG) in ethanol giving methyl [16-²H₂]oleate (13.0 g). The product contained 97.2% *cis* isomer, 1.6% *trans* isomer and 0.6% stearolate. MS showed that the ester was 98% dideuterated. The overall yield from butyric acid was 17.5%.

2. Methyl [18-²H₃]-cis-9-octadecenoate

Methyl 8-bromooctanoate was prepared in 59% yield from methyl hydrogen nonanedioate (ref. 12, procedure P), ¹H NMR δ 2.20 (t, -CH₂CO₂-), 3.32 (t, - CH₂Br), 3.56 (s, OCH₃). The bromoester (57.1 g) was reduced with lithium aluminum deuteride (6.6 g) as described above and gave $[1^{-2}H_2]$ -8-bromo-1-octanol (46.2 g), ¹H NMR δ 3.32 (t, -CH₂Br). The bromoalcohol (46.2 g) was converted to $[1^{-2}H_2]$ -8-bromo-1mesyloxyoctane (60.7 g), ¹H NMR δ 2.90 (s, -CD₂OSO₂CH₃), 3.32 (t, -CH₂Br). Reduction of the bromomesylate (60.7 g, 0.21 mol) with lithium aluminum deuteride (11.0 g, 0.26 mol) as before gave $[1^{-2}H_3]$ -8-bromooctane (33 g) b.p./23 mm 91–95°C, ¹H NMR δ 3.32 (t, -CH₂Br). By the reactions used in preparation of $[16^{-2}H_2]$ oleate, the bromooctane was converted to methyl $[18^{-2}H_3]$ oleate in an overall yield, from methyl hydrogen nonanedioate, of 17%. The product contained 97.2% *cis* isomer, 1.4% *trans* isomer and 1.1% stearolate, MS showed that it was 97% trideutero ester.

3. Methyl [14-²H₂]-cis-9-octadecenoate

 $[2-{}^{2}H_{2}]$ Hexylmalonic acid was prepared as described previously [3] and, by the procedures used to synthesize $[6-{}^{2}H_{2}]$ -1-bromooctane, was converted to $[4-{}^{2}H_{2}]$ -1-bromooctane. Methyl $[14-{}^{2}H_{2}]$ oleate was prepared from the bromooctane as before, the product contained 2% *trans* ester and was 96% dideuterated.

Methyl $[12-^{2}H_{2}]$ -cis-9-octadecenoate (by acetylenic route)

Using exchange conditions B, previously reported [3], $[2^{-2}H_2]$ octanoic acid was prepared and $[2^{-2}H_2]^{-1}$ -bromoctane was obtained by reduction and bromination as before, ¹H NMR δ 3.31 (s, -CD₂CH₂Br). Methyl $[12^{-2}H_2]$ oleate was then prepared as described above; the ¹³C NMR spectrum indicated that *trans* isomer content was less than 2% and the proportion of dideuterated ester was more than 95%.

III Results and Discussion

Certain readily available oxooctadecanoic acids were convenient starting materials for syntheses of gem dideutero stearic acids [3] but for oleic acid only two oxo acids, 17-oxo and 12-oxooleic acids, were obtainable by oxidation of naturally produced hydroxy acids. Crude 17-hydroxyoleic acid was prepared by hydrolysis of the glycolipid obtained by fermentation of oleic acid with the yeast *Torulopsis bombicola* [5]. Oxidation yielded 17-oxooleate which, following the procedure used with saturated oxo esters [3] was reduced with sodium borodeuteride to $[17-{}^{2}H]-17$ -hydroxyoleate, converted to 17-mesyloxyoleate, reduced to $[1,17-{}^{2}H_{4}]$ oleyl alcohol with lithium aluminum deuteride and finally oxidized to $[17-{}^{2}H_{2}]$ oleic acid. After purification the overall yield from 17-hydroxyoleate was 24%.

A report that 12-mesyloxyoleate was reduced by lithium aluminum hydride, in good yield, to oleyl alcohol [6] indicated that $[12^{-2}H_2]$ oleate could also be synthesized from ricinoleate by the above procedure. 12-Oxooleate was prepared from castor oil methyl esters [7] and reduced with sodium borodeuteride and $[12^{-2}H]$ -12-hydroxyoleate obtained after chromatographic separation from non-oxygenated castor oil esters. The 12-mesyloxyester was prepared in the usual way [3] but reduction with lithium aluminum deuteride gave numerous by-products, probably containing oxygenated compounds with *trans* double bonds) and the deuterated alcohol had to be purified by column chromatography. Due to the presence of readily oxidized impurities, oxidation to $[12^{-2}H_2]$ oleic acid was carried out twice and the final overall yield was only 2.5%.

