Sterol Lipids in Finger Millet *(Eleusine coracana)*

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ABSTRACT

Neutral lipids, glycolipids, and phospholipids (1.3%, 0.25%, and 0.10% of seed weight) were isolated from the total lipids (chloroform-methanol) of finger millet seeds (*Eleusine coracana*), and four sterol-containing lipids further isolated from neutral and glycolipids by preparative column and thin layer chromatography. On seed weight, these comprised: free sterols (S) 0.091%, sterol esters (SE) 0.013%, sterol glycosides (SG) 0.025%, acyl sterol glycosides (ASG) 0.020%, and total 0.149%. The major fatty acids, totaling 85-90%, were the same in both esterified sterols, but the proportions varied: 16:0, 18:1, and 18:2 comprising 24, 49, and 17% in SE (calculated iodine value 75) and 43, 36, and 7% in ASG (calculated iodine value 46). All four sterol lipids contained 80-84% of β-sitosterol, the remainder being stigmasterol. The only sugar in SG and ASG was D-glucose. It is deduced that the major representative species are: SE, β-sitosterol oleate/palmitate; SG, β-D-glucopyranosyl-(1 → 3)-β-sitosterol; and ASG, 6-0-palmitoyl-β-D-glucopyranosyl-(1 → 3)-β-sitosterol.

INTRODUCTION

Sterol lipids are known to be widely distributed in the plant kingdom. Earlier studies on the potato tuber, soybean, rice grain, alfalfa leaves, and pea seeds (1-5) have shown that sterols can be present in four forms: as free sterols (S), sterol esters (SE), sterol glycosides (SG), and acyl sterol glycosides (ASG). The present paper describes the isolation and chemical constituents of the sterol compounds present in finger millet seeds, an important food material in several regions in India.

EXPERIMENTAL PROCEDURES

Materials

Finger millet (variety Indaf I, 1976 crop) seeds were obtained from the University of Agricultural Sciences, Hebbal, India. Reagents and chemicals used were of analytical grade. Standard fatty acid methyl esters, β-sitosterol, stigmasterol, and boron trifluoride in methanol (14%) were obtained from Sigma Chemicals, St. Louis, MO, and standard cholesterol palmitate and silicic acid from the V.P. Chest Institute, New Delhi. Silica Gel G was from S.M. Chemicals, Baroda. Standard sterol glycoside and acyl sterol glycosides were prepared in purified form from pea seeds (5).

Extraction and Fractionation of Lipids

Millet seeds were ground in a mill and extracted three times with four volumes of chloroform-methanol (2:1, v/v) (6). Following Wuthier (7), the extract was concentrated and purified on a Sephadex G-25 column (particle size 100-300 μg), and the lipid further resolved into neutral lipid (1.3%), glycolipid (0.25%), and phospholipid (0.10%) on a silicic acid column by sequential elution with chloroform, acetone, and methanol (8). Recovery amounted to 85 to 90% of the weight of lipid applied to the column.

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Isolation of Sterol Lipids

The crude neutral lipid fraction in chloroform was subjected to silicic acid column chromatography. After removing hydrocarbons by hexane elution, sterol esters were eluted with 15% benzene in hexane (9). The crude sterol ester fraction was further purified on preparative thin layer chromatograms of Silica Gel G (1-mm) using a solvent system of petroleum ether-diethyl ether-acetic acid (80:20:1, v/v).

The crude neutral lipid was saponified with 0.3 N methanolic sodium hydroxide. Nonsaponifiables were extracted with three to four portions of light petroleum and concentrated in a rotary flash evaporator. Preparative thin layer chromatography, using the same solvent system as for sterol esters, yielded sterols (7% of the neutral lipid fraction) and sterol esters (1%).

The crude glycolipid was subjected to silicic acid column chromatography using stepwise elution with chloroform-acetone mixtures in different proportions as described earlier (10,11). The 9:1 and 7:3 chloroform-acetone eluates contained ASG and SG, respectively, slightly contaminated with glycerolipids and cerebrosides. Both fractions were rechromatographed repeatedly and finally purified on preparative thin layer chromatograms using chloroform-methanol (95:12). SG and ASG thus isolated in pure form amounted to 10% and 8% of the glycolipid, respectively.

Infrared Spectrophotometry

Infrared spectral characteristics of the isolated sterol compounds were obtained on a Hilger Watts Infragraph (1 mg sterol as 75 mg potassium bromide pellets).

