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THE STEROLS OF *NIGELLA SATIVA* SEED OIL

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Key Word Index—*Nigella sativa*; Ranunculaceae; free sterols; steryl esters; steryl glucosides; acylated steryl glucosides.

Abstract—Free sterols, steryl esters, steryl glucosides and acylated steryl glucosides were isolated from *Nigella sativa* seed oil and their components identified. Steryl glucosides comprised the major sterol form. Steryl esters appeared relatively richer in 4-methylsterols and triterpene alcohols than free sterols. Δ^7-Sterols were present only in the steryl ester fraction which was also richer in obfusifoliol (Δ^8-4α-methylsterol). The total fatty acids of the seed oil appeared richer in unsaturated acids than those of the acylated steryl glucosides and the steryl esters.

INTRODUCTION

Nigella sativa is a dicotyledon of the Ranunculaceae family which grows mainly in the areas around the Mediterranean. In this investigation the four sterol forms of *N. sativa* seed oil, free sterols (FS), steryl esters (SE), steryl glucosides (SG) and acylated steryl glucosides (ASG) were isolated. The 4-desmethyl-, and 4-methylsterols and the triterpene alcohols, which were isolated as free alcohols or which resulted from hydrolysis of the SE, SG and ASG were determined and identified. The fatty acids and sugars from hydrolysis of the SE, SG and ASG were also analysed. Some 4-desmethylsterols from *N. sativa* were described previously [1].

RESULTS AND DISCUSSION

Acetone extraction of the dry seeds of *N. sativa* yielded 38% w/w total lipids. The sterol fractions isolated from *N. sativa* seed oil are presented in Table 1. The steryl glucosides comprise the major sterol form, their sterol part accounting for 52.3% of total sterols (29.3% ASG and 23% SG). The FS account for 36.7% of the total followed by the sterols of the SE (11.0%). Potato tubers are another example where steryl glucosides are the major form [2]. The SE in *N. sativa* seed oil appear relatively richer in 4-methylsterols and triterpene alcohols than FS. Only 4-desmethylsterols were recovered from SG and ASG in accordance with other reports [3].

The 4-desmethylsterols from the four sterol fractions and the 4-methylsterols and triterpene alcohols from the FS and SE fractions were analysed by GLC and then acetylated and fractionated by argentation TLC. The resulting steryl acetates were analysed by GLC and combined GC-MS. The sterol components were identified by their RR_f and RR_t values relative to cholesteryl acetate, ΔR_{AC} values (RR_t of acetate/RR_t of alcohol), mass spectra and literature data [4–7] (Table 2).

Qualitative differences between the free and esterified 4-desmethylsterols concerned mainly the presence of Δ^7-sterols (Δ^7-stigmastenol and Δ^7-avenasterol) and of saturated sterols (campestanol and stigmastanol) only in the SE. In crude soyabean oil Δ^7-stigmastenol was also found only in the SE [8] and in sunflower oil, which was characterized by a relatively high percentage of Δ^7-sterols. Δ^7-Stigmastenol and Δ^7-avenasterol amounted to 22% in the SE while in the FS they were only 9% [9]. Concerning quantitative differences, the SE appeared poorer in stigmasterol and richer in Δ^5-avenasterol. Similar differences were reported in the literature for poppy seed oil, where the percentage of Δ^5-avenasterol was much higher in the SE [9], and for maize seedlings where the proportion of stigmasterol varied from SE (13–17%) to FS (20–30%) [10].

Comparison of the 4-methylsterols isolated from the FS and the SE showed that the ratio of obtusifoliol to citrostadienol was higher (3.8) in the SE than in the free 4-methylsterols (0.9). No appreciable differences were observed between the free and esterified triterpene alcohol fractions.

