Phytochemistry, Vol. 26, No. 3, pp. 731–733, 1987
Printed in Great Britain

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

DOMINANCE OF Δ^5-STEROLS IN EIGHT SPECIES OF THE CACTACEAE

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(*Received* 14 *May* 1986)

Key Word Index –Cactaceae; sterols; chemotaxonomy; 24α-ethylcholest-5-en-3β-ol; cholest-5-en-3β-ol; 24ξ-methylcholest-5-en-3β-ol.

Abstract –The sterols from eight species in seven genera of the Cactaceae are 24-alkyl-Δ^5-sterols. In all eight species, *Echinopsis tubiflora*, *Pereskia aculeata*, *Hylocereus undatus*, *Notocactus scopa*, *Epiphyllum* sp., *Schlumbergera bridgesii*, *Opuntia comonduensis* and *O. humifusa*, the dominant sterol is sitosterol (24α-ethylcholest-5-en-3β-ol) at 66–87% of the total sterol composition with the 24ξ-methylcholest-5-en-3β-ol present at 8–33%. Stigmasterol (24α-ethylcholesta-5,22*E*-dien-3β-ol) is present at 2–8% of the total sterol in *P. aculeata*, *H. undatus*, *N. scopa* and *Epiphyllum* sp. whereas cholesterol (cholest-5-en-3β-ol) is present in six species at levels of <0.1–5.0%. Avenasterol (24-ethylcholesta-7,24(28)*Z*-dien-3β-ol) and sitostanol (24α-ethyl-5α-cholestan-3β-ol) are each present in two species.

INTRODUCTION

Evidence that phytosterol composition may be potentially useful in plant chemosystematics is emerging within the order Caryophyllales [1–5]. The order consists of 12 families with about 10000 species [6] and is generally agreed [6–8] to include the following families: Phytolaccaceae, Achatocarpaceae, Nyctaginaceae, Aizoaceae, Didiereaceae, Cactaceae, Chenopodiaceae, Amaranthaceae, Portulacaceae, Basellaceae, Molluginaceae and Caryophyllaceae. Many species within three of the larger families, Caryophyllaceae (pink), Chenopodiaceae (goosefoot) and Amaranthaceae (amaranth), synthesize exclusively or predominately 24-alkyl-Δ^7-sterols. Species within the Caryophyllaceae and Amaranthaceae synthesize exclusively Δ^7-sterols or mixtures of Δ^7- and Δ^5-sterols [3, 4] whereas 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 [1, 2]. Within the order, the 24-alkyl-Δ^7-sterols are also the dominant sterols reported from other species and families which include *Phytolacca americana* and *P. esculenta* (Phytolaccaceae), [5, 9], *Tetragonia expansa* (Aizoaceae), *Basella alba* (Basellaceae), *Portulaca grandiflora* and *Claytonia virginica* (Portulacaceae) [5].

In the Cactaceae, Djerassi *et al.* [10] reported that *Lophocereus schottii* contained lophenol (4α-methyl-5α-cholest-7-en-3β-ol) and schottenol (24α-ethyl-5α-cholest-7-en-3β-ol) which were also isolated and characterized by Heed and Kircher [11]. More recently 5α-cholesta-8,14-dien-3β-ol, lathosterol (5α-cholest-7-en-3β-ol), 24-methyl-5α-cholest-7-en-3β-ol, spinasterol (24α-ethyl-5α-cholesta-7,22*E*-dien-3β-ol) and schottenol (24α-ethyl-5α-cholest-7-en-3β-ol) were all isolated from the sterol fraction of *Lophocereus schottii* [12]. The Δ^5-sterols cholesterol, campesterol and sitosterol in the proportions of 1:2:7 were also reported from the cacti *Stenocereus thurberi* and *S. gummosus* [13]. In order to characterize further the sterols of the Cactaceae we examined eight species in seven genera, several indigenous to South America, to determine if the sterol composition of this family is similar to that in the other families in the order.

RESULTS AND DISCUSSION

Individual sterols were characterized by their chromatographic properties on GLC and RPLC as well as their ^{1}H NMR and mass spectral characteristics. These properties were in agreement with authentic standards and with previously published values [1, 2, 4, 14]. The configuration of the C-24 alkyl substituents was determined by ^{1}H NMR as previously described [15, 16].

In all eight species examined, the dominant sterol was sitosterol (24α-ethylcholest-5-en-3β-ol) (66.9–87.0% of the 4-desmethylsterol), followed by 24ξ-methylcholest-5-en-3β-ol (8.0–33.1%) (Table 1). The ^{1}H NMR of the 24ξ-methylcholest-5-en-3β-ol isolated from these cacti exhibited broad signals for C-18 and a multiple of doublets in the 0.75–0.85 ppm region of the spectrum which is indicative of an epimeric mixture [15, 16] of 24α-methylcholesterol (campesterol) and 24β-methylcholesterol (22-dihydrobrassicasterol). Two species also contained the saturated analogues, sitostanol (24α-ethyl-5α-cholestan-3β-ol) (4.2 and 8.2%) and 24ξ-methyl-5α-cholestan-3β-ol (<0.1%). Only half of the species examined contained stigmasterol (24α-ethylcholesta-5,22-dien-3β-ol) (2.5–8.3%). Six of the eight species contained cholesterol (cholest-5-en-3β-ol) ranging from trace amounts (<0.1%) to 5.0% of the total 4-desmethylsterol. Two species contained avenasterol (24-ethyl-5α-cholesta-7,24(28)*Z*-dien-3β-ol) (8.4 and 8.5%).

