Phytochemistry, 1969, Vol. 8, pp. 207 to 214. Pergamon Press. Printed in England

THE STEROLS AND TRITERPENES OF BANANA PEEL

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(*Received* 21 *May* 1968)

Abstract—The following sterols and triterpenes have been identified in banana peel: β-sitosterol, stigmasterol, campesterol, cycloeucalenol, cycloartenol, and 24-methylene cycloartanol. 24-Methylene cycloartanol palmitate and an unidentified triterpene ketone were the major constituents. The ester represented approximately 30 per cent of the total extractable lipid.

INTRODUCTION

DURING the course of investigations concerned with phytosterol biosynthesis, it became of interest to examine the lipids of banana peel. Preliminary examination showed the tissue to contain a large amount of sterol, as had been suggested by previous communications.[1,2] No identification of the sterol components was made, however, by earlier workers. In examining the crude ethanol extract in more detail, our attention was directed toward an ester fraction which had limited solubility in the saponification mixture and was accordingly found in the non-saponifiable fraction. The major component of this fraction has been identified as 24-methylene cycloartanol palmitate, identical in physical and chemical behavior to a synthetic sample. Chemical reduction of this ester yielded a dihydro form with characteristics of a "liquid crystal". The probable presence of cycloeucalenol palmitate and cycloartenol palmitate has also been suggested by this study. The methylated sterols that have been found are of interest in relation to their proposed role as intermediates in phytosterol biosynthesis.[3,4] The complexity of the sterols and triterpenes of banana peel is indicated by this communication.

RESULTS

Non-saponifiable material (NS) obtained from dried banana peels by mild saponification (see "Experimental" section) was chromatographed on alumina (Merck, acid-washed). Material eluted with light petroleum consisted of a white wax, and contained triterpene esters along with a small amount of hydrocarbon.† This fraction represented approximately 30 per cent of the total NS material. Fractions were subsequently eluted with benzene, ethyl ether and ethanol. All fractions were monitored by thin-layer chromatography (TLC). The initial benzene fractions consisted of a thick, yellow oil, shown to be a complex mixture of

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† Esterified material was found in the NS fraction due to its insolubility in the saponification mixture, ethanol–water, 1:1 (v/v), containing 15% KOH.

[1] Annual Report, Tropical Products Institute, 56/62 Gray's Inn Road, London, W.C.1, England, p. 9 (1961).
[2] C. MOSS, *Analyst* **62**, 32 (1937).
[3] R. AEXEL, S. EVANS, M. KELLEY and H. J. NICHOLAS, *Phytochem.* **6**, 511 (1967).
[4] B. L. WILLIAMS, L. J. GOAD and T. W. GOODWIN, *Phytochem.* **6**, 1137 (1967).

sterol esters. Continued elution with benzene yielded an orange crystalline solid, containing primarily a single sterol ketone. The ethyl ether fractions contained the 4α-methyl and 4,4-dimethyl sterols, while the ethanol fractions contained the 4-desmethyl sterols. Throughout the text all TLC data refer to the S-1 solvent system unless otherwise specified (Table 1).

TABLE 1. THIN-LAYER CHROMATOGRAPHY SOLVENT SYSTEMS

Designation	Solvents	Solvent ratio (v/v)
S-1	Trimethyl pentane–ethyl acetate–acetic acid	40:20:0·4
S-2	Hexane–tetrahydronaphthalene	75:25
S-3	Acetonitrile–acetic acid (undecane impregnated reversed phase)	1:1
S-4	Acetonitrile–acetic acid (undecane impreganted reversed phase)	1:3

