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CHEMOSYSTEMATICS: SEED STEROLS IN THE CRUCIFERAE

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Abstract—Seed sterols of 56 representatives of the Cruciferae comprising 41 species from 21 genera and nine tribes have been analysed by GLC. Five classes can be recognised dependent upon the presence or absence of compounds corresponding in retention time to Δ^7-cholesten-3β-ol, 4α-methyl-Δ^7-cholesten-3β-ol, brassicasterol and stigmasterol. Comparison between these and recognised systematic categories are made.

INTRODUCTION

DURING studies relating to the host parasite relationship of members of the Cruciferae with *Plasmodiophora brassicae* Woron. it was found that seed sterol fractions could be divided into various groups. It was decided to investigate this phenomenon as a possible contribution to the chemosystematics of this family.

Sterols represent a well defined set of compounds which may be isolated as a group and are amenable to analysis by GLC and mass spectrometry. As a result, a number of recent reports describe the use of the analysis of sterols for classification. These include echinoderms,[1] green and brown algae,[2–4] red algae[5–7] and pollen.[8] The occurrence of related triterpenes in *Euphorbia*,[9] and sapogenins in *Dioscorea* and *Tamus* spp.[10] has also been studied. These, and a number of other less well defined reports, indicate that sterol composition may be used in chemosystematics in a way similar to that of other secondary compounds.

RESULTS

Table 1 lists the species, under tribe and subtribe headings according to Schulz,[11] investigated in this work, together with names of cultivars and alternative names given by seed suppliers when these were different. It should be stressed that no comparison has been made with voucher specimens and that it is therefore possible that some of the species examined have been incorrectly listed in the seed catalogues.[12] The group into which each

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TABLE 1. CRUCIFER SPECIES STUDIED

Tribe and subtribe[11]				Species		Seed source	GLC group based on seed sterol analysis	Alternative tribes (Refs. 13, 14)
VII	Brassiceae							
		VIIa	Brassicinae	*Brassica oleracea* L. f. *capitata*	[Primo]	4	3a	
				B. oleracea f. *cymosa*		?	3a	
				B. napobrassica (L.)	A [Balmoral]	6	3a	
				B. napobrassica	B [Wilhelmsburger]	6	3a	
				B. napus L. f. *annua*		6	5	
				B. campestris L. f. *rapifera*	A [Wallace]	6	3a	
				B. campestris f. *rapifera*	B [Golden Ball]	5	3a	
				B. campestris f. *sinensis*	[Wong Bok]	1	3a	
				Eruca sativa Mill.		1	3a	
				Sinapis alba L.		5	3a	
				S. arvensis L.		7	3a	
		VIIb	Raphaninae					
				Raphanus sativus L.	A [Cherry belle]	?	3a	
				R. sativus	B [French breakfast]	5	3a	
				R. sativus	C [Sparkler]	3	3a	
VIII	Heliophileae							
				Heliophila longifolia DC.		1	5	
X	Lepideae							
		Xb	Lepidiinae					
				Lepidium sativum L.		5	4	
				L. virginicum L.		8	4	
		Xd	Isatidinae					
				Isatis tinctoria L.		1	5	Arabideae,[13] Sisymbrieae[14]
		Xg	Iberidinae					
				Iberis gibraltarica L.		1	3b	
				I. sempervirens L.		2	1b	
				I. umbellata L.	A [Mixed]	2	3a	
				I. umbellata L.	B [Mixed]	3	3a	
				I. umbellata L.	C [Purple]	4	3a	
				I. umbellata L.	D [*I. coronaria* f. *hyacinthiflora*]*	1	3b	
				I. umbellata L.	E [*I. coronaria* f. *hyacinthiflora*]*	4	3b	

