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KOLAVANE DITERPENOIDS OF *VANCLEVEA STYLOSA*

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Key Word Index—*Vanclevea stylosa*; Asteraceae; Astereae; Solidagininae; kolavane diterpenoids; flavonoids.

Abstract—From the aerial parts of *Vanclevea stylosa*, 19 new kolavane diterpenoids were isolated as their methyl ester derivatives and two were also isolated as the free acids. Their structures were elucidated from the NMR and mass spectra and a few chemical transformations. All the compounds are kolavenol derivatives having a succinyloxy group either at C-17 or C-18, with widely differing C-9 side chains; in four cases carbons have been lost from this side chain. Three known flavonoids, identified as kaempferol 3,7,4′-trimethyl ether, quercetin 3,7,3′,4′-tetramethyl ether (retusine) and quercetin 3,3′-dimethyl ether, were also isolated.

INTRODUCTION

Vanclevea stylosa (Eastw.) Greene (Asteraceae, Astereae, Solidagininae), the sole representative of the genus, is distributed exclusively in sandy desert wastes in southeastern Utah and northeastern Arizona. The low tufted and slender shrubs are very resinous like members of the closely related genus *Grindelia*. Because nothing was known of the chemistry of this genus and also as part of our search for biologically active molecules from xerophytic Astereae, we have examined the chemical constituents of *V. stylosa*. We report here the isolation and identification of 22 compounds from the methylene chloride extract, 19 of which are new kolavane diterpenoids.

RESULTS AND DISCUSSION

The methylene chloride extract of the aerial parts of *V. stylosa* gave an ether-soluble fraction from which the sodium carbonate-soluble acid fraction was separated and methylated. Chromatography of the methylated product yielded 19 new diterpenoid methyl esters (**4**–**22**) and three flavonoids (**26**–**28**). The free acids **2** and **3** (vanclevic acids A and B, respectively) corresponding to the first two of these esters were isolated directly from the acid fraction. Except for **2**–**5**, **8** and **26**–**28**, these compounds were not separated as single entities but were isolated as unresolved TLC-homogeneous mixtures (I–VIII), six of which contained a pair of structural isomers (I–III and VI–VIII). These included **6** (80%) and **7** (20%) [I]; **8** (95%) and **9** (5%) [II]; **10** (90%) and **11** (10%) [III]; **12** (25%) and **13** (75%) [IV]; **8** (10%), **14** (20%), **15** (60%) and **16** (10%) [V]; **17** (35%) and **18** (65%) [VI]; **19** (90%) and **20** (10%) [VII]; and **21** (80%) and **22** (20%) [VIII]. The identity of each component and its percentage in the mixture were determined primarily by ^{1}H NMR analysis. All the diterpenoid esters (**4**–**22**) are derivatives of kolavenol (**1**) [1] oxidized and esterified with succinic acid either at C-17 or C-18. The majority retain the six-carbon C-9 side chain, but C-15 has been lost in compounds **14**–**16**, and C-14 as well in **13**. Six diterpenoids (**17**–**22**) were dimers, esterified either from two units of vanclevic acid A (**2**) or B (**3**) or one unit of **2** and **3**, with or without substitution at C-15. The flavonoids were identified as kaempferol 3,7,4′-trimethyl ether (**26**), quercetin 3,7,3′,4′-tetramethyl ether (retusine) (**27**) and quercetin 3,3′-dimethyl ether (**28**).

These compounds (**4**–**22** and **26**–**28**) were isolated from two different collections of *V. stylosa*. From the initial collection made in Utah in September 1985, **5**–**16**, **19**–**22** and **26**–**28** were isolated and the latter collection, made in Arizona in September 1986, yielded **4**–**9**, **17**, **18**, **26** and **27**. It is interesting to note that the C-18 substituted kolavanes were dominant in the Utah collection, while the Arizona collection was enriched in C-17 analogues. This could be due to seasonal and/or geographic differences in the plant collections.

The characterizations of these compounds are described below beginning with those isolated as single entities.

Structures of **2**–**5** and **23**–**25**

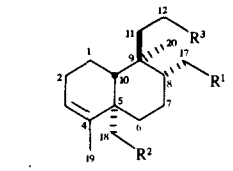
Vanclevic acids A (**2**) and B (**3**) were obtained as oils, $C_{24}H_{38}O_5$ by HRMS, $[\alpha]_D^{25}$ −43.5° ($CHCl_3$; *c*0.7) and −59.5° ($CHCl_3$; *c*5.1), respectively. Their ^{1}H NMR spectra (Table 1) show a geraniol-type side chain with an *E*-configuration [1–3] and a pattern of methyl and vinyl groups which suggests a kolavane carbon skeleton esterified with succinic acid (two slightly nonequivalent methylenes giving a complex symmetrical pattern for four protons at δ2.63). The points of attachment of the succinate groupings were clear from the splitting patterns for the methylenes attached to succinate and from the chemical shifts of the remaining methyl groups as compared to kolavenol (**1**) itself [1]; especially useful were the C-18 and C-20 methyl singlets at δ0.79 and 1.01, respectively, in the C-17 succinates, and the C-17 methyl

Table 1. ^{1}H NMR chemical shifts (δ, $CDCl_3$) and coupling constants (Hz, in parentheses) for compounds **2**, **3**, and **5**–**25**

