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THE BIOSYNTHESIS OF STEROIDAL SAPOGENINS IN *DIOSCOREA FLORIBUNDA* FROM DOUBLY LABELLED CHOLESTEROL

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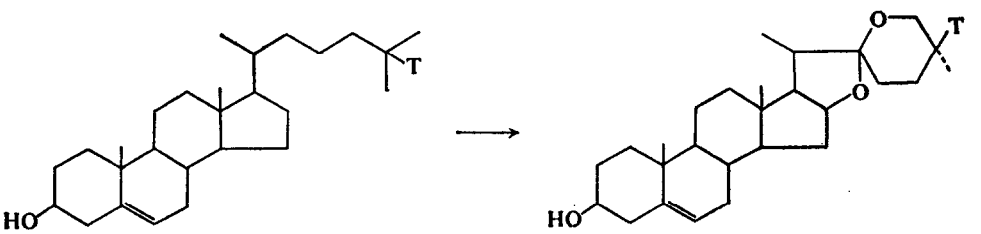
Abstract—Cholesterol-4-^{14}C-25-^{3}H was converted by *Dioscorea floribunda* plants to diosgenin, which had the same ^{3}H/^{14}C ratio as the administered cholesterol. The results indicate that cholesterol is not dehydrogenated at C-24 prior to forming diosgenin.

INTRODUCTION

ONE OF the last steps in the biosynthesis of cholesterol (I) involves the enzymatic reduction of a Δ^{24}-double bond.[1] However, recent experiments with *Digitalis* plants have raised the question of whether cholesterol, when administered to the plants, is directly converted to the sapogenins or first reconverted to a Δ^{24}-derivative before enzymatic oxygenation and hydrogenation to the final spirostanol structure takes place.[2,3] In order to decide between these two possibilities we have synthesized cholesterol-25-^{3}H[4] and have administered doubly labelled cholesterol-4-^{14}C-25-^{3}H to *Dioscorea floribunda*. If the ^{3}H/^{14}C ratio in the newly synthesized diosgenin (II) is the same as in the administered cholesterol, no dehydrogenation to a Δ^{24}-derivative is involved in the biosynthesis.



(I) Cholesterol-25-^{3}H (II) Diosgenin-25-^{3}H

RESULTS

Three *Dioscorea floribunda* plants were treated with a mixture of cholesterol-4-^{14}C and cholesterol-25-^{3}H (^{3}H/^{14}C ratio 1·35), twice a week for five weeks. Work-up by the usual

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[1] E. HEFTMANN and E. MOSETTIG, *Biochemistry of Steroids*, Reinhold, New York (1960).
[2] R. JOLY and CH. TAMM, *Tetrahedron Letters* 3535 (1967).
[3] G. M. JACOBSOHN and M. J. FREY, *Archs Biochem. Biophys.* **127**, 655 (1968).
[4] R. A. JOLY, H. H. SAUER and J. BONNER, *J. Labelled Compounds* **5**, 80 (1969).

methods gave, after acid hydrolysis, an extract containing the sapogenins and sterols, as well as nonpolar material. The hydrolyzate was subjected to preparative TLC, and a sterol-sapogenin fraction was isolated and acetylated. Diosgenin acetate was then separated from the sterols by TLC, diluted with carrier diosgenin acetate, and purified to constant specific radioactivity (Table 1). Furthermore, diosgenin acetate was converted to diosgenin by treatment with $LiALH_4$,[5] purified, and crystallized to constant specific activity (Table 1). The $^3H/^{14}C$ ratio of the radiochemically pure diosgenin was 1·23.

TABLE 1. RECRYSTALLIZATION OF DIOSGENIN ACETATE AND DIOSGENIN TO CONSTANT SPECIFIC ACTIVITY

Compound	Solvent used for crystallization	dpm/μmole*		$^3H/^{14}C$ ratio
		^{14}C	^3H	
Diosgenin acetate	Hexane	1577±28	2091±37	1·31
	Hexane	1540±30	1960±38	1·28
	Methanol	1605±32	2010±41	1·27
Diosgenin	Acetone	1682±48	2070±61	1·24
	Acetone	1655±43	1990±53	1·22
	Hexane–ether–CH_2Cl_2	1622±42	1970±51	1·23

* 90 per cent confidence level.

DISCUSSION

Joly and Tamm[2] previously found that in the biosynthesis of tigogenin from mevalonic acid-2-^{14}C in *Digitalis lanata*, C-26 was labelled but C-27 was not. This proved that both the saturation of the Δ^{24}-double bond and the oxygenation at C-26 are stereospecific enzymatic reactions. The selective oxygenation of one of the two terminal methyl groups could be attributed either to the presence of the Δ^{24}-double bond or, following reduction of the double bond, to the presence of a prochiral center at C-25.[6] The fact that cholesterol has been shown to be a precursor of the sapogenins would favor the latter theory. However, the former alternative would still be tenable if, as has been suggested,[2,3] cholesterol is reversibly dehydrogenated to a Δ^{24}-derivative by plants.

