The Synthesis of 11,11-Dideuterolinoleic Acid *

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SUMMARY

The synthesis of 11,11-dideuterolinoleic acid is described. The procedure employed resulted in a product of high isotopic purity. The NMR and mass spectral analyses of the product and intermediates confirm the position and extent of deuterium incorporation.

INTRODUCTION.

Linoleic acid (*cis,cis*-9,12-octadecadienoic acid), is a naturally-occurring material essential to animal nutrition and found in all normal animal tissues [1]. The rumen bacterium *Butyrivibrio fibrisolvens* catalyzes the isomerization of this acid to *cis*-9,*trans*-11-octadecadienoic acid by a reaction in which one of the hydrogen atoms on C-11 is removed [2]. As part of an effort to further elucidate the mechanism of the isomerization reaction, 11,11-dideuterolinoleic acid (*4a*) was synthesized and studied as a substrate [3]. The synthesis of this acid is described in this report.

DATA AND DISCUSSION.

Many syntheses of linoleic acid have been reported and are listed in Osbond's recent review [4]. The route chosen for the preparation of 11,11-dideuterolinoleic acid is based on that described by Osbond, Philpott, and Wickens [5] and later employed by Sgoutas and Kummerow [6] for the preparation of linoleic acid labeled with tritium in the 9, 10, 12, and 13 positions.

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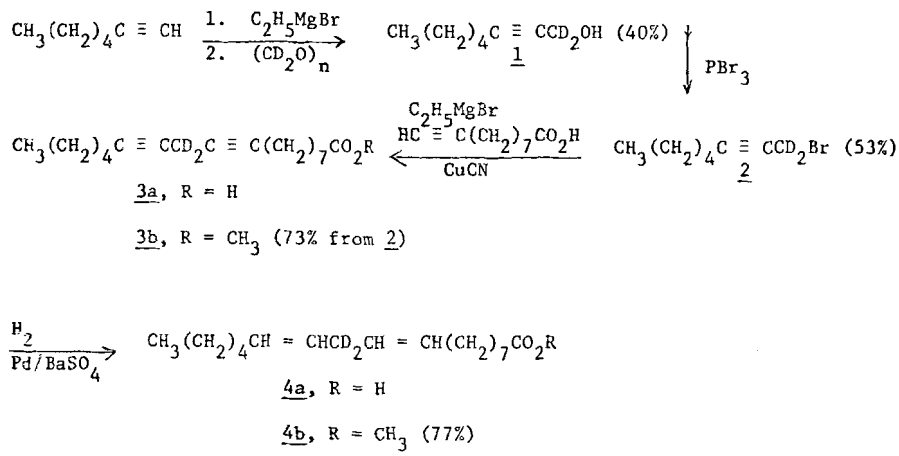
Some modifications of these procedures were made and are described in the Experimental. The full synthetic route is given in Scheme 1.

SCHEME 1.



The deuterium found in the final product was provided by paraformaldehyde-d_2 and was introduced into the reaction sequence during the synthesis of 2-octyn-1-ol (*1*) [6]. Preparation of primary alcohols via a Grignard reagent usually employs gaseous formaldehyde in rather large excess. We found, however, that a more efficient incorporation of the deuterium label was made possible by using solid paraformaldehyde-d_2 with excess heptynyl magnesium bromide. The alcohol was converted in 53 % yield to the bromide *2* by interaction with phosphorus tribromide [7], a procedure which minimizes the likelihood of H/D exchange.

The diynoic acid *3a* was prepared by condensing *2* with the di-Grignard derivative of 9-decynoic acid in anhydrous tetrahydrofuran with cuprous cyanide as catalyst [5]. This product was isolated in 73 % yield after conversion to *3b* by azeotropic methylation [6]. Stereospecific partial reduction of *3b* using a modified Lindlar's catalyst [8, 9] gave the desired methyl *cis,cis*-octadecadienoate-11,11-d_2 (*4b*) in 77 % yield. Small amounts of methyl oleate and methyl stearate were also formed. Final purification of the deuterated methyl linoleate (*4b*) was achieved by argentation column chromatography [10]. The free acid *4a* was obtained from the ester by standard saponification procedures.

Intermediates and products were examined for deuterium content by nmr spectroscopy and by mass spectral analysis. Neither technique gave evidence of any loss of deuterium in the reaction sequence and the final products (*4a* and *4b*) were at least 98 % dideuterated. Oxidative cleavage [11] of *4b* and mass spectral analysis of the cleavage products showed that the deuterium was located on carbon-11 and that no scrambling had occurred.