Degradation of Sterol Lipids

Sterol ester was reacted with 0.4 N potassium hydroxide in methanol for 4 hr at 37 C. The mixture was cooled and added to chloroform and water, where the sterol components passed into the chloroform layer. The aqueous methanol layer was acidified to pH 2 with 6 N hydrochloric acid, fatty acids extracted, isolated, and methylated with 14% boron trifluoride in methanol. Methyl esters were extracted with three to four portions of petroleum ether (40-60 C), concentrated under a stream of nitrogen, and stored at -20 C until analyzed by gas chromatography.

Sterol glycoside was refluxed with 5% methanolic HCl for 6 hr at 100 C, cooled, and the sterols extracted with hexane. The residual methanol layer was passed through a column of Amberlite IR-4B (OH^-) to obtain pure methyl glycoside.

TABLE I

Fatty Acid Composition of Sterol Ester (SE) and Acyl Sterol Glycoside (ASG) (% wt)

Fatty acid	SE	ASG
12:0	3.0	3.0
14:0	2.5	4.6
16:0	23.8	42.7
18:0	4.8	6.4
18:1	48.8	36.0
18:2	17.1	7.3
18:3	Trace[a]	Trace[a]
Calculated iodine value of acids	75.0	45.7

[a]Trace, below 0.5%.

TABLE II

Sterol Composition in Various Sterol Lipids
(% wt)

Sterols	Relative GLC retention time	Free sterols	Sterol esters	Sterol glycosides	Acyl sterol glycosides
β-Sitosterol	100	84.2	82.4	83.0	80.6
Stigmasterol	78	15.8	17.6	17.0	19.4

ASG was saponified with 0.4 N potassium hydroxide, and divided between chloroform and aqueous methanol fractions. From the latter, the fatty acid methyl esters were prepared as for sterol esters. The chloroform fraction yielded sterol and methyl glycosides.

Paper Chromatography

Methyl glycosides (syrup) prepared from ASG and SG were subjected to descending paper chromatography on Whatman No. 1 filter paper using n-butanol-pyridine-water (6:4:3) for 18 hr at room temperature. Spots were located with silver nitrate-sodium hydroxide reagent.

Gas Chromatography

Fatty acid methyl esters were analyzed by gas chromatography using a Varian Aerograph 1400 (flame ionization detector, nitrogen, 8 ft x 1/8 in. column with 15% (w/w) DEGS polyester on Chromosorb W, 185 C). For resolution of trimethyl silyl ether derivatives of sterols and methyl glycosides (12), an SE-30 column (5% on Chromosorb W, 230 C) was employed. Unknown peaks were identified by comparison with standards. Unsaturated fatty acid methyl esters were confirmed by bromination and subsequent disappearance on gas chromatograms.

RESULTS AND DISCUSSION

The four sterol lipid fractions isolated by column and thin layer chromatography from finger millet seeds each gave a single spot corresponding to reference sterols, sterol ester, sterol glycoside, and acyl sterol glycoside. IR spectra were likewise in agreement with published characteristics, suggesting that the compounds isolated were pure (1-5).

On total lipids, these sterol lipids consisted of: S 0.091, SE 0.013, SG 0.025, and ASG 0.020 (total 0.149) % wt respectively.

Table I shows that SE (iodine value 75.0) was considerably more unsaturated than ASG (iodine value 45.7). In both SE and ASG, oleic, linoleic, and palmitic together composed 85-90% of the total fatty acids, only the relative proportions varying.

Sterol composition in the S, SE, SG, and ASG fractions is presented in Table II. The gas chromatogram showed only two peaks, identified as β-sitosterol and stigmasterol, the former comprising 80-84% in all the four sterol types. Since the esterified sterols are rich in oleic and palmitic acid, β-sitosterol oleate and to a lesser extent palmitate would be the main esterified sterol species.

Paper chromatograms of the methyl glycosides prepared from SG and ASG indicated that D-glucose was the major sugar in both cases. Gas chromatographic resolution of the trimethyl silyl derivatives of these methyl glycosides yielded two peaks corresponding to α- and β-methyl glycosides, confirming that glucose is the only component sugar moiety of SG and ASG. Since β-sitosterol is the major component sterol in SG, the representative molecular specie here would be β-sitosterol glucoside. From this and earlier reports (5,13), the structure deduced would be β-D-glucopyranosyl-(1 → 3′)-β-sitosterol.

ASG has β-sitosterol and palmitic acid as major components, which implies that its representative molecular specie would be β-sitosterol-6-palmitoyl glucoside. Earlier reports (5,13) suggest the structure 6-0-palmitoyl-β-D-glucopyranosyl-(1 → 3′)-β-sitosterol.

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