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THE PENTACYCLIC TRITERPENE ESTERS AND THE FREE, ESTERIFIED AND GLYCOSYLATED STEROLS OF *SORGHUM VULGARE* GRAIN

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Key Word Index—*Sorghum vulgare*; Gramineae; phytosterols, triterpene esters.

Abstract—The free, esterified and glycosylated sterols and triterpene esters of *Sorghum vulgare* grains were analysed by GLC. 4-Demethylsterols were found free, esterified and glycosidated. 4-Monomethylsterols were found completely free whilst the triterpenes were completely esterified. Three quarters of the total sterols in the grain were located in the embryo whilst the triterpenes were equally distributed between the embryo and endosperm.

INTRODUCTION

The occurrence of phytosterols as glycosides [1–4], fatty acid esters [5–8] and the more abundant free form is now well documented and there have also been reports of acyl sterol glycosides [9,10] and water soluble sterol complexes [11,12]. Detailed information on the identities of naturally occurring combined triterpenes and sterols, their relative abundance, distribution and role in biosynthesis is still far from complete. This work is an attempt to provide as detailed a picture of these compounds in *S. vulgare* Pers. DC. 36* caryopses as present day chromatographic procedures will allow.

RESULTS AND DISCUSSION

Caryopses of *S. vulgare* were homogenized and extracted first with Me_2CO then $CHCl_3$–MeOH [13] mixture and the two combined to give a "total lipid" extract. The remaining grain residue was refluxed with water for 6 hr but after further purification of this extract no evidence could be found of the presence of water-soluble sterols or triterpenes such as were described by Pryce [11].

The "total lipid" extract was divided into two portions. The first was separated on silicic acid columns into neutral lipid (90%), glycolipid with sterol glycosides (6%) and phospholipid (4%) fractions. The phospholipid fraction was discarded. The sterol glycosides were isolated from the glycolipid fraction by TLC and then subjected to methanolysis [14] to release the sterols which were subsequently identified and quantified by TLC and GLC. The neutral lipid fraction was separated on an alumina column into fractions containing hydrocarbons (13%), steryl esters with triterpene esters (3%) and free sterols (84%). The free sterols were purified by digitonin precipitation then separated and identified by TLC, GLC and MS.

The second portion of "total lipid" and the steryl ester with triterpene ester fraction were both saponified and the unsaponifiable lipid from the latter fraction was separated by TLC into a pentacyclic triterpene band and a 4-demethylsterol band and further analysed and quantified by GLC.

The unsaponifiable lipid from the "total lipid" fraction was chromatographed by stepwise gradient elution on alumina columns [15] and the resulting fractions separated by TLC into penta-

* Also known as *S. bicolor* (Linn.) Moench. DC. 36.

Table 1. Percentage composition of the total sterols and triterpenes from whole *S. vulgare* grain

4-Demethylsterols* (394·0 μg/g dry wt)	4-Monomethylsterols (12·54 μg/g fry wt)
Cholesterol 0·4	24-Methylene lophenol 45·2
Campesterol 27·8	24-Ethylidene lophenol 54·7
Stigmasterol 15·0	Triterpenes (65·6 μg/g dry wt)
Sitosterol 49·6	Unknown triterpene (X) 6·8
28-Isofucosterol 5·0	δ-Amyrin 15·9
24-Ethylidene cholest-7-en-3β-ol 2·2	Lupeol 64·0
	Unknown triterpene (Y) 13·3

* Does not include 4-demethylsterols from glycosides. Results are the mean of three replicates.

cyclic triterpenes and 4-monomethyl and 4-demethylsterols which were identified and estimated by GLC (Table 1) and MS. The identification of the sterols and triterpenes of *S. vulgare* has already been reported [16–18].

The 4-demethylsterols were present as free sterols, esters and glycosides (Table 2), whereas 4-monomethylsterols were entirely free and the pentacyclic triterpenes completely esterified. Tetracyclic triterpenes such as cycloartenol were not detected [18].

The relative proportions of the various 4-demethylsterols in the free, esterified and glycosylated forms were similar (Table 2). However a higher proportion of stigmasterol appeared to be free than esterified or glycosidated. A similar situation between free and esterified sterols has been found in maize seedlings [5,19]. Conversely there was a lower proportion of free cholesterol than cholesteryl esters and glycosides. These differences appear to suggest the preferential esterification and glycosylation of some sterols.

Only those C_{28} and C_{29} sterols saturated at C24(28) were found as glycosides (Table 2). Their precursors, 28-isofucosterol, 24-ethylidene cholest-7-en-3β-ol and the related 4-monomethylsterols, 24-ethylidene and 24-methylene lophenol, were not found as glycosides. This suggests that the synthesis of sterol glycosides may be mediated by enzymes which are specific for the structure of the sterol side chain.

