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PROTON MAGNETIC RESONANCE SPECTRA OF LEUKOTRIENE A METHYL ESTER (I), LEUKOTRIENE B METHYL ESTER DIACETATE (II) AND 12-*epi*-6-*E*,8-*Z*-LEUKOTRIENE B METHYL ESTER DIACETATE (III; 5-*S*,12-*S*-di-HETE)

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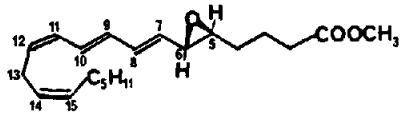
Abstract—High resolution proton magnetic resonance (pmr) spectra of derivatives of three important 5-oxygenated eicosanoids of synthetic origin, leukotriene A methyl ester (I), leukotriene B methyl ester diacetate (II) and 5-*S*,12-*S*-di-HETE methyl ester diacetate (III) have been obtained. In the case of II and III, good spectra could also be obtained for samples of biological origin although only microgram amounts were available, and their identity with those from synthetic material provided independent confirmation of stereochemistry and structure for leukotriene B and 5-*S*,12-*S*-di-HETE. The complex pmr spectra of I, II and III were also analyzed systematically to determine peak assignments and coupling constants, and from these, significant information with regard to molecular conformation was derived.

Recently a number of new biologically active substances derived from arachidonic acid by routes involving oxygenation at C-5 have been discovered. These include the initial product of the pathway, 5-*S*-hydroperoxy-6-*E*-8,11,14-*Z*-eicosatetraenoic acid (5-HPETE), and the further transformation products leukotrienes (LT's) A, B, C, D and E.[1-3] The native leukotrienes A–E have only been obtained in small (microgram or sub-microgram) amount in solution, usually aqueous. The structures of these substances were assigned on the basis of chemical and enzymic transformations and comparison with synthetic compounds whose structures and stereochemistry follow from unambiguous chemical syntheses.[3-7] The availability of synthetic LTA methyl ester (I) and LTB methyl ester diacetate (II) have permitted the determination of proton magnetic resonance (pmr) spectra of these important substances. These are reported herein along with chemical shift assignments and coupling constants. Although the amount of native LTA (or ester) available has been insufficient for such pmr studies, enough native LTB could be obtained to allow measurement of the pmr spectrum of its methyl ester diacetate derivative. Similarly, pmr spectra of the methyl ester diacetate of synthetic[8] and native[9] 5-*S*,12-*S*-di-HETE (III) could be determined and peak assignments made. These pmr studies provided independent confirmation of the identity of



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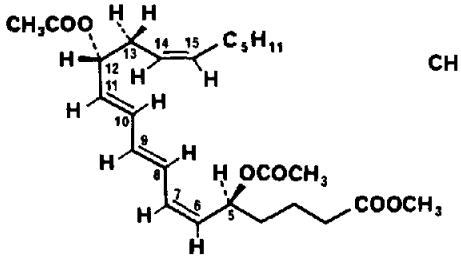

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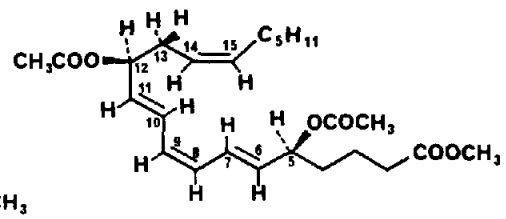

III

native LTB and 5-*S*,12-*S*-di-HETE with synthetic compounds which are parents of structures II and III, respectively.

The pmr data were obtained using three different spectrometers operating at fields from 250–500 MHz. The spectra, measured using as little as 15 μg of sample, are sufficiently definitive in our view to be very useful for the identification of biologically derived products, certainly when taken together with reversed phase HPLC data and bioassays. For this reason pmr spectra of I, II and III are reproduced herein.

The analysis of the spectra of I–III which led to peak assignments, chemical shifts, and coupling constants relied heavily on spin–spin decoupling experiments which are outlined in the following sections. The methods used can serve as a useful guide to the analysis of other linear polyunsaturated systems, but more importantly, they lead to information regarding the conformation of the conjugated polyene moieties of I–III which have important implications for the design of biologically active analogs.

