Biosynthesis of Yamogenin, Neotokorogenin, and Their (25*R*)-Isomers from [1,2-$^{13}C_2$]Acetate in *Dioscorea tokoro* Tissue Cultures

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Summary In the biosynthesis of (25*S*)-steroidal sapogenins in tissue cultures of *Dioscorea tokoro* Makino fed with sodium [1,2-$^{13}C_2$]acetate, the ^{13}C n.m.r. spectra of the ^{13}C-labelled (25*S*)-sapogenins (**3**) and (**4**) indicated (i) that a hydrogen atom at C-25 was introduced from the 25-*si* face of the Δ^{24} double bond of Δ^{24}-biosynthetic intermediates as in the case of the (25*R*)-sapogenins (**1**) and (**2**), and (ii) that oxidation of the *pro-R* methyl group (C-26) of cholesterol was accelerated by a higher concentration of sodium acetate giving (25*S*)-steroidal sapogenins.

ADDITION of the C-25 proton to the Δ^{24} double bond in the reduction of Δ^{24}-biosynthetic intermediates occurs on the *si*-face in the biosynthesis of cholesterol,[1] solasodine,[2] and tomatidine.[3] The hydrogen atom at C-25 of tigogenin

[(25*R*)-steroidal sapogenin] also enters from the same face of the double bond.[4] Tissue cultures of *Dioscorea tokoro* Makino were reported to produce furostanol derivatives which are converted into the (25*R*)-steroidal sapogenins diosgenin (**1**), yonogenin, and tokorogenin (**2**) by acid hydrolysis.[5] However, small quantities of the corresponding (25*S*)-steroidal sapogenins yamogenin (**3**), neoyonogenin, and neotokorogenin (**4**) were recently found together with (25*R*)-sapogenins in the acid hydrolysis product of a furostanol derivative mixture obtained from the tissue cultures;[6] (25*S*)-sapogenins are known to be only slightly isomerized to the (25*R*)-epimer by acid hydrolysis in methanol.[7,8]

should appear at δ 65·2 and 16·1 p.p.m. for (**3**), in $CDCl_3$, and 65·3 and 16·3 p.p.m. for (**4**) in C_5D_5N, respectively. In the completely ^1H-decoupled ^{13}C n.m.r. spectra of the ^{13}C-labelled compounds (**3**) and (**4**), the signals due to C-26 and C-27 appeared as a singlet and a doublet [^1J(CC) 35 Hz], respectively, as shown in the Figure. These facts demonstrated that C-26 and C-27 originated from C-2 and C-6, respectively, of mevalonic acid (MVA) in the biosynthesis of (25*S*)-sapogenins. In the case of the (25*R*)-sapogenins, C-26 and C-27 were confirmed to be derived from C-6 and C-2 of MVA, respectively, because the signals due to C-26 [δ 66·9 p.p.m. for (**1**) and 67·0 p.p.m. for (**2**)]