		Xh	Thlaspidinae					
				Aethionema creticum Boiss. et Heldr.		1	4	
				A. grandiflorum Boiss. et Hohen.		1	5	
				A. pulchellum Boiss. et Hohen.		1	4	
				A. schistosum Boiss. et Kotech.		1	4	
		Xk	Capsellinae					
				Hutchinsia alpina (L.) R.Br.		1	3a	
		Xl	Cochleariinae					
				Cochlearia acaulis	[*Ionopsidium acaule*]*	1	3c	
XIII	Lunarieae							
				Lunaria biennis Moench.		3	4	Alysseae[14]
XIV	Alysseae							
				Alyssum maritimum (L.) Lam	A [Carpet of snow]	3	3a	
				A. maritimum	B [Rosie O'Day]	3	3a	
				A. saxatile L.	[Compactum]	2	1a	
				A. argenteum Vitm.		1	4	
				A. montanum L.	A	1	4	
				A. montanum L.	B [*A. flexicaule*]*	1	4	
				A. alpestre L.	[*A. serpyllifolium*]*	1	4	
XV	Drabeae							
				Draba aizoon Wahl.		1	3b	Alysseae[13,14]
				D. pyrenaica L.		1	3b	Alysseae[13,14]
XVI	Arabideae							
				Arabis albida Stev.	[White *A. alpina*]*	2	1a	
				A. blepharophylla Hook. et Arn.		2	1a	
XVII	Matthioleae							
				Aubrieta deltoidea DC.	[Mixed]	3	4	Arabideae[13,14]
				Matthiola bicornis DC.		1	5	Alysseae,[13] Hesperideae[14]
				M. incana (L.) R. Br.	A [Brompton stock]	3	1a	Alysseae,[13] Hesperideae[14]
				M. incana	B [10 week stock]	3	1a	Alysseae,[13] Hesperideae[14]
XVIII	Hesperideae							
				Malcomia maritima (L.) R. Br.		3	3a	Alysseae[13]
				Hesperis matronalis L.		3	3b	Alysseae[13]
				Erysimum linifolium J. Gay.	[*Cheiranthus linifolius*]*	1	2a	Arabideae[13]
				Erysimum asperum DC.	[*E. arkansanum*]*	1	2b	Arabideae[13]
				E. marshallianum Andry.	[*Cheiranthus allionii*]*	2	1b	Arabideae[13]
				E. perofskianum Fisch. et Mey.		1	1a	Arabideae[13]
				Cheiranthus cheiri L.	A [Golden monarch]	4	2a	Alysseae[13]
				C. cheiri	B [Blood red]	?	2a	Alysseae[13]
				C. cheiri	C [Blood red]	3	2a	Alysseae[13]

* Purchased from the Seeds Merchant under this name.

Key to seed source: 1. Thompson & Morgan (Ipswich) Ltd., 2. D. R. Colegrave Seeds Ltd., 3. Ilotts Garden Centre., 4. Hurst, Gunson, Cooper, Taber Ltd., 5. Dobbie & Co. Ltd., 6. Scottish Agricultural Industries, 7. Collected by D. S. H. Drennan, 8. Collected in Jamaica and grown in greenhouse to produce new seed.

