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MONOCOTYLEDONAE

GRAMINEAE

STEROLS IN THE GENUS *TRITICUM*

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Key Word Index—*Triticum*; Gramineae; sterols; campesterol; 5α-dihydrocampesterol; sitosterol; stigmastanol.

Several reports of the analysis by GLC of sterols in species of *Triticum* have appeared[1–3] but no account seems to have been taken of the earlier reports of saturated sterols in this genus.[4,5] The present report describes the analysis by GLC of sterols from nine representatives of eight species of *Triticum*.

RESULTS AND DISCUSSION

In Table 1 the sterol content of grain from the eight species examined is listed and in Table 2 the sterol content from the whole ears of five species is listed. The yield of sterol from variety Jufy 1 of *Triticum aestivum* was 0·05%.

In all cases both saturated (5α-) and Δ^5-monounsaturated sterols were observed, the range of saturated sterol content being 26–40% in grain extracts[6] and 5–24% in whole ear extracts. In addition, whole ear (but not grain) contained stigmasterol as a significant component and there was also apparently relatively more cholesterol in the whole ear fractions. The occurrence of saturated sterols was confirmed by analytical TLC of the trifluoroacetate derivatives on silver nitrate impregnated silica gel (5% benzene in 40–60° petroleum: cholestanol T.F.A.: R_f 0·55 and cholesterol T.F.A.: R_f 0·50) and by oxidation to the corresponding ketones and separation by TLC (10% ethyl acetate in petroleum—saturated ketones: R_f 0·5 and Δ^4-3-ketones: R_f 0·3) and GLC [FS-1265: r.r.t. 9·5 (C_{28}) and 11·7 (C_{29})].

Since this genus and perhaps other genera of the family Gramineae contain saturated sterols,[6,7] the problem of separating unfractionated sterol mictures by GLC should be

[1] M. E. MCKILLICAN, *J. Am. Oil Chem. Soc.* **41**, 554 (1964).
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[3] D. V. MYHRE, *Can. J. Chem.* **46**, 3071 (1968).
[4] R. J. ANDERSON, R. L. SHRINER and G. O. BURR, *J. Am. Chem. Soc.* **48**, 2976 (1926).
[5] M. A. SPIELMAN, *Cereal Chemistry* **10**, 239 (1933).
[6] B. A. KNIGHTS, in *Memoirs for the Society for Endocrinology* (edited by J. K. GRANT), No. 16, p. 211, Cambridge University Press, Oxford (1967).
[7] F. RADT, *Elsevier's Encyclopedia of Organic Chemistry*, Vol. 14, pp. 1780–1828, Elsevier, New York (1954 and 1959).

TABLE 1. STEROLS IN GRAIN FROM *Triticum* SPECIES

Species	Genomic constitution	Sterols (% of total)					Ratio C_{29}:C_{28}		
		C_{27}	5α-C_{28}	Δ^5-C_{28}	5α-C_{29}	Δ^5-C_{29}	Total sterol	Δ^5	5α
T. monococcum L.	A	1·0	14·0	17·5	17·0	50·5	2·1	2·9	1·2
T. dicoccum Schübl.	AB	1·0	22·0	20·0	14·0	43·0	1·4	2·1	0·6
T. dicoccoides Kom.	AB	—	14·0	15·5	20·0	50·5	2·4	3·3	1·4
T. polonicum L.	AB	3·0	18·0	19·5	13·5	46·0	1·6	2·4	0·7
T. timopheevi Zhukov.	AG	4·0	18·0	14·5	22·5	41·0	2·0	2·8	1·25
T. spelta L.	ABD	0·5	18·5	17·5	15·0	48·5	1·7	2·8	0·8
T. aestivum L. cv. Kloka	ABD	—	21·5	26·5	6·5	45·5	1·1	1·7	0·45
T. aestivum L. cv. Jufy I	ABD	—	22·5	24·0	10·0	43·5	1·2	1·8	0·3
T. compactum Host.	ABD	4·0	13·5	16·5	13·0	53·0	2·2	3·1	0·95

discussed briefly, as methods for separating sterols into groups such as saturated, monounsaturated, etc. are likely to be subject to losses in handling so that comparative quantitative analyses would be liable to error. Sterols are commonly analysed either directly or after formation of acetate, trifluoroacetate, trimethylsilyl ether or methyl ether derivatives.[6,8] Saturated and Δ^5-monounsaturated sterols may be slightly resolved on stationary phases SE-30 or OV-101; DC-560 (as trifluoroacetates) and FS-1265 (QF-1) (using trifluoroacetate or trimethylsilyl ether derivatives), and on HiEFF-8B coated on polyvinylpyrrolidone (PVP) treated support[9] (as the free sterols only). The last named system represents the only