Identification of 24-Methylene Cycloartanol Palmitate

The light petroleum fractions were shown by TLC to contain a component that moved with the solvent front. In the S-2 solvent several minor spots were observed, the major component having an R_f of 0·40. Repeated crystallization of this material from acetone, ethyl acetate–ethanol and light petroleum–ethanol gave white granules, m.p. 54–56°; $[\alpha]_D^{26}+38{\cdot}2°$. The i.r. spectrum had major bands at 1732, 1640, 980 and 882 cm^{-1}, indicating an ester with strong methylene absorbance. This was confirmed by the NMR spectrum which indicated vinylic protons at τ 5·34 (CCl_4). Ozonolysis of the ester gave a volatile product identified as formaldehyde by its dimedone derivative. A dihydro derivative was prepared by reduction with H_2 in the presence of PtO_2 in ethyl acetate. Crystallization of the product to constant melting point from acetone gave white granules, m.p. 60–61°; $[\alpha]_D^{26}+37{\cdot}1°$. The vinyl bands were not found in the i.r. spectrum of this compound. The reduced product gave a transient and iridescent violet color when melted, a phenomenon indicative of a cholesteric phase.[5] This finding has been discussed in detail elsewhere.[6] The color phenomenon was reproducible in all preparations of the compound and reversible upon heating and cooling. After treatment in this manner no decomposition products were detected by TLC. A hydrochloride was also prepared from the ester by treatment of the parent compound with dry HCl gas in glacial acetic acid. Crystallization of the product from acetone gave small scales, m.p. 78–80°; $[\alpha]_D^{20}+42{\cdot}8°$. (Calcd. for $C_{47}H_{83}O_2Cl$: Cl, 4·95. Found: Cl, 4·15 per cent.) This derivative exhibited no properties associated with a cholesteric phase.

The ester was cleaved by either basic hydrolysis or lithium aluminium hydride reduction. In the first instance, hydrolysis was achieved by 2 hr reflux in ethanol:benzene:water (80:10:10, v/v/v) containing 15% KOH. This saponification mixture was required for maximal solubility of the esterified material. The non-saponifiable fraction was obtained in the usual manner. TLC indicated the presence of a single component with an R_f of 0·62 (4,4-dimethyl sterol region). Repeated crystallization of this material from acetone gave fine needles, m.p. 116–118°; $[\alpha]_D^{20}+45{\cdot}9°$. The i.r. spectrum had bands at 3600, 1639, 988, 887 and a definite shoulder at 3050 cm^{-1}, indicating a sterol containing a cyclopropane ring and a

[5] G. W. GRAY, *Molecular Structure and the Properties of Liquid Crystals*, p. 45, Academic Press, New York (1962).
[6] *J. Org. Chem.*, in press.

terminal methylene group. The NMR spectrum confirmed the presence of vinylic protons at τ 5·34. An acetate was prepared by reflux in acetic anhydride–pyridine. Crystallization of the product from acetone gave fine needles, m.p. 116° (R_f 0·90). The 3-keto derivative was obtained by CrO_3 oxidation in glacial acetic acid at room temperature. Crystallization from acetone gave needles, m.p. 116–117°; $[\alpha]_D^{20}$ +14·6°(R_f 0·94). These data are consistent with the identity of this 4,4-dimethyl sterol as 24-methylene cycloartanol.[7] Gas–liquid chromatographic analysis (GLC) indicated a single component with a retention time identical to that of the authentic compound (Table 2).

TABLE 2. RELATIVE RETENTION TIMES OF THE STEROLS AND TRITERPENES OF BANANA PEEL

	Relative retention time*		
	QF-1 (3%)	SE-30 (1%)	XE-60 (1%)
Cycloeucalenol	4·50	3·35	6·47
Cycloartenol	4·50	3·61	7·06
24-Methylene cycloartanol	5·42	4·02	7·77
Unknown 4α-methyl sterol ketone	7·42	—	8·18
Campesterol	3·75	2·52	—
β-Sitosterol	3·75	3·05	6·30
Stigmasterol	3·17	2·73	5·41
Cholestane (absolute retention time, min)	1·41	6·59	2·00

* Retention times relative to cholestane.