Leukotriene A methyl ester (I)

The pmr spectrum of LTA methyl ester at 270 MHz is reproduced in Fig. 1 along with an expanded representation of the olefinic proton region. Table 1 presents chemical shift values for individual protons in I and data on coupling constants. Assignment of the olefinic region of the pmr spectrum of leukotriene A methyl ester[4] was achieved as follows. Irradiation of the multiplet at δ3.03 (H-6 and H-13) caused both collapse of the signal at δ5.41 and simplification of the complex multiplet at δ5.53–5.63. It was expected that the nearly equivalent H-14 and H-15 were part of the δ5.53–5.63 signal suggesting that the resonance at δ5.41 was H-12 or H-7. As the multiplet δ5.41 consisted of a large (15.3 Hz) coupling suggesting a *trans* olefin, this resonance was assigned to H-7. Irradiation of H-7 caused the multiplet centered at δ6.44 to collapse identifying it then as H-8.

Irradiation of the δ5.53–5.63 region (now assigned to H-12, H-14, H-15) resulted in a simplification of only the signal at δ6.17 therefore assigned to H-11. Consequently, irradiation of H-11 enabled assignment of the downfield

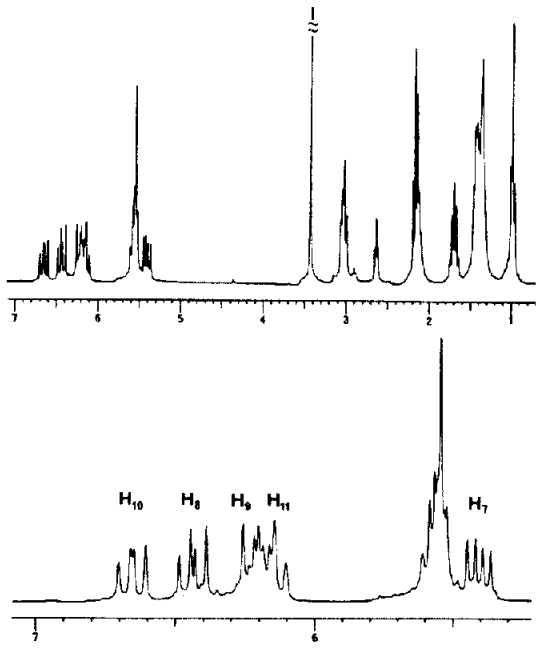

Fig. 1. Pmr spectrum of synthetic leukotriene A methyl ester (I) at 270 MHz in C_6D_6. Upper panel, full spectrum; lower panel, expanded spectrum in 5–7 δ region.

multiplet at δ6.23 to H-10. The remaining signal at δ6.23 was attributed to H-9 and indeed irradiation of H-9 caused both H-8 and H-10 to change their multiplicity.

Thus assignment of the triene region is complete and the olefin stereochemistry follows from the magnitude of the coupling constants. The method of synthesis in this case allowed the assumption of a trans stereochemistry for the 7,8 olefin linkage. In addition nmr data of diene systems[10] suggested that the outermost olefinic hydrogens would be furthest upfield and also that the vicinal couplings such as $J_{8,9}$ would be approximately the same as the cis-olefinic couplings $J_{11,12}$ resulting in triplet multiplicity for protons such as H-11 whereas H-7, H-8, H-9, H-10 appear as double doublets.

Table 1. 270 MHz ^{1}H NMR data for I in D_6-benzene

Proton	Chemical Shift (δ)	Coupling Constant (Hz)	
CO_2CH_3	3.43		
H_5	2.64	$J_{5,6}$	2.0
H_6	3.03	$J_{6,7}$	7.7
H_7	5.41	$J_{7,8}$	15.3
H_8	6.44	$J_{8,9}$	10.8
H_9	6.23	$J_{9,10}$	14.7
H_{10}	6.65	$J_{10,11}$	11.4
H_{11}	6.17	$J_{11,12}$	11.4
H_{12}	5.53 - 5.63		
H_{13}	3.03		
H_{14}	5.53 - 5.63		
H_{15}	5.53 - 5.63		

Leukotriene B methyl ester diacetate (II)

A 250 MHz pmr spectrum of II is shown in Fig. 2. The 270 MHz proton spectra of II in D_6-benzene confirmed the identity of the natural (15 μg sample, 23,500 transients, 18 hour total acquisition time) and synthetic (70 μg) samples, not only with regard to the methyl singlets (δ1.73, 1.80 and 3.40), but additionally in the olefin region.

Double resonance experiments conducted at 250 MHz on a 700 μg sample of synthetic II allowed assignment of all resonances above δ2.4, shown in Table 2. Irradiation of the multiplet centered at δ2.45, initially assumed to represent the geminally coupled nonequivalent protons H_{13}, produced changes in the low field region from δ5.5 to 5.6, leaving the multiplet at δ5.97 unchanged. The unaltered multiplicity of the signal at δ5.97 strongly supported its assignment as H-5.

