Fate of the 16β-Hydrogen Atom of Cholesterol in the Biosynthesis of Tomatidine and Solanidine

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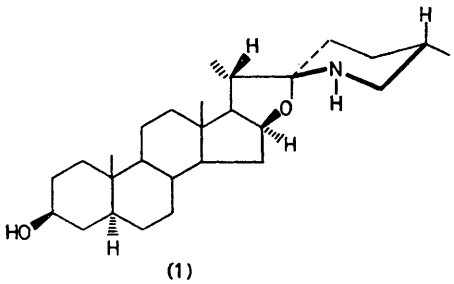
Summary During the biosynthesis of tomatidine (**1**) in *Lycopersicon pimpinellifolium*, the 16β-hydrogen atom of cholesterol is inverted to the 16α-position; the same hydrogen atom is lost during the biosynthesis of solanidine (**2**) in *Solanum tuberosum*.

SEVERAL aspects of the biosynthesis of steroidal alkaloids of the spirosolane [*e.g.*, tomatidine (**1**)] or solanidane type [*e.g.*, solanidine (**2**)] have not yet been fully elucidated. One of these is the closure of the cholesterol side chain to form rings E and F; useful information about this aspect may be obtained by determining the fate of the hydrogen atoms at C-16 of a known precursor of both alkaloids.

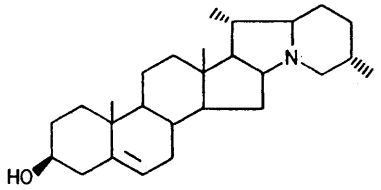
16β-[16-^{3}H]Cholest-5-en-3β-ol (**3**), a suitable substrate[1] for this purpose, was synthesized[2] from 3β-hydroxycholest-5-en-16-one (**4**), mixed with [4-^{14}C]cholest-5-en-3β-ol (1·42 × 10^{8} disint.^{14}C min^{-1}; ^{3}H:^{14}C, 2·84:1), and administered to *Lycopersicon pimpinellifolium*. Radioactive (**1**) was isolated and crystallized to constant specific activity, showing retention of the tritium (see Table).

This tritium is located in the 16α-position, as deduced from the structure of (**1**) and from the following reactions.[3]

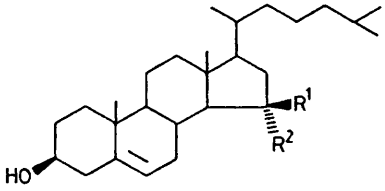
Hydrogenation of (**1**) yielded a mixture of (22*S*,25*S*)-22,26-epi-imino-5α-cholestane-3β,16β-diol (**5**) and its (22*R*)-stereoisomer (**6**), subsequent acetylation giving the triacetate



(1)



(2)



(3) $R^1 = T$; $R^2 = H$

(4) $R^1R^2 = O$

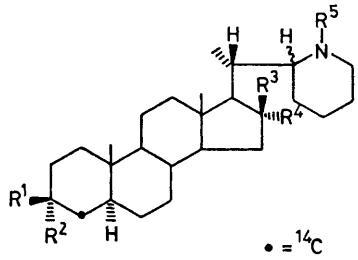
(**7**) and the diacetate (**8**), respectively, which were separated and hydrolysed to the respective isomeric *N*-acetyl-diols (**9**) and (**10**). Oxidation of (**9**) and (**10**) with CrO_3–dil.H_2SO_4 under non-equilibrating conditions yielded the isomeric diketones (**11**) and (**12**), which were completely devoid of tritium.

This leads to the conclusion that, during the biosynthesis of (**1**) from cholesterol, the 16β-hydrogen is retained, but is inverted to the 16α-position. This excludes the possibility that the oxygen function at C-16 of (**1**) derives from a 16β-hydroxy-group introduced by the usual mechanism of hydroxylation at saturated carbons.[4] In the light of these results, the presence of a keto-group at C-16 can also be excluded. One of the possible mechanisms would be nucleophilic attack on a cation derived from protonation of a Δ^{16} or a Δ^{15} double bond or from expulsion of a 16α-hydrogen atom or a leaving group.

TABLE

Incorporation of 16β-[16-^{3}H; 4-^{14}C]cholest-5-en-3β-ol ($1{\cdot}42 \times 10^8$ disint. ^{14}C min^{-1}; ^{3}H : ^{14}C, 2·84 : 1) into tomatidine in *Lycopersicon pimpinellifolium* and solanidine into *Solanum tuberosum*.

Compound	^{14}C Specific activity × 10^{-6} (disint. min^{-1} mmol^{-1})	^{3}H : ^{14}C
[16α-^{3}H; 4-^{14}C]-(**1**)	1·40	2·95 : 1
[16α-^{3}H; 4-^{14}C]-(**9**)	1·38	2·93 : 1
[16α-^{3}H; 4-^{14}C]-(**10**)	1·36	2·94 : 1
[4-^{14}C]-(**11**)	1·32	0·020 : 1
[4-^{14}C]-(**12**)	1·30	0
[4-^{14}C]-(**2**)	5·94	0·043 : 1



• = ^{14}C

(5) $R^1 = R^3 = OH$; $R^2 = R^5 = H$; $R^4 = T$; (22*S*)
(6) $R^1 = R^3 = OH$; $R^2 = R^5 = H$; $R^4 = T$; (22*R*)
(7) $R^1 = R^3 = OAc$; $R^2 = H$; $R^4 = T$; $R^5 = Ac$; (22*S*)
(8) $R^1 = OAc$; $R^2 = H$; $R^3 = OH$; $R^4 = T$; $R^5 = Ac$ (22*R*)
(9) $R^1 = R^3 = OH$; $R^2 = H$; $R^4 = T$; $R^5 = Ac$; (22*S*)
(10) $R^1 = R^3 = OH$; $R^2 = H$; $R^4 = T$; $R^5 = Ac$; (22*R*)
(11) $R^1R^2 = O$; $R^3R^4 = O$; $R^5 = Ac$; (22*S*)
(12) $R^1R^2 = O$; $R^3R^4 = O$; $R^5 = Ac$; (22*R*)

16β-[16-^{3}H; 4-^{14}C]Cholest-5-en-3β-ol was also administered to *Solanum tuberosum* and radioactive (**2**) was isolated, crystallized to constant specific activity, and counted. Complete loss of tritium (Table) indicates that during the biosynthesis of (**2**) in *Solanum tuberosum* the 16β-hydrogen of cholesterol is removed.

We thank Simes S.p.a. for growing the *Lycopersicon pimpinellifolium* plants.

(*Received*, 1*st February* 1977; *Com.* 085.)

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