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Gas chromatographic determination of sterols in fat-soluble concentrates obtained from plant materials

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ABSTRACT

Sterols in plant materials were indentified by gas chromatography using silicone stationary phases (SE-30, OV-17, etc.) and also as their trimethylsilyl and acetyl derivatives. Mainly β-sitosterol was found in all concentrates, with smaller amounts of campesterol, stigmasterol, Δ^7-stigmasterol and Δ^7-avenasterol. Quantitative analysis was performed by purification by column liquid chromatography, extraction of the sterols with eluents of increasing polarity and determination on a column packed with 3% SE-30 at 250°C. Cholesterol was used as the internal standard.

INTRODUCTION

The determination of this composition of phytosterols present in fat-soluble concentrates obtained as a result of the complete utilization of plant materials is of importance for their application as sources of biologically active substances in the cosmetic and pharmaceutical industries[1–3]. Methods involving gas chromatography[2–11], high-performance liquid chromatography[2,12,13] and spectrophotometry[2,14,15] are the most widely used for the determination of sterols in plant materials. Gas chromatographic methods, with high resolution and sensitivity, are usually preferred.

The aim of this work was to study the qualitative and quantitative composition of phytosterols in fat-soluble concentrates obtained from plant materials using gas–liquid chromatography (GLC).

EXPERIMENTAL

Reagents

Different batches of fat-soluble concentrates were studied: nettle (*Urtica dioica* L.), venetian sumach (*Cotinus coggygria* Scop.), pine (*Pinus silvestria* L.) and *Stellaria media* (L.) Vill. obtained in the periode 1986–1988. Standard solutions of concentration 1 mg/ml were prepared of campesterol, stigmasterol and β-sitosterol in hexane and of cholesterol (internal standard) in hexane–diethyl ether (3:1).

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Silica gel G (Merck) was used in thin-layer chromatography (TLC) and Florisil (Fluka), Fuller's earth (Bulgaria) and bentonite (deposit near Kardjali, Bulgaria) in column chromatography.

Separation of phytosterols by thin-layer chromatography

A 0.5-g sample of concentrate was dissolved in 2 ml of hexane and an aliquot (100–200 μl) was separated on a silica gel G plate with light petroleum (b.p.40–60°C)–acetone (10:1). A standard solution of β-sitosterol or campesterol (20 μl) was used as the marker. On spraying with methanol, the sterols were revealed as a white band on the wet plate. The sterols were extracted with 3 × 15 ml of hexane–diethyl ether (3:1). A standard solution of cholesterol (1 ml) was added to the combined extracts, the mixture was evaporated to dryness, the residue was dissolved in 2.5 ml of hexane and 1 μl was subjected to GLC.

Separation of phytosterols by column chromatography

A 0.1–0.2-g sample of concentrate was dissolved in 2 ml of hexane and passed through a column (1 cm I.D.) containing 10 g of adsorbent (Florisil, bleach earth or bentonite). Elution was effected using solutions of increasing polarity: hexane (60 ml), hexane–diethyl ether (9:1) (90 ml) and hexane–diethyl ether (3:1) (90 ml). The sterols were extracted in the last solvent, 4 ml of a standard solution of cholesterol were added to eluate and the mixture was evaporated to dryness. The residue was dissolved in 10 ml of hexane and 1 μl was subjected to GLC.

GLC determination of sterols

A Pye Unicam Series 304 (Philips) gas chromatograph was used, equipped with columns (1.5 m × 4 mm I.D.) containing 3% SE-30, OV-17 or 2.5% QF-1 + 5% DC-200 as the stationary and a flame ionization detector. The column temperature was 250°C, the injection port temperature 270°C and the detector temperature 290°C and the carrier gas (nitrogen) flow-rate was 32–35 ml/min.

The trimethylsilyl ethers (TMSE) and the acetyl esters (AE) of the sterols were prepared[4,6,10] and were chromatographed under the same conditions.

RESULTS AND DISCUSSION

The fat-soluble concentrates of nettle, sumach, stellaria and pine are, in fact, concentrates of the unsaponifiables present in the raw materials. In order to determine their sterol contents the most often recommended methode are TLC and column liquid chromatography, and also electrophoresis[2,7,16]. In this work, the sterols were succesfully separated by TLC on silica gel G, using methanol and iodine vapour for detection. Fig. 1 shows a chromatogram for a nettle concentrate.

