Phytochemistry, 1972, Vol. 11, pp. 3497 to 3500. Pergamon Press. Printed in England.

ISOLATION OF 14α-METHYL-9β,19-CYCLO-5α-ERGOST-24(28)-EN-3β-OL FROM *MUSA SAPIENTUM*

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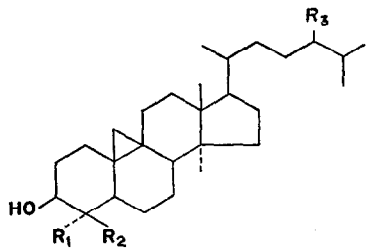
(*Received* 4 *July* 1972. *Accepted* 26 *July* 1972)

Key Word Index—*Musa sapientum*; Musaceae; banana; phytosterols; 24-methylenepollinastanol.

Abstract—14α-Methyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol has been isolated from *Musa sapientum* and is found primarily in the esterified sterol fraction. It is also released by saponification of tissue from which all solvent-extractable lipid has been previously removed. The relevance of these results is discussed in relation to the metabolism of sterols in this tissue.

INTRODUCTION

DURING an investigation of the sterols from the pollen of *Taraxacum densleonis* and *Hypochoeris radicata* an unusual sterol was isolated and identified as 14α-methyl-9β,19-cyclo-5α-cholestan-3β-ol (I).[1] This sterol was appropriately named pollinastanol and has since been found in a number of plants.[2–5] Although the biosynthesis of pollinastanol has not been studied, its conversion into cholesterol by *Nicotiana tabacum* has been demonstrated.[6] It is suggested that the presence of pollinastanol in some plants may be explained by its intermediary role in the conversion of cycloartenol into cholesterol.[5,7]



	R_1	R_2	R_3
(I)	H	H	H
(II)	H	H	$=CH_2$
(III)	CH_3	H	$=CH_2$

The present communication reports the identification of 14α-methyl-9β,19,cyclo-5α-ergost-24(28)-en-3β-ol (24-methylene pollinastanol) (II) in banana peel. This is present primarily in the esterified and tightly-bound sterol fractions. Its possible formation and further metabolism are discussed.

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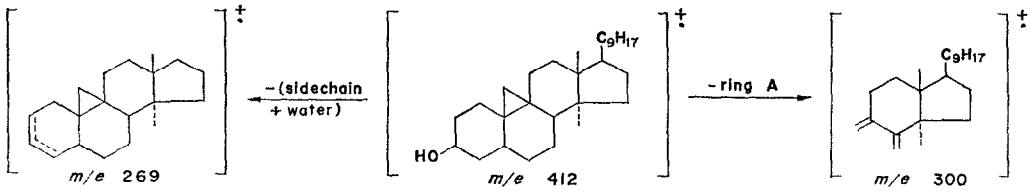
[1] M. F. HÜGEL, M. BARBIER and E. LEDERER, *Bull. Soc. Chim. Fr.* **81**, 2012 (1964).
[2] M. F. HÜGEL, W. VETTER, H. AUDIER, M. BARBIER and E. LEDERER, *Phytochem.* **3**, 7 (1965).
[3] M. DEVYS and M. BARBIER, *Bull. Soc. Chim. Biol.* **49**, 865 (1967).
[4] M. DEVYS, D. ANDRE and M. BARBIER, *Compt. Rend.* **269D**, 798 (1969).
[5] M. DEVYS, A. ALCAIDE, F. PINTE and M. BARBIER, *Compt. Rend.* **269 D**, 2033 (1969).
[6] M. DEVYS, A. ALCAIDE and M. BARBIER, *Phytochem.* **8**, 1441 (1969).
[7] M. DEVYS and M. BARBIER, *Compt. Rend.* **264**, 504 (1967).

RESULTS

Banana peel was extracted exhaustively with methanol until TLC indicated that no further sterol could be removed with this solvent. From this extract the unesterified sterols (fraction A) and the esterified sterol fraction were obtained. The latter material was saponified and the sterols released were then purified by TLC (fraction B). The residual plant tissue from the methanol extraction was saponified to release a tightly-bound sterol fraction which was purified by TLC (fraction C). Each of these three sterol fractions were then analyzed by GLC on 3% SE-30 and 1·5% OV-17 stationary phases (Table 1). The unesterified sterols (fraction A) consisted of the C_{28} and C_{29} sterols previously reported as constituents of banana peel and many other plant species.[8] The identification of these compounds was confirmed by GLC–MS analysis. However, the major component of the esterified sterols (fraction B) and also of the tightly-bound sterols (fraction C) was an unidentified compound. The increased relative retention time of this unknown sterol on OV-17 compared to that on SE-30 suggested that it possessed a side chain olefinic bond.[9]

