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^{13}C NMR STUDIES OF THE FOUR 20,22-EPOXYCHOLESTEROLS AND THE TWO 20(22)-DEHYDROCHOLESTEROLS

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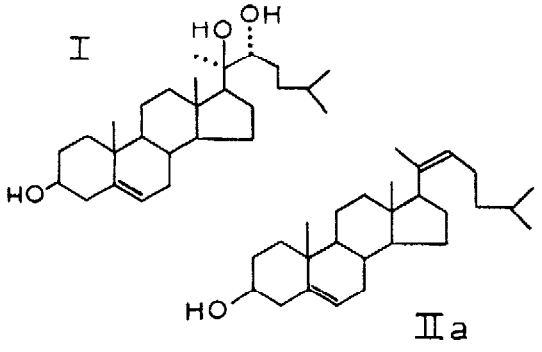
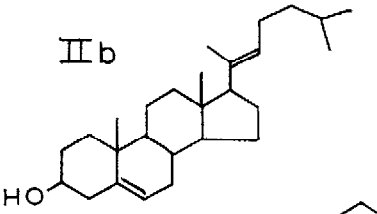
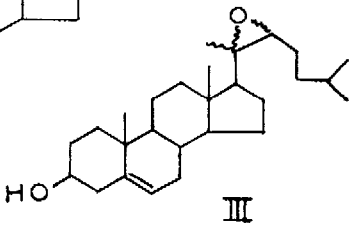
Recent investigators[1] of the biological conversion of cholesterol to pregnenolone via (20R,22R)-20,22-dihydroxycholesterol (I) have suggested 20(22)-dehydrocholesterol (IIa or IIb) and a 20,22-epoxycholesterol (III) as intermediates in the formation of I.



IIa

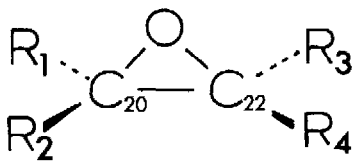




III

The stereochemistry of such intermediates is important since a biochemical opening of the epoxide ring would require a specific configuration (which would be formed from only one of the dienes IIa or IIb) in order to give the biologically correct 20R,22R configuration[2] for I.

The ^{13}C nmr technique has been shown to be useful as a probe of steric interactions of epoxide substituents[3-5]. However, the lack of data on complex tri-substituted acyclic epoxides and our interest in the possible use of ^{13}C nmr in conformational studies of various cholesterol side chain derivatives have led us to synthesize compounds IIa, IIb and IIIa-d. We wish to report here the synthesis and ^{13}C nmr data of these compounds.



	R_1	R_2	R_3	R_4
IIIa (20R,22S)	CH_3	Steroid nucleus	H	C_5H_{11}
IIIb (20S,22R)	Steroid nucleus	CH_3	C_5H_{11}	H
IIIc (20R,22R)	CH_3	Steroid nucleus	C_5H_{11}	H
IIId (20S,22S)	Steroid nucleus	CH_3	H	C_5H_{11}

The ^{13}C chemical shift assignments of IIa, IIb and IIIa-d have been made and together with those of cholesterol (IV)[10] are listed in the Table. Assignments are based on noise and CW decoupled spectra and on comparison with the cholesterol data.

In view of the planar olefinic structure and the near planarity of the epoxide carbon-substituent bonds, it was expected that some similarities in chemical shift trends within the two series would be observed. From the Table it can be seen in both series that geometrical changes about C-20 - C-22 are reflected primarily in the chemical shifts of C-17, C-18 and C-20 to C-23 with little or no effect apparent in the remaining carbon resonances. The effect of configuration on the shifts of C-17 and C-21 is consistent with previous results[4,9] and the cis shielding influence of the C-23 group is apparent. Thus, C-21 is more highly shielded in those configurations where it is cis to C-23 (IIb, IIIc, IIId) rather than trans (IIa, IIIa, IIIb). A similar influence is apparent in the C-17 data.