The types and amounts of phytosterols after TLC separation were determined by GLC on columns containing silicone stationary phases of different polarity : 3% SE-30, OV-17 and 2.5% QF-1 + 5% DC-200. Qualitative identification was achieved by comparing the relative retention times of campesterol, stigmasterol and β-sitosterol with respect to cholesterol (internal standard) obtained from the chromatogram of a standard mixture. Δ^5-avenasterol, Δ^7-avenasterol and Δ^7-stigmastenol were determined on the basis of data in the literature[6,8,11]. Table I gives the measured retention data.

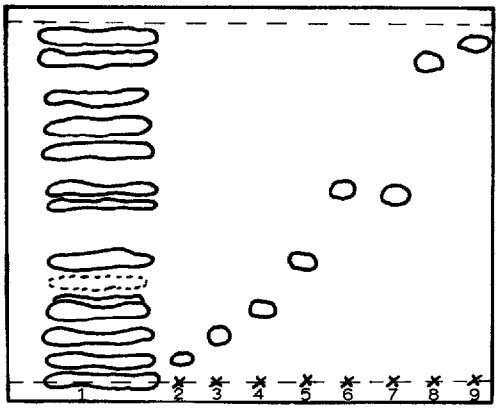

Fig. 1. TLC on silica gel G of a fat-soluble nettle concentrate using light petroleum–acetone (10:1) as the solvent and iodine vapour as spray reagent. Legend 1 = Nettle concentrate; 2 = xanthophyll of green leaves; 3 = β-sitosterol; 4 = stearyl alcohol; 5 = α-tocopherol; 6 = isopropyl stearate; 7 = isopropyl palmitate; 8 = β-carotene (from carrots); 9 = squalene.

TABLE I

RETENTION TIME (t_r') AND RELATIVE RETENTION TIMES WITH RESPECT TO CHOLESTEROL (r) OF FREE STEROLS IN NETTLE CONCENTRATE OBTAINED ON DIFFERENT STATIONARY PHASES.

Sterols	*3% SE-30*		*OV-17*		*2.5% F-1 + 5% DC-200*	
	t_r' *(min)*	r	t_r' *(min)*	r	t_r' *(min)*	r
Cholesterol	16.05	1.00	20.68	1.00	16.50	1.00
Campesterol	20.81	1.30	26.50	1.28	21.39	1.30
Stigmasterol	21.82	1.36	28.55	1.38	22.40	1.36
β-Sitosterol	25.70	1.58	32.68	1.58	25.40	1.54
Δ^7-Stigmastenol	29.10	1.82	40.12	1.94	29.70	1.80
Δ^7-Avenasterol	34.35	2.14	45.08	2.18	33.00	2.00

TABLE II

RETENTION TIMES (t_r') AND RELATIVE RETENTION TIMES WITH RESPECT TO CHOLESTEROL (r) OF FREE STEROLS AND THEIR DERIVATIVES IN NETTLE CONCENTRATES ON 3% SE-30 STATIONARY PHASE

Sterols	*Free*		*Trimethylsilyl ethers*		*Acetyl esters*	
	t_r' *(min)*	r	t_r' *(min)*	r	t_r' *(min)*	r
Cholesterol	16.05	1.00	20.05	1.00	24.18	1.00
Campesterol	20.18	1.30	25.25	1.26	31.45	1.30
Stigmasterol	21.82	1.36	27.46	1.37	32.88	1.36
β-Sitosterol	25.70	1.58	31.00	1.55	38.20	1.58
Δ^7-Stigmastenol	29.10	1.82	35.29	1.76	43.70	1.81
Δ^7-Avenasterol	34.56	2.15	43.90	2.19	51.50	2.13

TABLE III

RECOVERY OF β-SITOSTEROL AFTER TLC AND COLUMN LIQUID CHROMATOGRAPHIC (LC) SEPARATION

Raw material	*β-Sitosterol (%)*						*Recovery (%) after*	
	Determined after		*Expected quantity after 2% addition*		*Found after*			
	TLC	*LC*	*TLC*	*LC*	*TLC*	*LC*	*TLC*	*LC*
Nettles								
1	2.85	3.14	4.85	5.14	4.10	4.84	84.54	94.16
2	3.24	3.58	5.24	5.58	4.58	5.30	87.40	94.98
3	4.16	4.30	6.16	6.30	5.25	6.05	85.23	96.03
Sumach								
1	3.20	3.55	5.20	5.55	4.33	5.20	83.27	93.69
2	4.28	4.84	6.28	6.84	5.36	6.52	85.35	95.32

Trimethylsilylated and acetylated phytosterols were chromatographed on 3% SE-30 (Table II). Column liquid chromatography was also used for the separation of the sterols. The best results were achieved on columns containing Florisil, and were also good with Fuller's earth and bentonite. The most succesful fractional elution of the different concentrates was achieved with the above mentioned combination of solvents of increasing polarity.

