The Role of C-15 in the Biosynthesis of Digitoxigenin

By L. CANONICA,* F. RONCHETTI, and G. RUSSO
(*Istituto di Chimica Organica della Università di Milano, via Saldini* 50, 20133 *Milano, Italy*)

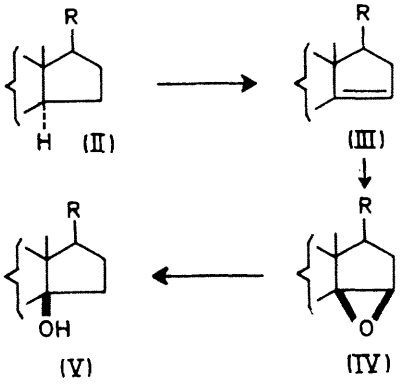
Summary The 15-position of pregnane-type precursors is not involved in the 14β-hydroxylation process during the biosynthesis of digitoxigenin.

THE biosynthesis of cardenolides, *e.g.* digitoxigenin (I), includes the unusual formation of a 14β-hydroxy-derivative from a 14α-H-precursor,[1,2] the whole process being opposite to the "normal" biological hydroxylation,[3] where the OH group introduced assumes the stereochemistry of the proton removed.

As Caspi and Lewis suggested,[4] the 14β-hydroxylation process could be due to the formation of a $\Delta^{14(15)}$ double-bond derivative (III) from a precursor (II) like progesterone, followed by oxidation to a 14β,15β-epoxide (IV) (a 14β, 15β-epoxy-moiety is present in some bufadienolides); reductive opening of the epoxide could provide the 14β-alcohol (V).

Quite recently, Tschesche *et al.*[5] found that $\Delta^{14(15)}$-progesterone is not converted into cardenolides by *Digitalis lanata* plants; however the lack of incorporation of a substance in complex organisms does not definitely demonstrate that it is not a biosynthetic precursor.

In order to investigate the possible role of C-15 of progesterone in the 14β-hydroxylation process during the incorporation of this precursor into cardenolides, we used 3*R*-[(2*R*)-2-^{3}H-2-^{14}C] MVA. This MVA is incorporated, with tritium in the configurations shown, into lanosterol (VI) whose 15β-H is retained during the elimination of the 14α-methyl group[6] and inverted, in liver homogenates, to the 15α-configuration during the *trans*-hydrogenation of the $\Delta^{14(15)}$ double bond intermediate[7] thus formed (VI → VII → VIII). Therefore the above precursor should originate from the plant progesterone, or its analogue (VIII), with the tritium in the 15α-position.



The radioactive MVA (0·1 mCi of ^{14}C; ^{3}H/^{14}C ratio 8·04) was dissolved in acetone and deposited on the leaves of *Digitalis lanata* plants. After four weeks the plants were harvested and the labelled digitoxigenin (IX) was isolated and purified. After dilution with carrier digitoxigenin and crystallization to constant specific activity, the material was acetylated and dehydrated with thionyl chloride in pyridine. Treatment of the anhydro-derivative (XI) with *m*-chloroperoxybenzoic acid and oxidation of the resulting

epoxide (XII) with chromic anhydride furnished 15-oxo-digitoxigenin (XIII). The specific activities of these compounds are summarized in the Table.

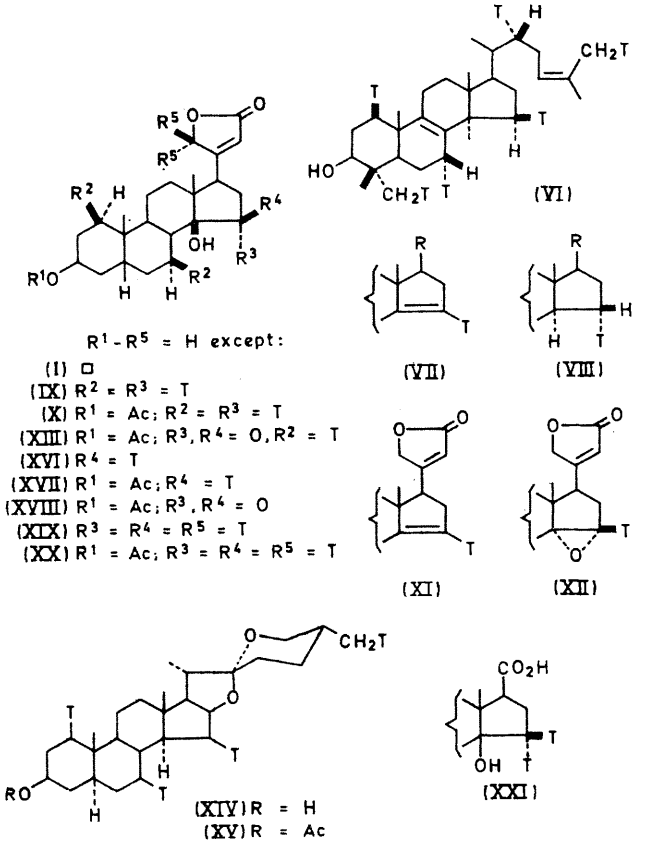
15-^{3}H coming from 3*R*-[(2*R*)-2-^{3}H-2-^{14}C]MVA is retained during the conversion of the precursor into cardenolides.