Combined gas–liquid chromatography–mass spectroscopy (GLC–MS) of this substance indicated a parent ion at *m/e* 440 with other peaks at *m/e* 425 (M-CH_3); 422 (M-HOH); 407 (M-CH_3-HOH); 379; 353; 313; 300 (M-ring A) and 297 (M-side chain-HOH). The spectrum was similar to that obtained by other workers for 24-methylene cycloartanol.[4, 8]

The aqueous phase of the saponification mixture was acidified with HCl and extracted with light petroleum. The petroleum layer was washed with water and distilled to dryness. The residue was crystallized from acetone to give flakes, m.p. 62–64°; no rotation in chloroform. This substance co-chromatographed with palmitic acid (R_f 0·58) in solvent S-3 (Table 1), a system which separates homologous fatty acids. The methyl ester was prepared by treatment with boron trifluoride–methanol. This substance had the same retention time as methyl palmitate on several different GLC columns. It may be significant that palmitic acid was the only acid found.

Alternatively, two spots were indicated by TLC of the lithium aluminum hydride reduction product (R_f 0·62 and 0·54). The former was identified as 24-methylene cycloartanol as described previously. The second component had an R_f identical to that of palmityl (cetyl) alcohol in solvent S-4 (R_f 0·60). The fatty alcohol was separated from the 4,4-dimethyl sterol by alumina column chromatography. The solvent was benzene containing increasing concentrations of diethyl ether. The sterol was eluted in fractions 44 through 46 (5% diethyl ether/benzene) while the fatty alcohol was eluted in fractions 65 through 69 (15% diethyl ether/benzene). The fractions containing the alcohol were pooled and crystallized from

[7] G. OHTA and M. SHIMIZU, *Chem. Pharm. Bull. (Tokyo)* **5**, 40 (1957).
[8] H. E. AUDIER, R. BEUGELMANS and B. C. DAS, *Tetrahedron Letters* **36**, 4341 (1966).

acetone to give pearly flakes, m.p. 56–58°; no rotation in chloroform. The i.r. spectrum was comparable to that of cetyl alcohol. The phenylurethan derivative, prepared by treatment with phenyl isocyanate in pyridine, crystallized as needles from ethanol, m.p. 78–79°. There was no depression upon admixture with the phenylurethan derivative of authentic cetyl alcohol. The i.r. spectra of these derivatives were essentially superimposable. Both alcohols also had identical retention times on an SE-30 (1%) column, a system that readily separates homologous fatty alcohols.

Synthesis of 24-Methylene Cycloartanol Palmitate

Palmitoyl chloride was refluxed for 2 hr with 24-methylene cycloartanol in benzene containing a trace of pyridine.[9] The product crystallized as white granules from acetone, m.p. 56–58°; $[\alpha]_D^{26} + 37{\cdot}5°$. The i.r. spectrum was superimposable on that of the naturally occurring ester. Both also co-chromatographed when subjected to TLC in several different solvent systems. The dihydro form of the synthetic ester was prepared in the same manner as described earlier. Crystallization from acetone gave an amorphous white solid, m.p. 63–64°; $[\alpha]_D^{26} + 35{\cdot}1°$. A deep violet color was observed upon melting this substance. The presence of an impurity to which this cholesteric behavior could be attributed would seem extremely unlikely from these results.

Identification of Cycloeucalenol and Cycloartenol

The mother liquors obtained from the crystallization of the naturally occurring 24-methylene cycloartanol palmitate were combined and saponified (ethanol: benzene: water, 80:10:10, v/v/v). The NS fraction gave two spots upon TLC analysis. One spot occurred in the 4,4-dimethyl sterol region, the other in the region of the 4α-methyl sterols (R_f 0·62 and 0·54, respectively). GLC on an SE-30 (1%) column showed three components with relative retention times (RRT) to cholestane of 3·35, 3·61 and 4·02. Preparative TLC was carried out and the two regions obtained analyzed by GLC in the same manner. The more polar material gave a single peak corresponding to cycloeucalenol (RRT 3·35) while the second region gave two peaks with retention times corresponding to cycloartenol and 24-methylene cycloartanol (RRT 3·61 and 4·02, respectively) (Table 2). There was no evidence for the presence of 24-methylene lophenol, 24-ethylidene lophenol (citrostadienol) or lanosterol.

