Pergamon

S0031-9422(96)00048-9

Phytochemistry, Vol. 42, No. 4, pp. 1075–1080, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0031-9422/96 $15.00 + 0.00

MOLECULAR SPECIES OF WAX ESTERS IN *CEREUS PERUVIANUS*

VALERY M. DEMBITSKY*† and TOMAS REZANKA‡

Natural Products Laboratory, Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel; ‡Institute of Microbiology, Videnska 1083, Prague 142 20, Czech Republic

(*Received in revised form* 18 *December* 1995)

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 C_{58} 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 C_{26} to C_{58} were detected. Fraction A was separated into three fractions. Fraction A_1 contained a mixture of esters of *n*-acids and *n*-alcohols. For example, the saturated C_{50} 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 A_2 was separated from A_3 by GC-mass spectrometry, as described earlier [16]. It contained a mixture of *br*- and *n*-isomers. C_{50} 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 A_3 comprised branched saturated isomers only; the C_{50} 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. B_1 consisted of *normal–monounsaturated* (acid–alcohol) and *monounsaturated–normal* (acid–alcohol) isomers. B_2 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 $[RCO_2H]^+$, $[RCO_2H_2]^+$ and $[R'\text{-}1)^+$ formed from esters of the general formula RCO_2R' (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 $[RCO\text{-}1]^+$ and $[RCO]^+$ which are much more intense than the ions

*Author to whom correspondence should be addressed.
†Present address: Department of Biotechnology, Moscow State Academy of Fine Chemical Technology, 86 Vernadsky Avenue, Moscow 117571, Russia.

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

Carbon number	A1			A2			A3			B1			B1			B2			B2			C		
	n–n	%	Total %	n–br + br–n	%	Total %	br–br	%	Total %	n–uns	%	Total %	uns–n	%	Total %	uns–br	%	Total %	br–uns	%	Total %	uns–uns	%	Total %
26	12–14	100	0.040																					
28	12–16	80		12–i16	100	0.085							14u–14	100	0.040									
	14–14	20	0.415																					
29	13–16	100	0.147	13–i16	100	0.038							15u–14	100	0.580									
30	12–18	12		12–i18	19		i14–i15	100	0.028	12–18u	100	0.167				14u–i16	100	0.085						
	14–16	55		14–i16 + i14–16	74								14u–16	57										
	16–14	33	1.258	i16–14	7	0.390							16u–14	43	0.260									
31	13–18	16		13–i18	17		i15–i16	100	0.024	13–18u	100	0.074	15u–16	71		15u–i16	100	0.047						
	14–17	9		15–i16 + a15–16	83	0.185							16u–15	17										