TABLE 2. STEROLS OF CRUCIFER SEEDS

Group		Species†	Sterol: Retention index								
			3255	3310	3325	3355	3385	3395	3440	3480	3505
Group 1											
	Section a	*Arabis albida*	1·3	—	—	22·7	—	—	76·0	*	—
		A. blepharophylla	1·0	—	—	15·7	—	—	81·1	2·2	—
		Alyssum saxatile	1·9	—	—	22·5	*	—	73·1	2·5	—
		Erysimum perofskianum	9·2	—	—	26·5	—	—	52·8	11·5	—
		Matthiola incana A	4·9	—	—	13·7	—	—	81·4	—	*
		M. incana B	0·9	—	—	12·3	—	—	84·6	—	2·2
	Section b	*Erysimum marshallianum*	8·8	—	*	28·1	—	*	53·1	10·0	—
		Iberis sempervirens	4·8	*	*	40·5	—	*	51·6	3·1	*
Group 2											
	Section a	*Cheiranthus cheiri* A	15·3	—	5·5	17·9	—	13·7	38·3	4·8	4·5
		C. cheiri B	15·0	—	3·2	19·5	—	4·5	51·4	3·7	2·7
		C. cheiri C	15·3	—	4·1	15·7	—	5·5	54·3	3·3	1·8
		Erysimum linifolium	4·5	—	1·3	34·9	—	1·7	48·0	9·6	*
	Section b	*Erysimum asperum*	13·8	—	0·7	37·8	—	—	35·0	11·8	—
Group 3											
	Section a	*Alyssum maritimum* A	1·1	4·3	—	14·3	—	—	78·8	—	1·5
		A. maritimum B	1·4	3·8	—	10·3	—	—	78·1	*	6·4
		Brassica oleracea f. *capitata*	*	12·5	—	26·4	—	—	61·1	—	—
		B. oleracea f. *cymosa*	1·2	22·7	—	23·4	*	—	51·8	—	—
		B. napobrassica A	*	19·1	—	38·2	—	—	42·7	—	—
		B. napobrassica B	*	8·2	—	34·8	—	—	57·0	—	—
		B. campestris f. *rapifera* A	0·3	19·6	—	25·2	—	—	53·4	1·5	—
		B. campestris f. *rapifera* B	2·7	13·4	—	22·4	—	—	61·5	*	—
		B. campestris (Chinese cabbage)	*	17·8	—	34·4	—	—	47·8	—	—
		Eruca sativa	6·6	12·0	—	32·2	—	—	49·2	*	—
		Hutchinsia alpina	12·5	6·0	—	38·9	—	—	42·6	—	—
		Iberis umbellata A	1·2	20·0	—	32·3	—	—	45·2	1·3	—
		I. umbellata B	*	17·7	—	33·8	—	—	43·8	2·4	2·3
		I. umbellata C	2·0	18·0	—	30·5	—	—	49·5	*	*
		Malcomia maritima	3·7	5·1	—	25·4	*	—	64·2	1·6	—
		Raphanus sativus A	2·5	15·1	—	30·1	—	—	52·3	—	—
		R. sativus B	2·4	8·4	—	24·2	—	—	62·0	3·0	—
		R. sativus C	1·5	8·6	—	25·7	—	—	59·8	4·4	*
		Sinapis alba	3·2	5·2	—	34·6	—	—	43·8	13·2	—
		S. arvensis	1·5	9·4	—	28·2	—	—	60·9	—	—

Section b	*Draba aizoon*	12·3	4·4	—	25·1	—	*	58·0	—	—
	D. pyrenaica	5·5	12·9	—	29·1	—	*	52·5	—	—
	Hesperis matronalis	1·3	7·0	—	23·0	—	*	66·3	2·3	—
	Iberis umbellata D	3·2	10·9	—	47·7	—	*	34·6	—	3·6
	I. umbellata E	5·3	8·8	—	42·7	—	*	39·3	2·2	1·7
	I. gibraltarica	7·6	8·8	—	36·6	—	*	36·3	*	10·7
Section c	*Cochlearia acaulis*	8·4	16·8	—	24·3	—	11·5	39·0	—	*
Group 4										
	Aethionema creticum	8·8	*	—	8·2	1·7	—	78·6	2·7	—
	A. pulchellum	3·8	*	—	6·5	4·5	—	85·2	*	—
	A. schistosum	4·0	—	—	6·0	1·0	—	89·0	—	—
	Alyssum argenteum	5·0	—	—	21·0	3·5	—	65·1	5·4	—
	A. montanum B	5·7	—	—	20·2	3·6	—	67·8	2·7	*
	A. montanum A	6·0	—	—	6·5	7·7	—	72·0	7·8	—
	A. alpestre	8·7	—	—	3·8	10·4	—	66·6	10·5	*
	Aubrieta deltoidea	3·0	—	—	17·5	2·6	—	76·9	*	—
	Lepidium sativum	5·8	—	—	11·2	4·2	—	52·8	26·0	—
	L. virginicum	2·5	—	—	30·9	12·1	—	51·4	3·1	—
	Lunaria biennis	0·8	—	—	29·8	2·3	—	67·1	*	*
Group 5										
	Aethionema grandiflorum	10·9	1·9	—	6·9	1·6	—	78·7	—	—
	Brassica napus f. *annua*	*	16·1	—	27·8	1·1	—	52·3	2·7	—
	Heliophila longifolia	2·1	17·6	—	25·8	4·3	—	49·1	1·6	—
	Isatis tinctoria	3·2	8·4	—	19·1	6·4	—	59·2	3·7	—
	Matthiola bicornis	3·1	3·1	—	20·3	3·1	—	70·4	*	—