Similar determinations of the total sterol and pentacyclic triterpene contents were made for the embryo and the endosperm plus scutellum. There was three times as much sterol present in the

Table 2. Percentage composition of the various sterol and triterpene fractions from whole *Sorghum vulgare* grain

Free 4-demethylsterols (86%)	Esterified 4-demethylsterols (13%)	Glycosylated 4-demethylsterols (1%)
Cholesterol 1·0	3·6	7·3
Campesterol 27·1	23·6	23·0
Stigmasterol 18·7	7·4	10·6
Sitosterol 47·7	55·1	58·8
28-Isofucosterol 4·0	6·5	ND*
24-Ethylidene cholesten-7-3β-ol 1·4	3·6	ND
Free 4-monomethylsterols	Esterified 4-monomethylsterols	Glycosylated 4-monomethylsterols
24-Methylene lophenol 38·8	ND	ND
24-Ethylidene lophenol 61·2	ND	ND
Free triterpenes	Esterified triterpenes	Glycosylated triterpenes
Unknown triterpenes (X) ND	17·3	ND
δ-Amyrin ND	14·5	ND
Lupeol ND	61·5	ND
Unknown triterpene (Y) ND	6·7	ND

Results are the mean of three replicates.
ND = none detected.
* 28-Isofucosterol may be denatured by methanolic HCl.

Table 3. Distribution of totals sterols and triterpenes between embryo* and endosperm

	Per seed	Per unit wt of tissue
Ratio of 4-demethylsterols in embryo to endosperm	2·6:1	18·9:1
Ratio of 4-monomethylsterols in embryo to endosperm	3·7:1	26·9:1
Ratio of triterpenes in embryo to endosperm	1·2:1	8·6:1

* Ratio of embryo to endosperm 1:7·2 w/w (moisture free basis).

Table 4. Percentage composition of the total sterols and triterpenes obtained from the embryo and endosperm fractions of *S. vulgare* grain

	Embryo	Endosperm
4-Demethylsterols*		
Cholesterol	0·6	1·0
Campesterol	23·7	26·7
Stigmasterol	12·9	17·7
Sitosterol	55·2	47·3
28-Isofucosterol	4·7	5·6
24-Ethylidene cholest-7-en-3β-ol	2·9	1·6
4-Monomethylsterols		
24-Methylene lophenol	62·9	61·8
24-Ethylidene lophenol	37·1	38·2
Triterpenes		
Unknown triterpene (X)	8·7	10·6
δ-Amyrin	13·9	14·6
Lupeol	64·9	64·9
Unknown triterpene (Y)	12·4	9·9

* Results are the mean of three replicates but do not include the 4-demethylsterols from the glycosides.

embryo plus scutellum, per seed, as in the endosperm (Table 3) but there was almost the same amount of pentacyclic triterpene in each tissue.

As the dry weight of the embryo plus scutellum was only one seventh of the dry weight of the endosperm there was more than 20 times the amount of sterol in the same dry weight of embryo as in the endosperm and 9 times as much pentacyclic triterpene. The percentage composition of the sterols and triterpenes in the embryo was not markedly different from that of the endosperm (Table 4).

EXPERIMENTAL

Lipid extraction. Sorghum vulgare Pers. DC. 36 grain obtained from Gunsons Seeds, Johannesburg, was dried to 8·3% H_2O content. It was homogenized with an Ultra Turrex, refluxed for 6 hr in Me_2CO and then for a further 4 hr in $CHCl_3$–MeOH (2:1). The extracts were evaporated to dryness, re-extracted with Et_2O, bulked and evaporated to dryness. Seed residue was refluxed for 6 hr with H_2O and aq. extract evaporated to dryness and then hydrolysed for 6 days with aq. methanolic HCl [11]. After extraction with Et_2O, neutralization and concn by evaporation an aliquot was treated with DMSO. Both the untreated and DMSO treated extracts were analysed for sterols and triterpenes. 0·005% BHT antioxidant was routinely added to all organic solvents used in extractions.

Column chromatography. Aliquots of lipid extract dried *in vacuo* were separated into neutral lipid, glycolipid and phospholipid fractions on Mallinckrodt SilicAR CC_7 200–325 mesh silicic acid columns [20]. Neutral lipid fractions were separated on Woelm anionotropic alumina (Brockman grade III) into hydrocarbon, triterpene and steryl ester, and free sterol fractions by successively eluting with petrol, 2% Et_2O in petrol and 40% Et_2O in petrol respectively. Unsaponifiable lipid was separated on alumina columns by stepwise elution with petrol containing 0, 2, 4, 6, 9 and 20% Et_2O.

Digitonin precipitation. Free sterols were precipitated overnight from the 40% alumina column fraction in 80% EtOH with 1% digitonin in 60% EtOH and regenerated from the digitonides by DMSO [21].

Saponification. Aliquots of the total lipid and the steryl ester with Triterpene ester fractions were refluxed for 90 min in 8% KOH in 80% MeOH under N_2.

TLC. Preparative TLC, sample application and visualization was generally as described previously [18]. The glycolipid fractions were separated on Chromolay plates coated with 0·75 mm Si gel G, activated for 90 min and developed with $CHCl_3$–MeOH–HOAc (65:25:8). The R_f's of sterol glycosides, mono- and digalactosyl diglycerides (0·86, 0·92, 0·45 respectively) were used as an aid to identification.

Methanolysis. The sterol glycoside fraction was mixed with 0·65 N methanolic HCl prepared from dry MeOH and AR acetyl chloride (20:1) and treated as previously described [22].

GLC. Glass columns (2 m × 1·5 mm) were used packed with 3% OV-17 on Gas chrom Q and operated at 225°. The carrier gas was N_2 (40 ml/min). Quantitative determinations were made by means of peak area triangulation calibrated against standard cholesterol.

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