	15–16	57											17u–14	12	2.466									
	16–15	18	0.418										14u–18	6										
32	12–20	2		14–i18 + i14–18	15		i14–i18	28		12–20u	6		16u–16	84		u14–i18	12		i14–u18	100	0.047	14–18	100	0.167
	14–18	8		16–i16 + i16–16	85	1.315	i16–i16	72	0.085	14–18u	94	0.376	18u–14	10	2.278	u16–i16	88	0.603						
	16–16	83											16u–18	4										
	18–14	7	4.122										16u–17	5										
33	13–20	7		15–18 + a15–18	48		a15–i18	59		15–18u	100	0.121	17u–16	90		u15–i18	40		a15–u18	100	0.047			
	15–18	19		17–i16 + a17–16	52	0.196	a17–i16	41	0.034				18u–15	1	3.043	u17–i16	60	0.101						
	16–17	32																						
	17–16	32																						
	18–16	10	0.570																					
34	12–22	1		16–i18 + i16–18	51		i16–i18	58		12–22u	3		14u–20	3		u16–i18	47		i16–u18	100	0.121	14–20	2	
	14–20	4		18–i16 + i18–16	47		i18–i16	42	0.090	14–20u	3		16u–18	31		u18–i16	53	0.960				16–18	98	1.074
	16–18	30		i14–20	2	1.667				16–18u	96	1.807	18u–16	65										
	18–16	58											20u–14	1	0.331									
	20–14	1	4.296																					
35	15–20	12		a15–20	12		a17–i18	100	0.012	15–20u	100	0.017	16u–20	14		u16–a19	47		a17–u18	100	0.028	17–18	100	0.121
	16–19	21		16–a19 + 19–i16	55								16u–19	21		u17–i18	53	0.097						
	17–18	16		17–i18 + a17–18	33	0.196							17u–18	33										
	18–17	26											18u–17	32	1.788									
	19–16	25	0.521																					
36	12–24	3		18–i18 + i18–18	72		i18–i18	53		12–24u	6		16u–20	30		u18–i18	86		i16–u20	19		14–22	2	
	14–22	3		20–i16 + i20–16	21		i20–i16	47	0.060	14–22u	4		18u–18	50		u20–i16	14	0.503	i18–u18	81	0.091	16–20	12	
	16–20	35		i16–20	7	0.852				16–20u	15		20u–16	15								18–18	86	1.180
	18–18	45								18–18u	76	1.650		22u–14	3	0.236								
	20–16	11																						
	22–14	3	2.520																					
37	13–24	7		a17–20	12					13–24u	30		14u–23	9		u18–a19	100	0.043	a19–u18	100	0.009	17–20	100	0.017
	14–23	11		18–a19	45					16–22u	17		16u–22	9										
	16–21	23		19–118	23					19–18u	63	0.123	16u–21	29										
	17–20	10		21–116	20	0.120							17u–20	26										
	18–19	18											18u–19	27	1.469									
	19–18	12																						
	21–16	19	0.476																					
38	12–26	8		14–i24 + i14–24	16		i20–i18	82		12–26u	21		14u–24	5		u14–i24	14		i14–u24	26		14–24	15	
	14–24	8		20–i18 + i20–18	30		i14–i24	18	0.029	14–24u	17		16u–22	16		u22–i16	86	0.115	i16–u22	20		16–22	33	
	16–22	18		22–i16	46					16–22u	30		18u–20	35					i20–u18	54	0.104	18–20	26	
	18–20	29		i16–22	8	0.366				18–20u	18		20u–18	9								20–18	26	0.544

