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SHORT COMMUNICATION

CONVERSION OF CHOLESTEROL TO AN OPEN-CHAIN SAPONIN BY *DIOSCOREA FLORIBUNDA*

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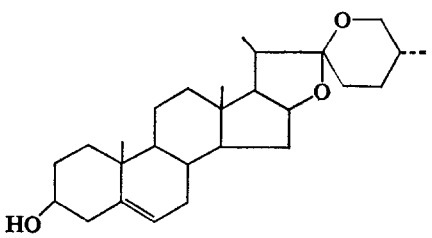
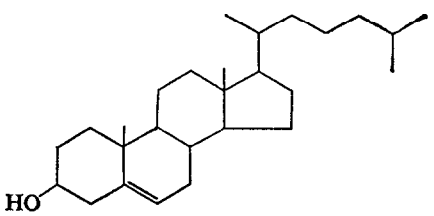
Abstract—In *Dioscorea floribunda*, cholesterol-4-^{14}C is a precursor of an open-chain glycoside of diosgenin, Δ^5-furostene-3β,22,26-triol 3-β-chacotrioside 26-β-D-glucopyranoside.

INTRODUCTION

AS EARLY as 1947 Marker and Lopez[1] postulated that steroidal saponins exist in plants in a form where the side chain is held open by glycoside formation. However, direct evidence for the existence of saponins with sugars attached to the side chain was lacking until recently.[2] Saponins lacking ring F (furostanols) have now been isolated from several species of plants.[3–6] Glycosides of both spirostanols, such as diosgenin (I), and furostanols give spirostanol aglycones when subjected to acid hydrolysis. Since cholesterol (II) is known to be a precursor



(I) Diosgenin



(II) Cholesterol

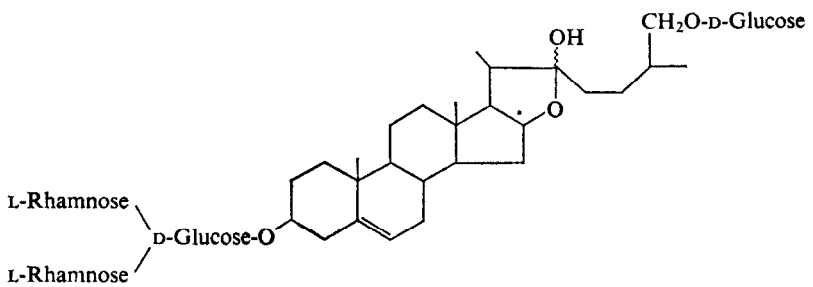
* A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Work conducted under a cooperative agreement with the California Institute of Technology. Requests for reprints should be addressed to E. H.

[1] R. E. MARKER and J. LOPEZ, *J. Am. Chem. Soc.* **69**, 2389 (1947).
[2] E. HEFTMANN, *Lloydia* **30**, 209 (1967).
[3] K. SCHREIBER and H. RIPPERGER, *Tetrahedron Letters* 5997 (1966).
[4] R. TSCHESCHE, G. LÜDKE and G. WULFF, *Tetrahedron Letters* 2785 (1967).
[5] S. KIYOSAWA, M. HUTOH, T. KOMORI, T. NOHARA, I. HOSOKAWA and T. KAWASAKI, *Chem. Pharm. Bull.* **16**, 1162 (1968).
[6] R. TSCHESCHE, B. T. TJOA, G. WULFF and R. V. NORONHA, *Tetrahedron Letters* 5141 (1968).

of the spirostanols,[7,8] it was of interest to determine whether the open-chain saponins are intermediates in this synthesis.

RESULTS AND DISCUSSION

A glycoside fraction was isolated from a *Dioscorea floribunda* plant that had been treated with cholesterol-4-^{14}C. TLC of this fraction showed radioactivity corresponding to dioscin (diosgenin 2′-*O*,4′-*O*-bis-α-L-rhamnopyranosyl-β-D-glucopyranoside) and to a substance which gave a red Ehrlich reaction characteristic of furostanol glycosides.[5] The latter material, which was isolated by column chromatography and preparative TLC, had the same mobility as an authentic sample of Δ^5-furostene-3β,22,26-triol 3-(2′-*O*,4′-*O*-bis-α-L-rhamnopyranosyl-β-D-glucopyranoside) 26-β-D-glucopyranoside (III) in three TLC systems. This compound had previously been isolated from *D. gracillima* and is known to form dioscin when treated with a glucosidase.[5] The radioactive material was further shown to be identical with the reference sample by the following methods. Both gave the same two products when acetylated, and in both cases the more polar peracetate (22-OH) was converted to the less polar one ($\Delta^{20,22}$) by dehydration with boiling acetic acid. Finally, acid hydrolysis of both substances gave diosgenin as the aglycone and D-glucose and L-rhamnose as the sugars. Thus, it appears evident that cholesterol was converted by the plant to the furostanol glycoside III.



