Fate of C-24 Hydrogen Atoms of Cholesterol during its Conversion into Tigogenin in *Digitalis lanata*

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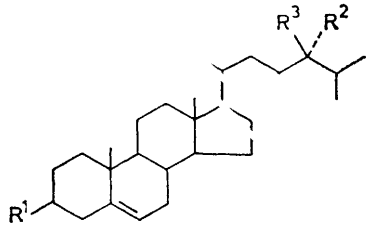
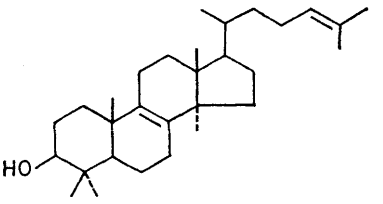
(24*R*)-[24-^{3}H]- and (24*S*)-[24-^{3}H]-cholesterols are transformed into tigogenin [(25*R*)-5α-spirostan-3β-ol] in *Digitalis lanata* without involvement of the hydrogen atoms at C-24; this confirms that the reduction of the Δ^{24}-precursor of cholesterol is a '*trans*' process.

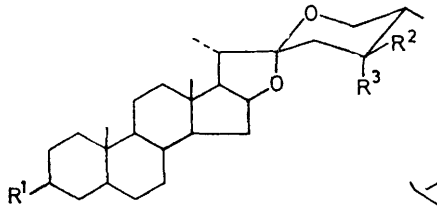
MANY of the stereochemical problems concerning the biological processes leading to cholesterol (I), have been studied;[1] one of these is the reduction of the 24,25-double bond of a Δ^{24}-precursor like lanosterol (II), which is reported to be '*cis*' in the rat both *in vitro* [2a] and *in vivo*.[2b]



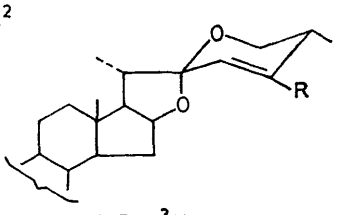
(I) R^1 = OH, R^2 = R^3 = H
(IV) R^1 = OH, R^2 R^3 = O
(V) R^1 = OBz, R^2 R^3 = O
(VI) R^1 = OBz, R^2 = OH, R^3 = H
(VII) R^1 = OBz, R^2 = H, R^3 = OH
(VIII) R^1 = R^2 = OH, R^3 = H
(IX) R^2 = H, R^1 = R^3 = OH
(X) R^1 = R^2 = OBz, R^3 = H
(XI) R^2 = H, R^1 = R^3 = OBz
(XII) R^1 = OH, R^2 = ^{3}H, R^3 = H
(XIII) R^1 = OH, R^2 = H, R^3 = ^{3}H



(II)



(III) R^1 = OH, R^2 = R^3 = H
(XIV) R^1 = OH, R^2 = ^{3}H, R^3 = H
(XV) R^1 = OH, R^2 = H, R^3 = ^{3}H



(XVI) R = ^{3}H
(XVII) R = H

We investigated the stereochemistry of the same reduction in plants [3] by studying the events occurring at C-24 in the biosynthesis of tigogenin [(25*R*)-5α-spirostan-3β-ol] (III), a steroidal sapogenin of *Digitalis lanata* derived from cholesterol,[4] and demonstrated that the hydrogen introduced at C-24 takes up the 24-*pro-R*-position. Since the hydrogen atom at C-25 is known to enter from the 24*si*,25*si*-face,[3] it was inferred that, unless stereochemical changes occur after the reduction of the Δ^{24}-intermediate, in *Digitalis lanata* the whole reduction is a '*trans*' process. The possibility of such stereochemical changes cannot be excluded completely, because the side chain of cholesterol undergoes several modifications when it is transformed into the spiroacetal system of sapogenins. We therefore wished to discover whether during the transformation of cholesterol

[1] L. J. Goad, 'Natural Substances formed Biologically from Mevalonic Acid,' ed. T. W. Goodwin, Academic Press, London and New York, 1970, p. 45; E. Heftmann, *Lloydia*, 1968, **31**, 293.

[2] (*a*) M. Galli Kienle, R. K. Varma, L. J. Mulheirn, B. Yagen, and E. Caspi, *J. Amer. Chem. Soc.*, 1973, **95**, 1996; (*b*) B. Yagen, J. S. O'Grodnick, E. Caspi, and Ch. Tamm, *J.C.S. Perkin I*, 1974, 1994.

[3] L. Canonica, F. Ronchetti, and G. Russo, *J.C.S. Chem. Comm.*, 1972, 1309; *J.C.S. Perkin I*, 1974, 1670.

[4] R. D. Bennett and E. Heftmann, *Phytochemistry*, 1965, **4**, 577.

into tigogenin the hydrogen atoms at C-24 retain their stereochemical identity.

