Pergamon

Tetrahedron Letters, Vol. 35, No. 51, pp. 9497-9500, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 \$7.00+0.00

0040-4039(94)02108-2

Synthesis of Deuterium Labeled Polyunsaturated Fatty Acids

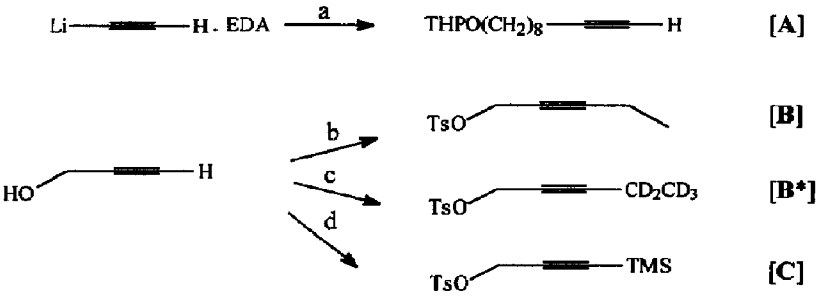
Aleš Svatoš,[1] Athula B. Attygalle, and Jerrold Meinwald*

Baker Laboratory, Department of Chemistry, Cornell University, Ithaca, New York 14853, USA

Abstract: Compounds containing carbon-carbon double bonds bearing *cis* deuterium atoms can be prepared conveniently by treating disubstituted acetylenes with bis(2-deuteriocyclohexyl)borane-B-D_1 [$DB(Cy)_2$] followed by reaction with CH_3CO_2D. As an example, the preparation of labeled linolenic acid is reported.

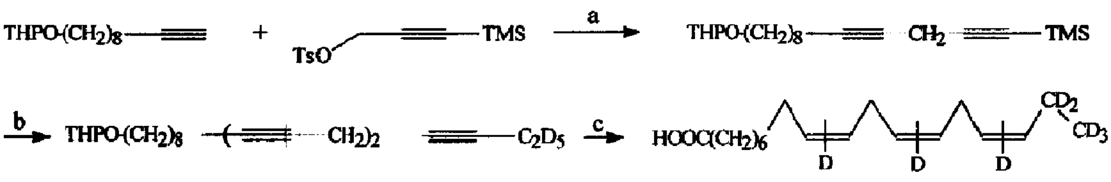
Recently we identified a new group of alkaloids, the azamacrolides, from the beetle *Epilachna varivestis,*[2] and we are now interested in elucidating the pathways by which these compounds are biosynthesized. Consideration of the structure of (5*Z*)-11-propyl-12-azacyclotetradec-5-en-14-olide (epilachnene), the major alkaloid in this family of compounds, suggests that its fourteen carbon chain may be derived from an unsaturated fatty acid such as linolenic acid by a process of chain shortening (*via* two β-oxidations), partial reduction, and an amination step. In order to explore this hypothesis, we have prepared a number of deuterium labeled linolenic acids. Although there is a growing interest in specifically labeled polyunsaturated fatty acids, particularly in connection with pheromone biosynthetic studies,[3] general methods for the synthesis of skipped dienes or polyenes bearing *cis* deuterium atoms are rather limited.[4] While the reduction of triple bonds to double bonds appears to be one of the more straightforward methods, after exploring many procedures we found that at least for compounds bearing three skipped carbon-carbon triple bonds, the use of heterogeneous catalysts,[5,6] or Cu/Zn couple[7] leads to products that are either contaminated substantially with *trans* isomers, or to partially under- and over-reduced material (cf. ref. 8). Moreover, the use of heterogeneous catalyts often results in deuterium scrambling. In fact, the hydroboration procedure described by Brown and Zweifel,[9] later modified by Brown and Molander,[10] for the *cis*-reduction of simple alkynes is the best technique available for this reduction. This procedure is very efficient since the reaction proceeds without over reduction, the products are stereochemically pure, and yields are high.[11] Here we described the use of the Brown-Zweifel procedure for the synthesis of deuterium labeled polyunsaturated fatty acids.