Sterols: — = not detected; * = trace detected,
I = 3255 cholesterol,
I = 3310 24-methyl-$\Delta^{5,22}$-cholestadien-3β-ol (brassicasterol),
I = 3325 Δ^{7}-cholesten-3β-ol,
I = 3355 24-methylcholesterol (campesterol) and 24-methylenecholesterol,
I = 3385 24-ethyl-$\Delta^{5,22}$-cholestadien-3β-ol (stigmasterol).
I = 3395 4α-methyl-Δ^{7}-cholesten-3β-ol.
I = 3440 24-ethylcholesterol (β-sitosterol).
I = 3480 Δ^{5}-avenasterol.
I = 3505 *cyclo*artenol; 24-ethyl-Δ^{7}-cholesten-3β-ol; 24-methylene-4α-methyl-Δ^{7}-cholesten-3β-ol.

† Seed sources of species sampled more than once are given in Table 1.

species fell when sterols were examined by GLC (Table 2) is included in Table 1 and reference is made to an earlier[13] and to a more recent classification.[14]

Sterols were isolated from seed material and subjected to analysis by GLC using previously described methods.[15] Analyses were obtained using a 3% OV-17 column under standard conditions. In five cases: *Brassica oleracea* L., *Sinapis alba* L., *Lepidium sativum* L., *Cheiranthus cheiri* L. and *Alyssum maritimum* (L) Lam. more detailed studies were made using combined gas chromatography-mass spectrometry (GC–MS). Because of the possibility of two or more compounds being eluted at the same time by GLC, results listed in the tables are described using retention indices to identify the peaks with an indication of possible compounds to which these peaks correspond (Table 2). Results are expressed on a percentage basis and values for individual sterols were obtained by triangulation of the peaks. Table 2 contains a list of the results obtained set out in groups dependent upon the presence or absence of peaks ascribed to brassicasterol (I = 3310), Δ^7-cholesten-3β-ol (I = 3325) and stigmasterol (I = 3385).

In addition to the data in Table 2, a number of reports of the finding of sterols in seeds from various species of the Cruciferae have been recorded. These include 'rape seed oil' (brassicasterol, 10%; campesterol, 33%; β-sitosterol, 57%; stigmasterol, trace)[16] [12·1%, 31·7% and 56·2% respectively][17] (some cholesterol by GC–MS);[18] *Cheiranthus cheiri* (α-sitosterol);[19] *Erysimum perofskianum* (β-sitosterol);[20] *Lepidium sativum* (β-sitosterol),[21] *Raphanus sativus* (β-sitosterol)[22] (cholesterol, campesterol, brassicasterol and others);[23] *Sinapis arvensis* (mol. wt. 398, 400, 414 by mass spectrometry)[24] *Sisymbrium loeselii* (β-sitosterol).[25] The results for the "oil seed rape" are comparable to the "fodder rape" *Brassica napus* f. annua studied in the present work and the finding of α-sitosterol by colour reaction in *C. cheiri*[19] is consistent with the finding of 4-methyl-Δ^7-sterols in this species in the present work. The compounds having molecular weights 398, 400 and 414 in *Sinapis arvensis* presumably correspond to brassicasterol, campesterol and β-sitosterol respectively, compounds recorded in this species in the present work.