A portion of the total NS material was subjected to GLC–MS. The mass spectrum of Peak 1 had a parent ion at *m/e* 426 with other peaks at *m/e* 411 (M-CH_3); 408 (M-HOH); 393 (M-CH_3-HOH); 365; 353; 343; 300 and 283 (M-side chain-HOH). This spectrum is similar to that obtained for cycloeucalenol by other workers.[8] Peak 2 exhibited a parent ion at *m/e* 426 with peaks at *m/e* 411 (M-CH_3); 408 (M-HOH); 393 (M-CH_3-HOH); 365; 339; 297 (M-side chain-HOH) and 286. This spectrum is the same as that obtained for an authentic sample of cycloartenol and to that described by other workers.[4, 8] The third peak had a spectrum identical to that described earlier for 24 -methylene cycloartanol. Palmitic acid was again the only component present in the saponifiable fraction. It seems probable, therefore, that these sterols were also present in the peel as palmitate esters.

The initial benzene fractions from the alumina chromatography of banana peel NS consisted of a thick oil. TLC in solvent S-2 indicated several components to be present. An additional passage through an alumina column and repeated attempts at crystallization were

[9] A. KUKSIS and J. M. R. BEVERIDGE, *J. Org. Chem.* **25**, 1209 (1959).

unsuccessful in yielding a crystalline product. This material was saponified in the same system used for saponification of the previous ester fraction. The NS fraction was shown by TLC and GLC to contain the same three sterol components as before. The acidic fraction was methylated by treatment with boron trifluoride–methanol and subjected to GLC analysis. Several recomponents were indicated. This material was not further investigated.

Triterpene Ketone Fraction

The orange crystalline material removed from the original column with benzene was shown by TLC to contain one major component (R_f 0·88). These fractions were accordingly combined and crystallized from acetone or ethanol to give needles, m.p. 133°; $[\alpha]_D^{20}+49{\cdot}0°$. The i.r. spectrum of this material had bands at 1700, 1640, 890 and a shoulder at 3020 cm^{-1}, indicating the presence of a ketonic carbonyl, a methylene group and a cyclopropane ring. This substance was pure as was indicated by GLC (Table 2). The mass spectrum exhibited a parent ion at *m/e* 424 with other peaks at *m/e* 409 (M-CH_3); 381; 367; 354; 328 and 298.

The corresponding alcohol was obtained by lithium aluminum hydride reduction of this substance in tetrahydrofuran. Recrystallization of the product from acetone yielded fine needles, m.p. 140°; $[\alpha]_D^{26}+40{\cdot}8°$. TLC indicated a major component in the 4α-methyl sterol region (R_f 0·54). A minor component (R_f 0·63) was assumed to represent the 3α isomer usually obtained in small yields from such reductions.[10] An acetate, prepared in the usual manner, was crystallized from acetone to yield needles, m.p. 110°; $[\alpha]_D^{26}+54{\cdot}7°$. The mass spectrum of the free alcohol had a parent ion at *m/e* 426. The fragmentation pattern was very similar to that obtained from cycloeucalenol. GLC also indicated a single component with the same retention time as cycloeucalenol. The melting point of cycloeucalenone, however, has been reported by several workers as being 84°.[11,12] Since an authentic sample was not available to us for comparison we cannot at this time conclude this compound to be identical to cycloeucalenone.