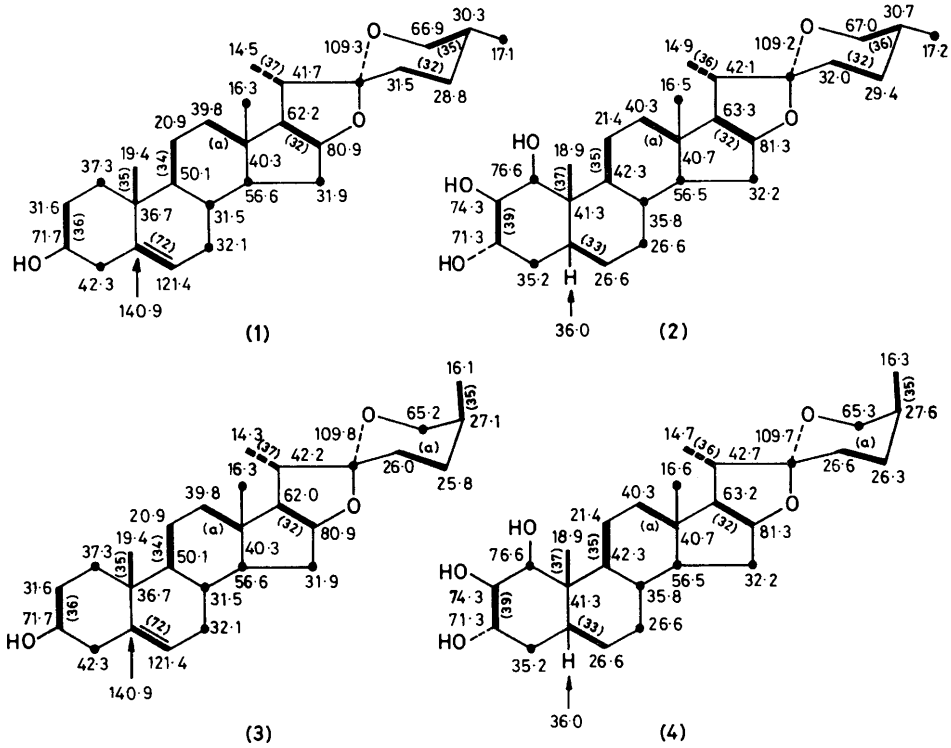


FIGURE. ^{13}C N.m.r. chemical shifts (δ/p.p.m.) and coupling constants [^1J(CC)/Hz, in parentheses] for the sapogenins (**1**)—(**4**). Spectra were recorded on a Varian XL-100-12A spectrometer at 25·160 MHz in $CDCl_3$ [(**1**) and (**3**)] at 30 °C and in C_5D_5N [(**2**) and (**4**)] at 80 °C using Me_4Si as internal reference. Accuracies in δ and ^1J(CC) values are ±0·1 p.p.m. and ±2 Hz, respectively. (a): The ^1J(CC) values could not be observed owing to the signals of both carbons being positioned very close to each other. ▬: ^{13}C labelled, formed from complete Me—CO_2H unit. ●: ^{13}C labelled, formed from isolated Me or CO_2H unit.

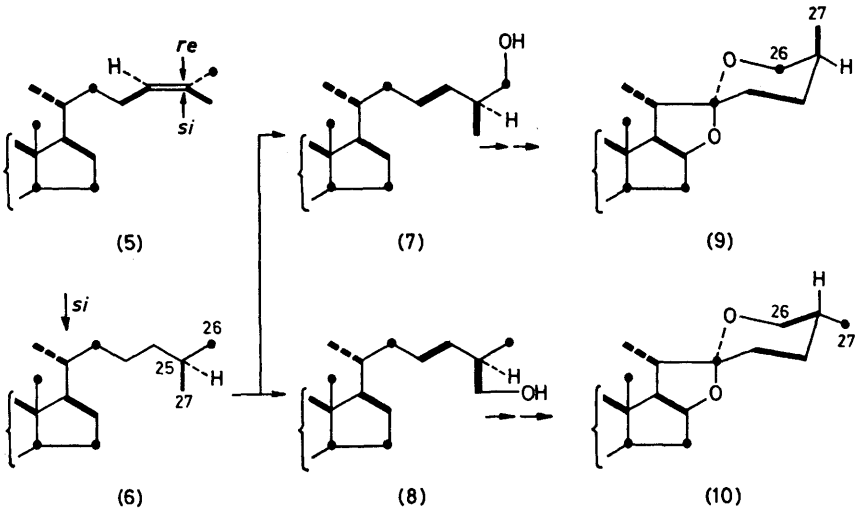
We studied the biosynthesis of the (25*S*)-steroidal sapogenins (**3**) and (**4**) and compared it with the case of their (25*R*)-epimers (**1**) and (**2**). Sodium [1,2-$^{13}C_2$]acetate (0·17 mg cm^{-3} of a 1 : 1 mixture of unlabelled and labelled sodium acetates) was added to two-week old tissue cultures of *D. tokoro* grown in a Linsmaier–Skoog medium. After two more weeks of culture a furostanol derivative mixture was obtained from the cultured cells, and then hydrolysed with hydrochloric acid in methanol giving the ^{13}C-labelled products (**3**) and (**4**) together with the corresponding (25*R*)-isomers (**1**) and (**2**), respectively.

All ^{13}C n.m.r. signals of the steroidal sapogenins have already been assigned;[8,9,†] the signals due to C-26 and C-27 and C-27 [δ 17·1 p.p.m. for (**1**) and 17·2 p.p.m. for (**2**)] appeared as a doublet and a singlet, respectively. The labelling patterns of all other carbons agreed well with the results reported for the biosynthesis of other sterols.[1b,10]

Another report has pointed out[11] that the metabolic pool size could be changed by using higher concentrations of precursors. Interestingly, the product ratio of (**2**) to (**4**) was increased from about 1 : 2 to 1 : 1 with an increase in concentration of sodium [1,2-$^{13}C_2$]acetate (0·17 and 0·44 mg cm^{-3}, respectively), but the labelling patterns were not affected.

Steroidal sapogenins are believed to be synthesized in plants *via* the following route: cycloartenol (**5**)[12] → chol-

† We have reassigned the C-23, C-24, and C-25 signals of the (25*S*)-steroidal sapogenins[8] which have so far been wrongly assigned.[9]



SCHEME

esterol (**6**) → 26-hydroxycholesterol[13] (**7**) or (**8**) →→ sapogenin (**9**) or (**10**) (Scheme). Our results indicated that in the biosynthesis of both the (25*R*)- and (25*S*)-sapogenins the introduction of the hydrogen atom at C-25 of the Δ^{24} double bond in cycloartenol occurred predominantly from the 25-*si* face, and the (25*S*)- and (25*R*)-sapogenins (**9**) and (**10**), respectively, were formed as a result of oxidation of the *pro-R* and the *pro-S* methyl group of (**6**) *via* (**7**) and (**8**), respectively. The process of the *pro-R* methyl oxidation might be accelerated by a high concentration of sodium acetate.

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