

MOLECULAR SPECIES OF WAX ESTERS IN *CEREUS PERUVIANUS*VALERY M. DEMBITSKY*[‡] and TOMAS REZANKA[‡]

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Key Word Index—*Cereus peruvianus*; Cactaceae; wax esters; fatty alcohols; fatty acids; molecular species.

Abstract—Alkyl esters were detected in the wax from *Cereus peruvianus* and their individual molecular species up to C₃₈ identified by means of capillary GC-mass spectrometry. The wax esters were composed of fatty acids up to 30:0 and alcohols up to *iso*-34:0. The main fatty acids were 16:0, 16:1, 18:0 and 18:1. The main fatty alcohols were 16:0, 18:0 and 18:1, together with 26:0 (9.8%) and 26:1 (12%). *Iso*- and *anteiso*-fatty acids (5.5%) and fatty alcohols (11.3%) were also found. More than 600 isomers of the wax esters were identified.

INTRODUCTION

Cactaceae are an interesting group of desert plants which have attracted the attention of chemists due to the broad diversity of their biologically-active compounds, such as alkaloids, saponins, steroids, triterpenes, glucosides, fats, oils and waxes, that they contain (see reviews [1–3]).

As recently reviewed, wax esters are important components in both the animal and plant kingdoms [4]. Detailed analyses of alkyl ester fractions of plant epicuticular waxes have been published recently [5–9]. Most xerophytic plants produce seeds which are good to excellent sources of oil. Also, the cuticular waxes of cacti are notably water-impermeable. Practically all true Cactaceae, as well as xerophytes, take up water during rainy or wet weather and conserve it. Their exterior cuticular waxes serve as an impervious moisture barrier [2, 3]. However, only very limited studies of the wax composition of cacti and succulents have so far been undertaken [10–15].

The present work reports on the composition of the molecular species of alkyl esters and their constituent fatty acids and alcohols in the cactus, *Cereus peruvianus*.

RESULTS AND DISCUSSION

The composition of the molecular species of wax esters is presented in Table 1. Esters from C₂₆ to C₅₈

were detected. Fraction A was separated into three fractions. Fraction A₁ contained a mixture of esters of *n*-acids and *n*-alcohols. For example, the saturated C₅₀ wax ester (0.8%, total wax esters) was composed of three isomers: *n*-22:0/*n*-28:0 (acid–alcohol, 6% total isomers), *n*-26:0/*n*-24:0 (4%) and *n*-24:0/*n*-26:0 (90%, major isomer). Fraction A₂ was separated from A₃ by GC-mass spectrometry, as described earlier [16]. It contained a mixture of *br*- and *n*-isomers. C₅₀ esters (0.13% total esters), included *n*-16:0/*iso*-34:0 (15%), *n*-18:0/*iso*-32:0 (11%), *n*-26:0/*iso*-24:0 (6%), *br*-25:0/*n*-25:0 (5%) and *iso*-24:0/*n*-28:0 (63%). Fraction A₃ comprised branched saturated isomers only; the C₅₀ ester consisted of two isomers: *iso*-20:0/*iso*-30:0 (50%) and *br*-25:0/*anteiso*-25:0 (50%).

Fraction B was separated into two parts. B₁ consisted of *normal*–*monounsaturated* (acid–alcohol) and *mono*–*unsaturated*–*normal* (acid–alcohol) isomers. B₂ consisted of *monounsaturated*–*branched* and *branched*–*monounsaturated* isomers. Fraction C consisted of esters of monounsaturated isomers only.

Proportions of individual isomers of identical chain length were quantified by the mass spectrometric methods of Aasen *et al.* [17]. These involve use of the relative intensities of the ions [RCO₂H]⁺, [RCO₂H₂]⁺ and [R'–1]⁺ formed from esters of the general formula RCO₂R' (where R and R' are alkyls of an acid or alcohol, respectively) to determine the percentage occurrence of each isomer. The relative percentage representation of any sum of the ions mentioned above corresponds to the relative occurrence of their combinations. This method can be used with unsaturated esters, as verified by Spencer [18]. However, with monoenoic acids it is necessary to use the ions [RCO–1]⁺ and [RCO]⁺ which are much more intense than the ions

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Table 1. Composition of wax esters from *Cereus peruvianus*