	20–18	6									20–18u	14	1.005	22u–16	26												
	22–16	31	2.157											24u–14	9	0.330												
39	13–26	10		a15–24	19		a23–i16	100	0.009		13–26u	47		14u–25	7		u16–a23	100	0.011	a15–u24	100	0.023	17–22	100	0.021			
	14–25	6		21–i18	17						15–24u	30		15u–24	13													
	15–24	8		23–i16 + a23–16	64	0.116					21–18u	23	0.202	16u–23	41													
	16–23	30												17u–22	6													
	18–21	11												18u–21	20													
	19–20	4												22u–17	6													
	21–18	6												24u–15	7	2.129												
	22–17	5																										
	23–16	20	0.742																									
40	18–22	15		16–i24 + i16–24	23		i16–i24	24			12–28u	2		14u–26	8		u16–i24	22		i14–u26	28		14–26	19				
	20–20	4		22–i18 + i18–22	17		i24–i16	76	0.049		14–26u	24		16u–24	23		u22–i18	19		i16–u24	60		16–24	46				
	22–18	15		24–i16 + i24–16	51						16–24u	44		18u–22	11		u24–i16	69	0.454	i18–u22	13	0.100	18–22	15				
	24–16	66	1.962	i14–16	6						18–22u	12		20u–20	3								20–18	3				
				i20–20	3	0.952					20–20u	1		22u–18	5								22–16	17	1.123			
											22–18u	17	1.931	24u–16	49													
														26u–14	1	0.463												
41	15–26	17		16–a25 + i16–25	23		a23–i18	22			15–26u	56		15u–26	21		u16–br25	63		a15–u26	76		17–24	100	0.060			
	16–25	29		23–i18 + a23–18	16		b25–i16	78	0.036		17–24u	17		16u–25	29		u17–i24	22		a23–u18	24	0.079						
	17–24	6		25–i16 + b25–16	41						23–18u	27	0.276	17u–24	10		u22–a18	15	0.054										
	18–23	21		a15–26	16									18u–23	28													
	23–18	9		a17–24	4	0.311								24u–17	12	2.383												
	24–17	8																										
	25–16		0.768																									
42	14–28	1		18–i24 + i18–24	23		i18–i24	18			14–28u	3		14u–28	2		u18–i24	26		i16–u26	58		14–28	2				
	16–26	55		24–i18 + i24–18	51		i24–i18	82	0.039		16–26u	60		16u–26	46		u24–i18	61		i18–u24	14		16–26	55				
	18–24	17		26–i16	6						18–24u	17		18u–24	19		u26–i16	13	0.372	i24–u18	28	0.267	18–24	20				
	20–22	1		i16–26	19						20–22u	1		20u–22	1								20–22	1				
	22–20	5		i20–22	2	0.685					22–20u	1		22u–20	4								22–20	1				
	24–18	17									24–18u	18	3.691	24u–18	20								24–18	21	2.483			
	26–16	4	3.394											26u–16	8	0.325												
43	16–27	16		24–a19	17		b25–i18	100	0.024		15–28u	31		16u–27	14		u18–br25	69		a15–u28	13		17–26	100	0.156			
	17–26	20		18–a25	25						17–26u	43		17u–26	29		u24–a19	41	0.055	a17–u26	34							
	18–25	34		25–i18 + b25–18	40						19–24u	12		18u–25	40					b25–u18	53	0.105						
	19–24	6		a17–26	18	0.164					25–18u	14	0.275	20u–23	6													
	23–20	8												24u–19	11	1.621												
	24–19	9																										
	25–18	7	0.479																									
44	16–28	11		14–i30	5		i20–i24	100	0.005		14–30u	1		16u–28	9		u20–i24	25		i16–u28	23		16–28	17				
	18–26	62		20–i24 + i20–24	18						16–28u	20		18u–26	66		u26–i18	75	0.054	i18–u26	60		18–26	73				
	20–24	3		26–i18	15						18–26u	67		20u–24	4					i20–u24	17	0.159	20–24	4				
	22–22	4		i16–28	8						20–24u	3		24u–20	17								22–22	2				
	24–20	15		i18–26	36						22–22u	2		26u–18	5								24–20	4	1.778			
	26–18	3		i24–20	18	0.216					24–20u	4				0.134												
	26–16	2	2.152								26–18u	3	2.450															
45	18–27	26		16–a29	40						17–28u	20		17u–28	10		u16–i29	100	0.011	b25–u20	100	0.010	17–28	100	0.035			
	19–26	33		b25–20	60	0.047					19–26u	63		18u–27	32													
	22–23	21									21–24u	17	0.134	20u–25	13													
	24–21	20	0.209											22u–23	19													
														24u–21	26	0.544	u16–i30	64		i18–u28	20		16–30	6				
46	18–28	27		16–i30	31		i16–i30	100	0.004		16–30u	12		18u–28	26		u22–i24	36	0.053	i20–u26	68		18–28	38				
	20–26	22		22–i24	18						18–28u	39		20u–20	27					i24–u22	12	0.106	20–26	26				