Synthetic Procedures The key step employed in the linolenic acid synthesis was the addition of bis-(2-deuteriocyclohexyl)borane-B-D_1 (formed *in situ* from $NaBD_4$, $BF_3.Et_2O$ and cyclohexene) to substrates with three carbon-carbon triple bonds. The resulting adducts were then subjected to deuterolysis with CH_3CO_2D.[12] In this way, two deuterium atoms can be added to the triple bond. Alternatively, we prepared derivatives bearing only one deuterium atom on each double bond by using bis-(cyclohexyl)borane and CH_3CO_2D. The appropriate triynes were prepared by the alkylation of terminal acetylenes with substituted propargylic tosylates.[13] The synthetic procedures we used are outlined below:



[a] $THPO(CH_2)_8Br$, DMSO; [b] (i) $LiNH_2$, NH_3, EtBr; (ii) TsCl/KOH, THF; [c] (i) $LiNH_2$, NH_3, CD_3CD_2Br; (ii) TsCl/KOH, THF; [d] (i) n-BuLi, THF; (ii) TMSCl; (iii) H^+; (iv) TsCl/KOH, THF. [EDA = ethylene diamine; THF = tetrahydrofuran; THP = tetrahydropyranyl; TMS = trimethylsilyl; Ts = *p*-toluenesulfonyl]

By using various combinations of labeled **B*** and nonlabeled **A-C** intermediates, and different reduction protocols, this synthetic route was used to assemble a variety of labeled linolenic acids and closely related derivatives. Spectral properties of some of the labeled fatty alcohols and methyl esters are given in Table 1, and ^{13}C-NMR data for the resulting alcohols are given in Fig. 1.

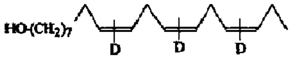
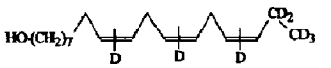
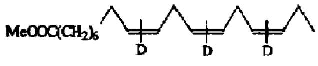
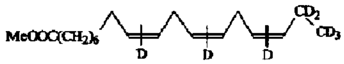


[a] $CuI/NaI/K_2CO_3$, DMF; [b] (i) KF, DMF; (ii) B^*, cond. [a]; [c] (i) $DB(Cy)_2$/ CH_3COOH; (ii) Dowex 50W-8X, MeOH; (iii) PDC, DMF; [THP = tetrahydropyranyl; TMS = trimethylsilyl; Ts = *p*-toluenesulfonyl; DMF = dimethylformamide]

Spectral Properties of Labeled Compounds: The olefinic region of the ^1H broad-band decoupled ^{13}C NMR spectrum of compound **3** bearing six deuterium atoms on the three double bonds, consists of five triplets of similar intensities [$^1J_{C,D} = 23.4$ Hz]. These signals appeared *ca.* 0.5 Hz upfield from those corresponding to the nonlabeled compound as a result of the combined α and β effects of deuterium. In contrast, spectra of compounds **1** and **2** are less complex and show only signals due to protonated carbons, while the triplets expected for deuteriated carbons are obscured by the noise. The five signals observed for **1**, compared to those of nonlabeled linolenyl alcohol, are shifted upfield by 0.1 ppm due the β -effect of the deuterium atom on the neighboring carbon of each double bond. Interestingly, the signal for the ^{13}C-16 atom in compound **2**, observed at 131.68 ppm, is shifted further by 0.1 ppm due to the β-effects of deuterium atoms attached to C-17 and broadened due to $^2J_{DC_{17},C_{16}}$ coupling. The existence of five signals (belonging to six ^{13}C) suggest that compounds **1** and **2** are, as expected, a mixture of isotopomers.

IR spectra of deuteriated linolenyl alcohols compared to that of unlabeled linolenyl alcohol show noteworthy differences in two specific regions. Linolenyl alcohol has an absorption at 3018 cm^{-1} characteristic for the =C-H stretch of skipped *cis* double bonds.[14,15] Introducing one deuterium atom at each double bond increases the frequency of this band by 5 cm^{-1}. Moreover, a new band appears at 2241 cm^{-1} and

Table 1. Spectral Data of Key Products.

