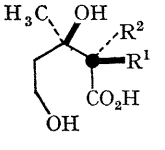
# The Stereochemical Origin of the C-22 Hydrogen Atoms of Cholesterol

By ELIAHU CASPI* and J. MALCOLM ZANDER
(*Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts* 01545)

*Summary* The 22-*pro-R* and 22-*pro-S* protons of cholesterol are derived from the 2-*pro-R* and 2-*pro-S* protons of mevalonic acid, respectively, and the protozoan *T. pyriformis* is shown to eliminate the 7$\beta$- and 22-*pro-R* hydrogens of cholesterol in the conversion into cholesta-5,7,22-trien-3$\beta$-ol.

THE formation of squalene from mevalonic acid (MVA) in the rat has been shown to be a stereospecific process.[1,2] Thus (2*R*)-[2-$^3H_1$; 2-$^{14}C$]-MVA (Ia) gives rise[3] to [1,5*R*,9*R*,16*R*,20*R*,24-$^3H_6$]squalene (IIa); conversely (2*S*)-[2-$^3H_1$; 2-$^{14}C$]-MVA (Ib) gives [1,5*S*,9*S*,16*S*,24-$^3H_6$]squalene (IIb). Subsequent cyclisation of (IIa) and (IIb), *via* squalene-2,3-oxide,[5] gives [$^3H_6$]lanosterol, which in turn leads to the stereospecifically labelled cholesterols (IIIa) and (IIIb).




(I) a; $R^1$=T, $R^2$=H
b; $R^1$=H, $R^2$=T

● = $^{14}C$

The stereochemistry of the protons at C-1, C-7, and C-15 of the cholesterols (IIIa) and (IIIb) has recently been established.[6–8]

indicating the loss of 88% of the tritium of (IIId). The location of the residual 12% tritium activity was established by degradation of (IV'c). Thus, reduction of (IV'c) with

$^3H:^{14}C$ *Ratios for synthetic and biosynthetic cholesterols and their derivatives*

| Compound | | $^3H:^{14}C$ (d.p.m.) | $^3H:^{14}C$ (atomic) Exp. | Theor. |
|---|---|---|---|---|
| [22*S*-22-$^3H_1$;4-$^{14}$C]Cholesterol | (IIIc) | 9·2[a] | | 1:1 |
| [22-$^3H_1$;4-$^{14}$C]Cholesta-5,7,22-trien-3$\beta$-yl acetate | (IVc) | 9·2 | 1·00:1·00 | 1:1 |
| [22*R*-22-$^3H_1$;4-$^{14}$C]Cholesterol | (IIId) | 9·7[a] | | 1:1 |
| [22-$^3H_1$;4-$^{14}$C]Cholesta-5,7,22-trien-3$\beta$-yl acetate | (IV'c) | 1·2 | 0·12:1·00 | 0:1 |
| [22-$^3H_1$;4-$^{14}$C]Cholesta-7,22-dien-3$\beta$-yl acetate | (V) | 1·1 | 0·11:1·00 | |
| [22-$^3H_1$;4-$^{14}$C]3$\beta$-Acetoxy-23,24-bisnor-5$\alpha$-chol-7-en-22-al | (VI) | 1·0 | 0·10:1·00 | |
| Methyl [4-$^{14}$C]3$\beta$-acetoxy-23,24-bisnor-5$\alpha$-chol-7-en-22-oate | (VII) | 0·2 | 0·02:1·00 | 0:1 |
| [$^3H_5$;$^{14}C_5$]-Cholesterol {from (2*R*)-[2-$^3H_1$;2-$^{14}$C]-MVA} | (IIIa) | 9·9[a] | | 5:5 |
| [$^3H_3$;$^{14}C_5$]Cholesta-5,7,22-trien-3$\beta$-ol acetate | (IVa) | 6·1 | 3·09:5·00 | 3:5 |
| [$^3H_3$;$^{14}C_5$]Cholesterol {from (2*S*)-[2-$^3H_1$;2-$^{14}$C]-MVA} | (IIIb) | 5·4[a] | | 3:5 |
| [$^3H_3$;$^{14}C_5$]Cholesta-5,7,22-trien-3$\beta$-yl acetate | (IVb) | 5·2 | 2·92:5·00 | 3:5 |

[a] In all four experiments with (IIIa—d), the $^3H:^{14}C$ ratio of the recovered cholesteryl acetate was, within experimental error, the same as that of the starting material.