DISCUSSION

The seed sterols of 41 species, in 21 genera, of the Cruciferae examined fell into five main groups as follows:

Group 1. Characterised by the nearly complete absence of Δ^{22}-sterols and Δ^7-C_{27} or -C_{28} sterols, included eight examples and could be sub-divided into two sections depending upon the presence or absence of detectable but unmeasurable peaks at I = 3325 (Δ^7-cholesten-3β-ol) and I = 3395 (4α-methyl-Δ^7-cholesten-3β-ol).

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[25] S. S. CHOUDARI, H. SINGH and K. L. HANDA, *Chem. Abs.* **51**, 10095c (1957).

Group 2. The five members of this group, from two genera (*Cheiranthus* and *Erysimum*) were characterized by the presence of a measurable peak at I = 3325. In addition all members except one exhibited peaks at I = 3395 and I = 3505 (4α-methyl-24-methylene-Δ^7-cholesten-3β-ol or 24-ethyl-Δ^7-cholesten-3β-ol). The exception, *Erysimum asperum*, not having these sterols was separated into a sub-group 2b and the absence of these peaks served to distinguish it from Group 1, Section b with which it had some relationship, e.g. *E. marshallianum.* Analyses by GC–MS for varieties A and B of *Cheiranthus cheiri* have confirmed structures for all the seven peaks observed. These were found to be I = 3255: cholesterol, I = 3325: Δ^7-cholesten-3β-ol (brassicasterol detected also), I = 3355: 24-methylcholesterol, I = 3395: 4α-methyl-Δ^7-cholesten-3β-ol, I = 3440: 24-ethylcholesterol (β-sitosterol), I = 3480: 24-ethylidenecholesterol (Δ^5-avenasterol), I = 3505: 4α-methyl-24-methylene-Δ^7-cholesten-3β-ol.

Groups 3–5. Were distinguished from Groups 1 and 2 in having peaks for Δ^{22}-sterols. Group 3 showed a peak corresponding to brassicasterol (I = 3310), Group 4 a peak corresponding to stigmasterol or its C-24 isomer (I = 3385), and Group 5 exhibited peaks for both compounds or their C-24 isomers.

The 27 examples in Group 3 could be divided into three sections. Of these sections, GLC traces from section b exhibited a detectable peak at I = 3395 and the one member of Section c (*Cochlearia acaulis* Desf.) was distinguished by a large proportion (11 %) of this compound in the mixture. Group 4 contained 11 examples. Analysis by GC–MS of the extract of *Lepidium sativum* L. verified the identity of the peak at I = 3385 as being 24-ethyl-$\Delta^{5,22}$-cholestadien-3β-ol (stigmasterol or its C-24 isomer poriferasterol). Further, it was found for this species that the peak (I = 3355) due to 24-methylcholesterol (campesterol) contained some 24-methylenecholesterol. Group 5 was found to contain five members.

In addition to the other qualitative differences listed it is noteworthy that Group 2 was further characterized by all members containing Δ^5-avenasterol and appreciable quantities of cholesterol; in other groups the occurrence of these two compounds was more variable.