Carbon number	A1			A2			A3			B1			B2			B2			C		
	n-n	%	Total %	n-br + br-n	%	Total %	br-br	%	Total %	n-uus	%	Total %	uns-br	%	Total %	br-uus	%	Total %	uns-uns	%	Total %
26	12-14	100	0.040										14u-14	100	0.040						
28	12-16	80	0.415	12-16	100	0.085				15u-14	100	0.580	14u-116	100	0.085						
29	13-16	100	0.147	13-16	100	0.038															
30	12-18	12		12-18	19					14u-16	57	0.260									
	14-16	55		14-16 + 14-16	74	0.390				15u-16	71	2.466	15u-116	100	0.047						
31	16-14	33	1.258	16-14	7					16u-15	17										
	13-18	16		13-18	17					17u-14	12										
	14-17	9		15-16 + a15-16	83	0.185				14u-18	6										
	15-16	57								16u-16	84	2.278	u14-118	12							
32	16-15	18	0.418							18u-14	10										
	12-20	2		14-18 + 14-18	15					16u-17	5		u16-116	88	0.603	u14-u18	100	0.047	14-18	100	0.167
	14-18	8		16-16 + 16-16	85	1.315				17u-16	90										
	16-16	83								18u-15	1	3.043	u15-118	40	0.101						
33	18-14	7	4.122	15-18 + a15-18	48																
	13-20	7		17-16 + a17-16	52	0.196				17u-16	90		u17-116	60							
	15-18	19								18u-18	4										
	16-17	32																			
	17-16	32																			
	18-16	10	0.570																		
34	12-22	1		16-18 + 16-18	51					12-22u	3		u16-118	47							
	14-20	4		18-16 + 18-16	47	0.090				14-20u	3	1.807	u18-116	53	0.960						
	16-18	30		14-20	2	1.667				16-18u	96										
	18-16	58																			
35	20-14	1	4.296																		
	15-20	12		a15-20	12					15-20u	100	0.017	u16-a19	47							
	16-19	21		16-a19 + 19-16	55					16u-19	21		u17-118	53	0.097						
	17-18	16		17-18 + a17-18	33	0.196				17u-18	33										
	18-17	26								18u-17	32	1.788									
36	19-16	25	0.821																		
	12-24	3		18-18 + 18-18	72					12-24u	6		u18-118	86							
	14-22	3		20-16 + 20-16	21	0.060				14-22u	4		u20-116	14	0.503	u16-u20	19				
	16-20	35		116-20	7	0.852				16-20u	15					u18-118	81	0.091	14-22	2	
	18-18	45								18-18u	76	1.650	0.246								
	20-16	11																			
37	22-14	3	2.520																		
	13-24	7		a17-20	12					14u-23	9		u18-a19	100	0.043	a19-u18	100	0.009	17-20	100	0.017
	14-23	11		18-a19	45					16u-22	9										
	16-21	23		19-118	23					16u-21	29	0.123									
	17-20	10	0.120	21-116	20					17u-20	26										
	18-19	18								18u-19	27	1.469									
	19-18	12																			
38	21-16	19	0.476																		
	12-26	8		14-24 + 14-24	16					14u-24	5		u14-u24	14							
	14-24	8		20-18 + 20-18	30	0.029				16u-22	16		u22-116	86	0.115	u16-u22	20				
	16-22	18		22-116	46					18u-20	35					u20-118	54	0.104	16-22	33	
	18-20	29		116-22	8	0.366				18-20u	18					120-u18	26				
																			20-18	26	0.544

[illegible]

$[\text{RCO}_2\text{H}]^+$ and $[\text{RCO}_2\text{H}_2]^+$ found in saturated acids [16, 19]. With diene wax esters, the situation is much simpler because both the alcohol and acid are monoenoic and, hence, the possibility of mutual combinations is much lower.

Compositions of fatty acids and fatty alcohols isolated from the total wax esters were studied and are listed in Table 2. Six major fatty acids were identified including, 16:0 (18.6%), 16:1 (11.3%), 18:0 (13.6%), 18:1 (11%), 24:0 (7.1%) and 24:1 (5.7%). Very long chain fatty acids from C_{25} to C_{30} comprised not more than 1% of the fractions. According to previous work

[20, 21], in which the fatty acid composition of more than 16 cactus species was studied, the two main fatty acids found were 18:2 (35–67%) and 18:1 (16–32%). Fatty alcohols were detected, of which more than 30% consisted of very long-chain alcohols from C_{23} to C_{34} .

We have identified more than 600 wax ester isomers from the leaves of *C. peruvianus*. This is the first time that such a diversity of wax esters has been found in a plant species. Thus, it will be of great interest to examine the content of wax esters in other species of cacti and variations with different climatic conditions.