The absolute configurations of the MVA-derived hydrogen atoms at C-22, although often assumed[6–10] to be as indicated in (IIIa) and (IIIb) [derived from (Ia) and (Ib), respectively], have remained unproven, largely because of the difficulties in establishing the configurations of the isotopic hydrogen atoms in an aliphatic side-chain. A solution to this problem has now been made possible by the recent important observation of Conner, Mallory, and their co-workers[11] that the protozoan *Tetrahymena pyriformis* converts cholesterol into cholesta-5,7,22-trien-3$\beta$-ol in high yield. We have therefore established the stereospecificity of the cholesterol $\Delta^{22}$-dehydrogenase of this organism, using cholesterol stereospecifically labelled with tritium[12] at C-22. Subsequent dehydrogenation of cholesterols (IIIa) and (IIIb) [derived from mevalonates (Ia) and (Ib)] by *T. pyriformis* showed that the isotopic hydrogen atoms at C-22 of (IIIa) and (IIIb) have the predicted stereochemistry.[6]

A mixture of radiochemically pure (22*S*)-[22-$^3H_1$]cholesterol and [4-$^{14}$C]cholesterol (IIIc; $^3H:^{14}C$ ratio 9·32; 0·28 $\mu$c $^{14}$C) was diluted with 66 mg. of inactive cholesterol and distributed equally between 6 flasks, each containing 1 l. of peptone based culture fluid.[13] The flasks were then inoculated with *T. pyriformis* and incubated at 28° for 66 hr. The cells were harvested and processed as previously described[13] to yield a non-saponifiable lipid fraction containing 71% (0·2 $\mu$c) of the $^{14}$C-activity initially added. This material was acetylated and subjected to t.l.c. on silver nitrate-impregnated silica gel, using ethyl acetate–hexane (1:9) as the developing solvent. Re-chromatography of the bands at $R_F$ 0·53 and 0·31 afforded 34·4 mg. of cholesteryl acetate and 18·7 mg. of cholesta-5,7,22-trien-3$\beta$-yl acetate (IVc). The identity of the latter was established on the basis of its physical and spectral properties[11] and also on the basis of the chemical degradation outlined below. The $^3H:^{14}C$ ratio of this triene (IVc) was identical to that of the starting material (Table), and hence the 22-*pro-S*-proton is retained.

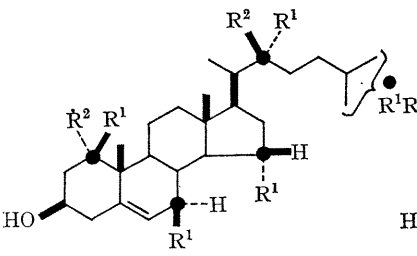
A similar experiment was carried out with [22*R*-22-$^3H_1$;4-$^{14}$C]cholesterol[12] (IIId; $^3H:^{14}C$ = 9·82; 0·045 $\mu$c $^{14}$C). The recovered triene (IV'c) had a $^3H:^{14}C$ ratio of 1·2, sodium in refluxing n-propanol gave 5$\alpha$-cholesta-7,22-dien-3$\beta$-yl acetate (V), $^3H:^{14}C$ = 1·1, m.p. 131—136°; $\nu_{max}$ (KBr) 1740, 965 ($\Delta^{22}$-*trans*-double bond) cm.$^{-1}$; *m/e* 426 ($M^+$), 411 ($M - CH_3$), 366 ($M - CH_3CO_2H$), 315 ($M - C_8H_{15}$), and 255 [$M - (CH_3CO_2H + C_8H_{15})$]. The u.v. spectrum showed end-absorption only. Dilution of (V) with carrier 5$\alpha$-ergosta-7,22-dien-3$\beta$-yl acetate[14] and selective ozonolysis[15] of the $\Delta^{22}$-double bond (zinc–acetic acid work-up) gave 3$\beta$-acetoxy-23,24-bisnor-5$\alpha$-chol-7-en-22-al (VI), $^3H:^{14}C$ = 1·0, m.p. 133—135°; $\nu_{max}$ (KBr) 2950, 2700, and 1740 cm.$^{-1}$; *m/e* 372 ($M^+$), 314 ($M - C_3H_6O$; McLafferty rearrangement[16]), 312 ($M - CH_3CO_2H$); n.m.r.: 1H multiplets at 648 (22-H) and 307 Hz. (7-H). Oxidation of (VI) with Jones reagent and esterification of the resulting C-22 acid gave methyl 3$\beta$-acetoxy-23,24-bisnor-5$\alpha$-chol-7-en-22-oate (VII), $^3H:^{14}C$ = 0·2, m.p. 142—143°; *m/e* 402 ($M^+$), 314 ($M - C_4H_8O_2$; *cf.* ref. 16), 342 ($M - CH_3CO_2H$); n.m.r.: 3H singlet at 218 Hz. (ester methyl), 1H multiplet at 308 Hz. (7-H) and 3H doublet at 71 Hz. ($J$ Hz.; C-21 methyl).