It should be emphasised that GLC analysis provides no evidence for the stereochemistry at C-24 of the 24-methyl and 24-ethyl substituted compounds corresponding to campesterol, brassicasterol, β-sitosterol and stigmasterol. From known findings[26] it seems clear that brassicasterol has the opposite configuration (24S) from campesterol (24R) and that stigmasterol and β-sitosterol have the same (24R) configuration. The co-occurrence of brassicasterol and a Δ^{22}-24-ethylsterol in the five species of Group 5 draws attention to a stereochemical question. Since campesterol (originally obtained from *Brassica campestris*)[27] and β-sitosterol are held to have the same configuration at C-24, and also that brassicasterol (originally obtained from *Brassica rapa*)[28,29] and stigmasterol are held to have the opposite configuration at C-24 the following statements may be presented as alternatives:

(a) Biosynthesis of both C_{28} and C_{29} Δ^5-sterols follows a stereochemically homogeneous path, whereas the corresponding biosynthesis of $\Delta^{5,22}$-sterols produces opposite isomers at C-24 (i.e. brassicasterol [24S] and stigmasterol [24R]).

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[28] A. Windhaus and A. Welch, *Chem. Ber.* **42**, 612 (1909).
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(b) Biosynthesis of C_{28} and $C_{29}\Delta^5$-sterols proceeds in the opposite sense from that for $\Delta^{5,22}$-sterols, in which case the C_{29} sterol found in the present work is poriferasterol and not stigmasterol.
(c) Biosynthesis proceeds to give the same stereochemistry in all four compounds (i.e. brassicasterol has the same stereochemistry as campesterol or else all four compounds in these examples have the 24S configuration).
(d) Biosynthesis varies from species to species and is therefore more variable than has hitherto been realised.

Whilst the fourth postulate must not be disregarded because of lack of evidence, the most likely of the other three would seem to be the second, and from the known stereochemical correlations,[26] the least likely seems to be the third. If the second postulate can be demonstrated, the finding of poriferasterol in higher plants appears to be novel since its recorded occurrence has so far apparently been confined to *Chlorella* and *Ochromonas* spp. If the first postulate holds true then the role of brassicasterol in these species must be considered to be unique and perhaps to fulfil some as yet unrecognized function. Should the third postulate prove to be correct, then it would seem that a reinvestigation of previous work would be required.

Although the data in Table 2 are limited in scale in relation to the size of the family Cruciferae, it is apparent from Table 1 that useful results may be obtained using sterols of seed as another factor to delineate taxa. Thus all members of the tribe Brassiceae examined in the present work were found to fall in Group 3a, except for *Brassica napus* (Group 5). However, since this species contained 16% of brassicasterol and only 1% of stigmasterol the inclusion of it in Group 5 is probably an arbitrary choice and results from the establishment of arbitrary groups based on qualitative differences in sterol composition. Similarly all members of the tribe Lepideae examined were found to fall in Groups 3–5, except for *Iberis sempervirens* (Group 1b), i.e. containing Δ^{22}-sterols. This tribe was much subdivided by Schulz[11] and it is notable that the divisions according to Schulz are also paralleled by the GLC data. The inclusion of *Aethionema grandiflorum* in Group 5 and the other examples of this genus in Group 4 is an arbitrary choice based on only a small difference. In addition, *A. grandiflorum* and *A. pulchellum* are considered to be synonyms for the same taxon and this genus is given a detailed treatment by Davis.[30] The only other species from the Lepideae examined in this work and found to fall into Group 5 was *Isatis tinctoria*. Thus, from the limited data available, this would suggest that a reclassification of the genus *Isatis* might be necessary. On the basis that no other genera held to belong to the tribe Arabideae by Von Hayek and by Manton[13] were found to belong to Group 5, the suggestion by Janchen[14] that the genus *Isatis* be included in the tribe Sisymbrieae seems the more probable of the two alternatives listed in Table 1. Members of the tribe Alysseae examined in this work have all been considered by some authorities to belong to the genus *Alyssum* and were found to fall into three groups (1a, 3a and 4). These results support a division of this genus. The description of *Alyssum maritimum* as *Lobularia maritima* and the proposed reclassification by Dudley[31] of *A. saxatile* as *Aurinia saxatilis* (L) Desv. affords a division whereby these genera coincide with the GLC division [*Alyssum*—Group 4; *Aurinia*—Group 1; *Lobularia*—Group 3a].

