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STEROLS AND STERYL ESTERS IN SOME *BRASSICA* AND *SINAPIS* SEEDS*

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Key Word Index—*Brassica*; *Sinapis*; Cruciferae; sterols; steryl esters; brassicasterol; Δ^5-avenasterol; cholesterol; chemotaxonomy.

Abstract—The qualitative and quantitative composition of sterols in the free form and esterified to fatty acids was studied in seed oils from *Brassica napus*, *B. campestris*, *B. juncea*, *B. nigra*, *Sinapis alba* and *S. arvensis* (*Brassica kaber*). Sitosterol, followed by campesterol, predominated in both the free and the esterified sterols. The free sterols were richer in brassicasterol (*ca* 10–20%) than the steryl esters (3–10%). Small amounts of Δ^5-avenasterol and Δ^7-stigmastenol were also found in the *Brassica* oils, often more in the esterified than in the free form. The quantity of sterols was studied only in *Brassica campestris*, which had *ca* 0.3% in the free as well as in the esterified form. In *Sinapis alba*, *ca* 10% of the sterols in the free form and 20% in the esterified sterols were Δ^5-avenasterol. This compared to only a few per cent in both the free and esterified sterols in the *Brassica* oils. Similarly, *ca* 2% of cholesterol was found among the sterols of *Sinapis alba* but only traces in the *Brassica* oils. The similarity of sterol compositions among the cultivated brassicas and wild mustard (*Sinapis arvensis*), and the specific characteristics of the sterols in white mustard (*Sinapis alba*) adds further weight to the suggestion that wild mustard should be treated as *Brassica kaber* and strengthens the generic separation of *Sinapis alba*.

INTRODUCTION

Recent research in our laboratory has revealed the qualitative and quantitative composition of the free sterols and the steryl esters of *Brassica napus* [1, 2]. The separate analyses of the two sterol classes yield data which, from a food technological, nutritional and a food legislative point of view, may be more useful than the total sterol pattern. In an extension of these studies to a second very important *Brassica* oil seed crop, viz. *B. campestris*, we considered it of importance to also include samples of *B. juncea*, *B. nigra*, *Sinapis alba* and *S. arvensis* since the total sterol composition obtained after saponification of *Brassica* oils has been utilized in taxonomic considerations [3].

RESULTS AND DISCUSSION

Rape and turnip rape

The qualitative and quantitative composition of the free and esterified sterols of low-erucic acid (LEAR) cultivars of *B. campestris* (turnip rape) appeared to be very similar to those of *B. napus* (Table 1 and ref. [1]). Besides the identified sterols listed in Table 1, a component with the same relative retention time as Δ^7-avenasterol was recognised in most of the investigated samples. However, the identity of this component could not be confirmed by MS. The peak size varied between different preparative TLC samples, as well as preparations of silyl ethers of the same sterol fraction. The component has therefore been omitted from the percentage distribution figures, since further studies are necessary to establish its identity and amount in the various samples.

Although the cultivars of each species were basically similar, there were some interesting inter-cultivar differences in each of the two *Brassica* species. An obvious difference in the content of Δ^5-avenasterol among the two cultivars of *B. napus* was found. Since the sterols have been suggested to play a role as precursors to plant hormones [4], we chose one extremely early maturing (WW 77-2902) and one extremely late maturing (WW 77-3185) genetic line of so called 'double-low' summer rape as the object of study. The markedly higher Δ^5-avenasterol content in line WW-3185 (6.6% vs 0.8% in free sterols and 5.1% vs 2.5% in the esterified fraction) was certainly beyond experimental error. The high Δ^5-avenasterol in WW 77-3185 was balanced mainly by a lower sitosterol percentage. Although no relationship between the sterol composition and the physiology of the plant can be drawn from these two analyses, it should be noted that Δ^5-avenasterol has been found to be the immediate precursor to sitosterol in higher plants [5]. Δ^5-Avenasterol occurred also at a high concentration in dry rape seeds, but 2 weeks after germination it had almost disappeared [6]. In a previous study on many cultivars of LEAR and 'double-low' *B. napus* [1], we reported only traces of Δ^5-avenasterol. However, levels from zero to 5.7% of Δ^5-avenasterol have been reported from studies of the total sterols of this species [7–11]. The percentages of brassicasterol, campesterol and sitosterol of the two samples of summer rape seed reported in this paper are similar to those reported earlier [1].

