Stereospecific Introduction of a 24-*pro-R*-Hydrogen in the Biosynthesis of Tigogenin in *Digitalis lanata*

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Summary In tigogenin biosynthesized in *Digitalis lanata* a 4-*pro-R*-hydrogen of mevalonic acid (MVA) occupies the 24-*pro-S*-position.

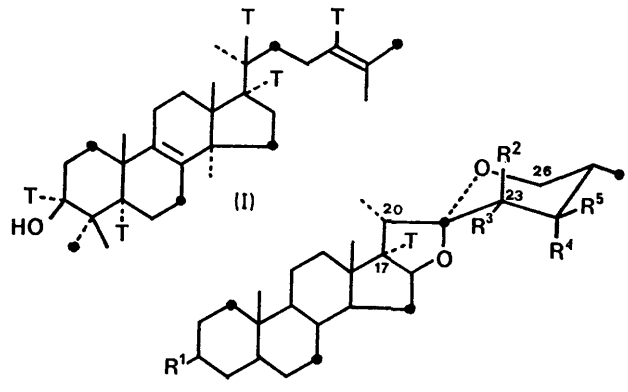
THE stereochemistry of the reduction of the C-24 double bond during the biosynthesis of cholesterol from Δ^{24}-precursors, *e.g.* lanosterol (I), has recently been studied in rat-liver homogenates.[1] We now report different results obtained with tigogenin (II), a steroidal sapogenin from *Digitalis lanata*.

The conversion of a Δ^{24}-precursor into tigogenin involves, *inter alia*, the introduction of two hydrogen atoms, one at C-24 and the other at C-25. The 25-*R*-configuration of tigogenin and the derivation of its C-26 atom bearing the oxygen function from C-3′ of MVA,[2] indicate that the hydrogen at C-25 entered from the rear [(IV) → (V)].

TABLE

Incorporation of 3R-[(4R)-4-^{3}H; 2-^{14}C]MVA *into tigogenin in* Digitalis lanata

Products	Specific activity $\times 10^{-5}$ (d.p.m. of ^{14}C/mmol)	^{3}H/^{14}C activity ratio
Tigogenin (II)	3·07	3·05
3-Deoxytigogenin (XI)	2·98	3·07
23-*R*-Bromo-(25*R*)-5α-spirostan (XIII)	2·95	3·09
(25*R*)-5α-Spirost-23-ene (XIV)	3·08	3·01
5α-Spirost-24-ene (XV)	3·10	3·0
3-Deoxytigogenin (XVI)	3·04	3·04
23-*R*-Bromo-(25*R*)-5α-spirostan (XVII)	3·06	3·01
(25*R*)-5α-Spirost-23-ene (XIX)	3·02	1·75



• = C-2 of MVA

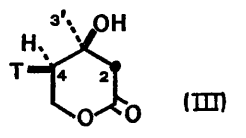
(II) R^1 = OH; R^2 = R^3 = H; R^4 = H (T); R^5 = H (T)
(VIII) R^1 = OH; R^2 = R^3 = R^5 = H; R^4 = T
(IX) R^1 = OH; R^2 = R^3 = R^4 = H; R^5 = T
(X) R^1 = OTs; R^2 = R^3 = R^4 = H; R^5 = T
(XI) R^1 = R^2 = R^3 = R^4 = H; R^5 = T
(XII) R^1 = R^2 = R^4 = H; R^5 = T; R^3 = Br
(XIII) R^1 = R^3 = R^4 = H; R^5 = T; R^2 = Br
(XVI) R^1 = R^2 = R^3 = R^5 = H; R^4 = T
(XVII) R^1 = R^3 = R^5 = H; R^4 = T; R^2 = Br
(XVIII) R^1 = R^2 = R^5 = H; R^4 = T; R^3 = Br

To examine the stereochemistry of the introduction of the hydrogen at C-24, we used 3*R*-[(4*R*)-4-^{3}H; 2-^{14}C]MVA (III), which is expected to give rise to a Δ^{24}-intermediate of tigogenin in which a tritium atom is present at C-24.