TABLE 2. STEROLS IN WHOLE EAR FROM *Triticum* SPECIES

Species	Sterol (% of total)					
	C_{27}	5α-C_{28}	Δ^5-C_{29}	5α-C_{29}	Δ^5-C_{29}	$\Delta^{5,22}$-C_{29}
T. dicoccum	4	6	19	7	47	17
T. durum. L.	4	12	23	12	31	18
T. polonicum	6	7	23	7·5	34	22
T. spelta	2	7	19	8	52	11
T. sphaerococcum Perc.	10	2·5	25	2·5	45	15

case we have found in which the dihydrosterol was eluted before the corresponding Δ^5-sterol but, since this column has a maximum operating temperature of 225°, retention times of free sterols are very long and the derived trifluoroacetates and trimethylsilyl ethers, having much shorter retention times, do not resolve. When stigmasterol is present in the extract (whole ear sterols) complete separation can only be achieved on the PVP column since, in all other systems, this sterol is eluted along with either the C_{28}-dihydrosterol or with the C_{28}-Δ^5-compound (FS-1265). For grain sterols, analysis of trifluoroacetate or trimethylsilyl ether derivatives on FS-1265 is satisfactory. Retention data are listed in Table 3.

[8] D. R. IDLER, L. M. SAFE and S. H. SAFE, *Steroids* **16**, 251 (1970).
[9] B. A. KNIGHTS, *J. Gas Chromatog.* **2**, 338 (1964).

TABLE 3. RELATIVE RETENTION TIMES (5α-CHOLESTANE = 1·0) FOR SOME STEROLS AND DERIVATIVES

Compound	Free sterol				Trimethylsilyl ethers			Trifluoroacetates		
	SE-30	DC-560	FS-1265	HiEFF-8B/PVP	DC-560	FS-1265	HiEFF-8B/PVP	SE-30	DC-560	FS-1265
Cholesterol	1·93	2·15	2·9	6·95	2·52	1·87	2·6	1·5	1·51	2·5
5α-Cholestan-3β-ol	2·04	2·22	3·2	6·34	—	—	—	—	—	—
Campesterol	2·56	2·77	3·84	9·35	3·35	2·5	3·44	2·06	1·98	3·15
5α-Dihydrocampesterol	—	—	—	8·7	—	2·65	—	—	2·08	3·55
Sitosterol	3·25	3·6	4·4	11·6	4·27	3·0	4·32	2·68	2·52	3·83
5α-Dihydrositosterol	—	—	—	10·8	—	3·2	—	—	2·64	4·28
Stigmasterol	2·82	3·1	3·9	9·9	3·71	2·5	3·82	2·24	2·14	3·3

The amount of available data (Tables 1 and 2) is insufficient to permit clear correlation between genomic constitution and derived data such as ratios of C_{29}:C_{28} for total sterol, Δ^5-sterol and 5α-dihydrosterol. Nevertheless, two cultivars of the bread wheat *T. aestivum* stand out as the extreme case in all three types of correlation, suggesting that unconscious selection for a high Δ^5-C_{28} and a low 5α-C_{29} content may have taken place during their breeding. The other extreme cases are represented by the tetraploid *T. timopheevi* (highest saturated sterol) and *T. sphaerococcum* (highest cholesterol content).

EXPERIMENTAL

Grain of *Triticum aestivum* varieties were obtained commercially. Other species were grown in the departmental garden from material originally obtained from the University of Reading wheat collection. Sterols were isolated from the nonsaponifiable fraction of petroleum extracts of milled grain using previously described methods.[6] Gas Chromatography was performed on a Pye 104 chromatograph, free sterols were analysed on a 3 m column packed with 1% w/w HiEFF-8B coated on 2% w/w polyvinylpyrrolidone treated acid washed Gas Chrom P at 225°.[9] Other sterol derivatives were analysed on columns packed with FS-1265 (1% w/w on siliconized Gas Chrom P — 3 m column at 225°), DC-560 (F-60) (1% w/w on siliconized Gas Chrom P — 6 m column at 250°) and SE-30 (5% w/w on siliconized Gas Chrom P — 3 m column at 240°).