The cultivars and lines of *B. campestris* investigated obviously did not display any marked difference between summer or winter types. In both summer and winter types

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Table 1. The free sterols and esterified sterols of some seeds of *Brassica* and *Sinapis* species

	Supplier and cultivar or line	Sterol composition (%)*											
		Cholesterol		Brassica-sterol		Campesterol		Sitosterol		Δ^5-Avena-sterol		Δ^7-Stigma-stenol	
		F†	E†	F	E	F	E	F	E	F	E	F	E
Brassica napus													
Summer rape	WW 77 2902	Tr	–	14.1	6.5	31.0	37.9	54.1	52.2	0.8	2.5	Tr	0.9
Summer rape	WW 77 3185	Tr	–	15.7	6.4	31.6	39.8	46.1	48.6	6.6	5.1	Tr	Tr
B. campestris													
Winter turnip rape	Sv 76 15069	Tr	–	12.7	5.5	28.2	36.3	58.0	52.6	1.1	5.6	Tr	–
Winter turnip rape	Sv 01030	Tr	–	13.8	6.1	28.8	37.1	55.8	52.0	1.6	4.7	Tr	Tr
Winter turnip rape	WW 78 2849	Tr	–	20.9	8.1	22.2	30.8	56.9	56.4	Tr	4.6	Tr	Tr
Summer turnip rape	CDA Span	Tr	–	12.8	4.8	26.7	35.2	60.5	57.3	Tr	2.7	Tr	–
Summer turnip rape	Sv 74 10105	Tr	–	13.8	4.2	26.6	34.0	59.6	57.8	Tr	3.1	Tr	0.7
Summer turnip rape	Sv 75 10223	Tr	–	10.4	4.8	29.0	38.8	60.6	53.4	Tr	2.9	Tr	Tr
Summer turnip rape	WW 77 5009	Tr	–	17.1	6.6	27.4	37.5	54.5	51.5	1.0	4.4	Tr	Tr
B. juncea	Sv	Tr	–	19.2	9.1	23.6	34.0	57.2	55.2	–	1.7	–	Tr
B. nigra	Sv	Tr	–	19.3	9.5	22.8	33.6	57.9	56.2	Tr	0.7	–	Tr
Sinapis alba	Sv Savor	2.0	2.3	10.2	2.6	24.5	32.7	52.0	41.7	11.3	20.7	–	–
Sinapis alba	Sv	2.4	1.2	11.4	3.4	25.3	34.2	48.4	44.4	12.5	16.8	–	–
S. arvensis (*Brassica kaber*)	SSTCI	–	Tr	7.8	5.9	25.8	32.5	66.4	56.1	–	4.4	–	1.1

* Traces of stigmasterol were observed in several but not all samples. Also, traces of a component with the RR_t of Δ^7-avenasterol were noted in a few free sterol samples. A component with the same RR_t as Δ^7-avenasterol was observed in many esterified sterol samples at levels from traces up to a few per cent.

† F = Free sterols, E = esterified sterols.

there was a much higher content of brassicasterol in the free than in the esterified form, whereas the Δ^5-avenasterol percentage was higher in the esterified form (Table 1). Attention should be given to these sterols since the former is used among the identity characteristics for low-erucic acid rapeseed oil [12] and the latter might be of technological significance if appearing in larger amounts (see below).

To the best of our knowledge, this is the first data on the patterns of the free and the esterified sterols of *Brassica campestris*. However, comparisons can be made to literature data on the total sterol compositions of this species obtained after saponification of the oil. It should also be noted that since *B. napus* and *B. campestris* are handled as one crop by the vegetable oil industry, data on commercial rapeseed oil could refer to either *B. napus*, *B. campestris*, or a mixture of the two [13]. Recent analyses of the sterols of specified Canadian cultivars of *B. napus* and *B. campestris* indicate similar sterol compositions for rapeseed (*B. napus*) and turnip rapeseed (*B. campestris*) [14]. Also data by Ingram *et al.* [6] on the sterols of *B. campestris* var. rapifera (in their text named *B. rapa*) are rather similar to our data on *B. campestris* var. oleifera, but the British authors noted very high total brassicasterol content in one sample.

It has earlier been suggested that *Brassica napus* should be placed in a taxonomic group separate from the other brassicas based on the presence of stigmasterol in *B. napus* but not in the other species [3]. The data in Table 1 on *B. campestris* and *B. napus*, together with previous data on *B. napus* [1], give no support for this.

The overall general similarity of the sterol compositions of *B. napus* and *B. campestris*, regardless of whether summer or winter type [1], place [1] and year of cultivation [1, 2] is of great interest to plant breeders as well as the edible oil industry. It should also be valuable for the Fats and Oils Committee of the Codex Alimentarius Commission, who have suggested the use of ranges in sterol composition among the identity characteristics for vegetable oils [15]. Other studies have indicated very small variations in the brassicasterol content between 'classical' high-erucic acid and the new low-erucic acid cultivars, although a trend towards lower brassicasterol content in the low erucic-acid types has been seen [14, 16–18]. We calculated the 'total' sterol composition and found the brassicasterol content to be 11.5–14.2% for low-erucic acid *B. napus* [1] and 8.6–9.5% for low-erucic acid *B. campestris* (from data in Tables 1 and 2).