EXPERIMENTAL.

Reactions were carried out under nitrogen in solvents dried by molecular sieves. The paraformaldehyde-d_2 used was purchased from Mallinckrodt Nuclear and guaranteed to have greater than 98 atom % deuterium. Gas-liquid chromatography was performed on an F and M Model 700 gas chromatograph equipped with a flame ionization detector using a 4 ft. 10 % diethylene glycol succinate column. Nuclear magnetic resonance spectra were obtained in deuteriochloroform on a Varian HA-100 spectrometer. Mass spectral analyses were obtained on an AEI MS-12 mass spectrometer.

1,1-Dideutero-2-octyn-1-ol (1).

A solution of 14.4 g (0.15 moles) of 1-heptyne (Columbia Organic) in 30 ml dry ether was slowly added at 0° to a solution of 0.14 moles of ethyl magnesium bromide in 100 ml of ether. After stirring for an additional hour, 3.0 g (0.1 mole) of paraformaldehyde-d_2 was added in one portion. The mixture was stirred cold for 2 hours, then at room temperature for 21 hours. The reaction mixture was hydrolyzed by 80 g of crushed ice and 60 ml 10 % sulfuric acid. After several minutes of vigorous stirring the organic layer was separated, washed with cold water, dried, and the solvent removed *in vacuo* at room temperature. This afforded 5.2 g (40 %) of *1* which was sufficiently pure (NMR spectrum and gas-liquid chromatographic analysis) for the next stage of the synthesis.

1,1-Dideutero-1-bromo-2-octyne (2).

Bromination of *1*, performed as reported by Taylor and Strong [7] provided *2* in 53 % yield. The material (bp 72°/3 torr) was shown by gas-liquid chromatography to be a single product. NMR analysis showed the complete absence of absorption at τ 4.01, where the 1-methylene group of the non-labeled material absorbs.

Methyl 11,11-dideuterooctadecadiynoate (3b).

The procedure of Osbond *et al.* [5] was used for the condensation of *2* (1.91 g, 0.01 mole) and 9-decynoic acid (3.36 g, 0.02 moles) in anhydrous tetrahydrofuran and cuprous cyanide as catalyst. The product was isolated as the methyl ester *3b* (after azeotropic methylation [6]) and was purified by vacuum distillation [2.12 g (73 %) based on *2*]. Gas-liquid chromatography showed that this product was >97 % pure and NMR analysis indicated that the deuterium was retained.

Methyl 11,11-dideuterooctadecadienoate (Methyl 11,11-dideuterolinoleate) 4b.

Following the procedure of Augustine [9], 2.04 g (7 mmoles) of *3b* in 25 ml of methanol was reduced using freshly prepared 5 % palladium on barium sulfate catalyst (60 mg) poisoned with two drops of synthetic quinoline. Uptake of hydrogen abruptly slowed after very nearly the theoretical amount was consumed (approximately 2 hr). The catalyst was filtered and the solvent evaporated *in vacuo*. The residue was taken up in hexane and the hexane solution was washed twice with 5 % hydrochloric acid, twice with water, and then dried. Evaporation of the hexane gave 1.80 g of product which was shown by gas chromatography to be approximately 85 % (77 % yield) of the desired *4b*, contaminated with small amounts of methyl oleate and methyl stearate.

Purification of this mixture of methyl esters was accomplished by argentation column chromatography according to the procedure of De Vries [10]. The material obtained in this fashion was greater than 98 % pure by gas chromatography and gave only a single spot on thin layer chromatography (Silica gel; heptane; isopropyl ether; acetic acid 6 : 4 : 0.3 and Silica gel impregnated with silver nitrate : Skellysolve B : ether; 85 : 15 v/v). These methods and the absorption spectrum confirmed the absence of any *trans* isomer.

The position of the deuterium label and the degree of incorporation were established by NMR spectroscopy and mass spectral analysis of *4b* and by mass spectral analysis of its oxidative cleavage products [11].

11,11-Dideuterooctadecadienoic Acid (Linoleic Acid) (4a).

Purified *4b* was saponified under nitrogen and at room temperature by a 10-fold excess of potassium hydroxide in 50 % ethanol. The resulting *4a* was analyzed by thin layer chromatography and, after remethylation, by gas chromatography. The chromatographic behavior of these materials was identical with that of authentic samples of linoleic acid and methyl linoleate.

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