Identification of Unesterified 4α-Methyl and 4,4-Dimethyl Sterols

Free methylated sterols were eluted from the column with ethyl ether. Only a small amount of 4-desmethyl sterol was eluted with this material. TLC indicated two major spots, one in the region of the 4α-methyl sterols the other in the region of the 4,4-dimethyl sterols (R_f 0·54 and 0·62, respectively). This material was crystallized several times from acetone to remove the organe coloring material. Only three components were indicated by GLC. These were identified as cycloeucalenol, cycloartenol and 24-methylene cycloartanol.

Identification of Campesterol, Stigmasterol and β-Sitosterol

TLC of the material eluted from the original alumina column with ethanol showed a large amount of 4-desmethyl sterol (R_f 0·47). Three peaks were indicated by GLC on SE-30 (1%) with relative retention times to cholestane of 2·52, 2·73 and 3·05. These were identified as campesterol (2%), stigmasterol (92%) and β-sitosterol (6%), respectively. The mixture was acetylated in the usual manner. Crystallization from acetone or ethanol yielded flakes, m.p. 137–140°; $[\alpha]_D^{20}-49{\cdot}0°$. A portion of this material was saponified to give a substance that crystallized from ethanol as flakes, m.p. 170–175°; $[\alpha]_D^{20}-36{\cdot}5°$. Another portion of the acetate was reduced with H_2 in the presence of PtO_2 in glacial acetic acid. Crystallization of

[10] L. F. FIESER and M. FIESER, *Steroids*, p. 269, Reinhold, New York (1959).
[11] J. S. G. COX, F. E. KING and T. J. KING, *J. Chem. Soc.* 1384 (1956).
[12] L. AMOROS-MARIN, W. I. TORRES and C. F. ASENJO, *J. Org. Chem.* **24**, 411 (1959).

the product from ethanol gave fine needles, m.p. 135–136°; $[\alpha]_D^{20}+24{\cdot}2°$. The remaining acetate was brominated in glacial acetic acid with Br_2. After standing overnight at room temperature the insoluble bromo acetate was filtered off and crystallized from chloroform–methanol to give very fine needles, m.p. 200–204° (dec.). Literature values are as follows: stigmasterol acetate, m.p. 141°; $[\alpha]_D-49{\cdot}5°$: stigmastanol acetate, m.p. 137°; $[\alpha]_D+15{\cdot}4°$; stigmasterol, m.p. 170–171°; $[\alpha]_D-45{\cdot}0°$ (Et_2O): stigmasterol acetate tetrabromide, m.p. 204° (dec.).[13]

A portion of the crude 4-desmethyl sterol fraction was analyzed by GLC–MS. Peak 1 had a parent ion at *m/e* 400 with other peaks at *m/e* 385 (M-CH_3); 382 (M-HOH); 367 (M-CH_3-HOH); 315; 289; 273 (M-side chain); 261; 231; 229 and 213. This spectrum is comparable to that reported by other workers for campesterol.[14] The second peak exhibited a parent ion at *m/e* 412 with peaks at *m/e* 397 (M-CH_3); 394 (M-HOH); 369; 351; 300; 271 (M-side chain-2H); 255 (M-side chain-HOH); 231; 229 and 213. This spectrum is the same as that obtained for stigmasterol.[14] The third peak had a parent ion at *m/e* 414 with other fragmentation peaks at *m/e* 399 (M-CH_3); 396 (M-HOH); 381 (M-CH_3-HOH); 329; 303; 275; 273 (M-side chain); 255 (M-side chain-HOH) 246; 231; 229 and 213. This spectrum is the same as that for β-sitosterol.[14]

DISCUSSION

The identification of cycloeucalenol, cycloartenol and 24-methylene cycloartanol together with the apparent absence of lanosterol would seem to support the proposed pathway of phytosterol biosynthesis.[3,4] It should be noted, however, that neither 24-methylene lophenol nor 24-ethylidene lophenol (citrostadienol) was found in the present study. These compounds may have been present in small amounts not detectable by the methods employed.