The content of free sterols and steryl esters was determined for only one sample of the winter type and for one of the summer types of *B. campestris* (Table 2). Comparing these to previous data on *B. napus*, it would appear that the content of free sterols was similar, *ca* 0.3% of the oil. The esterified sterols on the other hand were definitely lower in *B. campestris* than in *B. napus*, *ca* 0.4% compared to *ca* 0.6%, respectively. The figures for total sterols were similar to those recently reported for *Brassica* oils [14, 18].

Mustard seed (B. juncea, B. nigra, Sinapis alba, S. arvensis)

There is considerable confusion and misunderstanding regarding the proper identification of seeds usually named

Table 2. Content of free sterols, esterified sterols and steryl esters* in oils from *Brassica campestris* and *B. napus* seeds

Species	Cultivar	% of the oil		
		Free sterols	Esterified sterols	Calculated steryl esters
B. campestris				
Winter type	Sv 76-15069	0.27	0.36	0.6
Summer type	CDA Span	0.35	0.25	0.4
B. napus				
Winter type†	Sv Brink	0.31	0.51	0.9
Summer type†	WW Olga	0.36	0.71	1.2

* Assuming that the esters are all sitosteryl linoleate, cf. ref. [1].
† From ref. [1].

'mustard'. *Brassica juncea* which is grown both as a condiment and as an oilseed crop (mainly in India and China) is called brown, oriental or Indian mustard. *Brassica nigra* is used only as a condiment and is called black mustard, true mustard or table mustard [19]. The qualitative and quantitative composition of the free, as well as the esterified, sterols of *B. juncea* and *B. nigra* were rather similar and generally resembled those of *B. napus* and *B. campestris* (Table 1). However the two *Brassica* mustards were found to be high in brassica-sterol both in the free and esterified form (19 and 9%, respectively). They were very low (*ca* 1%) in Δ^5-avenasterol in the steryl esters.

Another mustard species of commercial interest is *Sinapis alba* (sometimes named *B. hirta* or *B. alba* [13, 19]) which is generally used as a condiment under the trade names of white or yellow mustard. However, this species is also used to a small extent as an oilseed crop with quality characteristics slightly different from those of rapeseed oil [13]. *S. alba* had a composition of free sterols and esterified sterols which was considerably different from those of rape and turnip rape, brown and black mustard. It had a remarkably high content of Δ^5-avenasterol, *ca* 12% in the free sterols and *ca* 19% in the esterified sterols (Table 1). Further, there was *ca* 2% cholesterol among the free sterols and 1–2% in the esterified sterols. The percentage of campesterol was about the same in *S. alba* as in the *Brassica* species, but the percentage of brassicasterol and sitosterol were generally lower than in the brassicas.

Generally, wild mustard or charlock is not grown as a crop but is a prolific weed [19, 20]. It is sometimes utilized as a condiment substitute since it has a volatile oil which is similar to black mustard [10]. Its common systematic name is *Sinapis arvensis* L. but an alternative name is *B. kaber* (DC) L. Wheeler. It was obvious that the sterol patterns of *S. arvensis* do not have the characteristics of *S. alba*, but resembled those of the brassica mustards (Table 1). This observation was in agreement with the compositions of the total sterols after saponification of the oils reported by British scientists [3, 6], except that they reported some cholesterol to be present among the total sterols of the *S. arvensis* species. Our sterol data (Table 1) and those of the previous British study add further support to the generic status of *S. alba* and strongly contradict the name of *B. hirta* for white or yellow mustard. Similarly wild mustard or charlock should not be named *S. arvensis*. In agreement with accumulated data for hybridization experiments [21–23] and analyses of the phenol patterns in various organs of *Brassica* and *Sinapis* species (B. Mattson, personal communication), the alternative name *B. kaber* Boissier [23] appears most appropriate.

In view of the aforementioned confusion regarding 'mustard oil', literature data on the sterols of mustard oil must be interpreted with caution. However, the results from an Italian study of the total sterols after saponification of 'black' mustard and 'white' mustard are in good agreement with our data [24]. As regards other literature data on the sterols of mustard seed, the low percentages of cholesterol and Δ^5-avenasterol noted in American [25] and Japanese [26], as well as German studies [27], clearly indicate that their samples were mainly from *B. juncea*, the major source for the commodity sold as 'mustard oil'.

