Structure of a Potential Intermediate in Cholesterol Biosynthesis

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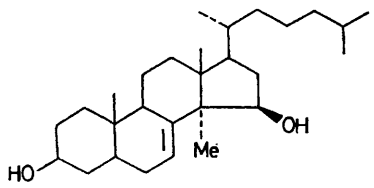
Summary The structure of the epimer (at C-15) of 14α-methylcholest-7-ene-3β,15-diol which is convertible into cholesterol upon incubation with rat liver homogenate preparations has been established as 14α-methylcholest-7-ene-3β,15β-diol.

THE possibility of 15-oxygenated sterols as intermediates in the overall enzymatic removal of the C-32 methyl group of Δ^8 and Δ^7-sterol precursors of cholesterol has been considered by several groups.[1–4] In previous work[3] we have shown that hydride reduction of 3β-benzoyloxy-14α-methylcholest-7-en-15-one yields two epimers (at C-15) of 14α-methylcholest-7-ene-3β,15-diol. The two epimers have been arbitrarily designated as Diol A and Diol B. Only one



FIGURE

(Diol A) of the two epimers was found to be convertible into cholesterol upon incubation with rat liver homogenate preparations.[3] We now report that Diol A is the 15β-diol.

Diols A and B were synthesised and purified as previously described.[3] The absolute configuration at C-15 was investigated through *X*-ray crystallographic analysis. Upon reaction of Diol A with *p*-bromobenzoyl chloride in pyridine, three products (R_f 0·9, 0·7, and 0·5) were obtained and purified by preparative t.l.c. on silica gel with benzene as solvent. The major product (R_f 0·5) formed plates from EtOAc–MeOH, m.p. 193—195°, clearing at 219—220°. N.m.r. and low-resolution mass spectral analyses were compatible with a 3β-*p*-bromobenzoate ester of Diol A. High-resolution mass spectrometry showed two molecular ions at *m/e* 600·2998 ($C_{35}H_{51}{}^{81}BrO_3$) and 598·2971 ($C_{35}H_{51}{}^{79}BrO_3$).

The crystals were suitable for *X*-ray analysis. *Crystal data:* $C_{35}H_{51}O_3Br$, M 599, monoclinic, $a = 6{\cdot}819(3)$, $b = 9{\cdot}513(5)$, $c = 25{\cdot}272(11)$ Å, $\beta = 94°32(3)'$, $U = 1639{\cdot}2$ Å^3, $\mu = 17{\cdot}5$ cm^{-1} (Cu-K_α), F (000) = 664, $D_m = 1{\cdot}19$ g cm^{-3}, $Z = 2$, $D_c = 1{\cdot}21$ g cm^{-3}, space group $P2_1$.

2101 nonzero reflections were measured on a Picker FACS-1 diffractometer using Cu-K_α radiation. The structure was solved by the heavy atom method and has been refined by full-matrix least-squares methods on positional and anisotropic thermal parameters for all but three of the non-hydrogen atoms to an R factor of 0·091 on all observed data.† The *X*-ray data indicated that the C-15 hydroxy-function has the β-configuration.

This work establishes that the epimer (at C-15) of 14α-methylcholest-7-ene-3β,15-diol which is convertible into cholesterol upon incubation with rat liver homogenate preparations has the 15β-configuration (Figure). In consideration of this finding, we note reports from three laboratories[5] which are compatible with a stereospecific loss of the 15α-hydrogen of lanosterol upon enzymatic formation of cholest-7-en-3β-ol, 7-dehydrocholesterol, and cholesterol. Since all hydroxylation reactions at saturated carbon atoms in the sterol nucleus studied to date have been shown to involve introduction of the hydroxyl-function with 'retention of configuration,' the findings that the hydroxy-group at C-15 in the epimer which is convertible into cholesterol has the β-configuration while the hydrogen that is lost from C-15 in the overall conversion of lanosterol into cholesterol has the α-configuration strongly indicates that the sterol under consideration may not be a significant intermediate in cholesterol biosynthesis. However, such a conclusion may be premature since the possi-

† In the crystal there is a rotational disorder around the C(24)–C(25) bond such that the two carbon atoms of the terminal *gem*-dimethyl group occupy three sites. These atoms were assigned isotropic temperature factors and the occupancy of the three sites was varied to give final values of 0·74(4), 0·61(5), and 0·66(6).

bility exists that not all hydroxylation reactions procede with retention of configuration. This latter possibility has been suggested by other studies[6] of the biosynthesis of non-steroidal natural products in plants.

Additional information is clearly required to satisfy criteria[4] for the assignment of an intermediary role of 14α-methylcholest-7-ene-3β,15β-diol in cholesterol biosynthesis.

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