Table 1. (*Continued*)

Carbon number	A1			A2			A3			B1			B1			B2			B2			C		
	n–n	%[a]	Total %[a]	n–br + br–n	%	Total %	br–br	%	Total %	n–uns	%	Total %	uns–n	%	Total %	uns–br	%	Total %	br–uns	%	Total %	uns–uns	%	Total %
	22–24	23		28–i18	4					20–26u	19		22u–24	17								22–24	15	
	24–22	22		i18–28	6					22–24u	18		24u–22	21								24–22	13	
	26–20	6	0.665	i20–26	32					24–22u	12	0.938	26u–20	9	0.091							26–20	2	0.680
				i24–20	9	0.182																		
47	21–26	23		18–a29	29		a17–i30	54		21–26u	45		22u–25	27		u18–a29	63		a23–u24	49				
	22–25	20		22–a25	23		a23–i24	46	0.004	23–24u	27		24u–23	73	0.080	u22–br25	37	0.017	b25–u22	51	0.019			
	23–24	16		b25–22	27					25–22u	28	0.134												
	24–23	41	0.212	i24–23	21	0.047																		
48	20–28	3		16–i32	11		i18–i30	25		18–30u	9		20u–28	4		u16–i32	12		i20–u28	31		18–30	10	
	22–26	52		18–i30	25		i24–i24	75	0.010	20–28u	5		22u–26	42		u18–i30	34		i24–u24	69	0.053	20–28	6	
	24–24	45	0.678	24–i24 + i24–24	59					22–26u	49		24u–24	50		u24–i24	54	0.095				22–26	40	
				i20–28	5	0.166				24–24u	37	0.881	26u–22	4	0.491							24–24	41	
																						26–22	3	0.636
49	23–26	41		24–a25 + i24–25	40		b25–i24	100	0.005	23–26u	85		24u–25	85		u24–br25	100	0.017	a23–u26	47				
	24–25	45		a23–26	26					25–24u	15	0.113	26u–23	15	0.080				b25–u24	53	0.052			
	25–24	9		b25–24	34	0.076																		
	26–23	5	0.190																					
50	22–28	6		16–i34	15		i20–i30	50		18–32u	1		22u–28	4		u16–i34	36		i24–u26	100	0.096	18–32	2	
	24–26	90		18–i32	11		b25–a25	50	0.004	22–28u	10		24u–26	89		u18–i32	35					22–28	7	
	26–24	4	0.777	26–i24	6					24–20u	85		26u–24	7	0.629	u26–i24	29	0.031				24–26	85	
				i24–28	63					26–24u	4	1.000										26–24	6	0.864
				b25–25	5	0.125																		
51	23–28	12		22–a29	6					23–28u	31		24u–27	66		u26–br25	100	0.003	b25–u26	100	0.072	22–30	4	
	24–27	32		b25–26	94	0.062				25–26u	69	0.070	26u–25	34	0.035							24–28	54	
	25–26	45																				26–26	42	0.287
	26–25	11	0.088																					
52	24–28	54		18–i34	39					22–30u	7		24u–28	43		u18–i34	63		i24–u28	100	0.022			
	26–26	46	0.171	22–i30	40					24–28u	60		26u–26	57	0.173	u22–i30	37	0.017						
				24–28	31	0.035				26–26u	30													
										28–24u	3	0.319												
53	27–26	100	0.010	23–i30	14					25–28u	47		26u–27	100	0.004	u24–a29	100	0.006	b25–u28	100	0.016			
				24–a29	41					27–26u	53	0.023												
				b25–28	45	0.017																		
54	26–28	35		24–i30	100	0.021	i24–i30	100	0.002	24–30u	48		26u–28	100	0.013	u24–i30	100	0.017	i24–u30	100	0.005	24–30	56	
	28–26	65	0.030							26–28u	24											26–28	44	0.061
										28–26u	28	0.088												
55				25–i30	100	0.001	b25–i30	100	0.002	24–32u	29								b25–u30	100	0.004			
56	30–26	100	0.010	24–i32	75					28–28u	22					u24–i32	66							
				26–i30	25	0.010				30–26u	49	0.026				u26–i30	34	0.009						
58				24–i34	100	0.007				30–28u	100	0.003				u24–i34	100	0.006						
sum of fraction			29.477			8.650			0.555			17.893			22.006			3.836			1.644			11.226

Abbr.: i = iso; a = anteiso; b = branched; u = unsaturated; n = normal.

