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SHORT COMMUNICATION

CONVERSION OF AN OPEN-CHAIN SAPONIN TO DIOSCIN BY A *DIOSCOREA FLORIBUNDA* HOMOGENATE

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Abstract—A *Dioscorea floribunda* leaf homogenate converted an open-chain glycoside of diosgenin, Δ^5-furostene-3β,22,26-triol 3β-chacotrioside 26β-D-glucopyranoside, to dioscin.

INTRODUCTION

STEROIDAL saponins in which the side chain is held open by glycoside formation (furostanols) have now been isolated from several species of plants.[1-4] Although these glycosides have been converted to the normal saponins (spirostanols) by treatment with glucosidases,[1-3] this conversion has not yet been demonstrated to occur in the plants containing the spirostanols. We previously prepared 4-^{14}C-labeled Δ^5-furostene-3β,22,26-triol 3-(2′-*O*,4′*O*-bis-α-L-rhamnopyranosyl-β-D-glucopyranoside) 26-β-D-glucopyranoside (I) by treatment of *Dioscorea floribunda* plants with cholesterol-4-^{14}C,[5] and we have now investigated its conversion to dioscin (II) by a homogenate of the same plant.

RESULTS AND DISCUSSION

Radioactive I was incubated with a homogenate of a *Dioscorea floribunda* leaf for 1·5 hr. TLC of the product showed essentially complete conversion of I to dioscin. To demonstrate that the steroid nucleus was not changed by this treatment, a portion of the dioscin was subjected to acid hydrolysis. The product, diosgenin (III), was acetylated, and the diosgenin acetate was shown to be radiochemically pure by dilution with carrier and crystallization (Table 1). This experiment, together with our previous findings,[5] demonstrates the biosynthesis of dioscin from cholesterol via the furostanol I in *D. floribunda*. The question of whether I is an obligatory intermediate can only be answered by further investigations into the biosynthetic steps between cholesterol and the saponins.

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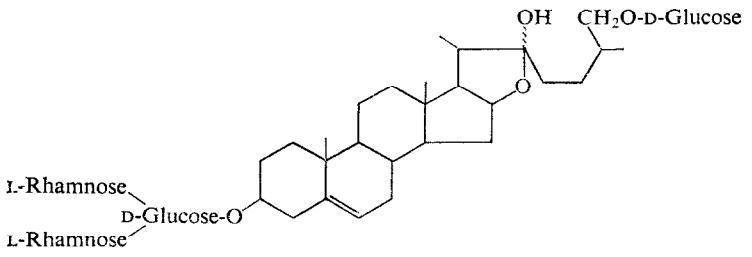
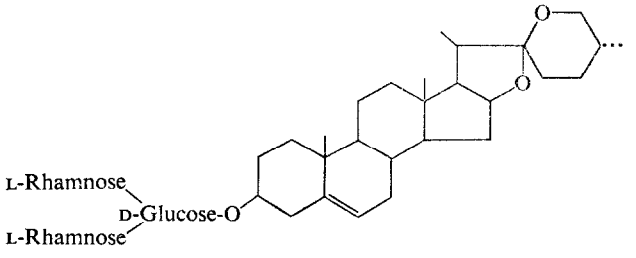
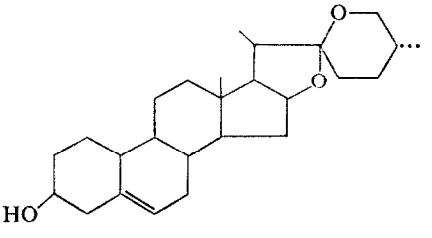
[5] R. A. JOLY, J. BONNER, R. D. BENNETT and E. HEFTMANN, *Phytochem.*, in press.



(I) Δ^5-Furostene-3β,22,26-triol 3β-chacotrioside 26β-D-glucopyranoside



(II) Dioscin



(III) Diosgenin

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

Solvent used for crystallization	Counts/min/μmole†
Hexane	965 ± 22
Hexane	970 ± 23
Methanol	962 ± 22

* Portions of 0·2 mg or less were plated from solution on ringed planchets over an area of 12·7 cm^2 and counted in duplicate on a Beckman Widebeta II instrument. Counter efficiency was 34 per cent and background was 2 counts/min.

† 90 per cent confidence level.

EXPERIMENTAL

TLC techniques were as described previously.[6] All chromatograms were run on Silica Gel G plates, purchased from Analtech, Inc., Wilmington, Delaware.* Aliquots of radioactive samples were counted on planchets at infinite thinness under a gas-flow detector (see Table 1, legend, for details). All m.ps. were determined on a Kofler block and are corrected.

The homogenate (10 ml) of a fresh *Dioscorea floribunda* leaf (0·81 g) was incubated at 25° with $1{\cdot}93 \times 10^4$ counts/min of the glycoside I. After 1·5 hr, the mixture was worked up by extraction with five 10-ml portions of butanol, which were washed with water and combined. Evaporation of the extract gave 124 mg of radioactive material ($1{\cdot}76 \times 10^4$ counts/min). When submitted to radiochromatography in CH_2Cl_2–MeOH–H_2O (28:12:3), only one sharp peak, having the same mobility as a reference sample of dioscin, was observed. Therefore, the conversion rate of the open-chain glycoside to dioscin can be estimated as $92{\cdot}5 \pm 5$ per cent.

A portion ($4{\cdot}28 \times 10^3$ counts/min) of the crude dioscin was further purified by repeated preparative TLC with CH_2Cl_2–MeOH–H_2O (28:12:3) and $CHCl_3$–MeOH–H_2O (13:7:2) until a chromatographically pure sample of dioscin (3·3 mg, $1{\cdot}83 \times 10^3$ counts/min) was obtained. Another portion ($1{\cdot}21 \times 10^4$ counts/min) was hydrolyzed with 5·5 ml of 3 N HCl in 2·0 ml of MeOH–H_2O (1:1) by refluxing for 1·5 hr. The hydrolyzate was worked up by adding ice, extracting with ether–CH_2Cl_2 (3:1), and washing the extracts, in succession, with H_2O, 2 N Na_2CO_3, and H_2O. Evaporation of the extracts yielded a radioactive residue ($1{\cdot}05 \times 10^4$ counts/min) from which diosgenin ($8{\cdot}50 \times 10^3$ counts/min) was isolated by preparative TLC with CH_2Cl_2–Me_2CO (9:1). When acetylated, this material corresponded to diosgenin acetate by TLC with CH_2Cl_2–ether (97:3). The diosgenin acetate was purified by TLC in the same system, yielding chromatographically homogeneous material ($8{\cdot}09 \times 10^3$ counts/min). This was then diluted with 3·5 mg of carrier diosgenin acetate and recrystallized to constant specific activity as shown in Table 1. No depression of the m.p. was observed when the product was mixed with authentic diosgenin acetate; m.p. 192–193°.

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* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

[6] R. D. BENNETT and E. HEFTMAN, *Phytochem.* **5**, 747 (1966).