Numerous morphological and chemical studies over the past several decades have firmly established the

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Table 1. Percentage composition of the sterols in the Cactaceae

	Cholesterol	Avenasterol	24ζ-Methyl-cholesterol	Stigmasterol	Sitosterol	24ζ-Methyl cholestanol	Sitostanol
Echinopsis tubiflora			33.1		66.9		
Epiphyllum sp.		8.4	9.4	2.5	75.5	T	4.2
Hylocereus undatus	T		18.5	8.3	73.2		
Notocactus scopa	2.6		12.5	2.8	73.9	T	8.2
Opuntia comonduensis	4.4		8.8		86.7		
Opuntia humifusa	5.0		8.0		87.0		
Pereskia aculeata	2.5		18.7	6.3	72.5		
Schlumbergera bridgesii	T	8.5	10.2		81.3		

T = Trace amount detected at less than 0.1 % of total sterols.

taxonomic position of the Cactaceae in the Caryophyllales [6]. We selected a diverse range of species within this family to investigate sterol composition. While the genus *Pereskia*, with its wooden stems and succulent leaves, is considered to be the most archaic genus [6, 17], subdivisions and taxonomic relationships between the other genera are difficult to establish [17]. *Opuntia* (prickly pear cactus) and *Schlumbergera* (Christmas cactus) have stems consisting of a series of broad, flattened joints, while *Echinopsis*, *Hylocereus*, *Notocactus* and *Epiphyllum* are barrel, columnar or flattened stem cacti. No correlation between the cacti morphology and the sterol composition is evident (Table 1). In the Chenopodiaceae [1] and the Caryophyllaceae [4] where respectively eight and 11 genera were analysed for sterol composition, the diversity of sterol production was, in most cases, consistent at the tribal and subfamilial level suggesting similarities in sterol biosynthesis, end product requirements and taxonomic relatedness. No such delineation is evident in the Cactaceae. With the exception of *Lophocereus schottii* [10, 11], all species in the Cactaceae produce Δ^5-sterols and are thus like the 'main line' angiosperms [18 20]. However, the Cactaceae are unique in the Caryophyllales which, to date, are a predominantly Δ^7-sterol producing order [1–5]. The examination of additional cacti, especially in the genus *Lophocereus* or closely related genera, may yield additional Δ^7-sterol producing species or species which produce mixtures of Δ^7- and Δ^5-sterols.

The origin of the Cactaceae is postulated to be in or near the Phytolaccaceae, the basal family of the Caryophyllales [6]. The sterol composition from only two species in the Phytolaccaceae, *Phytolacca americana* and *P. esculenta*, are reported [5, 9, 21, 22], and both produce exclusively Δ^7-sterols. Since sitosterol is the dominant sterol in the Cactaceae and because these cacti have a sterol composition that is sufficiently different from that reported for other species and families in the order, examination of the Phytolaccaceae, a small family of 18 genera, may reveal a group of Δ^5-sterol producing species whose ancestors may have given rise to the present day Cactaceae.

EXPERIMENTAL

Opuntia humifusa (Raf.) Raf. was field-collected in mid-summer in southern New Jersey. The remaining species, *Echinopsis tubiflora* (Pfeiff.) Zucc., *Pereskia aculeata* Mill., *Hylocereus undatus* Britt. and Rose, *Notocactus scopa* (Spreng) Berg., *Epiphyllum* sp., *Schlumbergera bridgesii* (Lem.) Lofgren and *Opuntia comonduensis* (Coulter) Britt. and Rose, were acquired through the generosity of Dr. Donald G. Huttleston, Longwood Gardens, Kennett Square, PA.

The plants were washed and cleaned of all necrotic tissue and the mature photosynthetic tissue was finely chopped and extracted with Me_2CO in a Soxhlet apparatus for 48 hr. The extract was reduced to dryness and saponified by refluxing in 5% KOH in 70% EtOH. The neutral lipids were extracted with Et_2O (4 ×) and then chromatographed on a neutral Al_2O_3 column as previously described [1, 2, 4]. The sterols were isolated and purified by preparative RPLC on a Perkin Elmer high efficiency C_{18} column with MeCN–MeOH (9:1) 7.5 ml/min at 35° as previously described [1, 2, 4]. GLC analysis was performed on a 2 m × 2 mm 1% XE-60 or on a 0.75% SE-30 column at 235° with He at 35 ml/min. Analytical RPLC was performed with a Zorbax ODS column (30 cm × 3 mm) at 45° with MeCN–*iso*-PrOH (8:2) at 1.5 ml/min. EIMS (probe) was performed at 70 eV. ^{1}H NMR spectroscopy was performed at 360 MHz at ambient temp. in $CDCl_3$ with TMS as an internal standard. Authentic standards were obtained and purified as previously reported [1, 2, 14].

Acknowledgements –The authors wish to thank Mrs. J. R. Landrey for performing ^{1}H NMR and mass spectroscopy and Dr. Donald G. Huttleston of the Longwood Gardens for his generosity in providing many of the plant samples. Use of the ^{1}H NMR facility at the University of Pennsylvania is gratefully acknowledged. This study was supported in part through a grant from the Martin Marietta Corporation.

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