To do this, we synthesized stereospecifically labelled (24*R*)- and (24*S*)-[24-^{3}H]cholesterols from 3β-hydroxycholest-5-en-24-one [5] (IV). The benzoate (V) was reduced with sodium borohydride to a *ca.* 3 : 2 mixture of (24*S*)- and (24*R*)-cholest-5-ene-3β,24-diol 3-benzoates, (VI) and (VII), which were separated by repeated preparative t.l.c. on silica gel and characterized. The less polar monobenzoate (VI) (of higher m.p.) was shown to have the (24*S*)-configuration on the basis of its conversion into the known [6] (24*S*)-cholest-5-ene-3β,24-diol (cerebrosterol) (VIII) and its dibenzoate (X); the (24*R*)-configuration was analogously assigned to the more polar monobenzoate (VII) (of lower m.p.) by correlation with the corresponding known [6] (24*R*)-3β,24-diol (IX) and -3β,24-dibenzoate (XI).

(24*R*)-Cholest-5-ene-3β,24-diol 3-benzoate (VII) was transformed into (24*S*)-[24-^{3}H]cholesterol (XII) by tosylation, reduction of the tosylate (with $LiAl^3H_4$), purification by preparative t.l.c. of the acetate, and removal of the ester group (with $LiAlH_4$).

The same procedure, afforded (24*R*)-[24-^{3}H]cholesterol (XIII) from (24*S*)-cholest-5-ene-3β,24-diol 3-benzoate (VI).

(24*S*)-[24-^{3}H]Cholesterol (XII) (3.45×10^7 disint. min^{-1}) was mixed with [4-^{14}C]cholesterol (4.17×10^7 disint. min^{-1}) and administered to three young *Digitalis lanata* plants; the experiment, carried out as previously described,[3] afforded doubly labelled tigogenin (XIV), which was purified, diluted with carrier material, and crystallized to constant specific activity (see Table). Elimination of the 24-axial hydrogen atom from (XIV) by transformation [3] into the (25*R*)-5α-spirost-23-ene (XVI) proceeded with retention of 93% of the original tritium (see Table). The retention of tritium shows that it occupied the 24-equatorial position in the tigogenin (XIV), *i.e.* the same 24-*pro*-*S*-position as in the starting cholesterol (XII).

In contrast, (24*R*)-[24-^{3}H;4-^{14}C]cholesterol (5.32×10^7 disint. min^{-1} of ^{14}C; ^{3}H : ^{14}C 0.88 : 1) gave a doubly labelled tigogenin (XV) which lost 87% of its tritium on transformation into the (25*R*)-5α-spirost-23-ene (XVII) (see Table).

These data show that the hydrogen atoms at C-24 are not involved in the conversion of cholesterol into tigogenin by *D. lanata*; this means that no stereochemical changes occur at C-24 after the reduction of the Δ^{24}-intermediate, so confirming that this reduction is a '*trans*' process.

Incorporation of (24*S*)-[24-^{3}H;4-^{14}C]cholesterol (4.17×10^7 disint. ^{14}C min^{-1} ; ^{3}H : ^{14}C ratio 0.83 : 1) into *Digitalis lanata*

Product	^{14}C Specific activity × 10^{-4} (disint. min^{-1} mmol^{-1})	^{3}H : ^{14}C activity ratio
Tigogenin (XIV)	4.16	0.70 : 1
(25*R*)-5α-Spirost-23-ene (XVI)	3.88	0.65 : 1

Incorporation of (24*R*)-[24-^{3}H;4-^{14}C]cholesterol (5.32×10^7 disint. ^{14}C min^{-1} ; ^{3}H : ^{14}C ratio 0.88 : 1) into *Digitalis lanata*

Product		
Tigogenin (XV)	5.57	0.84 : 1
(25*R*)-5α-Spirost-23-ene (XVII)	5.30	0.11 : 1

EXPERIMENTAL

M.p.s were determined by use of a silicone oil bath. I.r. spectra were recorded with a Perkin-Elmer 257 spectrophotometer for solutions in chloroform. Optical rotations were determined for 1% solutions in chloroform. T.l.c. was carried out on Merck Kieselgel HF_{254} (0.25 mm thick). Radioactive samples were counted on a Packard Tri-Carb 3320 liquid scintillation counter.

(24S)- *and* (24R)-*Cholest-5-ene-3β,24-diol* 3-*Benzoates* [(VI) *and* (VII)].—3β-Hydroxycholest-5-en-24-one [5] (IV) (0.5 g) was benzoylated with benzoyl chloride–pyridine by the usual procedure, to yield the 3-benzoate (V) (0.62 g). The product (V) was dissolved in dioxan (20 ml), sodium borohydride (400 mg) in isopropyl alcohol (20 ml) was added with stirring, and the mixture was stirred for 90 h at room temperature. The solution was then evaporated under vacuum and the residue shaken with water (30 ml) and diethyl ether (60 ml). The water layer was extracted twice with ether (60 ml) and the combined organic layers were washed with sodium chloride solution, dried, and evaporated to yield a residue (0.61 g). The (24*S*)- and (24*R*)-24-hydroxy-3-benzoates were separated by multiple t.l.c. (20 elutions with benzene–light petroleum, 3 : 2).