The contents of phytosterols in concentrates from nettle and sumach are presented in Table III. They show that the analytical recovery of sterols after TLC is about 85% and after column liquid chromatography about 95%. For this reason a combination of column liquid chromatography and GLC was used for the determina-

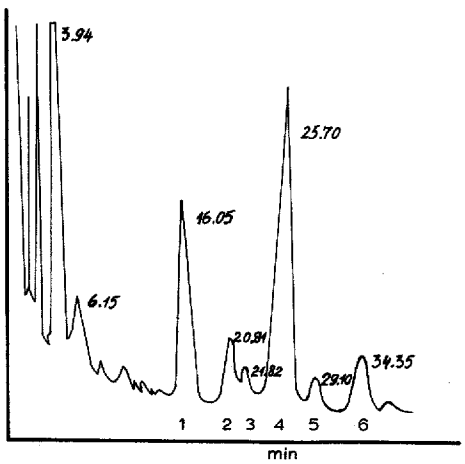

Fig. 2. Gas chromatogram of phytosterols in nettle concentrate obtained after column liquid chromatography on Florisil and GLC on an 3% SE-30 column (1.5 m × 4 mm I.D.). 1 = Cholesterol; 2 = campesterol; 3 = stigmasterol; 4 = β-sitosterol; 5 = Δ^7-stigmastenol; 6 = Δ^7-avenasterol.

TABLE IV

CONTENT OF PHYTOSTEROLS IN FAT-SOLUBLE CONCENTRATES DETERMINED BY LIQUID CHROMATOGRAPHIC SEPARATION GLC

Raw material	*Phytosterols (%)*					
	Campesterol	*Stigmasterol*	*β-Sitosterol*	*Δ^7-Stigmastenol*	*Δ^7-Avenasterol*	*Total*
Nettles						
1	0.40	0.24	2.19	Trace	0.40	3.23
2	0.40	0.24	2.50	Trace	0.42	3.66
3	0.60	0.35	0.03	Trace	0.42	4.40
4	0.60	0.20	3.14	Trace	0.50	4.44
5	0.70	0.30	4.55	Trace	0.50	6.05
Sumach						
1	0.40	0.26	2.50	Trace	0.20	3.36
2	0.64	0.28	4.20	Trace	0.25	5.37
3	0.64	0.28	3.55	Trace	0.25	4.72
4	0.67	0.30	4.84	Trace	0.25	6.06
5	0.65	0.25	5.26	Trace	0.40	6.56
Stellaria						
1	0.10	Trace	0.60	–	Trace	0.70
2	0.25	0.10	1.20	–	Trace	1.55
3	0.20	Trace	0.82	–	Trace	1.02
Pine						
1	0.20	Trace	0.58	–	Trace	0.78
2	0.20	Trace	0.60	–	Trace	0.80
3	0.25	Trace	1.50	–	Trace	1.75

tion of sterols in the different fat-soluble concentrates, despite the longer time required. Fig. 2 shows a chromatogram of the sterols in a concentrate of nettle.

Results for the contents of sterols in the different types of concentrates are given in Table IV. They indicate that the nettle and sumach concentrates are richer in sterols than those of stellaria and pine. The major sterol in all samples is β-sitosterol, its concentration varying from 67.8 to 75.2% in nettle, from 74.4 tot 80.2% in sumach, from 77.4 to 85.7% in stellaria and from 74.4 tot 85.7% in pine concentrates. The total concentration of the other sterols (campesterol, stigmasterol, Δ^7-avenasterol and Δ^7-stigmastenol) is between 14.3 and 32.2% of the total quantity of sterols.

CONCLUSIONS

A method has been developed for the determination of phytosterols in fat-soluble concentrates obtained from different plants, involving purification by column liquid chromatography and subsequent GLC determination with a recovery of about 95%. A relatively high concentration of phytosterols was found in concentrates from nettle and sumach (3.2–6.6%), which justifies their use as sterol sources for the production of various cosmetic and pharmaceutical preparations.

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