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BIOSYNTHESIS OF STEROIDAL SAPOGENINS. INCORPORATION AND DISTRIBUTION OF RADIOACTIVITY OF [2-^{14}C]- AND [3-^{14}C]-MEVALONATE IN TIGOGENIN.

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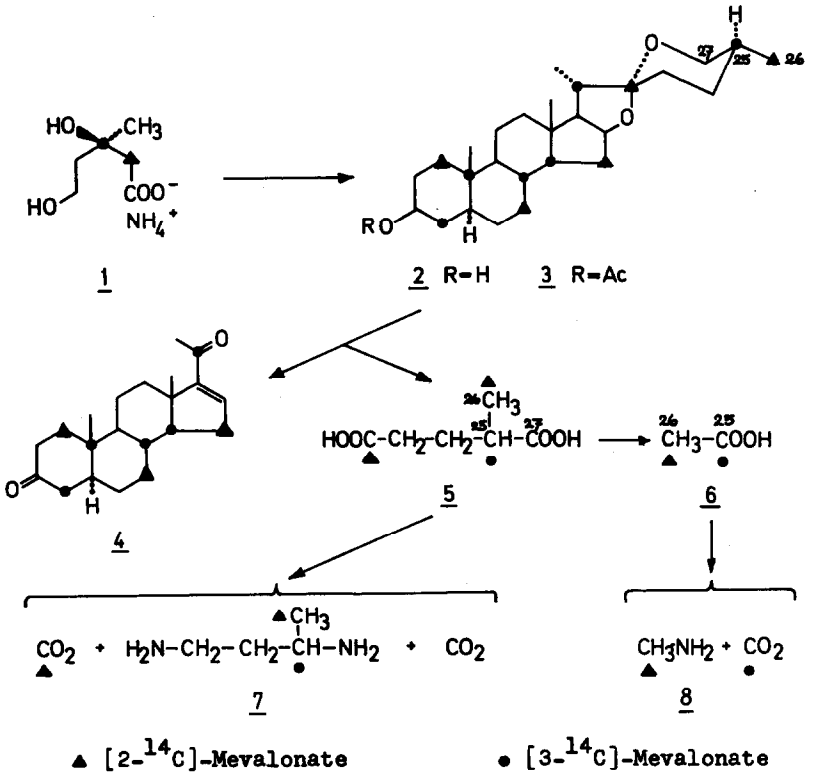
Little is known about the biosynthesis of steroidal sapogenins (spirostanols). Bennett et al.[1] have shown that [2-^{14}C]-mevalonic acid is incorporated into diosgenin, yamogenin, gentrogenin and correllogenin in Dioscorea spiculiflora when administered to the leaves. After administration of [4-^{14}C]-cholesterol to seedlings of the same plant diosgenin and kryptogenin were found to be radioactive[2]. In Digitalis lanata, Tschesche and Hulpke[3] observed the conversion of [4-^{14}C]-cholesterol-glucoside into tigogenin, gitogenin and diosgenin, when the radioactive compound was applied to the leaf surfaces. It was concluded from these experiments that the plants can utilize cholesterol for the biosynthesis of the spirostanols. Both cholesterol and the sapogenins possess the same skeleton of 27 carbon atoms, while the cardenolides occuring simultaneously in Digitalis contain only 23 carbon atoms. It has been demonstrated that their biosynthesis proceeds via a pregnane derivative (C_{21}) to which one mole of acetate is attached to form the butenolide ring[4-7]. The C_{21}-intermediate is considered to arise from cholesterol or a C_{27}-equivalent.

Since the distribution of the radioactivity of the incorporated precursors, especially that of mevalonic acid,