Fraction B was acetylated and analysed by TLC on silica gel impregnated with silver nitrate. After development with alcohol-free $CHCl_3$, three bands were observed corresponding to Δ^5 (band *a*), C-29 $\Delta^{5,22}$ (band *b*) and $\Delta^{24(28)}$ (band *c*) steryl acetates. GLC analysis (1·5% OV-17) showed that band *a* contained cholesteryl acetate (1·9%), campesteryl acetate (21·5%) and sitosteryl acetate (76·5%). Band *b* consisted of stigmasteryl acetate while band *c* contained the unknown steryl acetate (95%) and a small amount of 28-isofucosteryl acetate (< 5%). Saponification of this material gave the unknown sterol (II) which had an IR spectrum indicating a cyclopropane ring (3040 cm^{-1}) and in particular a side chain methylene group (1620 and 886 cm^{-1}) which is consistent with the behaviour of this compound upon GLC analysis on SE-30 and OV-17. The mass spectrum had a molecular ion at *m/e* 412 indicating a C_{29} sterol. A fragmentation ion at *m/e* 269 for loss of the side chain and water[10] revealed the presence of a C_9 side chain and an additional methyl group in the nucleus, probably located at C-14, since substitution at C-4 can be eliminated on the basis of TLC mobility. 9β,19-Cyclopropane sterols are characterized by MS fragmentation involving loss of a ring A.[11,12] In the MS of the new sterol an ion at *m/e* 300 can be assigned to this fragmentation and in addition to confirming the presence of a 9β,19-cyclopropane ring it established that the additional nuclear methyl group was located at C-14.



The MS and IR data are therefore in accord with a 14α-methyl-9β,19-cyclo-5α-ergostanol skeleton for the new sterol. These data, however, could not be used to differentiate between a 24-methylene or 25-methylene side chain. The location of the side chain methylene group

[8] F. F. KNAPP and H. J. NICHOLAS, *Phytochem.* **8**, 207 (1969).
[9] B. A. KNIGHTS and C. J. W. BROOKS, *Phytochem.* **8**, 463 (1969).
[10] P. BENVENISTE, P. HIRTH and G. OURISSON, *Phytochem.* **5**, 31, 45 (1966).
[11] H. AUDIER, R. BEUGELMANS and B. C. DAS, *Tetrahedron Letters* No. 36, 4341 (1966).
[12] R. T. APLIN and G. M. HORNBY, *J. Chem. Soc.* 1078 (1966).

was established by NMR spectroscopy. The NMR spectrum of the steryl acetate contained a signal at 5·35 τ integrating for the two protons of the methylene group. The absence of a methyl resonance at 8·4–8·5 τ eliminated the presence of a 25-methylene group[13,14] and therefore established that the sterol must be a 24-methylene compound. In addition, signals at 9·35 and 9·53 τ for the non-equivalent protons of the cyclopropane ring[15] further confirmed the proposed nuclear structure. The structure of this sterol is therefore established as 14α-methyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol (II).

TABLE 1. STEROL COMPOSITION OF THE FRUIT PEEL OF *Musa sapientum*

Sterol	R_t Relative to cholesterol 3% SE-30	1·5% OV-17	% Composition* Fraction A	B	C	Sterol	R_t Relative to cholesterol 3% SE-30	1·5% OV-17	% Composition* Fraction A	B	C
Cholesterol	1·00	1·00	0·54	0·34	0·55	Unknown sterol	1·46	1·64	1·00	74·50	59·10
Campesterol	1·30	1·33	11·10	3·85	6·19	Sitosterol	1·63	1·69	43·00	13·70	22·00
Stigmasterol	1·41	1·46	42·00	3·85	6·19	28-Isofucosterol	1·63	1·87	3·38	3·71	5·96

* Calculated from data from both SE-30 and OV-17 analyses.

DISCUSSION

The presence of a 14α-methyl group in a sterol which does not contain C-4 groups is relatively unique and pollinastanol[1] (I) and macdougallin[16] (14α-methylcholest-8(9)-en-3β,6β-diol) are two sterols which contain this structural feature. The present communication represents the first report of 24-methylenepollinastanol (II) in a higher plant. A recent report, however, has described the identification by GLC and MS of small amounts of this sterol and other 14α-methyl sterols in triparanol-treated cultures of *Chlorella emersonii*.[17,18]

The absence of any detectable amount of 24-methylenepollinastanol in the unesterified sterol fraction of banana peel, while it is the major component of the esterified sterol (74·5%) and tightly-bound sterols (59·1%) may be of some significance. The similarity between the composition of the esterified and tightly-bound sterol fraction perhaps suggests that the latter represents steryl ester tightly-bound within the tissue. The 24-methylenepollinastanol is presumably formed by C-4 demethylation of cycloeucalenol[17] (III) the major 4α-methyl sterol of banana tissue.[8]

The role of 24-methylenepollinastanol is obscure but it may perhaps act as a precursor of C-28 and C-29 sterols in this tissue as suggested for sterol formation in *Chlorella emersonni*.[17] Such transformations would entail opening of the cyclopropane ring, followed by double bond migration, C-32 demethylation and either 24-methylene reduction (C-28 sterols) or a second alkylation (C-29 sterols). All of these transformations are of course known to occur in plants.[19] Alternatively, the possibility exists that 24-methylene pollinastanol is not further metabolized, and represents an end product of sterol biosynthesis which for some reason accumulates in the steryl ester pool as the banana fruit matures.

