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SHORT COMMUNICATION

ISOLATION OF A NEW STEROL FROM POTATO LEAVES

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Abstract—A new sterol of probable structure 4α,14α-dimethylcholesta-8,24-dien-3β-ol has been identified in potato leaves. A possible intermediate role for this compound in cholesterol biosynthesis in plants is discussed.

INTRODUCTION

Previous investigators have demonstrated the presence of cycloartenol, 24-methylenecycloartanol, 24-methylenelophenol, 24-ethylidenelophenol, lophenol, β-sitosterol, stigmasterol, campesterol and cholesterol in leaves of *Solanum tuberosum* var. "Aquila".[1–3] Because of the reported high content of cycloartenol in this plant, we have used it to study cycloartenol biosynthesis.[4] However, during a preliminary examination of the sterols of *S. tuberosum* var. "Majestic", we observed a new 4-methyl sterol in addition to those previously reported. The present communication reports some observations on the nature of this sterol.

RESULTS AND DISCUSSION

The sterols obtained from *Solanum tuberosum* var. "Majestic" leaves via their digitonides were chromatographed on alumina (Brockmann Grade III) and the 4-methyl sterols further purified by preparative thin-layer chromatography on Kieselgel G. Gas–liquid chromatography (GLC) revealed one major component with a retention time which did not correspond to any available 4α-methyl sterol. There were also a number of minor peaks. Acetylation, followed by chromatography on $AgNO_3$– silica gel thin layers, resulted in the separation of the 4-methyl steryl acetate mixture (28 mg) into four component bands: (A) R_f 0·49 (4·9mg); (B) R_f 0·43 (11·4 mg); (C) R_f 0·36 (2·4 mg); (D) R_f 0·32 (0·6 mg). Bands B, C and D co-chromatographed with the acetates of 24-ethylidenelophenol, cycloeucalenol and 24-methylenelophenol respectively. GLC of Band B showed a major component (85 per cent) and a minor component (15 per cent), neither of which corresponded to any available 4α-methyl steryl acetate, but the comparatively short retention time of the main component indicated that it had an unsubstituted side-chain. The main component was further purified by preparative GLC on 3 per cent XE-60 and a portion gave a yellow colour, λ_{max} 460 nm

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[1] K. Schreiber and G. Osske, *Die Kulturpflanze* **10**, 372 (1962); *Tetrahedron* **20**, 1803 (1964).

[2] G. Osske and K. Schreiber, *Tetrahedron* **21**, 1559 (1965).

[3] M. von Ardenne, G. Osske, K. Schrieber, K. Steinfelder and R. Tümmler, *Die Kulturpflanze* **13**, 101, 115 (1965).

[4] H. H. Rees, L. J. Goad and T. W. Goodwin, *Biochem. J.* **107**, 417 (1968).

after 25 min, with the Liebermann Burchard reagent. This response appears to be characteristic of sterols containing a C-14 methyl group.[5] Analysis by mass spectrometry (Fig. 1) showed a molecular ion at *m/e* 454 ($C_{31}H_{50}O_2$) and other peaks at *m/e* 439 (M^+-CH_3), 394 (M^+-acetate) and 379 (M^+-acetate-CH_3). A prominent peak at *m/e* 287 (M^+-$C_{12}H_{23}^{\cdot}$) was present and could arise by the same fragmentation as that previously observed in obtusifoliol acetate[6, 7] (4α,14α-dimethylergosta-8,24(28)-dien-3β-yl acetate). This fragmentation has been assigned to loss of the side-chain plus part of ring D by fission of bonds 13–17 and 14–15, accompanied by a methyl migration from the remaining ring fragment. The peak at *m/e* 227 (Fig. 1) can arise by the same fragmentation but with the additional loss of acetate. The above data indicate that the most probable structure for the new potato leaf sterol is 4α,14α-dimethylcholesta-8,24-dien-3β-ol (see Fig. 1) but it is emphasized that the double-bond positions and configuration at present are based solely on GLC retention data and biogenetic considerations.

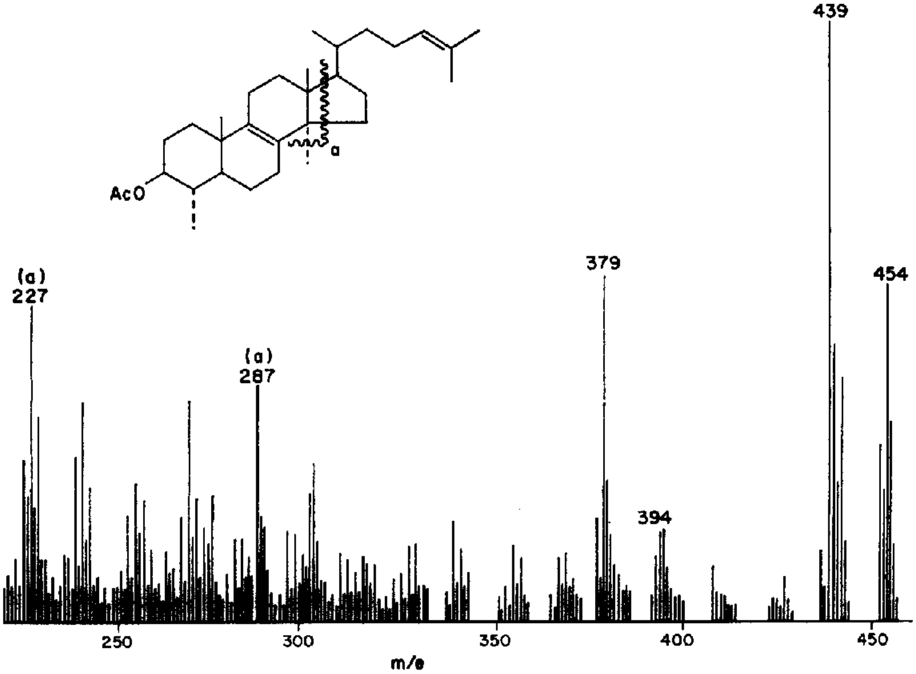

FIG. 1. MASS SPECTRUM OF 3β-ACETOXY-4α,14α-DIMETHYLCHOLESTA-8,24-DIENE.