has not been determined in the sapogenins, an analogous pathway (degradation and resynthesis) can not be excluded a priori for the formation of the spiroketal system, at least in Digitalis. We have therefore applied ammonium [3-^{14}C]-D,L-mevalonate (1)[5,8] to young plants of Digitalis lanata EHRH. using the wick method. (Details cf[5]). The saponins were isolated and hydrolysed as described[5*]. The following sapogenins and incorporations were obtained: tigogenin (2) (0.84 %), gitogenin (0,41 %) and digitogenin (0.11 %). Tigogenin (2) was acetylated and 3-O-acetyl-tigogenin (3) converted to Δ^{16}-pregnene-3,20-dione (4) and α-methyl-glutaric acid (5) by subsequent treatment with acetic anhydride in n-octanoic acid, CrO_3 and NaOH. The relative activities of the transformation products are listed in the table. Both Δ^{16}-pregnene-3,20-dione (4) and α-methyl-glutaric acid (5) were radioactive in a ratio of 5:1. Schmidt degradation of 5 gave 2 moles of inactive CO_2 and labelled 1,3-diamino-butane (7) (isolated as dipicrate). Kuhn-Roth oxidation of 5 yielded 1 mole of acetic acid (6) which contained the total activity of the starting material. The Schmidt degradation to methylamine (8) (isolated as picrate; inactive) and CO_2 (isolated as $BaCO_3$; active) showed, as expected, that only the carboxyl group, which corresponds to C-25 of tigogenin (2), was labelled. This degradation demonstrates that six mevalonate units are incorporated into tigogenin (2), one of them being used for the formation of the spiroketal system.

In order to establish the origin of C-26 and C-27 of the spirostanols, ammonium [2-^{14}C]-mevalonate (1) was fed to Digitalis plants. Again tigogenin (2) (0.05 % incorp.), gitogenin (0.02 % incorp.) and digitogenin (0.04 % incorp.) were isolated. The degradation of 3-O-acetyl-tigogenin (3) was carried out as described above and the ratio of activity

* The extraction and separation was carried out by Dres. A. Lardon and J. von Euw.



of Δ^{16}-pregnene-3,20-dione (4) and α-methyl-glutaric acid (5) showed fair agreement with the calculated values. The acetic acid (6) formed by Kuhn-Roth oxidation of 5 contained 50 % of the activity. The Schmidt degradation revealed that the activity is concentrated exclusively in the methyl group which corresponds to C-26 of tigogenin (2), as methylamine (8) carries the total activity and CO_2 is practically inactive. The Schmidt reaction of α-methyl-glutaric acid (5) led to labelled 1,3-diamino-butane (7) and to active CO_2. According to the values measured only one of the two moles of CO_2 carries a label.

T A B L E

Relative Radioactivities in Per Cent

Compound	Precursors			
	[3-^{14}C]-Mevalonate		[2-^{14}C]-Mevalonate	
	Calc.	Found	Calc.	Found
Tigogenin (2)	100	100	100	100
3-O-Acetyl-tigogenin (3)	100	100	100	100
Δ^{16}-Pregnene-3,20-dione (4)	83.3	81.6	60	70
α-Methyl-glutaric acid (5)	16.7	15.3	40	38.6
CO_2 ($BaCO_3$) from acetic acid (6)	16.7	16.3	0	2.3
Methylamine (8) (picrate) from acetic acid (6)	0	0	20	19.3
1,3-diamino-butane (7) (dipicrate)	16.7	17.2	20	19.5
CO_2 ($BaCO_3$) from α-methyl-glutaric acid (5)	0	0	10	9.2

We conclude from these experiments which show excellent agreement between the theoretical and measured values, that the C_{27}-intermediate formed in the course of the well established biogenetic sequence[9] is converted into the spirostanols directly, i.e. without degradation and resynthesis. No randomization of the terminal methyl label has taken place in the precursors where the carbon atoms No. 20 to 27 have not cyclized. The equatorial 26-methyl group of tigogenin is derived from C-2 of mevalonate and C-27 to which the oxygen bridge is attached, corresponds to the methyl group of mevalonate. The non-equivalence of the 26- and 27-methyl groups**

** Non-equivalence was also observed in the case of C-23 and C-24 of soyasapogenol D[10]. On the other hand randomization of the terminal methyl label of the isoprenoid portion has been observed recently in plumieridl[11], verbenalin and aucubin[12], in several indol alkaloids[13] and in β-skytanthine[14].

in the open chain intermediate excludes cholesterol as an intermediate in the main biogenetic pathway of the sapogenins since a Δ^{24}-double bond is required for the selective enzymatic oxygenation of C-27 and the stereospecific enzymic hydrogenation leading to the final spiroketal structure. Therefore the essential intermediate is rather Δ^{24}-desmosterol or an analogous compound***.

The details of this investigation will be reported in Helv. Chim. Acta.

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