The decrease in the ^{3}H/^{14}C ratio (from 8·04 to 6·34)

Incorporation of 3R-[(2R)-2-^{3}H-2-^{14}C]*MVA*, [15*β*-^{3}H,4-^{14}C]*progesterone, and* [15*α*,15*β*,21,21,21-^{3}H$_5$,4-^{14}C]*pregn-5-en-3β-ol-20-one into digitoxigenin in* Digitalis lanata

Products	Specific activity (d.p.m. of ^{14}C/mmole)	^{3}H/^{14}C activity ratio	^{3}H/^{14}C atomic ratio
3*R*-[(2*R*)-2-^{3}H-2-^{14}C]MVA (0·1 mCi of ^{14}C; ^{3}H/^{14}C ratio 8·04)			
Digitoxigenin (IX)	$6·78 \times 10^4$	6·34	3 : 3
3-Acetyldigitoxigenin (X)	$6·90 \times 10^4$	6·30	2·95 : 3
14,15-Didehydro-3-acetyldigitoxigenin (XI)	$6·81 \times 10^4$	5·19	2·47 : 3
15-Oxodigitoxigenin (XIII)	$6·85 \times 10^4$	4·18	2 : 3
Tigogenin (XIV)	$2·86 \times 10^4$	5·34	4 : 5
3-Acetyltigogenin (XV)	$2·93 \times 10^4$	5·31	3·95 : 5
[15*β*-^{3}H,4-^{14}C]Progesterone (0·1 mCi of ^{14}C; ^{3}H/^{14}C ratio 4·23)			
Digitoxigenin (XVI)	$1·21 \times 10^6$	4·18	0·99 : 1
3-Acetyldigitoxigenin (XVII)	$1·18 \times 10^6$	4·21	0·99 : 1
15-Oxodigitoxigenin (XVIII)	$1·12 \times 10^6$	0·15	0·03 : 1
[15*α*,15*β*,21,21,21-^{3}H$_5$,4-^{14}C]Pregn-5-en-3*β*-ol-20-one (0·1 mCi of ^{14}C; ^{3}H/^{14}C ratio 45)			
Digitoxigenin (XIX)	$2·42 \times 10^5$	15·68	—
3-Acetyldigitoxigenin (XX)	$2·68 \times 10^5$	14·99	—
3*β*-Acetoxy-14*β*-hydroxy-5*β*,14*β*-etianic acid (XXI)	$2·86 \times 10^5$	9·52	—

The ^{3}H/^{14}C ratio of the anhydro-derivative (XI) shows that the dehydration is not stereospecific; the ^{3}H/^{14}C ratio



of 15-oxodigitoxigenin (XIII), when compared with that of digitoxigenin (IX) or its acetate (X), indicates that the observed upon conversion of the doubly labelled MVA into digitoxigenin can be explained by loss of tritium caused by the reversibility of the isopentenyl pyrophosphate–dimethylallyl pyrophosphate enzymic isomerization.[8] If this is the case, the biosynthesized cholesterol would have the same decreased ^{3}H/^{14}C ratio as digitoxigenin (IX). To confirm this hypothesis we isolated, from the same source, labelled tigogenin (XIV). In fact, in the biosynthesis of tigogenin from 3*R*-[(2*R*)-2-^{3}H-3-^{14}C]MVA only the 22*R*-H of cholesterol, which is a precursor of both spirostanols and cardenolides, is lost; therefore the ^{3}H/^{14}C ratio of cholesterol must be 5/4 of the ^{3}H/^{14}C ratio of tigogenin (XIV).

The labelled tigogenin (XIV) was purified, diluted with non-radioactive material, and crystallized to constant specific activity. The ^{3}H/^{14}C ratios of tigogenin (XIV) and its acetate (XV) indicate that cholesterol biosynthesized in *Digitalis lanata* had a ^{3}H/^{14}C ratio of $5/4 \times 5·34 = 6·64$, in accordance with that found for digitoxigenin (IX).

In order to investigate the possible role of the 15*β*-H of the precursors in the 14*β*-hydroxylation process we administered to *Digitalis lanata* plants [15*β*-^{3}H,4-^{14}C]progesterone (0·1 mCi of ^{14}C; ^{3}H/^{14}C ratio 4·23), synthesized according to the method of Ramm and Caspi.[9] The isolated digitoxigenin (XVI), its acetate (XVII), and the ketol (XVIII) obtained from this last compound had the radioactivity values shown in the Table. These values indicate that the 15*β*-H is not involved in the 14*β*-hydroxylation process.

To confirm these results [15*α*,15*β*,21,21,21-^{3}H$_5$,4-^{14}C]-pregn-5-en-3*β*-ol-20-one (0·1 mCi of ^{14}C; ^{3}H/^{14}C ratio 45) was synthesized[10] and fed to *Digitalis lanata* plants. The administered precursor had, after back-exchange with NaOH in CH_3OH/H_2O, a ^{3}H/^{14}C ratio of 9·49, due only to the tritium atoms in the 15-position, 77·4% of which had the 15*β*- and 22·6% the 15*α*-orientation.[10] After four weeks the radioactive digitoxigenin (XIX) was isolated and converted into the etianic acid (XXI) by acetylation, ozonolysis, hydrolysis, and oxidation with periodic acid. The etianic acid (XXI) had a ^{3}H/^{14}C ratio of 9·52, confirming that the hydrogen atoms at C-15 are completely retained.

The above results indicate that neither 15α-H and 15β-H of pregnane-type precursors is in any way involved in the biosynthesis of cardenolides.

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