Examination of the $^3H:^{14}C$ ratios for (IV'c—VII) (Table) shows that the C-22 aldehyde (VI) contains 10% of the tritium activity of the starting cholesterol (IIId) and that this activity falls to 2% of (IIId) on oxidation of (VI) to the C-22 acid (VII).

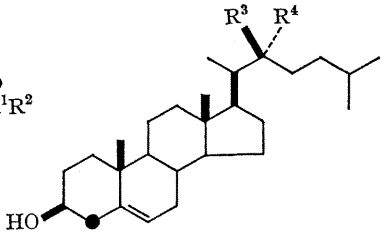
The results obtained with both the (22*S*)-[22-$^3H_1$]- and (22*R*)-[22-$^3H_1$]-cholesterols reveal the stereospecificity of the $\Delta^{22}$-dehydrogenase of *T. pyriformis* for the 22-*pro-R*-hydrogen. The residual activity at C-22 of (IV'c) is ascribed to some randomisation of the label in the starting material (IIId); it follows from the degradation of (IV'c) that the (22*R*)-[22-$^3H_1$]cholesterol (IIId) had 88% of the tritium in the 22*R*-configuration, 8% in the 22*S*-configuration, and 4% divided between C-20 and C-23. The result is not surprising in view of the fact that the [22-$^3$H]cholesterols were prepared[12] by hydrogenolysis of the [22-$^3H_1$] methanesulphonyl esters with $LiAlH_4$, a reaction which precedent suggests may lead to such distribution of isotopic hydrogens.[17]

We next prepared biosynthetic cholesterol (IIIb) by

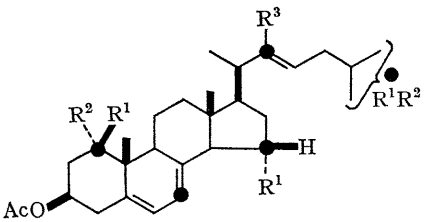
incubating (2*S*)-[2-$^3H_1$; 2-$^{14}C$]-MVA (Ib) with a rat-liver preparation.[18] After dilution with inactive cholesterol, this was incubated with *T. pyriformis* as described above. The $^3H:^{14}C$ ratios for the cholesterol (IIIb) and the triene (IVb) are given in the Table, and are virtually identical. Thus the tritium atom at C-22 is wholly retained, and must therefore have the 22-*pro*-*S*-configuration. This was confirmed in a complementary experiment with the biosynthetic cholesterol (IIIa),† derived from (2*R*)-[2-$^3H_1$; 2-$^{14}C$]-MVA (Ia), which was converted by *T. pyriformis* into the triene (IVa) with the loss of 39% of the tritium activity (Table). This corresponds to the removal of isotopic hydrogen atoms at both C-7($\beta$) and C-22. Since we have proved that the $\Delta^{22}$-dehydrogenase is stereospecific for the 22-*pro*-*R*-hydrogen, this establishes as 22-*pro*-*R* the configuration of the tritium in the cholesterol derived from the (2*R*)-[2-$^3H_1$;-2-$^{14}C$]-MVA. It follows also that the introduction of the $\Delta^{7(8)}$-double bond into cholesterol by *T. pyriformis* entails the elimination of the 7$\beta$- and 8$\beta$-hydrogen atoms.[19]



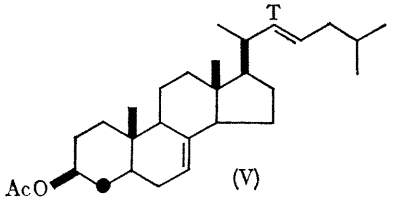
(III) a; $R^1$=T, $R^2$=H
b; $R^1$=H, $R^2$=T



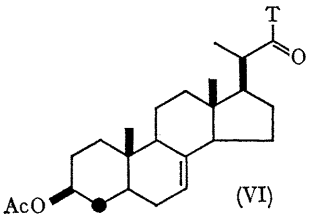
(III) c; $R^3$=T, $R^4$=H
d; $R^3$=H, $R^4$=T



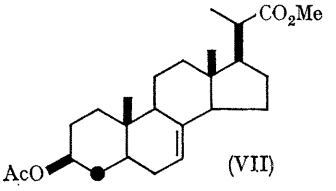
(IV) a; $R^1$=T, $R^2$=$R^3$=H
b; $R^1$=H, $R^2$=$R^3$=T
c; $R^1$=$R^2$=H; $R^3$=T;
● at C—4 only.



(V)



(VI)



(VII)

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