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BIOGENESIS OF SPIROSTAN SAPOGENINS: STEREOCHEMISTRY OF REDUCTION OF THE 24,25 DOUBLE BOND IN THE BIOSYNTHESIS OF SARSASAPOGENIN

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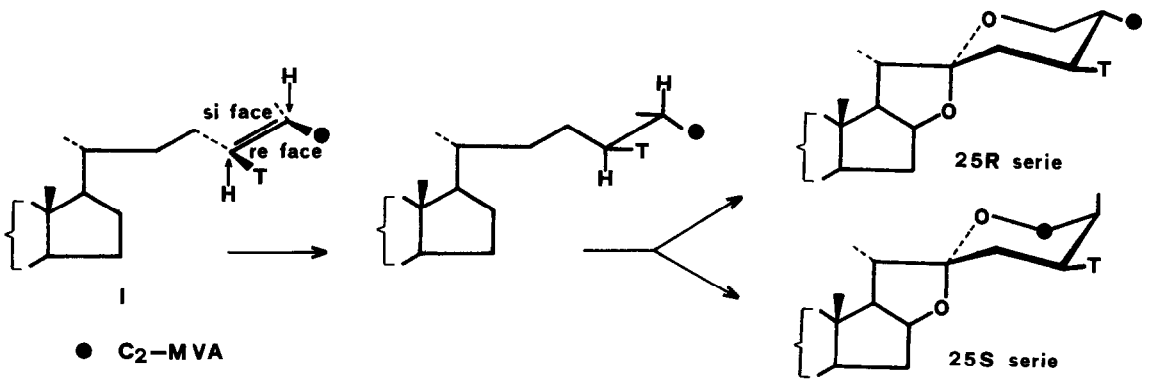
It is well known that reduction of the 24,25 double bond of the Δ^{24}-sterols such as lanosterol or cycloartenol (I) is a key step in the biosynthesis of many steroid components of plants [1]. Several papers have been published in recent years concerning the stereochemistry of the reduction of this double bond; it has been reported to be "cis" in rat liver homogenates [2,3] and "trans" in the biosynthesis of tigogenin [4,5] (25R-spirostan sapogenin, "iso" series) in Digitalis lanata [6].



SCHEME 1

We have shown in a previous paper [7] that, in the biogenesis of sarsasapogenin (IIa) [25S-spirostan sapogenin, "neo" series] in Agave attenuata, the C-26 is derived from the C-2 of mevalonic acid [8], while the C-27 of tigogenin (25R) in

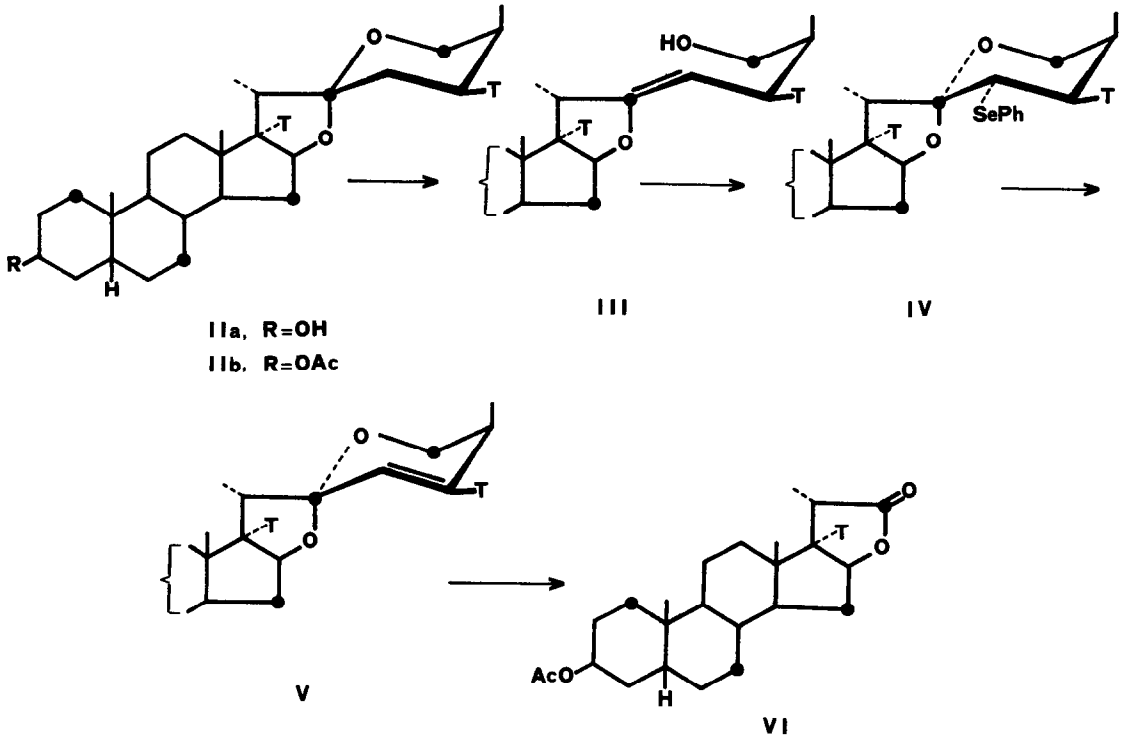
Digitalis lanata also originates from the C-2 of MVA [9]. However, the stereochemistry in the reduction of the 24,25 double bond of the intermediates in the "neo" spirostan sapogenins is so far unknown.