The fatty acid compositions of *N. sativa* seed oil, SE and ASG are shown in Table 3. There is a progressive increase

Table 1. Sterol fractions from *N. sativa* seed oil

Fraction		Mg of compound per 100 g seed oil
FS	4-Desmethylsterols	40.0
	4-Methylsterols	4.5
	Triterpene alcohols	8.2
SE	4-Desmethylsterols	2.6
	4-Methylsterols	3.7
	Triterpene alcohols	9.5
ASG	4-Desmethylsterols	42.0
SG	4-Desmethylsterols	33.0

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Table 2. Sterols and triterpenes in *N. sativa* seed oil

Acetate	RR_f* OV-17	ΔR_{Ac}†	Percentage composition FS	SE	SG	ASG
4-Desmethylsterols						
Cholesterol	1.00	1.33	0.6	1.1	0.2	0.3
Campesterol	1.32	1.33	11.9	15.2	9.6	10.9
Campestanol	1.34		—		—	—
Stigmasterol	1.44	1.32	20.8	8.8	18.5	16.1
Sitosterol	1.66	1.33	61.8	60.0	69.9	72.7
Stigmastanol	1.69		—		—	—
Δ^5-Avenasterol	1.84	1.32	4.9	11.7	1.8	tr
Δ^7-Stigmastenol	1.96	1.34	—	tr	—	—
Δ^7-Avenasterol	2.19	1.33	—	3.2	—	—
4-Methylsterols						
Lophenol	1.34	1.30	—	tr		
Obtusifoliol	1.48	1.29	26.0	40.0		
24-Methyllophenol	1.77	1.30				
Cycloeucalenol	1.77	1.30	45.6	48.2		
Gramisterol	1.77	1.30				
24-Ethyllophenol	2.15	1.30	—	1.3		
Citrostadienol	2.40	1.30	28.4	10.5		
Triterpene alcohols						
Tirucallol	1.47	1.21	tr	tr		
Taraxerol	1.57	1.19	1.3	1.0		
β-Amyrin	1.66	1.19	7.1	3.2		
Butyrospermol	1.70	1.18				
Cycloartenol	1.88	1.23	39.6	31.6		
Unidentified	1.96	1.20	52.0	64.2		
24-Methylenecycloartanol	2.09	1.22				

*Relative to cholesteryl acetate.
†RR_f of acetate/RR_f of alcohol.

Table 3. Fatty acids in *N. sativa* seed oil

Methyl ester	Percentage composition Seed oil	SE	ASG
Myristic acid	tr	tr	tr
Palmitic acid	14.3	22.0	36.5
Palmitoleic acid	3.0	3.7	2.0
Stearic acid	2.7	6.7	8.8
Oleic acid	20.7	25.3	28.4
Linoleic acid	55.8	39.0	19.0
Linolenic acid	0.6	1.8	2.3
Arachidic acid	2.9	1.5	3.0

of the proportion of saturated fatty acids from the seed oil total fatty acids (20% of total acids) to SE (30%) and ASG (48%). Similar results were reported in the literature for other plants and vegetable fats, but were not evaluated [9, 11, 12]. D-Glucose was found to be the only sugar in the glucosidic sterols. This is similar to other plants and plant products [3].

EXPERIMENTAL

Extraction and fractionation. The seed oil was extracted from 150 g dried and pulverized seeds in a Soxhlet apparatus with 700 ml Me_2CO for 24 hr and fractionated by CC and TLC into SE, FS, SG and ASG as described previously [13].

Hydrolysis of SE, ASG and SG and analysis of fatty acid methyl esters and sugars. Each of the esterified and the glucosidic fractions was hydrolysed [13] and the fatty acid methyl esters were analysed by GLC, dual FID, N_2 at 30 ml/min, isothermal 180°, 1.8 m × 3 mm packed with 10% EGSS-X. Qualitative analysis of sugar was performed by silica gel–H_3BO_3 TLC [13].