While configurational changes about C-20 - C-22 result in a maximum variation of only 1 ppm in the C-20 chemical shift, very substantial differences (6.7 ppm) are seen in the C-22 data. In IIa and IIb the monosubstituted C-22 is shielded relative to the disubstituted C-20, as would be expected from previous work[9]. However, while a similar relationship could be expected for the epoxides[4,5], the opposite is seen to be the case and in IIIa and IIIb the C-22 (doublet in SFORD) is in fact well downfield of C-20 (singlet in SFORD). In IIIc and IIId C-20 and C-22 have similar shifts. The deshielding effect on C-22 (and possibly the increased shielding of C-18 in IIIc and IIId) may well reflect the influence of the epoxide group on conformational preference about the C-17 - C-20 bond.

TABLE. $^{13}C^{a,b}$ nmr chemical shifts of compounds IIa, IIb, IIIa-d, IV

Carbon	IV	IIa	IIb	IIIa	IIIb	IIIc	IIId
13	42.3	45.9	43.6	42.5	42.4	43.2	42.5
14	56.8	55.9	56.5	56.4	56.2	56.7	56.8
15	24.3	24.7	24.5	24.3	24.7	23.9	24.1
16	27.9	26.3	26.0	26.9	26.3	26.2	26.6
17	56.3	51.2	59.1	53.3	50.5	56.7	56.8
18	11.8	14.0	13.0	14.7	14.4	13.2	13.5
20	35.8	134.2	134.1	61.1	61.0	60.0	60.4
21	18.7	22.8	17.8	22.6	22.7	17.3	20.3
22	36.5	129.6	126.1	66.5	65.1	60.4	59.8
23	23.8	24.7	24.9	23.4	24.7	21.9	22.4
24	39.9	38.5	38.9	36.4	35.8	35.6	35.7

[a] In ppm (± 0.1 ppm) downfield from TMS, 0.3M in $CDCl_3$. Spectra were taken in FT mode on a Varian HA100 modified with Digilab NMR-3HC.

[b] Remaining carbon resonances had similar δ's in all seven compounds: C1 (δ 37.4 ± 0.1), C2 (δ 32.1 ± 0.3), C3 (δ 71.8 ± 0.2), C4 (δ 42.4 ± 0.1), C5 (δ 141.0 ± 0.2), C6 (δ 121.6 ± 0.2), C7 (δ 31.9 ± 0.2), C8 (δ 31.9 ± 0.2), C9 (δ 50.4 ± 0.2), C10 (δ 36.6 ± 0.2), C11 (δ 21.0 ± 0.3), C12 (δ 39.6 ± 0.3), C19 (δ 19.4 ± 0.1), C25 (δ 28.0 ± 0.2), C26 (δ 22.6 ± 0.2), C27 (δ 22.6 ± 0.2)

The synthesis of IIb has already been described[6]. Following the modified method by Josan and Eastwood[7], the 3β-acetate of IIa was synthesized from (20R,22S)-3β,20,22-trihydroxycholesterol 3β-acetate and the resulting acetate was hydrogenolyzed with LAH to give the corresponding dehydrocholesterol IIa.

Oxidation of the 3β-acetate of IIb with m-chloroperbenzoic acid gave the 3β-acetates of IIIc and IIId which were separated on preparative tlc, followed by hydrogenolysis of their acetates with LAH to give the corresponding epoxycholesterols, IIIc and IIId.

(20R)-3β-acetoxy-20(2'-tetrahydropyranyloxy)-pregn-5-ene-20-carbonitrile was converted to the corresponding aldehyde by reduction with diisobutylaluminum hydride to the imine, followed by aqueous acetic acid hydrolysis in ethanol. Grignard reaction of this 3β-acetate aldehyde with isoamylmagnesium bromide in CH_2Cl_2 at -70° and cleavage of the THP ether under acidic conditions gave the 3β-acetate of I which was converted to its 22-mesylate. IIIa was obtained by treatment of this mesylate with aqueous KOH in pyridine.

Oxidation of 3β-acetate of IIb with OsO_4 and hydrolysis of the osmate with aqueous $NaHSO_3$ yielded the (20S,22S)- and (20R,22R)- glycol acetates which were separated by HPLC. The major fraction (20S,22S) glycol was transformed to IIIb as described above. The full details of these syntheses will be described elsewhere[8].

Acknowledgments

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