Our present results indicate that cholesterol-25-^3H (I) is directly converted to diosgenin-25-^3H (II) by *Dioscorea floribunda*. No loss of tritium was observed, as shown by the constant $^3H/^{14}C$ ratio. Our experiments, which showed agreement between the theoretical and experimental values for this ratio, permit the conclusion that cholesterol undergoes a direct conversion to diosgenin without dehydrogenation to a Δ^{24}-intermediate.

EXPERIMENTAL

TLC techniques were as described previously.[7] All chromatograms were run on Silica Gel G plates, purchased from Analtech, Inc., Wilmington, Delaware. Aliquots of radioactive samples were counted either on planchets at infinite thinness under a gas-flow detector with a Beckman Widebeta II instrument, having an efficiency of 34 per cent, or with a liquid scintillation counter, Beckman Model LS-200B, having an efficiency

[5] R. D. BENNETT, E. HEFTMANN, W. H. PRESTON, JR. and J. R. HAUN, *Archs Biochem. Biophys.* **103**, 74 (1963).
[6] R. JOLY and CH. TAMM, *Tetrahedron Letters* 898 (1968).
[7] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **5**, 747 (1966).

of 59·3 per cent for ^{14}C and of 72·1 per cent for ^{3}H. Cholesterol-4-^{14}C, having a specific activity of 50 $\mu c/\mu$mole, was purchased from New England Nuclear Corporation. Cholesterol-25-^{3}H was synthesized as described previously.[4] *Dioscorea floribunda* plants were raised from seeds in a greenhouse.

A mixture of cholesterol-4-^{14}C ($2{\cdot}61 \times 10^7$ dpm) and cholesterol-25-^{3}H ($3{\cdot}52 + 10^7$ dpm) was administered in doses of $0{\cdot}87 \times 10^6$ dpm [^{14}C] and $1{\cdot}17 \times 10^6$ dpm [^{3}H] ($^{3}H/^{14}C$ ratio 1·35) to each of three potted *D. floribunda* plants, 4 months old, by the technique previously described.[8] A total of ten such treatments were given to the plants, twice a week.

Seven days after the last treatment, two of the plants were cut off at the soil line, frozen in liquid N_2 and lyophilized, yielding 10·7 g of dry material. Only the dried material of the leaves (753 mg) was homogenized with 50 ml H_2O in a blender and refluxed for 1·5 hr after adding 15·5 ml conc. HCl. The hydrolyzate was worked up by adding ice and extracting with six 20-ml portions of CH_2Cl_2. The CH_2Cl_2 extracts were washed with H_2O, 10 per cent $KHCO_3$, and H_2O in succession, combined and evaporated. Nonpolar and very polar material were separated from the sterols and sapogenins by subjecting the residue to preparative TLC with CH_2Cl_2–acetone (9:1). The sterol-sapogenin zone was eluted and yielded 12·7 mg ($4{\cdot}5 \times 10^6$ dpm of ^{14}C). This material was acetylated and rechromatographed with CH_2Cl_2–ether (97:3). In this system, cholesterol acetate runs as a zone near the solvent front, while diosgenin acetate (R_f 0·43) is completely separated from the isomeric yamogenin acetate (R_f 0·36).[9] The diosgenin acetate zone was removed and rechromatographed in the same system, yielding 2·0 mg of chromatographically homogeneous diosgenin acetate. No radioactive impurities were observed by TLC with CH_2Cl_2–ether (97:3).*

Crystallization from hexane then gave 0·2 mg of pure diosgenin acetate, m.p. 193–194°.† No depression was observed when a mixed m.p. was taken. The crystals and mother liquor were then combined and diluted with 10·3 mg of carrier material and recrystallized to constant specific activity as shown in Table 1. A 6·6 mg portion of the diosgenin acetate was reduced with an excess of $LiALH_4$ by the previously described procedure[5] and gave 5·9 mg diosgenin (99 per cent yield). No radioactive impurities were detected by TLC with CH_2Cl_2–MeOH (24:1). Upon crystallization from acetone–ether pure diosgenin was obtained; m.p. 203·5–205°. No change in specific acitivity was observed (Table 1).

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[8] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 475 (1965).
[9] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 577 (1965).
* Radiochromatograms were scanned on a Packard Model 7201 Scanner.
† All m.p.s. were determined on a Kofler block and are corrected.