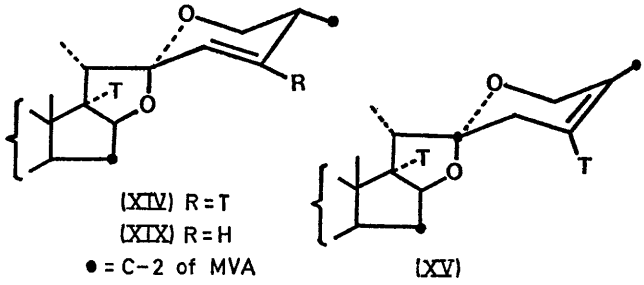
Front attack of hydrogen on this carbon atom will produce the 24-*S*-configuration (VII), corresponding to the

tigogenin (IX) with equatorial tritium, while attack from the rear will lead to the opposite configuration (VI) and to the tigogenin (VIII). Consequently, the direction of attack at C-24 can be studied by determining the axial or the equatorial orientation of the tritium on that carbon.

For this, we administered 3*R*-[(4*R*)-4-^{3}H;2-^{14}C]MVA (0·1 mCi of ^{14}C; ^{3}H/^{14}C ratio 7·65) to *Digitalis lanata* plants. After four weeks the plants were harvested and the biosynthesized doubly labelled tigogenin was isolated and purified by usual procedures. After dilution with carrier tigogenin and crystallization to constant specific activity,



(III)



(XIV) R = T
(XIX) R = H
● = C-2 of MVA

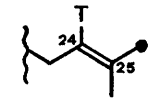
(XV)

the product exhibited a ^{3}H/^{14}C ratio of 3·05 (see Table), showing the presence of two tritium atoms in the molecule (^{3}H : ^{14}C atomic ratio 2 : 5; ^{3}H : ^{14}C ratio = 7·65 × 2 : 5 = 3·06) presumably located at C-17 and at C-24, the loss of tritium at C-20 being expected from earlier work.[3]

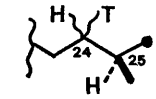
The doubly labelled tigogenin (II) was transformed, by reduction of the corresponding tosyl ester (X) with $LiAlH_4$,[4] into 3-deoxytigogenin (XI); from the latter the corresponding Δ^{23}-olefin (XIV) was obtained as follows:[5] bromination of (XI) yielded a mixture of the mono-bromides (XII) and (XIII), which were separated by chromatography. The equatorial 23*S*-bromo-derivative (XII) could not be dehydrobrominated with Bu^{t}OK in DMSO–benzene, whereas under the same conditions the axial derivative (XIII) easily yielded, *via* a *'trans'* elimination process,† the Δ^{23}-olefin (XIV), which exhibited the same ^{3}H/^{14}C ratio and specific activity as that of the starting bromide (XIII).

The Δ^{23}-olefin (XIV) was isomerized, with retention of tritium, into the Δ^{24}-olefin (XV) using Bu^{t}OK–DMSO at 110° and this, in turn, was converted into the **25**-*R*-5α-spirostan (XVI) by stereospecific homogeneous catalytic hydrogenation.

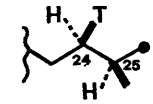
Since this hydrogenation occurs on the β-side,[5] the sequence (XI) · · · → (XIV) → (XV) → (XVI) causes inversion of the 24-equatorial hydrogen of (XI), which assumes the 24-axial orientation in (XVI).



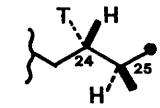
(IV)



(V)



(VI)



(VII)

● = C-2 of MVA

Bromination of (XVI), separation of the isomeric bromides (XVII) and (XVIII), and dehydrobromination of the axial bromide (XVII) yielded a Δ^{23}-olefin (XIX) with loss of 0·85 tritium atoms, thus confirming that a tritium atom is present at C-24. The retention of tritium during the dehydrobromination of the bromide (XIII) to the Δ^{23}-olefin (XIV), coupled with all the above results, demonstrates that in tigogenin biosynthesized in *Digitalis lanata* from 3*R*-[(4*R*)-4-^{3}H; 2-^{14}C]MVA the tritium atom at C-24 is equatorial.

Unless unlikely stereochemical changes occur at some stage of the biosynthesis after the reduction of the Δ^{24}-intermediate, it follows that the incoming hydrogen at C-24 assumes, through a front attack [(IV) → (VII)], the *pro-R*-position, the whole reduction of the Δ^{24}-double bond occurring with *'trans'* stereochemistry.

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† Experiments on deuteriated samples to prove that the above dehydrobromination occurs with *'trans'* stereochemistry will be reported in the full paper.

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