Fractionation, determination and analysis of sterols and triterpenes. Each of the free alcohol fractions was separated by TLC into 4-desmethyl- and 4-methylsterols and triterpene alcohols which were determined by Thin Layer-Flame Ionization Detector Chromatography, Iatroscan TH-10, Iatron Lab., Inc. [13] and analysed by GLC, dual FID, N_2 at 30 ml/min, isothermal 255°, 1.8 m × 3 mm glass column packed with 3% OV-17. Steryl acetates were prepared and separated by $AgNO_3$–silica gel TLC [5]. The recovered fractions were analysed by GLC (3% OV-17) and combined GS-MS, 78 eV, 25 m, glan WCOT SE-54, isothermal 200°, split ratio 1:10.

The 4-desmethylsteryl acetates from the FS, the SG and the ASG were each separated into three major bands upon argentation TLC. The least polar band-1 (RR_f 1.21) contained cholesteryl, campesteryl and sitosteryl acetates. Band-2 (RR_f 0.86) yielded stigmasteryl acetate, and band-3 (RR_f 0.18) 24-

ethylidenecholesteryl acetate (Δ^5-avenasteryl acetate). Fractionation of the 4-desmethylsteryl acetates from the SE afforded five major bands. Band-1 (RR_f 1.32) contained campestanyl and stigmastanyl acetates. Band-2 (RR_f 1.21) contained cholesteryl, campesteryl and sitosteryl acetates, and Δ^7-stigmastenyl acetate; MS *m/z* (rel. int.): 456 $[M]^+$ (100), 441 (13), 396 $[M-HOAc]^+$ (21), 381 (20), 315 $[M-$side chain$]^+$ (14), 288 $[M-$side chain $-C_3H_5]$ (17), 273 (13), 255 (99), 228 (42), 213 (62). Band-3 (RR_f 0.89) contained stigmasteryl acetate and band-4 (RR_f 0.20) yielded an unidentified component (RR_f 1.64), Δ^5-avenasteryl and Δ^7-avenasteryl acetates. Band-5 (RR_f 0.11) contained two components (RR_t 1.34 and 1.61) which remained unidentified.

The 4-methylsteryl acetates from the FS were separated into four major bands upon argentation TLC. Band-1 (RR_f 1.11) yielded an unidentified component (RR_t 1.74). Band-2 (RR_f 0.38) contained citrostadienyl acetate, band-3 (RR_f 0.32) obtusifoliyl and cycloeucalenyl acetates, and band-4 (RR_f 0.12) gramisteryl acetate. The 4-methylsteryl acetates from the SE were fractionated also into four major bands. Band-1 (RR_f 1.16) contained lophenyl, 24-methyl-, and 24-ethyllophenyl acetates. Otherwise, the bands contained the same components as in the case of the FS.

Fractionation of the triterpene alcohol acetates (from the FS and the SE) and argentation TLC resulted in four major bands. Band-1 (RR_f 1.42) contained β-amyrin acetate. Band-2 (RR_f 0.84) probably contained a 3-ketone, fridelan-3-one [7]. Band-3 (RR_f 0.76) contained tirucallyl acetate; MS *m/z* (rel. int.): 468 $[M]^+$ (21), 453 (62), 393 $[M-HOAc-Me]^+$ (100), 355 $[M-$side chain $-2H]^+$ (5), 297 (4), 295 (10), 255 (10), 241 (35), taraxeryl acetate, MS *m/z* (rel. int.): 468 $[M]^+$, 453, 408 $[M-HOAc]^+$, 344 (17), 329 (9), 269 (13), 228 (37), 204 (100), and cycloartenyl acetate. Band-4 (RR_f 0.42) contained butyrospermyl acetate; MS *m/z* (rel. int.): 468 $[M]^+$ (33), 453 (82), 393 $[M-HOAc-Me]^+$ (100), 355 $[M-$side chain $-2H]^+$ (11), 297 (15), 295 (11), 255 (21), 241 (32), an unidentified component (RR_t 1.96, ΔR_{AC} 1.20) and 24-methylenecycloartanyl acetate.

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