Biosynthesis of Sapogenins in Tissue Cultures of *Dioscorea tokoro* Makino

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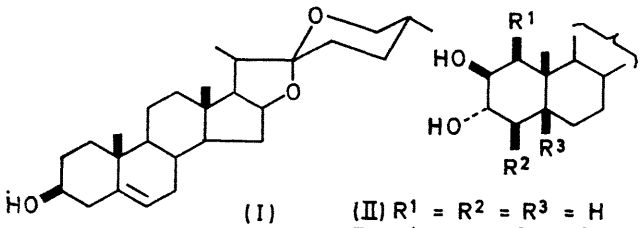
Summary The five intermediates in sapogenin biosynthesis from cycloartenol in *Dioscorea tokoro* tissue cultures have been established.

IT has previously been reported that the callus derived from seedlings of *Dioscorea tokoro* retains the ability to synthesize diosgenin (I), yonogenin (II), and tokorogenin (III), though isodiotigenin (IV) and kogagenin (V), formed in the intact plant, are not produced in tissue cultures.[1] Therefore, the pathway of sapogenin biosynthesis in tissue cultures must differ from that in the intact plant.



(I) (II) $R^1 = R^2 = R^3 = H$
(III) $R^1 = OH, R^2 = R^3 = H$
(IV) $R^1 = R^3 = H, R^2 = OH$
(V) $R^1 = R^3 = OH, R^2 = H$

In order to establish the general pathway of sapogenin biosynthesis in the tissue cultures, five labelled compounds were incubated with the tissue cultures in liquid medium. [24-$^3H_1$]Cycloartenol (VI) was synthesized by reduction of 24-oxocycloartanyl acetate with tritiated $NaBH_4$ followed by dehydration with $POCl_3$ in pyridine and deacetylation by treatment with $LiAlH_4$ in absolute ether. Treatment of 3,16,26-trihydroxycholest-5-ene[2] with triphenylmethyl chloride in pyridine, followed by acetylation and detritylation by treatment with hydrochloric acid yielded 3,16-diacetoxycholest-5-en-26-ol which was oxidized with chromic acid–pyridine in methylene chloride to give the corresponding aldehyde. The aldehyde was reduced with tritiated $NaBH_4$ to the alcohol, which was converted into [26-$^3H_1$]-3,16,26-trihydroxycholest-5-ene. [16-$^3H_1$, 22-$^3H_1$]-3,16,22,26-tetrahydroxycholest-5-ene was synthesized by reduction of kryptogenin with tritiated $NaBH_4$ in ethanol followed by addition of an excess of $NaBH_4$ to complete the reduction. Reduction of kryptogenin with an equivalent of tritiated $NaBH_4$ in ethanol yielded [16-$^3H_1$]-3,16,26-trihydroxycholest-5-en-22-one with a small amount of tetrahydroxycholest-5-ene, and the former compound was converted into [16-$^3H_1$]diosgenin by treatment with acetic acid. Therefore, the hydroxy-group at C-16, formed by reduction of the carbonyl group with tritiated $NaBH_4$, has the β-configuration. These labelled compounds were purified by preparative t.l.c. on silica gel and were added to the liquid medium of *D. tokoro* tissue cultures. Cells were harvested after two weeks and were extracted with 70% methanol. Diosgenin, yonogenin, and tokorogenin were isolated as described previously[1] and were recrystallised to constant specific radio activity after addition of the pure carrier sapogenins. The radioactivities of the sapogenins are listed in the Table.

Incorporation of the labelled precursors into sapogenins in Dioscorea tokoro *tissue cultures*

Precursors added	(Total radioactivity, d.p.m.) Diosgenin	Yonogenin	Tokorogenin
Cycloartenol[b]	7.59×10^3	2.13×10^2	6.16×10^3
[4-^{14}C]Cholesterol[a]	3.73×10^4	1.09×10^3	2.14×10^4
3,16,26-Trihydroxycholest-5-ene[a] ..	6.21×10^4	4.93×10^3	8.13×10^4
3,16,22,26-Tetrahydroxycholest-5-ene[a]	14.4×10^4	5.09×10^3	7.35×10^4
Diosgenin[c]	—	3.10×10^3	2.01×10^4

Radioactivity: [a] 100 μCi; [b] 50 μCi; [c] 10 μCi.

These results lead to the following conclusions. (i) Cycloartenol is not only a key intermediate of phytosterol biosynthesis but also a precursor of sapogenins in *D. tokoro* tissue cultures. (ii) Hydroxylation of the side-chain in cholesterol occurs prior to that of the A-ring in biosynthesis of yonogenin and tokorogenin. (iii) The following pathway for sapogenin biosynthesis in the tissue cultures would be the most plausible.

Mevalonic acid[3] → cycloartenol → cholesterol → 3,16,26-trihydroxycholest-5-ene → 3,16,22,26-tetrahydroxycholest-5-ene → diosgenin → yonogenin ⇄ tokorogenin ?

However, it is not yet clear whether tokorogenin is synthesized *via* yonogenin or not.

We thank Dr. H. Minato for helpful discussions.

(*Received, October 26th*, 1970; *Com.* 1838.)

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[3] Y. Tomita and A. Uomori, to be published.