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POLYGONALES AND PLUMBAGINALES: STEROL COMPOSITION IN RELATION TO THE CARYOPHYLLIDAE

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Key Word Index—Polygonales; Plumbaginales; Caryophyllidae; sterols; chemotaxonomy; 24-ethylcholest-5-enol; 24-ethylcholesta-5,22-dienol; 24-methylcholest-5-enol.

Abstract—The dominant sterols from five genera in the Polygonales and three genera in the Plumbaginales are 24-alkyl-Δ^5-sterols. In all 18 species examined, 24-ethylcholest-5-enol was the dominant sterol followed by lesser amounts of 24-ethylcholesta-5,22-dienol and 24-methylcholest-5-enol. The sterol profiles of these species are distinctly different from those reported for the associated order Caryophyllales. This supports the uncertain affinity of these two orders to the subclass Caryophyllidae.

INTRODUCTION

The Polygonales and Plumbaginales are both monofamilial orders (Polygonaceae and Plumbaginaceae) with an uncertain taxonomic relationship to the order Caryophyllales [1]. Collectively, these three orders comprise the angiosperm subclass Caryophyllidae. In our previous work in the Caryophyllales, eight of the 12 families have been investigated for sterol composition [2–7]. Species in the four largest families, Chenopodiaceae [3], Caryophyllaceae [4], Amaranthaceae [5] and Cactaceae [6], have been examined in greater detail. Many species within the Chenopodiaceae, Caryophyllaceae and Amaranthaceae synthesize exclusively or predominantly 24-alkyl-Δ^7-sterols. Other species within the Caryophyllaceae and Amaranthaceae synthesize mixtures of Δ^7- and Δ^5-sterols. Additionally, some species in the Chenopodiaceae synthesize Δ^7-sterols, or Δ^5-sterols, or mixtures of Δ^7- and Δ^5-sterols in relatively fixed proportions. Within the Cactaceae, with the exception of *Lophocereus schottii* [8, 9], all species examined synthesize Δ^5-sterols [6]. Within the other four families examined, the 24-alkyl-Δ^7-sterols are the dominant sterols from species which include *Phytolacca americana* and *P. esulenta* (Phytolaccaceae) [7, 10], *Tetragonia expansa* (Aizoaceae), *Basella alba* (Basellaceae), *Portulaca grandiflora* and *Claytonia virginica* (Portulacaceae) [7].

We have examined species in five genera in the Polygonales and three genera in the Plumbaginales to determine if the sterol composition of these two orders is as diverse as the affiliated order Caryophyllales.

RESULTS AND DISCUSSION

The individual sterols were characterized by their chromatographic properties on GLC as well as their mass spectral characteristics. These properties were in agreement with authentic standards and with previously published values [2–5, 11].

All 18 species contained 24-ethylcholesterol (24ξ-ethylcholest-5-en-3β-ol; 54.6–90.8% of the desmethylsterol), with lesser amounts of 24-ethylcholesta-5,22-dienol (24ξ-ethylcholesta-5,22-dien-3β-ol; 2.1–17.4%), and 24-methylcholesterol (24ξ-methylcholest-5-en-3β-ol; 1.9–10.2%) (Table 1). Five species also contained the saturated analogues 24-methylcholestanol (2.9–10.8%) or 24-ethylcholestanol (7.1%). Eight of the 18 species also contained cholesterol (cholest-5-en-3β-ol; 1.0–10.4%). The remaining isolated sterols are known intermediates in sterol biosynthesis and were not isolated consistently from any one genus (Table 1).

Evidence that phytosterol composition may be potentially useful in plant chemosystematics is emerging within the order Caryophyllales [2–7]. Species within eight of the 12 families examined to date have exhibited a diversity of sterol synthesis previously unknown in angiosperms. Species within this order have been shown to synthesize exclusively Δ^7-sterols, mixtures of Δ^7- and Δ^5-sterols in relatively fixed proportions or Δ^5-sterols. In two of the largest families (Chenopodiaceae, Caryophyllaceae), where a sufficient number of species have been examined [3, 4], the diversity of sterol production was, in most cases, consistent at the tribal and subfamilial level. It was therefore of interest to extend this study to incorporate the two smaller orders (Polygonales, Plumbaginales) to determine if this diversity of sterol composition was characteristic of the subclass, Caryophyllidae.

The species examined in the Polygonales and Plumbaginales all synthesize Δ^5-sterols from 87–100% of the desmethylsterols (Table 1). The 24-methylcholesterol, 24-ethylcholesterol and 24-ethylcholesta-5,22-dienol were isolated consistently from all species in ratios generally characteristic of 'main line' angiosperms [12–14]. Species in the Polygonales and Plumbaginales, therefore, are distinctly different with respect to their sterol compositions than the majority of species examined thus far in the Caryophyllales.