Table 2. Fatty acids and fatty alcohols identified from wax esters of *Cereus peruvianus*

Alkyl chain	Acid, wt %	Alcohol, wt %
<i>n</i> -12:0	1.9	—
<i>n</i> -13:0	0.8	—
<i>n</i> -14:0	4.0	2.2
<i>n</i> -15:0	1.3	0.4
<i>n</i> -16:0	18.6	18.4
<i>n</i> -17:0	1.0	1.0
<i>n</i> -18:0	14.6	8.3
<i>n</i> -19:0	0.7	0.6
<i>n</i> -20:0	1.5	4.7
<i>n</i> -21:0	0.5	0.6
<i>n</i> -22:0	3.6	2.1
<i>n</i> -23:0	0.8	1.2
<i>n</i> -24:0	7.1	4.3
<i>n</i> -25:0	0.4	1.2
<i>n</i> -26:0	0.8	9.8
<i>n</i> -27:0	0.1	0.4
<i>n</i> -28:0	0.2	1.3
<i>n</i> -30:0	0.1	—
Normal saturated	57.0	56.5
<i>iso</i> -14:0	0.6	—
<i>iso</i> -16:0	1.5	4.7
<i>iso</i> -18:0	0.8	4.0
<i>iso</i> -20:0	0.6	—
<i>iso</i> -24:0	0.8	0.9
<i>iso</i> -30:0	—	0.3
<i>iso</i> -32:0	—	0.4
<i>iso</i> -34:0	—	0.1
<i>anteiso</i> -15:0	0.5	—
<i>anteiso</i> -17:0	0.3	—
<i>anteiso</i> -19:0	0.1	0.4
<i>anteiso</i> -23:0	0.2	0.1
<i>anteiso</i> -25:0	0.1	0.4
<i>anteiso</i> -29:0	—	0.1
Branch saturated	5.5	11.3
5-14:1	1.8	—
6-15:1	1.0	—
7-16:1	11.3	—
8-17:1	1.3	—
9-18:1	11.0	9.3
11-20:1	1.3	1.3
13-22:1	2.1	1.6
15-24:1	5.7	4.6
17-26:1	1.0	12.0
19-28:1	—	2.7
21-30:1	—	0.6
23-32:1	—	0.1
Monoenoic	36.5	32.2

EXPERIMENTAL

Cereus peruvianus 'Monstrosus' was obtained from the Plant Adaptation Unit, Institute of Desert Research, and was collected in September 1993. Sampled leaves (860 g, fr. wt) were blended immediately and then placed in a Soxhlet extractor for refluxing with hexane-*iso*-PrOH (2:1). After 24 hr, solvent was removed from the extract by heating and evaporating under a stream of N_2 . The combined extracts were evapd to dryness. The residue obtained was dissolved in hexane. This extract was then sepd on prep. TLC plates in petrol (40–60°)– Et_2O –HOAc (80:20:1). The bands corresponding to wax esters were removed and extracted with hexane.

Total wax esters were further sepd by prep. TLC on silica gel G containing 10% AgNO_3 in hexane– Et_2O (4:1). After visualization with 2,7-dichlorofluorescein under UV, the bands corresponding to components having from zero to two double bonds were removed and extracted with Et_2O . The extract was dissolved in 3 ml MeOH, 500 mg urea added and the mixt. dissolved by warming. The soln crystallized at -15° within 3 hr. After filtration, the filtrate was acidified and extracted with hexane to isolate the *br*-wax esters. The crystals were redissolved in an acidic mixt. (pH 4) of H_2O –MeOH (1:1) and extracted $\times 3$ with an equal vol. of CHCl_3 . After Ag-TLC, 3 frs were obtained A ($\text{O} \times \text{C}=\text{C}$, saturated only), B ($1 \times \text{C}=\text{C}$, wax esters with one double bond in acid or alcohol), C ($2 \times \text{C}=\text{C}$, wax esters with two double bonds, monoenoic acids and monoenoic alcohols).

GC and GC-MS. Injection temp. (splitless) was 100° and a fused-silica capillary column (Supelcowax 10; 15 m $\times$ 0.25 μm ID, 0.25 μm film thickness) was used. The temp. prog. was as follows: 100° for 1 min, then increased at 20°min^{-1} to 230° and at 2°min^{-1} to 280° , maintained for 10 min. The carrier gas was H_2 at a flow-rate of 120 cm s^{-1} . Ammonia (0.6 torr) was used as the CI (positive and/or negative mode) reagent gas. Spectra were scanned within the range m/z 200–750.

TMSi derivatives of alcohols. A fused-silica (0.25 μm film thickness) capillary column DC-510 (Macherey-Nagel) (40 m $\times$ 0.25 μm ID) was used. The temp. was programmed from 100 to 280° at 1°min^{-1} . Analysis of fatty acid Me esters was carried out under the same conditions.

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