	Structure	Spectral data
1		^{1}H NMR spectra (δ, ppm) 5.27 (3H, m); 3.62 (2H, t, *J*=7.5 Hz); 2.81 (4H, m); 2.08 (4H, m); 1.58 (2H, m); 1.4-1.2 (12H, m); 0.97 (3H, t, *J*=7.4Hz); MS spectra (m/z) 267 (M$^+$); IR spectra (cm^{-1}) 3664, 3023, 2934, 2867, 2241, 1636, 1455, 1353, 1269, 1047.
2		^{1}H NMR spectra (δ, ppm) 5.25 (3H, m); 3.62 (2H, t, *J*=7.5 Hz); 2.81 (4H, m); 2.08 (2H, m); 1.58 (2H, m); 1.4-1.2 (12H, m); MS spectra (m/z) 272 (M$^+$); IR spectra (cm^{-1}) 3666, 3023, 2934, 2866, 2231, 2077, 1635, 1454, 1351, 1268, 1048.
3		^{1}H NMR spectra (δ, ppm) 3.62 (2H, t, *J*=7.5 Hz); 2.80 (4H, m); 2.07 (4H, m); 1.58 (2H, m); 1.4-1.2 (12H, m); 0.97 (3H, t, *J*=7.4Hz); MS spectra (m/z) 270 (M$^+$); IR spectra (cm^{-1}) 3666, 2934, 2866, 2252, 1626, 1455, 1352, 1175, 1046.
4		^{1}H NMR spectra (δ, ppm) 5.37 (3H, m); 3.67 (3H, s); 2.81 (4H, m); 2.30 (2H, t, *J*=7.3 Hz); 2.06 (4H, m); 1.60 (2H, m); 1.4-1.2 (10H, m); 0.97 (3H, t, *J*=7.4Hz); MS spectra (m/z) 295 (M$^+$); IR spectra (cm^{-1}) 3022, 2936, 2866, 2242, 1759, 1441, 1353, 1173, 1023.
5		^{1}H NMR spectra (δ, ppm) 5.38 (3H, m); 3.66 (3H, s); 2.81 (4H, m); 2.32 (2H, t, *J*=7.5 Hz); 2.04 (2H, m); 1.60 (2H, m); 1.4-1.2 (10H, m); MS spectra (m/z) 300 (M$^+$). IR spectra (cm^{-1}) 3023, 2935, 2865, 2230, 2076, 1759, 1441, 1352, 1173, 1024, 932.

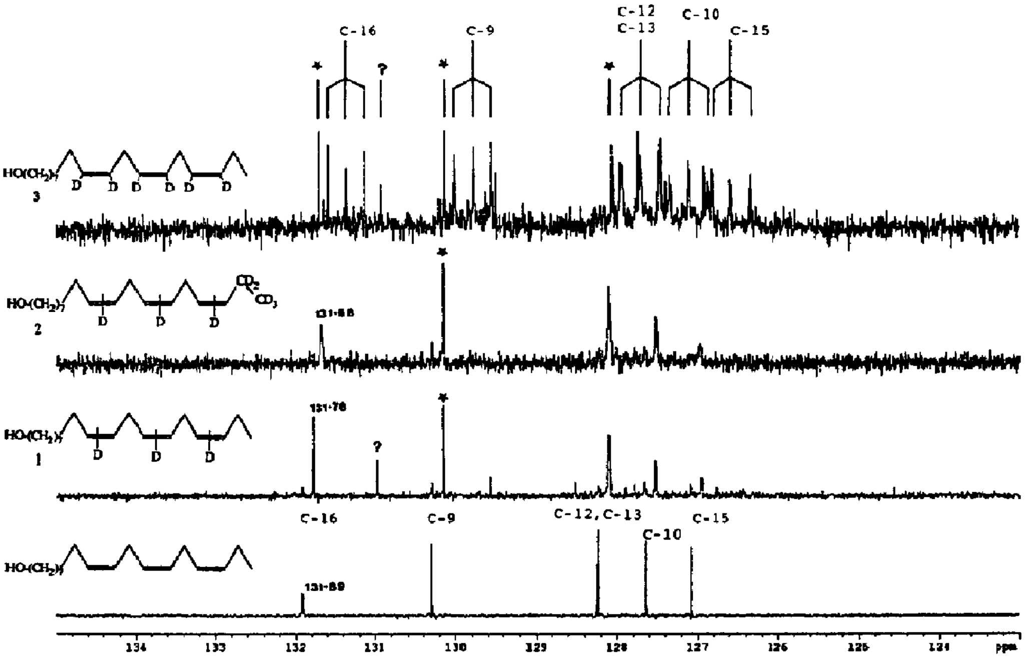


Figure 1. Olefinic region of ^{1}H broad-band decoupled ^{13}C NMR spectra of linolenyl alcohol and three labeled alcohols (**1** - **3**) measured in $CDCl_3$ at 100.6 MHz.

(The peaks marked with * represent =C(H) signals arising from incompletely deuterated congeners. Although these impurities are <5%, their =C(H) signals are deceptively intense due to the disproportionate NOE enhancement they receive during the decoupling experiment; the peak marked "?" is due to an unidentified impurity; spectra are plotted on different scales to normalize peak intensities).

