

SHORT COMMUNICATION

THE DISTRIBUTION OF STEROLS AND STERYL ESTERS IN THE BANANA PLANT

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Abstract—The sterol and steryl ester compositions of the stalk, leaves and rhizome of the banana plant are very similar, although the relative amounts vary between tissues. Cycloeucalenol, cycloartenol and 24-methylenecycloartanol are found in the free form and in the esterified form, primarily as esters of palmitic acid. Stigmasterol, campesterol and β -sitosterol are also present, primarily in the free form.

Plant. Banana plants, cultivars of *Musa sapientum* L., were used.

Source. The plant material was generously supplied by the Missouri (Shaw's) Botanical Gardens, St. Louis, Missouri.

Previous work. The sterol composition of banana peel¹ and pulp² has been reported. The major 4,4-dimethyl sterols of these tissues are cycloartenol and 24-methylenecycloartanol. The sole 4 α -methyl sterol identified is cycloeucalenol, while an unidentified 4 α -methyl sterol ketone has been found in large amounts in banana peel and in lesser amounts in banana pulp. The 4-des methyl sterols consist of stigmasterol, campesterol and β -sitosterol. The steryl ester composition of both tissues consists primarily of the palmitic acid esters of cycloeucalenol, cycloartenol and 24-methylene cycloartanol. In contrast to the steryl ester composition of some other plants that have been recently investigated,^{3,4} very little 4-des methyl sterol is esterified in banana peel and pulp. Other workers have reported a 3 β -OH- Δ^5 sterol in banana leaves, presumably β -sitosterol.⁵

Identification of sterols. The dried plant material of the different organs was shredded and extracted exhaustively with hot ethanol. The extracts were concentrated to low volume, dissolved in ether and washed with 5% KOH. The neutral fractions obtained by this procedure consisted in most cases of an orange wax. In the case of the rhizome it was a red oil. The lipid fractions were separated by preparative TLC. Four areas were scraped from the plates and eluted with CHCl₃-MeOH (1:1, v/v) as follows: Band A, R_f 0.46 (4-des methyl sterols); Band B, R_f 0.54 (4 α -methyl sterols) and Band C R_f 0.62 (4,4-dimethyl sterols), eluted together as one zone; Band D, R_f 0.82 (4 α -methyl sterol ketone); and the solvent front

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¹ F. F. KNAPP and H. J. NICHOLAS, *Phytochem.* **8**, 207 (1969).

² F. F. KNAPP and H. J. NICHOLAS, *J. Food Sci.*, in press.

³ R. J. KEMP and E. I. MERCER, *Biochem. J.* **110**, 111 (1968).

⁴ R. J. KEMP and E. I. MERCER, *Biochem. J.* **110**, 119 (1968).

⁵ V. CARELLI, P. MARCHINI and A. TUCCI, *Ann. Chim. (Rome)* **45**, 1133 (1955), (*Chem. Abs.* 3925e, 1957).

(predominantly steryl esters). These eluates were then analyzed by gas-liquid chromatography (GLC). Material at the solvent front was divided into two portions. The first of these was analyzed by GLC. The remainder of these steryl esters were saponified with ethanolic KOH and the sterols in the neutral fractions then analyzed by GLC. The acidic fractions from these saponified samples contained primarily palmitic acid and were not analyzed in detail.

Results. The results of the GLC analyses are summarized in Table 1. The unidentified 4 α -methyl sterol ketone was a major component of the stalk, rhizome and peel, but was a minor component of the pulp and leaves. As is indicated in Table 1, the steryl ester fractions

TABLE 1. GLC ANALYSES OF THE FREE AND ESTERIFIED STEROLS OF THE BANANA PLANT*

	Per cent ethanol-extractable lipid per dry weight†				
	Peel 7	Pulp 0.003	Stalk 0.9	Rhizome 0.6	Leaves ...
4-Des methyl sterols					
Campesterol	2	12	14	16	12
Stigmasterol	92	16	40	54	35
β -Sitosterol	6	72	46	30	53
Methylated sterols					
Cycloeucalenol	53	60	27	32	40
Cycloartenol	13	12	14	41	...
24-Methylenecycloartanol	34	28	59	27	...
Unidentified material	21 (0.9)‡ 13 (1.1) 26 (1.8)
Esterified sterols					
Cycloeucalenol	42	45	...	17	...
Cycloartenol	17	24	...	63	...
24-Methylenecycloartanol	40	31	...	20	...
Stigmasterol	36
β -Sitosterol	1	1
Unidentified material	64 (1.8)

* Numbers refer to the per cent of each component in that sterol class; ... = not detected.

† Calculated on a wet weight basis for banana pulp.

‡ Numbers in parentheses refer to retention times relative to cholestane (RT 3.8 min).

consisted mainly of the palmitic acid esters of cycloeucalenol, cycloartenol and 24-methylene cycloartanol. This was determined by GLC analyses of the ester fractions as well as analyses of the sterol fractions obtained by saponification of these esters. The ester fraction from the stalk was an exception, containing primarily stigmasterol and an unidentified component. In particular, there was essentially no esterified sterol in banana leaves, while a large amount of hydrocarbon was present. The sterol and steryl ester composition was very similar in most cases, although the relative amounts differed markedly. In general, campesterol was the minor component of the 4-des methyl sterol fraction while cycloartenol was the minor constituent of the methylated sterols. These results constitute one of the first complete studies of the sterol and steryl ester composition of all parts of the same plant.

EXPERIMENTAL

General procedures were performed as previously described.^{1,2} The TLC solvent was trimethylpentane-ethyl acetate-acetic acid (40:20:0.4, v/v/v). Steryl esters were saponified in ethanol-benzene-water (80:10:10, v/v/v) containing 15% KOH. For the GLC analyses, a Barber-Colman model 5000 instrument was used. Free sterols were separated on an SE-30 (3%) column at 250° under the conditions previously described.^{1,2} Sterols had the following retention times relative to cholestane (RT 3.8 min): cycloeucaleanol, 2.84; cycloartenol, 3.16; 24-methylenecycloartanol, 3.48; stigmasterol, 2.42; campesterol, 2.23; β -sitosterol, 2.71. Steryl esters were analyzed by modifications of the methods of Kuksis and Swell.⁶⁻⁹ Glass columns, 600 \times 4 mm, were packed with Gas Chrom Q (100/120 mesh) coated with SE-30 (3%). The column was conditioned for 72 hr at 320° (thermal stripping) with the carrier gas (N₂) flowing at 100 cm³/min. Steryl esters were analyzed at a column temperature of 290° and a carrier gas flow rate of 100 cm³/min. Palmitic acid esters had the following absolute retention times: cycloeucaleanol palmitate, 7.0 min; cycloartenol palmitate, 7.5 min; 24-methylene cycloartanol palmitate, 8.1 min.

⁶ A. KUKSIS and M. J. MCCARTHUR, *Can. J. Biochem.* **40**, 679 (1962).

⁷ A. KUKSIS, *Can. J. Biochem.* **42**, 407 (1964).

⁸ A. KUKSIS, *Can. J. Biochem.* **42**, 419 (1964).

⁹ L. SWELL, *Soc. Exp. Biol. Med. Proc.* **121**, 1290 (1966).