In this paper we show that during reduction of this 24,25 double bond in the biosynthesis of sarsasapogenin in Agave attenuata the incoming hydrogen at C-24 enters from the 24re,25re face, so that the whole reduction is a "trans" process (Scheme 1), unless stereochemical changes occur after reduction.

[2-^{14}C,(4R)-4-^{3}H] MVA (5 μCi of ^{14}C and 50 μCi of ^{3}H) was administered to the plant and three days later the saponins were extracted with ethanol. After hydrolysis of the extract with 1.5 N HCl, the biosynthesized sarsasapogenin IIIa was isolated, purified, (incorporation 1.7%), diluted with carrier material and crystallized to its constant specific activity: 6.87×10^5 dpm mmol^{-1} of ^{14}C and atomic ratio, ^{3}H/^{14}C 2.09/5.

Sarsasapogenin acetate IIb (0.2 mmol) reacts with phenylselenyl chloride (0.3 mmol) in acetic acid at room temperature during 20 minutes to produce the (23S)-phenylselenide derivative IV (specific activity: 6.93×10^5 dpm mmol^{-1} of ^{14}C, atomic ratio, ^{3}H/^{14}C 1.96/5) stereoselectively in high yield (96%). This reaction proceeds by trapping the vinyl ether intermediate III formed in the acid-catalyzed opening of ring F [10]. The ^{1}H-NMR spectrum of IV, ($C_{35}H_{50}O_4Se$, M^+ 613, mp 189-190° C, $[\alpha]_D$ -24°), in CCl_4/D_6C_6: 3/2 shows the signal of H-C$_{23}$ to be an ABX system at δ 3.50 with J_{aa} = 13 Hz and J_{ae} = 5 Hz (spin decoupling experiment), establishing the equatorial configuration of the phenylselenide at C-23.

The elimination of phenylselenide was accomplished by oxidation with a large excess of H_2O_2 in THF followed by pyrolysis of the resulting phenylselenoxide derivative at room temperature for 48 hr to give the olefin V [11] (yield 85%, $C_{29}H_{44}O_4$, M^+ 456, mp 147-148° C, $[\alpha]_D$ +6°). Its MS displays the expected pattern for a 24-dehydro spirostan sapogenin, m/e: 113, 137 and 315 [12]. The doublet in the ^{1}H-NMR spectrum ($CDCl_3$) at δ 5.50 (J = 10 Hz) corresponds to H-C$_{23}$ and the four peaks at 6.08, 6.01, 5.98 and 5.91, to H-C$_{24}$. As, on one hand, the specific activity (6.98×10^5 dpm mmol^{-1} of ^{14}C) and the atomic ratio (^{3}H/^{14}C, 2.01/5) of V are the same as for IV, and, on the other, this elimination is known to take place in syn form [13], we conclude that the tritium at C-24 in sarsasapogenin IIIa has a β-equatorial configuration. Degradation of V, by ozonolysis in CH_2Cl_2 at -78° C and oxidation with Jones' reagent, gives the known lactone VI with the expected atomic ratio, ^{3}H/^{14}C, 1.20/4.



SCHEME 2

From what we know at present, we may deduce that the stereochemistry of the reduction of the 24,25 double bond is the same in the biosynthesis of both 25R and 25S spirostan sapogenins from Δ^{24}-precursors (see Scheme 1) and that the first divergence in the biogenesis of the side chain (in the two series of sapogenins) occurs according to whichever terminal isopropyl methyl is oxidized.

All compounds gave correct quantitative elemental analyses. Optical activities were measured in $CHCl_3$.

REFERENCES AND FOOTNOTES

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4 L Canonica F Ronchetti & G Russo: J C S - Perkin I 1670 (1974)

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6 This follows the currently accepted hypothesis that the geometry of the 24,25 double bond of the Δ^{24}-precursor is that represented in I (Scheme 1); K J Stone W R Roeske & R B Clayton: J C S - Chem Commun 530 (1969), and references (4) and (5).

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8 The same conclusion was reached subsequently and independently by Ronchetti and Russo in the biosynthesis of neotigogenin (25S) in Lycopersicon pimpinellifolium; F Ronchetti & G Russo: Tetrahedron Letters 85 (1975).

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10 Longer reaction time leads quantitatively to the re-formation of sarsasapogenin acetate IIb because the acid-catalyzed opening of ring F gives vinyl ether III again with loss of phenylselenide.

11 This sequence permits a high yield synthesis of the Δ^{23}-derivatives of 25S-spirostan sapogenins which are difficult to obtain by other known chemical procedures. The scope of the reaction of organoselenides with ketals is being studied.

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