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FREE AND BOUND STEROLS IN SEEDLINGS OF *CUCUMIS SATIVUS*

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Abstract—Free sterols, sterol esters, sterol monoglucosides and sterol acylmonoglucosides have been obtained from 10 days old seedlings of *Cucumis sativus*. Free sterols and sterol esters consist mainly of Δ^7 di- and triunsaturated sterols, whereas Δ^7 mono-unsaturated and Δ^5 mono- and diunsaturated sterols predominate in the glucosides and acylglucosides. Both acetates and derivatives of higher fatty acids, mainly linoleic and linolenic acids, have been found in the sterol esters. Sterol acylglucosides contain mostly saturated fatty acids, palmitic and stearic acids being the main components.

INTRODUCTION

THERE are some uncertainties about the sterols of *Cucumis sativus*. Sucrow and Reimerdes[1] analyzed the non-saponifiable lipid fraction from the seeds and found, as in certain other members of the Cucurbitaceae, mostly Δ^7 sterols (stigmasta-7,22-dien-3β-ol, stigmasta-7,25-dien-3β-ol and stigmasta-7,22,25-trien-3β-ol) accompanied by small quantities of some unidentified Δ^5-sterols. On the other hand, Tunmann and Frank[2,3] who analyzed both fruits and unripe seeds reported that the non-saponifiable lipid fraction contained only sitosterol,[2] whereas the sterol acylglucoside fraction contained a mixture of 6′-*O*-palmityl- and 6′-*O*-stearyl-3β-glucosides of sitosterol, stigmasterol and stigmastanol.[3] The incomplete nature of this data stimulated us to undertake a more detailed qualitative and quantitative reexamination of free and bound sterols in *C. sativus* seedlings.

RESULTS AND DISCUSSION

The chloroform–methanol extract from 2 kg of fresh *C. sativus* seedlings yielded, after silica gel column chromatography (see Experimental), the following crude sterol fractions: sterol esters (2·72 g) in the light petroleum–diethyl ether, 98:2 fraction; free sterols (2·82 g) in light petroleum–diethyl ether, 84:16; sterol acylmonoglucosides (0·52 g) in light petroleum–diethyl ether, 30:70; and sterol monoglucosides (0·85 g) in diethyl ether–methanol, 95:5. Fractions containing the sterol esters, free sterols and sterol acylmonoglucosides were then rechromatographed separately on alumina columns. This operation gave partly purified sterol esters (470 mg) in the light petroleum–diethyl ether, $^{98:2}$ fraction; free sterols (470 mg) in light petroleum–diethyl ether, 84:16; and sterol acylmonoglucosides

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(150 mg) in diethyl ether–methanol, 98:2. The fraction of free sterols was further purified by preparative TLC on silica gel using the solvent system 3 (see Experimental) and fraction of acylmonoglucosides in the solvent system 2.

The fraction of sterol esters chromatographed on silica gel TLC plates (system 1) formed two bands: R_f ~0·4 (12 mg)—corresponding to sitosterol acetate and R_f ~0·6 (280 mg)—corresponding to the sitosterol palmitate standard. Direct GLC comparison of the less mobile fraction with standards showed that, in fact, this fraction contains sterol acetates. Occurrence of small amounts of sterol acetates together with higher fatty acid sterol esters presents, perhaps, a more general regularity in higher plants. Similarly, in the course of our earlier studies on sterols of *Calendula officinalis* flowers[4,5] we found sterol acetates as a relatively small part of the whole sterol ester fraction.

Crude sterol monoglucosides obtained by column chromatography were further purified by crystallization from diethyl ether and repurified by TLC on silica gel in system 2 (64 mg, m.p. 285°). The product was chromatographically identical with a standard mixture of 3β-D-monoglucosides of sitosterol and stigmasterol.