Sequential irradiation at δ5.97 (H-5), δ5.38 (H-6), δ6.08 (H-7) and δ6.84 (H-8) resulting in the partial collapse of signals at δ5.38 (H-6), δ6.08 (H-7), δ6.84 (H-8) and δ6.15 (H-9), respectively, permitted direct assignment of resonances to H-6, H-7, H-8 and H-9. Although irradiation of δ6.15 did not permit the direct assignment of H-10 due to the close proximity of the H-9 and H-10 signals, the failure of irradiation at δ6.15 (H-9) to alter the signal at δ5.71 (H-11), together with irradiation at δ6.40 (H-10) resulting in collapse of the signal at δ5.71 (H-11), unequivocally completed assignment of the trieneic region. Careful reinspection of the original irradiation at δ2.45 (H-13) and one further experiment, irradiation at δ2.1 (H-16) then allowed assignment of H-12, H-14 and H-15.

The availability of a small amount of time on a 500 MHz pmr instrument permitted the acquisition of a higher field spectrum. At 500 MHz only the nearly coincident resonances of H-12 and H-15 remained unresolved, and the above assignment was fully supported.

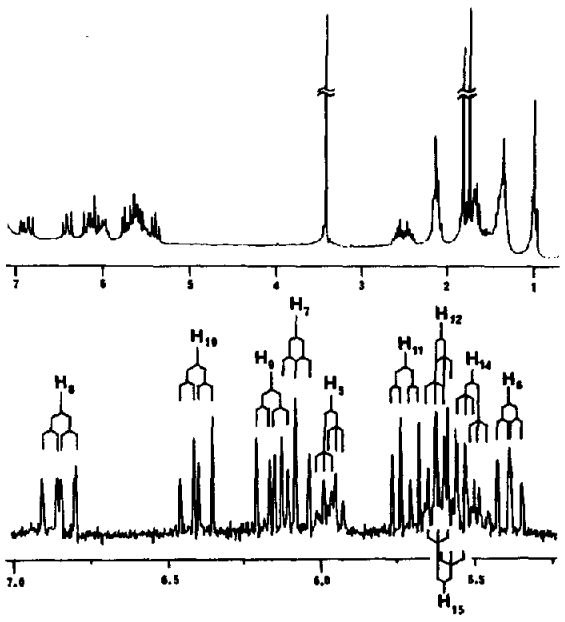

Fig. 2. Pmr spectrum of synthetic leukotriene B methyl ester diacetate (II) at 250 MHz in C_6D_6. Upper panel, full spectrum; lower panel, 5–7 δ region expansion.

5-S,12-S-di-HETE methyl ester diacetate (III)

Synthetic (70 μg) and natural (10 μg) samples of III provided identical 300 MHz pmr spectra. The internal symmetry of the trieneic moiety of III complicated the

Table 2. 250 MHz ^{1}H NMR data for II in D_6–benzene

Proton	Chemical Shift (δ)	Coupling Constant (Hz)	
H_5	5.97	$J_{5,6}$	9.5
H_6	5.38	$J_{6,7}$	11.0
H_7	6.08	$J_{7,8}$	11.3
H_8	6.85	$J_{8,9}$	14.8
H_9	6.15	$J_{9,10}$	10.5
H_{10}	6.40	$J_{10,11}$	14.9
H_{11}	5.72	$J_{11,12}$	6.9
H_{12}	5.61	$J_{12,13}$	6.3
H_{13}	2.42	$J_{13,13}$	16.1
H_{13}	2.48	$J_{13,14}$	7.5
H_{14}	5.50	$J_{14,15}$	10.7
H_{15}	5.61	$J_{15,16}$	7.7
H_{16}	2.1		
H_{16}	2.1		
$-CO_2CH_3$	3.40		
5,12-OAc	1.73 1.80		

assignment of the proton spectrum considerably relative to that of II. A pmr spectrum obtained with synthetic III at 300 MHz is shown in Fig. 3. An assignment (Table 3) was devised as follows.