The less polar (24S)-*derivative* (VI) (335 mg) had m.p. 168—170° (from chloroform–methanol), $[\alpha]_D^{20}$ −23.4°, $\nu_{max.}$ 3 620, 3 540—3 380, and 1 715 cm^{-1} (Found: C, 80.4; H, 9.8. $C_{34}H_{50}O_3$ requires C, 80.6; H, 9.9%). The more polar (24R)-*derivative* (VII) (260 mg) had m.p. 158—160° (from chloroform–methanol), $[\alpha]_D^{20}$ −9.9°, $\nu_{max.}$ 3 620, 3 540—3 380, and 1 715 cm^{-1} (Found: C, 80.15; H, 10.05%).

Further benzoylation of the monobenzoates (VI) and (VII) afforded the dibenzoates (X) and (XI), identical (m.p. and $[\alpha]_D^{20}$) with the known [6] materials.

Hydrolysis of the benzoates (VI) and (VII) yielded the diols (VIII) and (IX), identical (m.p. and $[\alpha]_D^{20}$) with the known diols.[6]

(24R)-[24-^{3}H]*Cholesterol* (XIII).—A solution of the (24*S*)-24-hydroxy-3-benzoate (VI) (5 mg) in dry pyridine (0.5 ml) was cooled to 0 °C, and freshly crystallized toluene-*p*-sulphonyl chloride (5 mg) was added. The mixture was kept at 0 °C for 16 h, then poured into ice–water and extracted with ether (15 ml). The ether layer was washed with cold 0.5N-hydrochloric acid, cold dilute aqueous sodium hydrogen carbonate, and cold sodium chloride solution, dried, and evaporated. To a solution of the crude tosylate in dry ether (2 ml), were added lithium aluminium hydride (1 mg) and, after 5 min, lithium aluminium tritiide (New England Nuclear; 16.7 mCi; specific activity 158 mCi mmol^{-1}). The mixture was refluxed for 12 h. Inactive lithium aluminium hydride was then added, followed after stirring for 36 h, by 5% hydrochloric acid until two layers separated. The water layer was extracted with ether (10 ml) and the ether solution was washed with dilute sodium hydrogen carbonate and sodium

[5] B. Riegel and I. A. Kaye, *J. Amer. Chem. Soc.*, 1944, **66**, 723; J. Cason, *ibid.*, 1946, **68**, 2708.

[6] A. Ercoli and P. De Ruggieri, *Gazzetta*, 1953, **83**, 720; N. Koizumi, M. Morisaki, and N. Ikekawa, *Tetrahedron Letters*, 1975, **26**, 2203.

chloride solutions, dried, and evaporated. The crude (24*R*)-[24-^{3}H]cholesterol (XIII) was purified by acetylation, preparative t.l.c. (benzene) of the acetate on silica gel–silver nitrate, and reduction with an excess of lithium aluminium hydride in ether. The product (XIII) exhibited a total activity of 4.7×10^7 disint. min^{-1}.

(24S)-[24-^{3}H]*Cholesterol* (XII).—The (24*R*)-24-hydroxy-3-benzoate (VII) (2.5 mg) was transformed into the labelled product (XII) as above, by use of 8.3 mCi of lithium aluminium tritiide. The purified compound had a total activity of 3.45×10^7 disint. min^{-1}.

Administration of (24S)-[24-^{3}H;4-^{14}C]*Cholesterol to* Digitalis lanata, *and Extraction of* (24S)-[24-^{3}H;4-^{14}C]*Tigogenin* (XIV).—A mixture of (24*S*)-[24-^{3}H]cholesterol (XII) and [4-^{14}C]cholesterol (4.17×10^7 disint. min^{-1}) was administered to three young *Digitalis lanata* plants. The experiment, effected as previously described,[3] afforded radioactive tigogenin (XIV) which was diluted (to 100 mg) with carrier tigogenin and repeatedly crystallized from methanol and counted (see Table).

(25R)-[24-^{3}H;4-^{14}C]-5α-*Spirost*-23-*ene* (XVI).—The active tigogenin (XIV) was transformed into the olefin (XVI) by the reactions previously described.[3] The product (XVI) was repeatedly crystallized from chloroform–methanol and counted (see Table).

Administration of (24R)-[24-^{3}H;4-^{14}C]*Cholesterol to* Digitalis lanata, *and Extraction of* (24R)-[24-^{3}H;4-^{14}C]*Tigogenin* (XV).—The experiment was repeated with (24*R*)-[24-^{3}H;4-^{14}C]cholesterol (5.32×10^7 disint. ^{14}C min^{-1}; ^{3}H : ^{14}C ratio 0.88 : 1). The recovered (24*R*)-[24-^{3}H;4-^{14}C]tigogenin (XV) was purified, diluted, and counted (see Table).

(25R)-[4-^{14}C]-5α-*Spirost*-3-*ene* (XVII).—The labelled tigogenin (XV) was transformed into the olefin (XVII) by the reactions previously described.[3] The product (XVII) was repeatedly crystallized and counted (see Table).

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