The purified fractions containing bound sterols were hydrolyzed and the sterol mixtures obtained from the individual fractions were acetylated and then separated by TLC on $AgNO_3$ impregnated silica gel (system 4). Each fraction, with a known amount of cholesterol as an internal standard, was then assayed by GLC. Identification of the individual sterols was carried out by TLC and GLC comparison with authentic standards except for the $\Delta^{7,25}$, $\Delta^{7,24(28)}$ and $\Delta^{7,22,25}$ C_{29} sterols which because of lack of appropriate standards were identified on the basis of m.p. and MS alone.[6-8] The following compounds were obtained after TLC on $AgNO_3$ impregnated silica gel as homogeneous substances: 24-ethylcholesta-7,25-dien-3β-ol acetate; m.p. 150–154° (lit.[1] 149–153°), MS 454 (M^+) −45, 439 (M–Me) −46, 425 (M–C_2H_5) −3, 394 (M–acetate) −18, 379 (M–acetate–Me) −24, 313 (M-side chain–2H) −100, 288 (M-side chain–27) −12, 273 (M-side chain–part of ring D) −24, 255 (M-side chain–acetate) −56, 253 (M-side chain–2H–acetate) −4, 229 (M-side chain–27–MeCOO) −28, 213 (M-side chain–part of ring D–acetate) −62; 24-ethylcholesta-7,22,25-trien-3β-ol acetate; m.p. 174–176° (lit.[1] 169–176°), MS 452 (M^+) −82, 437 −30, 423 −40, 392 −13, 377 −13, 368 (M–C_{24-27}–H) −21, 363 (M–acetate–C_2H_5) −24, 342 (M–C_{22-27}–H) −70, 313 −100, 288 −32, 273 −18, 255 −90, 253 −24, 229 −72, 213 −61; 24-ethylcholesta—7,24(28)-dien-3β-ol acetate MS 454 (M^+) −40, 439 −22, 394 −8, 356 (M–C_{23-27}–H) −52, 313 −100, 255 −40, 229 −12, 213 −12.

The results of qualitative and quantitative sterol determinations in the individual fractions obtained are summarized in Table 1. It is apparent that both in the fraction of free sterols and in the fractions of sterol esters (both acetates and esters with higher fatty acids) C_{29} Δ^7-dienes and trienes markedly predominate (95% of total free sterols, 75% of total sterols from acetates and 71% of total sterols from higher fatty acid esters). Differences between the free sterol fraction and ester sterol fractions concern mainly the higher content of Δ^5 sterols in the latter. Sterols acetates are distinguished from free sterols and sterols esters of the higher fatty acid for having a relatively high content of C_{29} $\Delta^{7,24(28)}$ sterol. On the other hand, sterols of higher fatty acid esters and acetates differ from the free sterols

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in their C_{29} $\Delta^{7,25}$-diene content as compared to their C_{29} $\Delta^{7,22,25}$-triene content. In contrast to the free sterol fraction only small amount of C_{29} $\Delta^{7,22,25}$-triene was found in the monoglucosides. In acylmonoglucosides this sterol has not been found at all. Δ^7 monoenes and Δ^5 sterols (sitosterol, cholesterol and stigmasterol) markedly predominate in the glucoside and acylglucoside fractions.

TABLE 1. STEROL COMPONENTS OF THE SEEDLINGS OF *Cucumis sativus* L.

Sterol component	R_f* (acetate)	R_t† (acetate)	Concentration (μg per 100 g fr. wt) Free sterols	Acetates	Higher fatty acid esters	Monoglucosides	Acylmonoglucosides
C_{27}, Δ^0		1·38	—	—	trace	trace	—
C_{28}, Δ^0	0·84	1·69	—	—	1	25	—
C_{29}, Δ^0		2·11	trace	—	3	67	—
C_{27}, Δ^7		1·43	—	—	—	930	85
C_{28}, Δ^7	0·80	1·77	—	—	—	trace	—
C_{29}, Δ^7		2·34	15	—	7	212	—
C_{27}, Δ^5		1·34	34	3	10	126	—
C_{28}, Δ^5	0·74	1·71	trace	trace	5	—	—
C_{29}, Δ^5		2·10	357	3	180	250	28
C_{29}, $\Delta^{7,22}$		2·05	7	1	23	75	4
C_{29}, $\Delta^{5,22}$	0·66	1·85	12	trace	51	125	9
C_{29}, $\Delta^{7,25}$	0·47	2·41	361	—	328	—	—
C_{29}, $\Delta^{7,24(28)}$	0·42	2·18	7	5	—	—	—
C_{29}, $\Delta^{7,22,25}$	0·22	2·12	4911	12	292	50	—
Total sterols			5740	24	900	1860	126