This study represents the first reported identification of 24-methylene cycloartanol palmitate and may be important with respect to the possible role of esterified intermediates in phytosterol biosynthesis. The possibility that formation of these compounds proceeds by way of such intermediates has been discussed elsewhere.[15] Studies of the sterol composition of various plants have shown these substances often exist in the esterified form. These tissues include birchwood,[16] grapefruit peel,[4] maize[17] and peas.[17] No identification of the acyl portions of these esters was made, however, in this earlier work. The present investigation has illustrated that in banana peel the majority of the methylated sterols are present as palmitate esters. Several studies have shown that label from 2-^{14}C-mevalonate is rapidly incorporated into phytosterol esters.[18] Preliminary studies with banana peel have given similar results.[19]

With respect to sterol biosynthesis, Lindenberg and co-workers have presented evidence indicating that during cholesterol biosynthesis a 3-ketone may be an obligatory intermediate.[20] Such an intermediate could presumably be formed at the step involving removal of the C4 methyl groups. A similar type of intermediate may be involved in β-sitosterol bio-

[13] *Elsevier's Encyclopedia of Organic Chemistry* (edited by F. RADT), Vol. 14, Elsevier, New York (1954).
[14] B. A. KNIGHTS, *J. Gas Chromatog.* 273 (1967).
[15] L. J. GOAD, *Terpenoids in Plants* (edited by J. B. PRIDHAM), p. 185, Phytochemical Group Symposium, Academic Press, New York (1967).
[16] J. BERGMAN, B. O. LINDGREN and C. M. SVAHN, *Acta. Chem. Scand.* **19**, 1661 (1965).
[17] R. J. KEMP, S. A. HAMMAM, L. J. GOAD and T. W. GOODWIN, *Phytochem.* **7**, 447 (1968).
[18] D. R. THRELFALL, W. T. GRIFFITHS and T. W. GOODWIN, *Biochem. J.* **92**, 56P (1964).
[19] F. F. KNAPP and H. J. NICHOLAS, unpublished observations.
[20] M. LINDENBERG, F. GAUTSCHI and K. BLOCH, *J. Biol. Chem.* **238**, 1661 (1963).

synthesis in peas.[21] The isolation of cycloartenone[22] and results presented here for the presence of a 4α-methyl sterol ketone similar to cycloeucalenone may lend credence to this proposal.

A more recent report by Goodwin[17] has shown that label from 2-^{14}C-mevalonate is rapidly incorporated into both free and esterified sterols in maize and peas. What these findings mean with respect to phytosterol biosynthesis is not known. The identification of the esters present in banana peel make this tissue an excellent system in which to study the turnover of these substances. Studies are now in progress in an effort to explain the significance of esterified or ketonic intermediates in phytosterol biosynthesis.

EXPERIMENTAL

Solvents and Chemicals

All solvents were A.R. grade, distilled before use, except the ethanol used in the initial extraction. Anhydrous benzene was prepared by standing over Na prior to distillation. Light petroleum (b.p. 30–60°) was allowed to stand over conc. H_2SO_4 before distillation. Pyridine was distilled over BaO. The palmitoyl chloride was purchased from Fischer Scientific (95 per cent pure).

Preparation of Non-Saponifiable Material (NS)

The dried peels (4·5 kg) from 227 kg of locally purchased bananas were finely ground and extracted exhaustively with hot ethanol. The extract was concentrated to small volume and saponified in aqueous ethanol (1:1, v/v) containing 15% KOH. The saponified mixture was extracted thoroughly with diethyl ether. The ether phase was then washed with water and distilled. The residue thus obtained (360 g) represented the non-saponifiable (NS) fraction.