It is suggested[3, 8, 9] that cycloartenol may play an important role in phytosterol biosynthesis by replacing lanosterol, the established intermediate in animal sterol biosynthesis. Following the intermediate formation of 24-methylenecycloartanol and cycloeucalenol, it is postulated that the cyclopropane ring is opened to give obtusifoliol[5,7,9] which has been isolated from various plant sources and which can subsequently be metabolized to the

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[5] B. L. WILLIAMS, L. J. GOAD and T. W. GOODWIN, *Phytochem.* 6, 1137 (1967).

[6] J. B. BARRERA, J. L. BRETÓN, J. D. MARTIN and A. G. GONZÁLEZ, *Anal. Fis. Quim.* 623, 191 (1967).

[7] B. L. WILLIAMS, L. J. GOAD and T. W. GOODWIN, *European J. Biochem.* 3, 232 (1967.)

[8] P. BENVENISTE, L. HIRTH and G. OURISSON, *Phytochem.* 5, 31, 45 (1966).

[9] L. J. GOAD, in *Terpenoids in Plants* (edited by J. B. PRIDHAM), p. 159, Academic Press, London (1967).

phytosterols. The present tentative identification of 4α,14α-dimethylcholesta-8,24-dien-3β-ol suggests a similar intermediate role for this compound during cholesterol formation in plants according to the sequence: Cycloartenol → 31-norcycloartenol → 4α,14α-dimethylcholesta-8,24-dien-3β-ol → lophenol → cholesterol. The presence of relatively large amounts of cholesterol in potato leaves [3, 10, 11] and the probable role of cholesterol as an intermediate in the formation of steroid alkaloids and saponins[12] make such a scheme more attractive. It is also notable that although 31-norcycloartenol does not appear yet to have been reported in nature, the closely related 31-norcycloartanol has been isolated from plant sources.[13, 14]

EXPERIMENTAL

Extraction of Non-saponifiable Lipid

1100 g of potato leaves (*Solanum tuberosum* var. "Majestic") were crushed and then saponified directly by refluxing for 3 hr with 1400 ml of ethanol, 140 ml of water and 168 g of NaOH. The non-saponifiable material (4·43 g) was extracted from the filtered mixture with diethyl ether in the usual manner.

Separation of Sterols

The sterols (0·23 g) were precipitated from the non-saponifiable material with digitonin,[15] regenerated with pyridine[16] and chromatographed on a Brockmann Grade III alumina column (20 g). 200-ml volumes of the following fractions were collected: (I) petrol. ether (b.p. 40–60°); (II) 2 per cent (v/v) diethyl ether in petrol. ether (III) 6 per cent (v/v) diethyl either in petrol. ether (123 mg); (IV) 9 per cent (v/v) diethyl ether in petrol. ether (45 mg); (v) 20 per cent (v/v) diethyl ether in petrol. ether (52 mg). 4, 4′-Dimethyl sterols, 4α-methyl sterols and the 4-demethyl sterols were mostly present in fractions III, IV and V, respectively.

Thin-layer Chromatography

This was carried out as described previously.[5]

Gas–Liquid Chromatography

A Varian-Aerograph 1522B instrument with hydrogen flame ionization detectors and on column injection was used. Stainless-steel columns (1·8 m × 3 mm) were packed with 80–100 mesh silanized chromosorb W coated with (*a*) 1 per cent QF-1, (*b*) 0·7 per cent HiEFF 8B, (*c*) 3 per cent XE-60, (*d*) 3 per cent SE-30.

Preparative gas–liquid chromatography was carried out on a 3 per cent XE-60 column, fitted with an effluent-splitter and compounds were collected as they eluted from the column in glass capillary tubes at ambient temperature. The collected sample was rendered free of any traces of stationary phase by TLC on silica gel developed with 40 per cent benzene in petrol. ether (60–80°).

The Liebermann–Burchard Reaction

This was carried out as previously described.[17]

Mass Spectrometry

The mass spectrum was kindly determined by Mr. J. Berkley of the Department of Chemistry, The University, Liverpool 3, using an AEI MS 9 mass spectrometer.

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[10] H. H. Rees, L. J. Goad and T. W. Goodwin, unpublished observations.

[11] D. F. Johnson, R. D. Bennett and E. Heftmann, *Science* **140**, 198 (1963).

[12] R. D. Bennett and E. Heftmann, *Phytochem.* **4**, 577 (1965).

[13] G. Berti, F. Bottari, A. Marsili, I. Morelli, M. Polvani and A. Mandelbaum, *Tetrahedron Letters*, 125 (1967).

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[15] A. Windaus, *Ber. Chem. Dtsch. Ges.* **42**, 238 (1909).

[16] R. Schoenheimer and H. Dam, *Hoppe-Seylers Z. Physiol. Chem.* **215**, 59 (1933).

[17] B. L. Williams and T. W. Goodwin, *Phytochem.* 4, 81 (1965).