The Fats and Oils Committee of the Codex Alimentarius Commission has suggested the use of sterol compositions as markers of commercial vegetable oils [15]. They propose ranges of 5.2–11.8% brassicasterol in mustard oil compared to 4.2–19.6 in rapeseed oil, tr–12.8% Δ^5-avenasterol in mustard oil vs tr–5.7% in rapeseed oil and $<2.7\%$ cholesterol in rapeseed compared to tr–2.7% in mustard, all established by use of data from OV-17 columns. It should be noted that sterol data from GLC analyses on packed SE-30 columns or other very non-polar columns lack figures for Δ^5-avenasterol because of peak overlapping with sitosterol. Therefore, only results from analyses on OV-17 columns or columns of similar polarity yielding data on Δ^5-avenasterol and Δ^7-sterols are relevant in these discussions.

The presence of small amounts of cholesterol in white mustard seed oil, compared to traces in *Brassica* seed oils, is considered of no practical interest. However, the substantial levels of Δ^5-avenasterol in white mustard seed oil could be of interest from a food technological point of view. It is known that white mustard seed oil has a better stability towards oxidation than rapeseed oil [28]. Since their linoleic and linolenic acid contents are very similar [13], other natural constituents appear to be involved. The high Δ^5-avenasterol is of interest since it has been shown to delay or minimize darkening and polymerization of vegetable oils during heating [29–31]. Whether this sterol is active as an anti-oxidant also at room temperature remains to be investigated. It should also be noted that the content of tocopherols is generally similar (*ca* 0.08%) in rapeseed oil and white mustard seed oil. However, the percentage of α-tocopherol, which is more

powerful as an anti-oxidant than α-tocopherol, is considerably higher in white mustard seed oil than in rapeseed oil [13].

EXPERIMENTAL

Seed samples were from the Swedish Seed Association, Svalöv, Sweden (samples marked Sv in Table 1), from the Weibullsholm Plant Breeding Institute, Landskrona, Sweden (samples marked WW) and from the Swedish Seed Testing and Certification Institute, Lund (sample marked SSTCI). *Brassica napus* L. var. oleifera was of the so-called double-low type, which means very low in erucic acid content (*ca* 1% vs typically 40–50% of the oil) and with strongly reduced levels of glucosinolates in the seeds. Line WW 77-2902 was extremely early maturing and line WW 77-3185 was very late. *B. campestris* L. var. oleifera had normal levels of seed glucosinolates but reduced levels of erucic acid in the oil (*ca* 5% vs typically 30–50%). *B. juncea* Coss and *B. nigra* (L.) Koch were of an unknown cultivar, but were harvested from plots at Svalöv and consequently checked for species identity. *Sinapis alba* L. One registered cultivar, Sv Savor, and one genetic line selected for high oil content were used. All the seed samples marked Sv and WW were carefully selected, pure samples which were analysed within some months from the harvest in 1978. *S. arvensis* L. (*B. kaber* (DC) L. Wheeler) was gathered as weed seed in the inspection of samples of *B. napus* and *B. campestris* submitted for approval as certified seeds by the SSTCI.

Extraction of dry seeds was performed with hexane–EtOH (3:1) as presented earlier [1]. TLC of the lipids was performed on Si gel plates (Anasil H, 0.5 mm, from Analabs, Inc.) with hexane–Et_2O–HOAc (70:30:1). After development, a reference lane was sprayed with dichlorofluorescein (0.025% in EtOH), and the sterols and steryl esters were located under UV-light (365 mm). The corresponding areas from the actual sample were quickly removed from the plate, and the sterols and the steryl esters were eluted from the Si gel with Et_2O. When quantifications were made, *ca* 10% cholesterol was added as an internal standard to a duplicate sample. Steryl esters (~1 mg) were refluxed for 2–3 hr in 5 ml 1 M methanolic NaOH. The soln was then acidified with conc HCl, the sterols and the free fatty acids were extracted with hexane (2 × 2 ml) and separated in the same TLC system as previously described.

GLC of the sterols was undertaken after silylation by heating with Tri-Sil at 60° for 45 min. The silyl ethers were analysed at 270°, on 180 cm × 2 mm glass columns packed with 3% OV-1 and 1% OV-17, respectively [1].

The TMS-sterols were identified by comparison of the R_fs with those of known standards and by GC–MS. Possible differences in GLC response for different sterols have been ignored in the calculation by direct normalization.

Combined GC–MS was performed using a glass column filled with 1% OV-17. Details have been previously presented [1].

Sterol nomenclature used in this report is brassicasterol = ergosta-5,22-dien-3β-ol; Δ^5-avenasterol = stigmasta-5,-*Z*24(28)-dien-3β-ol; Δ^7-avenasterol = 5α-stigmasta-7,*Z*24(28)-dien-3β-ol; Δ^7-stigmasterol = 5α-stigmast-7-en-3β-ol.

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