[30] P. H. DAVIS, *Flora of Turkey*, Vol. 1, p. 263 (1965).
[31] T. R. DUDLEY, *J. Arn. Arb.* **45**, 390 (1964).

Results for the tribe Matthioleae suggest that the genus *Matthiola* might need to be re-examined (cf. Refs. 13, 14), since *M. bicornis* falls into Group 5 whilst *M. incana* was found in Group 1a.

The value of sterol analysis in determining taxonomic relations at the level of the genus can be seen best when comparison is made between *Cheiranthus* and *Erysimum*. The GLC data indicate the close similarity of these two genera and Snogerup[32] has suggested on other grounds that the genus *Cheiranthus* should be discarded and its members be included in *Erysimum* (sectio Cheiranthus). The chemotaxonomy of the sterols supports this view. In contrast, the difference between these results and those for *Malcomia maritima* and *Hesperis matronalis* suggest that they should be separated at least into a different subtribe from *Erysimum* and possibly into a separate tribe.

Thus, bearing in mind the limited amount of data available and also the possibility of misidentification of some of the more difficult species such as *Aethionema*, the present work supports the overall classification of the Cruciferae with respect to the species studied. The tribe Brassiceae appears to be largely homogeneous and the subtribal divisions of the Lepideae[11] are supported. The proposal by Janchen[14] to reclassify the genera *Isatis* and *Lunaria* and the proposal by Snogerup[32] to discard the genus *Cheiranthus* are all supported. It also seems probable that the Matthioleae and Hesperideae need further study to complete their classification, but the data for *Draba* species do not strongly uphold the belief that they be included in the Alysseae.[13,14]

EXPERIMENTAL

Isolation of sterols. Seed was milled in a coffee grinder and then extracted in a Soxhlet using petrol (b.p. 40–60°). Following evaporation of the petrol, sterols were obtained by saponification of the residue and precipitation with digitonin from the non-saponifiable material.[15] The obtained sterol fractions (*ca.* 0·1%) were dissolved in *bis*-trimethylsilylacetamide (100 μl) in order to convert them to the trimethylsilyl derivative prior to GLC.

Gas chromatography of the sterol fractions. This was carried out using a Pye 104 model 14 chromatograph. A 274-cm column packed with a commercially prepared packing (Applied Science Laboratories) of 3%, w/w OV-17 coated on Gas Chrom Q was used with N_2 carrier gas (60 ml/min) at 256°. Compounds were identified by comparison with GLC data from authentic compounds and by combined GLC-mass spectrometry. In the latter case an LKB 9000 mass spectrometer was used equipped with a 304 cm column packed with 0·5%, w/w OV-17 coated on Gas Chrom Q. Operating conditions were He carrier gas at 250° with molecular separators at 270°, ion source at 290° and a scan voltage of 70eV. Sterol fractions from *Brassica oleracea* f. capitata, *Sinapis alba*, *Cheiranthus cheiri* (varieties A and B), *Lepidium sativum* and *Alyssum maritimum* (Rosie O'Day) have been analysed in this way and for these cases have confirmed, as far as possible, the assignments indicated in Table 2.

Quantitative estimation of proportions of individual sterols was carried out by triangulation of peaks from the GLC traces. Two assumptions were made, the first that all sterols were eluted from the column with equal efficiency and secondly that mass response within the detector was the same for all compounds detected (see Refs. 33, 34 for detailed discussion prompting these assumptions).

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[32] S. Snogerup, *Opera Botany* **13**, 1 (1967).
[33] P. G. Simmonds and J. E. Lovelock, *Anal. Chem.* **35**, 1345 (1963).
[34] B. A. Knights in *Steroid Hormone Analysis* (edited by H. Carstensen), pp. 380–383 and references cited therein, Marcel Dekker Inc., New York (1967).