Since lithium triethylborohydride had been reported to reduce mesylates readily [8], the reaction sequence was repeated and lithium triethylborodeuteride employed to reduce the mesylate, the yield, however was no better than before. A small improve-

$$\begin{array}{c} \text{CH}_{3}\text{CH}_{2}\text{CH}_{2}\text{CO}_{2}\text{Na} \xrightarrow{D_{2}0,\text{Na}\text{OD}} \text{CH}_{3}\text{CH}_{2}\text{CD}_{2}\text{CO}_{2}\text{H} \xrightarrow{\text{LiAll}_{4}} \text{CH}_{3}\text{CH}_{2}\text{CD}_{2}\text{CD}_{2}\text{CH}_{2}\text{OH} \\ \xrightarrow{\text{CH}_{3}\text{SO}_{2}\text{Cl}} \text{CH}_{3}\text{CH}_{2}\text{CD}_{2}\text{CD}_{2}\text{CH}_{2}\text{OSO}_{2}\text{CH}_{3} \xrightarrow{\text{Na},\text{CH}_{2}(\text{CO}_{2}\text{C}_{2}\text{H}_{5})_{2}} \text{CH}_{3}\text{CH}_{2}\text{CD}_{2}\text{CD}_{2}\text{CH}_{2}\text{CH}_{2}\text{OH}_{2} \\ \xrightarrow{\text{CH}_{3}\text{CH}_{2}\text{CD}_{2}(\text{CD}_{2}\text{C$$

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Carbon	Oleate [1]	Position of deuterons				
		18	17	16	14	12
18	14.09	I	13.84 (-0.25)	14.01 (-0.08)	14.07	14.07
17	22.70	22.41 (-0.29)	1	22.47 (-0.23)	22.69	22.67
16	31.90	31.82 (-0.08)	31.69 (-0.21)	1	31.86 (-0.04)	31.90
15	29.33	29.32	29.26 (-0.07)	29.13 (-0.20)	29.14 (-0.19)	29.31
14	29.52	29.52	29.53	29.47 (-0.05)		29.46 (-0.06)
13	29.33	29.32	29.32	29.32	29.14 (-0.14)	29.13 (-0.20)
12	29.76	29.77	29.77	29.77	29.72 (-0.04)	I
11	27.22	27.21	27.21	27.21	27.23	27.03 (-0.19)

ment in yield (to 4%) was obtained when $[12-^{2}H]-12$ -tosyloxyoleate was intermediate instead of mesylate.

Because other oxygenated oleates were not available and because of the very low yields obtained in the reduction procedure when the hydroxyl group was close to the double bond, attention was turned to the well established acetylenic route [9,10]. The procedure adopted for synthesis of $[18-{}^{2}H_{3}]$, $[16-{}^{2}H_{2}]$, $[14-{}^{2}H_{2}]$ and $[12-{}^{2}H_{2}]$ oleates is shown below for $[16-{}^{2}H_{2}]$ oleate.

Deuterated octanols were prepared first. Exchange of sodium octanoate at 200°C [3], recovery of $[2-{}^{2}H_{2}]$ octanoic acid and reduction with lithium aluminum hydride gave $[2-{}^{2}H_{2}]$ octanol which was the starting material for synthesis of $[12-{}^{2}H_{2}]$ oleate. $[4-{}^{2}H_{2}]$ octanol, for preparation of $[14-{}^{2}H_{2}]$ oleate, was prepared by malonate chain extension from $[2-{}^{2}H_{2}]$ hexanol obtained from $[2-{}^{2}H_{2}]$ hexanoic acid [3]. Preparation of $[6-{}^{2}H_{2}]$ octanol from $[2-{}^{2}H_{2}]$ butyric acid [3] is outlined in Fig. 1. These three octanols were converted to the corresponding deuterated octyl bromides by bromination with triphenylphosphine dibromide [11]. To synthesize $[8-{}^{2}H_{3}]$ -1-bromooctane, methyl 8-bromooctanoate was prepared by a modified Hunsdieker reaction [12], reduced with lithium aluminum deuteride to $[1-{}^{2}H_{2}]$ -8-bromooctanol and converted to the mesylate. Reduction of the mesylate with lithium aluminum deuteride gave the trideutero octyl bromide. There was no appreciable reduction of the aliphatic bromide group in the absence of lithium deuteride [13,14].

Acetylenic C_{16} alcohols were obtained (see Fig. 1) by reaction between the deuterated octyl bromides and the lithium derivative from the tetrahydropyranyl ether of 7-octyn-1-ol [15]. The chain was lengthened through the C_{16} mesylate and the hexynylmalonic acid to the stearolic acid; all three of these intermediates could be purified by crystallization. The pure stearolate, thus obtained, was semihydrogenated to the deuterated oleate and the products contained less than 2% of *trans* isomer. Overall yields from undeuterated starting materials were about 17%.

¹³C NMR Spectra of the products were measured to confirm the positions of the deuterons and to estimate purity and extent of deuteration; chemical shifts are listed in Table 1. The second and third atom isotope effects, which are similar to those previously observed [16,17] together with the marked increase in line width of signals of affected carbons [1,16,17] confirmed the structures of the deuterated oleates. The absence of signals in the 32.6 p.p.m. region (chemical shifts of C-8 and C-11 of *trans* isomers) showed that no more than traces of *trans* isomers were present. Triplets due to the -CDH- grouping were also absent indicating at least 95% deuteration at the desired position. Finally mass spectrometry showed that deuteration was 96–98% and hence that there had been no loss of deuterium at any stage in the acetylenic route.

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