Homodecoupling experiments at 300 MHz rapidly established that the lowest field signals were due to the olefinic protons H-7, H-8, H-9 and H-10, and additionally allowed signals due to H-6 and H-11 to be identified in the complicated δ5.5 to 5.7 region. However, differentiation of the two sets of symmetrically related protons was not yet possible; for example, did the coupled protons at δ5.60, 6.90 and 5.96 represent H-6, H-7 and H-8 or H-11, H-10 and H-9? As with II, the solution was again sequential irradiation of H-13 and H-16. In neither case was the multiplet at δ5.48 altered. Since the only unassigned signals represented H-5, H-12, H-14 and H-15, this clearly implicated δ5.48 as H-5. Irradiation of δ5.48 (H-5) allowed assignment of H-6, and thus H-7, H-8, H-9 and H-10. Close examination of the remaining unassigned lines in the δ5.5 to 5.7 region produced an internally consistent assignment for all lines.

The assignment was verified by computer simulation using a minicomputer version of the LAOCOON program on an ASPECT 2000 computer. The calculated and observed spectra were in substantial agreement, confirming the assignment.

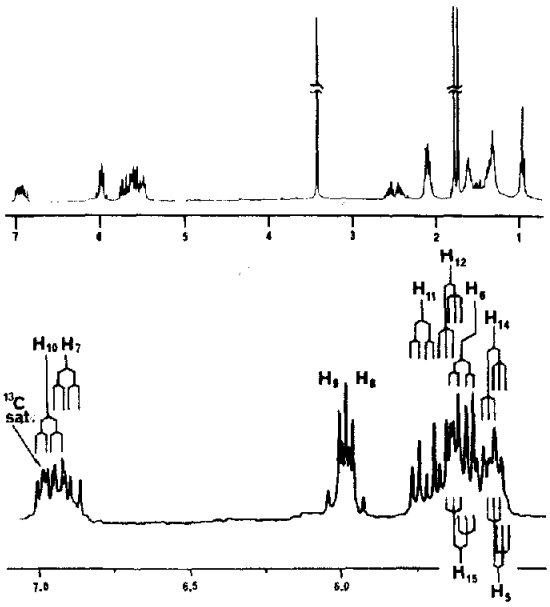

Fig. 3. Pmr spectrum of synthetic 5-*S*,12-*S*-di-HETE methyl ester diacetate at 270 MHz in C_6D_6. Upper panel, full spectrum; lower panel, expanded 5–7 δ region with illustrated pattern of spin–spin splittings.

EXPERIMENTAL

Spectra. Pmr spectra were measured using one or more of the following spectrometers: Bruker WM-250 (250 MHz) and WM-300 (300 MHz) instruments, JEOL FX-270 (270 MHz), or the 500 MHz instrument of the Bitter National Magnet Laboratory, M.I.T. All samples (10–100 μg) were purified by high performance liquid chromatography (hplc) and residual traces of proton-containing solvent were removed by repeated addition of 99.96 at.% D_6-benzene (Merck) and evaporation under argon in a 5 mm pmr tube which had been prewashed with D_2O and dried.

Table 3. 300 MHz ^{1}H NMR data for III in D_6-benzene

Proton	Chemical Shift (δ)	Coupling Constant (Hz)	
H_5	5.48	$J_{5,6}$	7.4
H_6	5.60	$J_{6,7}$	14.9
H_7	6.90	$J_{7,8}$	10.2
H_8	5.96[a]	$J_{8,9}$	11[a]
H_9	6.00[a]	$J_{9,10}$	10.2
H_{10}	6.96	$J_{10,11}$	15.0
H_{11}	5.73	$J_{11,12}$	7.4
H_{12}	5.64	$J_{12,13}$	6.4
H_{13}	2.43	$J_{13,13}$	14.0
H_{13}	2.56	$J_{13,14}$	7.0
H_{14}	5.51	$J_{14,15}$	11.2
H_{15}	5.60	$J_{15,16}$	7.4
H_{16}	2.1		
H_{16}	2.1		
$-CO_2CH_3$	3.43		
5,12-OAc	1.75 1.79		

[a]Calculated

Spectral data in Tables 1–3 are not corrected for non-first order effects. Reported chemical shift values represent the geometric center of the observed multiplet relative to residual protons in D_6-benzene as $\delta 7.25$. Tabulated coupling constants are ± 0.7 Hz.