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

EXPERIMENTAL

TLC techniques were as described previously.[9] Silica Gel G plates* were used for TLC of all compounds except sugars, for which cellulose MN 300 plates were used. Aliquots of radioactive samples were counted on planchets at infinite thinness with a Beckman Widebeta II instrument, having an efficiency of 34 per cent. Cholesterol-4-^{14}C (50 μc/μM) was purchased from New England Nuclear Corporation. *Dioscorea floribunda* plants were raised from seeds in a greenhouse.

A *D. floribunda* plant, about 8 months old, was treated with 10 μc of cholesterol-4-^{14}C by the technique previously described.[10] A total of ten such treatments were given, twice a week. Thirteen days after the last treatment the plant was cut off at the soil line, frozen in liquid N_2, and lyophilized. The dried material (22 g) was homogenized with 550 ml of 95 per cent aqueous EtOH and filtered. The filter cake was then reextracted by refluxing for 5 min with 400-ml portions of 95, 90, 70, 60, 55, and 50 per cent aqueous EtOH in succession.

* Analtech, Inc., Wilmington, Delaware. 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.

[7] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 577 (1965).
[8] R. TSCHESCHE and H. HULPKE, *Z. Naturforsch.* **21b**, 494 (1966).
[9] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **5**, 747 (1966).
[10] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 475 (1965).

Each extract was filtered, and the filtrates were finally combined and evaporated. The residue was taken up in 75 ml H_2O, and nonpolar material was removed by extraction with five 30-ml portions of benzene, which were washed with 20 ml H_2O. The aqueous layer was then extracted with six 20-ml portions of butanol. The extracts were washed with the same 20 ml of H_2O as above, combined, and evaporated, giving 1·35 g of a polar fraction ($8{\cdot}68 \times 10^6$ counts/min). A portion (800 mg) of this fraction was freed of some cholesterol by chromatography on a 350-g column of silica gel (0·05–0·2 mm).* Elution with CH_2Cl_2–MeOH–H_2O (6:4:1) gave 367 mg of a glycoside fraction ($9{\cdot}96 \times 10^5$ counts/min), which represents a 2·22 per cent incorporation of cholesterol into the crude saponins. A portion (136 mg) of this material was further purified by chromatography on a 150-g column of silica gel (100–200 mesh),† which was eluted with 10-ml fractions of CH_2Cl_2–MeOH–H_2O (67:23:7). Fractions 16–25 contained dioscin (16·0 mg, $1{\cdot}02 \times 10^5$ counts/min) and fractions 50–72 the furostanol glycoside (III) (26·1 mg, $1{\cdot}57 \times 10^5$ counts/min). The latter was further purified by preparative TLC with CH_2Cl_2–MeOH–H_2O (28:12:3), giving 20·0 mg ($1{\cdot}51 \times 10^5$ counts/min). This material corresponded in mobility to a reference sample of compound III in three TLC systems: CH_2Cl_2–MeOH–H_2O (28:12:3), CH_2Cl_2–MeOH–H_2O (10:10:3), and $CHCl_3$–MeOH–H_2O (13:7:2).

A 5-mg portion of the pure radioactive saponin was peracetylated and compared with the acetylation product of authentic compound III by TLC with CH_2Cl_2–MeOH (24:1) and benzene–isopropyl alcohol (10:1). The same two products were observed in both cases. When a portion of the radioactive peracetate was refluxed for 2 hr with AcOH, TLC in the same two systems as above showed only one product, corresponding in mobility to the less polar of the two products. The same result was observed when the peracetate of compound III was subjected to this reaction.

A 5-mg portion of the radioactive saponin was dissolved in 0·5 ml MeOH and refluxed with 1·0 ml of 3 N HCl for 3·5 hr. The reaction was worked up by adding ice, extracting with ether–CH_2Cl_2 (3:1), and washing the extracts with H_2O, 2 N Na_2CO_3, and H_2O. Evaporation of the extracts gave 2·1 mg of material, from which diosgenin was isolated by preparative TLC with CH_2Cl_2–acetone (9:1). A TLC scan in the same system showed a single radioactive peak corresponding to authentic diosgenin. When acetylated, this material corresponded to diosgenin acetate by TLC with CH_2Cl_2–ether (97:3). Hydrolysis of authentic compound III by the same method also gave diosgenin.

The sugars were identified as follows. The aqueous solution from the acid hydrolysis above was neutralized with freshly prepared $BaCO_3$, filtered, evaporated to dryness, taken up in acetone, filtered, and again evaporated to dryness. TLC of the residue with butanol–AcOH–H_2O (3:1:1) and spraying of the chromatogram with $AgNO_3$–NaOH[11] showed two spots, corresponding in mobility to authentic samples of D-glucose and L-rhamnose, respectively.

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* Brinkmann Instruments, Westbury, New York.

† Grace-Davison Chemical Co., Baltimore, Maryland.

[11] E. STAHL, editor, *Dünnschicht-Chromatographie*, 2nd edition, p. 851, Springer-Verlag, Heidelberg (1967).