* On silica gel impregnated with $AgNO_3$ (system 4).
† Retention time of free cholesterol = 1·00.

The differences observed between sterol components of free and bound sterol fractions may be caused either by substrate specificity of the acylating and glucosylating enzymes or they may result from spatial segregation of some processes leading to sterol transformations (isomerization or introduction of double bonds), esterification and glucosylation, within the plant cell.

TABLE 2. COMPOSITION OF THE FATTY ACID MIXTURES OBTAINED FROM STEROL ESTER AND STEROL ACYLMONOGLUCOSIDE FRACTIONS OF *Cucumis sativus* L.

	Fatty acids (%) 14:0	15:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:0	20:2	20:3	22:0	22:2	24:0	24:1
Sterol acylmonoglucosides	2·8	3·0	26·4	2·5	4·7	18·0	5·3	11·0	0·9	6·1	4·5	1·5	4·3	2·3	3·1	3·6
Sterol esters	1·0	—	13·8	1·5	0·6	6·2	2·9	57·0	14·3	1·0	1·7	—	—	—	—	—

The fatty acid acyl components of the esters were assayed by GLC (Table 2). Again considerable differences were found between the simple and glucoside esters. The results show that although both fractions contain complex mixtures of fatty acids, simple sterol esters contain considerably higher amounts of unsaturated acids (> 72% of total acids), linoleic and linolenic acid being the main components. On the other hand, saturated acids predominate (> 68% of total acids) in acylglucosides, palmitic and stearic acids being the main components.

EXPERIMENTAL

Extraction. 10-days old seedlings of *C. sativus* var. Wisconsin (2·0 kg) were homogenized with MeOH, filtered and the insoluble parts were extracted 2 × boiling $CHCl_3$–MeOH (2:1). From the combined extracts after evaporation of the solvents (19·5 g) free and bound sterols were isolated chromatographically.

Column chromatography. Preliminary separations of sterol fractions were obtained on silica gel column (type 5024 H, 100–200 mesh, Koch-Light). The following solvents were used consecutively as eluents: light petroleum (40–60°), light petroleum: Et_2O (98:2, 96:4, 92:8, 84:16, 68:32 and 30:70), Et_2O alone, Et_2O–MeOH (98:2, 95:5 and 90:10). Rechromatography was carried out on neutral alumina (type 507 C, act. III, Fluka) using the same elution systems.

Thin-layer chromatography. Purification of the sterol fractions was carried out on silica gel (Kieselgel G, Merck) using the following solvent systems: benzene:Et_2O, 1:1 (1), $CHCl_3$–MeOH, 9:1 (2), light petroleum (40–60°): $CHCl_3$–MeOH–HO, 20:10:2 (3). Preliminary separation of the mixtures of sterol acetates was obtained on silica gel containing 10% of $AgNO_3$ using EtOH free $CHCl_3$ (4) as solvent.

Gas–liquid chromatography. Sterol acetates were separated on a Pye 104 Chromatograph equipped with FID using 7 ft all-glass columns filled with 1% SE-30 on 80–120 mesh Chromosorb W, at 250°. Fatty acid methyl esters were separated on a 9 ft column filled with 10% PEGA on Diatomite CQ 100–120 mesh, at 200°.

Other methods. Mass spectra were taken using a LKB 900 apparatus. Glucosides and acylglucosides were hydrolyzed with boiling 10% H_2SO_4 in 80% MeOH for 3 hr. Sterol esters were hydrolyzed with boiling 10% NaOH in 85% MeOH for 1 hr.