2230 cm^{-1} in the spectra of 1 and 2 respectively. A complete substitution of olefinic hydrogen by deuterium replaces the *cis* =C-H stretch band by a new absorption at 2252 cm.$^{-1}$ The mass spectra of **4** and **5** exhibited ions and losses characteristic of methyl esters (M^+-31 and *m/z* 59), in addition to the expected molecular ions.

We conclude that deuterioboration is uniquely suitable for the preparation of skipped polyenes labeled with deuterium at the carbon-carbon double bonds. Our most important observation in this context is that the final products were virtually free (< 0.5% by GC) of *trans* isomers and of over- or under-reduced material. Consequently, no further purification steps, which are essential for products prepared by other reduction procedures, are necessary.

Acknowledgment: The partial support of this work by NIH grant AI 12020 and NSF grant MCB-9221084 is acknowledged with pleasure.

REFERENCES AND NOTES

1. Permanent address: Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic.
2. Attygalle, A. B.; McCormick, K. D.; Blankespoor, C. L.; T. Eisner, T.; Meinwald, J. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5204-5208.
3. Jurenka, J. A.; Roelofs, W. L. In *Insect Lipids: Chemistry, Biochemistry, Biology* Stanley-Samuelson, D. W.; Nelson, D. R. Eds.; Univ. of Nebraska Press: Lincoln, Nebraska, 1993; pp. 353-388.
4. Tulloch, A. P. *Chem. Phys. Lipids* **1979**, *24*, 391-406.
5. Hydrogenation with nonmodified Lindlar catalyst (Pd/$CaCO_3$) poisoned by quinoline gave a product contaminated with other isomers [Reference 8]. Column chromatography with $AgNO_3/SiO_2$ allowed the isolation of the desired product. However, the yield was poor (40%).
6. We used the modified P2-Ni reduction procedure described by Byrne, B.; Lafleur Lawter, L. M.; Wengenroth, K. J. *J. Org. Chem.* **1986**, *51*, 2607-2609. Even from a substrate bearing only one triple bond, a mixture containing the desired product (90%), starting material (5%) and completely reduced (5%) compounds was obtained.
7. Näf, F.; Decorzant, R.; Thommen, W.; Willhalm, B.; Ohloff. G. *Helv. Chim. Acta* **1975**, *58*, 1016-1037.
8. Huang, W.; Pulaski, S. P.; Meinwald, J. *J. Org. Chem.* **1983**, *48*, 2270-2274.
9. Brown, H. C.; Zweifel, G. *J. Amer. Chem Soc.* **1959**, *81*, 1512.
10. Brown, H. C.; Molander, G. A. *J. Org. Chem.* **1986**, *51*, 4512-4514, and references cited therein.
11. Millar, J. G; Underhill, E. W. *Can J. Chem.* **1986**, *64*, 2427-2430.
12. A modified hydroboration procedure [Zweifel, G.; Brown, H. C. *Organic Reactions* **1963**, *13*, 1-54] was used. A suspension of $NaBD_4$ (293 mg; 6.5 mmol) in THF (2 ml) and cyclohexene (940 µl, 9.3 mmol) was treated with boron trifluoride etherate (570 µl; 4.6 mmol) at 0°C. The resulting white suspension was stirred at room temperature for 1.5 h, and a THF solution of tetrahydropyranyloxy-9,12,15-octatriyne (348 mg, 1 mmol) was added at 0°C. The reaction mixture was stirred overnight, and hydrolyzed with CD_3CO_2D (Aldrich, 1.9 ml). It was made basic with NaOH solution, and oxidized with H_2O_2 (30%, 1.9 ml). Usual work up followed by flash chromatography afforded 300 mg of a yellow oil, which was deprotected by treating with Dowex 50w-8X in methanol to give the alcohol **3** (66% yield).
13. Lapitskaya, M. A.; Vasiljeva, L. L.; Pivnitsky, K. K. *Synthesis* **1993**, 65-66.
14. Attygalle, A. B.; Svatoš, A.; Wilcox, C.; Voerman, S. *Anal. Chem.* **1994**, *66*, 1696-1703.
15. Attygalle, A. B.; Svatoš, A.; Wilcox, C.; Voerman, S. (in preparation)

(*Received in USA* 21 *June* 1994; *revised* 11 *August* 1994; *accepted* 21 *October* 1994)