LTB methyl ester diacetate (II). Ethereal diazomethane was vaporized into a cold solution (0°) of 20 μg of LTB in 4 ml of 1:1 methanol–ether until a definite yellow color of the reagent persisted, and the mixture was stored for 1 h at 0° and concentrated to dryness under a stream of argon. The residue was transferred into a 5 mm culture tube with a little ether and the methyl ester was freed of solvent *in vacuo* (0.1 mm, 15 min to remove last traces). Pyridine (20 μl) and acetic anhydride (2 μl) were added and the mixture was stored at 25° for 6 h. Excess anhydride was hydrolyzed by exposure to 5 μl of water at 25° for 10 min and the mixture was diluted with 1 ml of pentane and washed with 0.25 ml of water. The pentane layer was concentrated under argon and the residual pyridine was removed under high vacuum. Hplc purification on a μ-Porasil column using 0.5% isopropyl alcohol-hexane for elution afforded 16.5 μg or pure II (64% by ultraviolet assay), as a single peak by analytical hplc (retention vol. 7.5), ultraviolet maxima at 261.4, 270.5, 281.6 nm.

The same procedure was used for the conversion of 5-*S*,12-*S*-di-HETE to the methyl ester diacetate III (ultraviolet maxima at 260, 269.5, 280 nm; hplc retention vol. 5.8 under the above described conditions) except that the acetylation step was conducted at 35° for 17 h.

DISCUSSION

The first total syntheses of leukotrienes A and B and 5-*S*,12-*S*-di-HETE were carried out in these laboratories[4–8] and as part of this work the pmr spectra reported herein were measured. The identity of pmr spectra of derivatives II and III derived from synthetic material and native sources provide an independent confirmation that the synthetic and natural compounds are the same. It is noteworthy that good spectra could be obtained on II and III from native sources though only 15 μg were available. Such a comparison could not be made for the case of I because not even 1 μg of that substance of natural origin could be obtained. The pmr spectra of I–III should assist in future identification of leukotrienes and di-HETEs from biological experiments.

The pmr data, after analysis and peak assignment, also reinforce the conclusions reached on the basis of methods of synthesis with regard to the exact formulation of LTA, LTB and 5-*S*,12-*S*-di-HETE. The coupling constants for *trans* CH=CH units fall in the expected range 15.0 ± 0.4 as do those for *cis* CH=CH units, 11.0 ± 0.4.[11,12] In general, protons at the end carbons of the conjugated triene unit (HC=C–C=C–C=CH) showed chemical shifts upfield from protons attached to the inner carbons of the triene unit (C=CH–CH=CH–CH=C), again in line with previous experience.[11,12]

The coupling constants observed for olefinic protons across a connecting single bond, i.e. C=CH–CH=C, were uniformly observed to be 11 ± 0.5 Hz for the triene units in I, II and III. This fact indicates that in I–III the triene units are essentially planar with a transoid arrangement of olefinic protons about interconnecting single bonds. This conformational preference for the triene moiety, illustrated in the drawings displayed herein for I, II and III, is of special interest in connection with the design of biologically active analogs of LTA and LTB. Studies on analogs generated from this approach, which have been underway for some time, have already yielded significant results which will be dealt with in forthcoming publications.

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REFERENCES

[1]P. Borgeat and P. Sirois, *J. Med. Chem.* **24**, 121 (1981) (Review).
[2]D. M. Bailey and L. W. Chakrin, *Ann. Rept. Med. Chem.* **16**, 213 (1981) (Review).
[3]E. J. Corey, Y. Arai and C. Mioskowski, *J. Am. Chem. Soc.* **101**, 6748 (1979).
[4]E. J. Corey, D. A. Clark, G. Goto, A. Marfat, C. Mioskowski, B. Samuelsson and S. Hammarström, *Ibid.* **102**, 1436, 3663 (1980).
[5]E. J. Corey, S. Hashimoto and A. E. Barton, *Ibid.* **103**, 721 (1981).
[6]E. J. Corey, D. A. Clark, A. Marfat and G. Goto, *Tetrahedron Letters* **21**, 3143 (1980).
[7]E. J. Corey, A. Marfat, J. E. Munroe, K. S. Kim, P. B. Hopkins and F. Brion, *Ibid.* **22**, 1077 (1981).
[8]E. J. Corey, A. Marfat and B. C. Laguzza, *Ibid.* **22**, 3339 (1981).
[9]P. Borgeat, S. Picard, P. Vallerand and P. Sirois, *Prostaglandins and Medicine* **6**, 557 (1981).
[10]H. W. Gardner and D. Wisleder, *Lipids* **5**, 678 (1970).
[11]A. V. Cunliffe, R. Grinter and R. K. Harris, *J. Magn. Res.* **2**, 200 (1970).
[12]P. Albriktsen and R. K. Harris, *Acta Chem. Scand.* **27**, 1875 (1973).