$[RCO_2H]^+$ and $[RCO_2H_2]^+$ 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 %
n-12:0	1.9	—
n-13:0	0.8	—
n-14:0	4.0	2.2
n-15:0	1.3	0.4
n-16:0	18.6	18.4
n-17:0	1.0	1.0
n-18:0	14.6	8.3
n-19:0	0.7	0.6
n-20:0	1.5	4.7
n-21:0	0.5	0.6
n-22:0	3.6	2.1
n-23:0	0.8	1.2
n-24:0	7.1	4.3
n-25:0	0.4	1.2
n-26:0	0.8	9.8
n-27:0	0.1	0.4
n-28:0	0.2	1.3
n-30:0	0.1	—
Normal saturated	**57.0**	**56.5**
iso-14:0	0.6	—
iso-16:0	1.5	4.7
iso-18:0	0.8	4.0
iso-20:0	0.6	—
iso-24:0	0.8	0.9
iso-30:0	—	0.3
iso-32:0	—	0.4
iso-34:0	—	0.1
anteiso-15:0	0.5	—
anteiso-17:0	0.3	—
anteiso-19:0	0.1	0.4
anteiso-23:0	0.2	0.1
anteiso-25:0	0.1	0.4
anteiso-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-dicloroflurescein 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 ×3 with an equal vol. of $CHCl_3$. After Ag-TLC, 3 frs were obtained A (O× C=C, saturated only), B (1× C=C, wax esters with one double bond in acid or alcohol), C (2× C=C, wax esters with two double bonds, monoenic acids and monoenic alcohols).

GC and GC-MS. Injection temp. (splitless) was 100° and a fused-silica capillary column (Supelcowax 10; 15 m × 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 × 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.

REFERENCES

1. Cruse, R. R. (1959) *Economic Botany* **13**, 243.
2. Cruse, R. R. (1973) *Economic Botany* **27**, 210.
3. Gibson, A. G. and Nobel, P. S. (1992) *The Cactus Primer*.
4. Hamilton, R. J. (Ed.) (1995) *Wax: Chemistry, Molecular Biology and Functions*. The Oily Press, Dundee.
5. Tulloch, A. P. (1987) *Phytochemistry* **26**, 1041.
6. Gulz, P.-G., Markstadter, C. and Riederer, M. (1994) *Phytochemistry* **35**, 79.
7. Stevens, J. F., Hart, H., Bolk, A., Zwaving, J. H. and Malingre, T. M. (1994) *Phytochemistry* **35**, 389.
8. Rieley, G., Welker, J. M., Callaghan, T. V. and Eglinton, G. (1995) *Phytochemistry* **38**, 45.
9. Shephard, T., Robertson, G. W. and Griffiths, D. W. (1995) *Phytochem. Anal.* **6**, 65.
10. Herbin, G. A. and Robins, P. A. (1968) *Phytochemistry* **7**, 239.
11. Herbin, G. A. and Robins, P. A. (1968) *Phytochemistry* **7**, 256.
12. Kurtz, E. B., Jr. (1958) *J. Amer. Oil Chem. Soc.* **35**, 465.
13. Miwa, T. K. (1971) *J. Amer. Oil Chem. Soc.* **48**, 259.
14. Hughes, J., Ramos, G. and Moyna, P. (1980) *J. Nat. Prod.* **43**, 564.
15. Serrano, C. E. and Guzman, C. A. (1991) *An Asoc. Quim. Argent.* **79**, 245.
16. Rezanka, T. and Podojil, M. (1986) *J. Chromatogr.* **362**, 399.
17. Aasen, A. A., Hofstetter, H. H., Iyengar, B. T. R. and Holman, R. T. (1971) *Lipids* **6**, 502.
18. Spencer, G. F. (1979) *J. Amer. Oil Chem. Soc.* **56**, 642.
19. Vajdi, M., Nawar, W. W. and Merritt, C. (1981) *J. Amer. Oil Chem. Soc.* **58**, 106.
20. Serrano, C. E. and Guzman, C. A. (1991) *An. Asoc. Quim. Argent.* **79**, 237.
21. Pieters, E. P. (1972) *Phytochemistry* **11**, 2623.