Studies on the Distribution of Phytosterols in *Dioscorea* Species Tubers

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A survey was made of the distribution of sterols in five *Dioscorea* species tubers. All contained β-sitosterol, stigmasterol, cholesterol and campesterol, whereas cycloartenol was detected only in two *Dioscorea* species.

Introduction

To the Dioscoreaceae family belong several plant species, which are vines with large and voluminous, generally cordate leaves and small unisexual flowers arranged in spikes or racemes. The interest for these plants has been tremendous as they have been the traditional source of diosgenin, the steroidal sapogenin used as the principal raw material in the synthesis of several very potent medicinal steroids [1].

Despite considerable interest in the biosynthesis of sterols, comparatively few detailed investigations concerning the distribution of sterols in *Dioscorea* spp. tubers have appeared in the literature [2–5]. Especially there is a lack of quantitative data on the composition and content of sterols in wild species of *Dioscorea* tubers. The objective of this study is to report the results obtained from five *D.* species tubers.

Materials and Methods

Tubers of *Dioscorea* species were collected from the southern and northeastern zones of Guatemala as described elsewhere [6]. These were thoroughly washed, ground into powder and dried in an oven at 70 °C for six days prior to extraction for sterols.

Extraction and separation of sterols

The total, free, glycosidic and esterified sterols were isolated by the method of Bae and Mercer [7]. The sterol fractions were purified prior to gas liquid chromatographic analysis. The fractions were dissolved in 40 ml of boiling absolute ethanol and added to this mixture were 20 ml of hot 2% digitonin in 80% (W/V) ethanol and 10 ml of hot water. The samples were allowed to cool and remain at room temperature overnight. The precipitate was washed three times with 80% ethanol and three times with diethyl ether. The white steroldigitonide precipitate was dried overnight at room temperature.

The dried precipitate was cleaved with pyridine containing a known amount of internal standard cholestane, heated at 70 °C for 2 h and subsequently left at room temperature for 16 h. The digitonin was removed by precipitation with diethyl ether [8].

The qualitative and quantitative sterol analysis was performed by gas chromatography with a Perkin-Elmer gas chromatograph, Model 402, equipped with a flame-ionization detector. The column was a 6-ft U-shaped glass tube with 1/2-*in.* internal diameter, packed with Diaport, 80/100-mesh, coated with 1% SE-30. The column temperature was 275 °C, and the flash heater and detector temperatures were at least 35 °C above that of the column. N_2 was the carrier gas at a flow rate of 100 ml/min.

Results and Discussion

Table I shows the results on the distribution of total free, glycosidic and esterified sterols from five *D.* species tubers. Free sterols represented the largest fraction of the total sterols in all *D.* spp. examined.

Table I. Sterol distribution in five *D.* species tubers[a].

D. Species[b]	Sterols			
	Total	Free	Glycoside	Esters
D. belizensis	4.81	2.62	0.81	1.38
D. nelsonii	1.64	0.89	0.20	0.55
D. bartletti	0.66	0.36	0.06	0.24
D. floribunda	3.25	1.65	0.34	1.26
D. convolvulaceae	0.20	0.09	0.03	0.08

[a] Units expressed as mg sterols/g dry weight of tuber.
[b] Voucher specimens are on deposit in the herbarium of Missouri Botanical Garden, St. Louis, Missouri, USA.

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Table II. Sterol composition of the free sterols, steryl esters and steryl glycosides from *D.* spp. tubers.

D. Species	Sterol Form	Relative Percent Composition[a]				
		Cholesterol	Campesterol	Cycloartenol	β-Sitosterol	Stigmasterol
D. belizensis	Free	8.92	22.77	2.97	37.62	27.72
	Ester	17.77	24.44		37.68	20.11
	Glycoside	11.07	17.48		30.32	41.13
D. nelsonii	Free	12.42	16.96	6.13	47.57	16.92
	Ester	9.68	13.42		42.26	34.64
	Glycoside	6.96	8.16		24.13	60.75
D. bartletti	Free	16.40	20.47		40.98	22.15
	Ester	13.48	24.12		32.56	29.84
	Glycoside	12.39	13.71		11.25	62.65
D. floribunda	Free	19.05	25.40		29.36	26.19
	Ester	9.38	28.71		38.29	23.62
	Glycoside	20.13	33.12		39.15	7.6
D. convolvulaceae	Free	14.79	18.35		41.57	25.29
	Ester	19.49	21.10		36.28	23.13
	Glycoside	14.32	10.43		31.16	44.09

[a] Individual sterols are expressed in percent of each sterol form.

Table II shows approximate compositions of the sterol fractions of five *D.* spp. tubers, determined by GLC. Estimations of these approximate compositions were carried out by measuring the peak areas of the sterols and the internal standard, and making corrections for the differences in relative weight responses [8]. The sterol fractions consisted mainly of cholesterol, campesterol, β-sitosterol and stigmasterol, among which β-sitosterol was the predominant sterol. The next highest concentration in most fractions was that of stigmasterol, followed by campesterol and cholesterol, respectively. Cycloartenol was found only in the three form in *D. belizensis* and *D. nelsonii.* The sterol glycoside fraction also showed a large variation in sterol composition. Stigmasterol accounted for the larger fraction of the sterol glycoside in *D. bartletti* and *D. nelsonii.*

The absence of cycloartenol in certain *D.* spp. tubers may be due to the physiological state of the tubers analyzed. Cycloartenol is considered to be the key intermediate in the plant sterol biosynthesis [9] and has been identified in potato tubers [4, 7]. The membrane stability effects are very specific with respect to the configuration of the sterols present and it has been shown that slight changes in sterol concentration influence membrane permeability [10]. The differences in sterol composition and contents suggest their varying requirements for the formation of structural components of membranes among *D.* spp. tubers.

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