Additionally, numerous morphological and chemical differences have suggested that the relationship between these three orders is somewhat dubious. The Polygonales and Plumbaginales have embryos with a true endosperm, not a perisperm and S-type sieve-tube plastids instead of the P-type plastid characteristic of the Caryophyllales [1]. Furthermore, the Plumbaginales has pollen which is

Table 1. Percentage composition of the desmethylsterols isolated from Polygonales and Plumbaginales

Species	A	B	C	D	E	F	G	H	I	J	K	L
Polygonaceae												
Atraphaxis muschketowii	2.0	—	—	6.2	2.8	7.6	69.8	6.5	—	0.9	2.2	2.0
Muehlenbeckia axillaris	1.2	—	—	—	3.4	3.4	86.7	3.2	—	—	2.1	—
Muehlenbeckia varians	3.0	1.7	—	—	2.9	8.4	76.8	—	—	1.4	5.8	—
Polygonella articulata	—	—	—	—	8.8	2.1	89.1	—	—	—	—	—
Polygonum amphibium	—	—	—	—	9.6	16.7	73.7	—	—	—	—	—
P. aviculare	—	—	—	—	6.6	3.3	90.1	—	—	—	—	—
P. cuspidatum	—	—	—	—	1.9	7.7	90.4	—	—	—	—	—
P. pensylvanicum	—	—	—	—	7.3	5.1	87.6	—	—	—	—	—
P. punctatum	—	—	—	—	7.1	6.5	86.4	—	—	—	—	—
Rumex acetosella	—	—	—	—	8.6	4.2	87.2	—	—	—	—	—
R. crispus	—	—	—	—	5.0	5.6	89.4	—	—	—	—	—
R. sp.	—	—	—	—	5.7	5.5	88.8	—	—	—	—	—
Plumbaginaceae												
Acantholimon glumaceum	1.0	4.1	4.1	—	10.2	4.1	69.4	—	7.1	—	—	—
Limonium europea	1.0	—	—	7.7	3.7	5.3	71.5	10.8	—	—	—	—
L. nashii	—	—	—	—	5.1	4.1	90.8	—	—	—	—	—
L. thouinii	5.5	7.0	—	2.0	5.0	17.4	54.6	—	—	8.0	0.5	—
Plumbago rosea	10.4	—	—	3.2	2.3	10.4	73.7	—	—	—	—	—
P. zeylanica	5.5	—	—	—	5.2	6.8	69.8	2.9	—	7.4	2.4	—

A. Cholest-5-en-3β-ol.
B. 24-Methylcholesta-5,22-dien-3β-ol.
C. 24-Methylcholesta-5,24(28)-dien-3β-ol.
D. 24-Ethylcholesta-5,24(28)-dien-3β-ol.
E. 24-Methylcholest-5-en-3β-ol.
F. 24-Ethylcholesta-5,22-dien-3β-ol.
G. 24-Ethylcholest-5-en-3β-ol.
H. 24-Methyl-5α-cholestan-3β-ol.
I. 24-Ethyl-5α-cholestan-3β-ol.
J. Cholest-7-en-3β-ol.
K. 24-Ethylcholest-7-en-3β-ol.
L. 24-Ethylcholesta-7,24(28)-dien-3β-ol.

morphologically distinct from either of the other two orders [15]. Chemically, the Polygonales and Plumbaginales produce anthocyanins, not betalains, contain ellagic acid and are tanniferous unlike the Caryophyllales [1]. The difference in the sterol profile of species in these two orders along with other morphological and chemical differences support the belief that the Polygonales and the Plumbaginales should be divorced from the Caryophyllales.

EXPERIMENTAL

Plant material. Polygonum amphibium (Coleman) Fern., *P. aviculare* Meisn., *P. cuspidatum* Sieb. and Zucc., *P. pensylvanicum* L., *P. punctatum* Ell., *Polygonella articulata* (L.) Meisn., *Rumex acetosella* L., *R. crispus* L., *Rumex* spp. and *Limonium nashii* Small were field-collected in mid-summer in Maryland and Pennsylvania. *Atraphaxis muschketowii* Krassn., *Muehlenbeckia axillaris* Walp., *M. varians* Meisn., *Acantholimon glumaceum* (Jaub. and Spach) Boiss., *Limonium europea* L., *L. thouinii* (Viv.) Kuntze, *Plumbago rosea* L. and *P. zeylanica* L. were acquired from the Royal Botanic Garden (Kew, England).

Extraction and isolation of sterols. The plants were washed and cleaned of all necrotic tissue. The mature photosynthetic tissue was oven dried at 80° and then ground to pass a 20-mesh screen. The dry powder was extracted with $CHCl_3$–MeOH (2:1) in a Soxhlet for 48 hr. The extract was reduced to dryness under red. pres. and the residue saponified for 45 min. at reflux in 10% KOH in 70% EtOH–H_2O. The neutral lipids were extracted with Et_2O (4 ×) and then chromatographed on an Al_2O_3 column as previously described [2–4]. The desmethylsterols were separated by prep TLC on silica gel G TLC plates (E. Merck, Darmstadt). Samples were streaked at the origin and the plates were developed in Et_2O–hexane (2:8). Standards run concurrently were sprayed with 1% 2′,7′-dichlorofluorescein in EtOH and visualized under long wave UV. Bands co-migrating with cholesterol standards were scraped from the plate and the desmethylsterols were extracted with Et_2O (4 ×).

GLC was performed on a glass column (2 m × 2 mm) packed with 3% SE-30 and on a SP-2330 capillary column (30 m × 0.25 mm, 25 μm film, Supelco, Bellefonte, PA) in a Varian Model 3700 gas chromatograph equipped with a flame ionization detector. Retention times and area units were recorded with a Varian CDS 401 data system. GLC/MS was performed at 70 eV on a Finnigan model 4510 equipped with a J&W DB-1 fused silica capillary column (30 m × 0.32 mm, 25 μm film) and interfaced with an Incos Data System.

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