Column Chromatography

In a typical preparation, 150 g of banana peel NS material was chromatographed on 1800 g of alumina (Merck, acid-washed) on a 6 cm dia. column. The large ester fraction was eluted with light petroleum (fractions 18 through 69). The initial benzene fractions consisted of a thick oil and were shown to contain a complex mixture of sterol esters. Continued elution with benzene removed the triterpene ketone (fractions 103 through 119). Free triterpene alcohols were eluted with ethyl ether. The final ethanol wash removed the 4-desmethyl sterols. The waxy ester material removed with light petroleum was rechromatographed on a 2 cm dia. column containing 400 g of alumina. Elution was performed with light petroleum, 24-methylene cycloartanol palmitate being removed from the column in fractions 30 through 34. The separation of 24-methylene cycloartanol from palmityl alcohol was performed on the same type of column, with increasing concentrations of diethyl ether in benzene as solvent.

Thin-Layer Chromatography (TLC)

Silica gel G spread 250 μ thick on 20 × 20 cm glass plates was activated immediately before use by heating. For preparative purposes, Silica gel H 500 μ thick was used. Long-chain fatty alcohols were stained using a phosphomolybdic acid spray (10% in ethanol). Other plates were stained using a variety of reagents including anisaldehyde (5% in 95% ethanol–H_2SO_4, 19:1, v/v), H_2SO_4 (50% in water) and $SbCl_5$ (20% in $CHCl_3$). Colors were developed by heating for a short period at 150°. Several solvent systems were used (Table 1).

Gas–Liquid Chromatography (GLC)

A Barber–Colman Model 5000 Gas Chromatograph was used in all cases except for the combined GLC–mass spectral analyses. This model uses a H_2 flame detector. 180-cm glass columns were used containing either QF-1 (3%), SE-30 (1%) or SE-60 (1%), on Gas Chrom Q, 100/120 mesh (Applied Science Laboratories, Inc.). The column temperature in most cases was 240°. Fatty acid methyl esters and fatty alcohols were run at a column temperature of 130°. The carrier gas was argon, with a flow rate of 55 ml/min, inlet pressure 24 psi. Samples were injected in benzene solution using a Hamilton Syringe with a Chaney adaptor.

Melting Points

Melting points were determined on a Fischer-Johns hot plate and are uncorrected.

[21] H. H. Rees, E. I. Mercer and T. W. Goodwin, *Biochem. J.* **99**, 726 (1966).
[22] C. Djerassi and R. McCrindle, *J. Chem. Soc.* 4034 (1962).

Optical Rotations

Optical measurements were made in $CHCl_3$ in a 5 cm cell using a Model 200-S Rudolph photoelectric polarimeter.

Infra-Red Spectroscopy

I.r. spectra were recorded in $CHCl_3$ using a Model 21 Perkin-Elmer double-beam spectrophotometer.

Combined Gas Chromatography–Mass Spectroscopy (GLC–MS)

Mass spectra were determined using a LKB Model 9000 single focusing gas chromatograph–mass spectrometer. An OV-1 (3%) column was used in all cases at a temperature of 255°. The mass spectra were obtained under the following conditions: flash heater, 240°; molecular separator, 258°; ion source, 260°; ionizing current, 60 μA; ionizing energy, 70 eV.

Acknowledgements—We should like to thank Dr. W. H. Elliott (St. Louis University School of Medicine, St. Louis, Missouri) for aid in interpreting the mass spectra and Mr. H. Robinson for technical assistance. We are grateful to the following individuals for gifts of reference compounds: Dr. K. Schreiber (24-methylene lophenol and 24-ethylidene lophenol), Dr. G. Ourisson (cycloartenol, cycloeucalenol and 24-methylene cycloartanol) and Dr. A. Kuksis (campesterol). This work was supported by Grants from the National Science Foundation (NSF GB-4673) and the National Institutes of Health (NIH AM-09992).

Note added in proof—After this manuscript was accepted for publication the authors became aware of the paper "Banana peel wax" by K. V. RAO and S. D. RAO, *Indian J. Appl. Chem.* **28**. 210 (1965). This paper described the composition of sterols and esters in banana peel but mentions no specific compounds.