[13] N. S. BHACCA, D. P. HALLIS, J. F. JOHNSON and E. A. PIER, in *Varian NMR Spectra Catalog*, National Press, New York (1963).
[14] F. F. KNAPP and H. J. NICHOLAS, *Steroids* **16**, 329 (1970).
[15] R. MCCRINDLE and C. DJERASSI, *Chem. Ind.* 1311 (1961).
[16] C. DJERASSI, J. G. KNIGHTS and D. I. WILKINSON, *J. Am. Chem. Soc.* **85**, 835 (1963).
[17] P. J. DOYLE, G. W. PATTERSON, S. R. DUTKY and M. J. THOMPSON, *Phytochem.* **11**, 1951 (1972).
[18] P. J. DOYLE, G. W. PATTERSON, S. R. DUTKY and C. F. COHEN, *Phytochem.* **10**, 2093 (1971).
[19] L. J. GOAD and T. W. GOODWIN, *Progress in Phytochemistry*, Vol. 3, p. 113, Academic Press, New York, (1972).

EXPERIMENTAL

TLC plates were spread with silica gel H (500 μ) and were developed in $CHCl_3$. For analytical purposes plates were sprayed with a 10% H_2SO_4 and heated briefly at 80° to develop the spot colour. Preparative plates were sprayed with a solution of Rhodamine 6G in acetone and bands visualized at 366 nm. GLC was performed using a Pye Model 104 instrument equipped with a flame ionization detector. Columns (152 × 0·64 cm) were packed with either 3% SE-30 or 1·5% OV-17 on 100/120 mesh Gas-Chrom Q (Applied Science Laboratories, Inc., State College, Pa.). Analyses were usually performed at 235° with carrier gas (Argon) flow rate of 60 ml/min. IR spectra were determined as either KBr discs or, in the case of semisolids, as a thin film. MS were determined with an AEI MS-12 instrument and NMR spectra were obtained with a Varian Model 60 spectrometer with tetramethylsilane as an internal standard in $CDCl_3$ solution.

Banana peel from fruit (*Musa sapientum*) purchased locally was dried thoroughly and the tissue (104 g) extracted 4 × with MeOH (1 l.) overnight. The residual tissue was then saponified with 12% KOH in 80% EtOH and the non-saponifiable lipid obtained in the usual manner (77 mg). From this material were obtained the tightly-bound sterols (fraction C, 3·4 mg). Combination of the MeOH extracts yielded 4·0 g of lipid. Sterols (fraction A) from an aliquot of this material (50 mg) were obtained by TLC for GLC analysis. The remainder of the MeOH extractable lipid was chromatographed on Brockman Grade III alumina. Esterified material and hydrocarbons were eluted with 2% ether in light petrol. (2·86 g). A portion of this material (1·43 g) was saponified to yield 934 mg of non-saponifiable material from which the sterol (fraction B) was purified by TLC. Acetylation of fraction B with pyridine–Ac_2O gave the steryl acetates (22 mg). Silica-gel–$AgNO_3$ TLC developed with alcohol-free $CHCl_3$[20] gave Δ^5-steryl acetates (band *a*, R_f 0·47, 1·8 mg), $\Delta^{5,22}$-steryl acetates (band *b*, R_f 0·40, 1·0 mg) and $\Delta^{24(28)}$-steryl acetates (band *c*, R_f 0·26, 6·7 mg). Band *c* was a semi-solid and could not be adequately crystallized from a variety of solvents, IR ν_{max}^{film} cm^{-1} 3050 (cyclopropane ring), 1720 (acetate carbonyl) and 1620 and 886 (=CH_2); MS, $C_{31}H_{50}O_2$, with ions at *m/e* 454 (M^+, 12%), 439 (M^+–CH_3, 7%), 411 (M^+-43, 3%), 394 (M^+-acetate, 100%), 379 (M^+–CH_3-acetate, 8%), 329 (5%), 300 (M^+-ring A; 5%), 296 (10%) and 269 (M^+-acetate-side chain, 10%); NMR (τ) 5·35 (2H, =CH_2), 8·02 (3H,–OCH_3) and 9·35 and 9·53 (2H, cyclopropane).

Saponification of this acetate (4·2 mg) yielded (II); needles from MeOH–H_2O (2 mg), m.p. 115–117°, IR ν_{max}^{KBr} cm^{-1} 3250 (–OH), 3040 (cyclopropane), 1640 and 892 (=CH_2); MS $C_{29}H_{48}O$, with ions at 412 (M^+, 79%), 397 (M^+–CH_3, 84%), 394 (M^+–H_2O, 100%) 379 (M^+–CH_3-H_2O, 68%), 300 (M^+-ring A, 57%), 287 (34%) and 269 (M^+-side chain–H_2O, 28%).

Acknowledgements—F.F.K. was the recipient of an NIH Postdoctoral Fellowship during the course of this work. We would also like to thank the S.R.C. for financial assistance.

[20] Z. A. WOJCIECHOWSKI, personal communication.