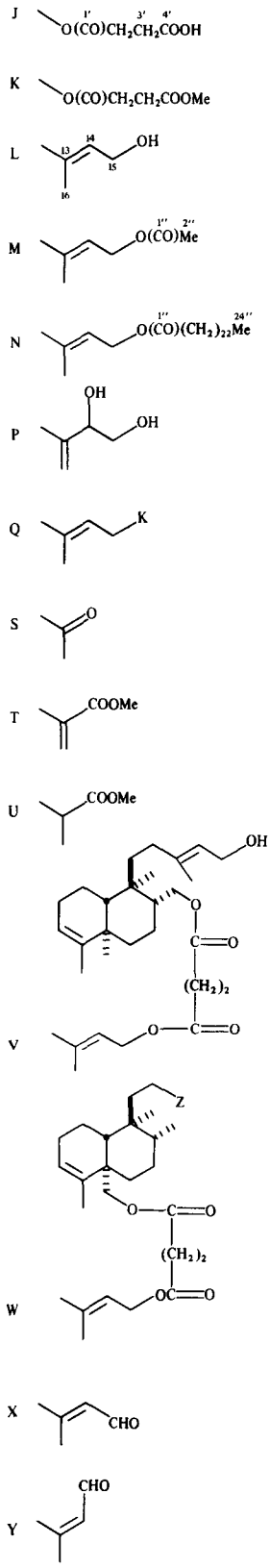
H	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
2	2.03 *m*	2.00	2.00	2.00		2.00		2.00		2.00	2.00	
3	5.20 *br s*	5.21	5.21	5.21	5.20	5.21	5.21	5.21	5.21	5.21	5.21	5.21
12			1.87 *m*	1.89		1.89					2.30	
14	5.40 *td* (6.6, 1.0)	5.41 *t* (7.0)	5.43 *td* (6.9, 1.1)	5.35 *br t* (6.6)	5.34 *br t* (6.5)	5.35 *br t* (6.6)	5.35 *br t* (6.6)	4.25 *m*		5.35 *br t* (7.0)		
15	4.14 *d* (6.6)	4.16 *d* (7.3)	4.15 *d* (7.4)	4.59 *d* (7.1)	4.57 *d* (7.0)	4.59 *d* (7.1)		3.53 *dd* (11.1, 7.4), 3.67 *m*		4.62 *d* (7.1)		
16	1.67 *s*	1.69 *d* (1.0)	1.70 *s*	1.73	1.71	1.73		4.99*s* 5.14*s*	4.97 5.09	1.73	2.18	5.57 6.15
17	3.76 *dd* (10.9, 8.1), 4.29 *dd* (10.9, 3.6)	0.91 *d* (6.3)	0.88 *d* (6.4)	0.88 *d* (6.3)	3.85 *dd* (11.0, 8.5), 4.26 *dd* (11.0, 3.0)	0.88 *d* (6.3)	3.85 *dd* (11.0, 8.5), 4.26 *dd* (11.0, 3.0)	0.89 *d* (6.3) 4.13		0.87 *d* 6.3)	0.87 *d* (6.3)	0.85 *d* (6.2)
18	0.79 *s*	4.11 *d* (11.5) 4.27 *d* (11.5)	4.12 *d* (11.6) 4.20 *d* (11.6)	4.11 *d* (11.6) 4.18 *d* (11.6)	0.79	4.11 *d* (11.6) 4.18 *d* (11.6)	0.79	4.15 *d* (11.5) 4.23 *d* (11.5)	0.79	4.12 *d* (11.5) 4.18 *d* (11.5)	4.12 *d* (11.7) 4.19 *d* (11.7)	4.06 *d* (11.6) 4.13 *d* (11.6)
19	1.59 *d* (1.3)	1.58 *d* (1.3)	1.58 *d* (1.5)	1.58 *d* (1.5)	1.59 *br s*	1.58	1.58	1.58 *d* (1.2)	1.58 *d* (1.2)	1.58 *d* (1.5)	1.58 *d* (1.5)	1.58 *br s*
20	1.01 *s*	0.98	0.99	0.99	1.01	0.99	1.01	0.99	1.01	0.99	0.99	0.99
2′, 3′	2.64 *m*	2.62	2.63 *s*	2.63	2.63	2.63	2.63	2.63	2.63	2.64	2.64	2.62
OMe			3.69 *s*	3.69	3.70	3.69	3.70	3.69	3.70	3.69, 3.70	3.70	3.68, 3.77
2″				2.07 *s*	2.06	2.30 *t* (7.5)	2.30 *t* (7.5)					
3″–23″						1.25 *br s*	1.25					
24″						0.88 *t* (6.6)	0.88 *t* (6.6)					

H	**15***	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
2	2.01		2.02	2.02	2.00	2.00	2.00	2.00	2.00	2.00	2.00
3	5.21	5.21	5.20	5.20	5.21	5.21	5.21	5.21	5.20	5.22	5.22
12							1.89			2.03	2.43 *t* (8.7)
14			5.33 *d* (6.6) 5.41 *d* (7.1)	5.33 *d* (6.6) 5.41 *d* (7.1)	5.35 *d* (7.0)	5.35 *d* (7.0)	5.35 *d* (6.6)	5.35 *d* (6.6)	5.35 *d* (7.0) 5.90 *d* (8.1)	5.91 *d* (8.0)	5.88 *d* (8.1)
15			4.14 *d* (6.6) 4.61 *d* (7.0)	4.14 *d* (6.6) 4.59 *d* (7.0)	4.59 *d* (6.6) 4.61 *d* (6.6)	4.59 *d* (6.6)	4.58 *d* (6.7) 4.61 *d* (6.2)	4.58 *d* (6.7)	4.60 *d* (7.1) 9.99 *d* (8.1)	9.99 *d* (8.0)	9.93 *d* (8.1)
16	1.17 *d* (7.0)	1.16 *d* (7.0)	1.68, 1.72	1.68, 1.70	1.72	1.70, 1.72	1.72	1.70, 1.72	1.71 2.20 *d* (1.2)	2.21 *d* (1.2)	2.02 *d* (1.0)
17	0.84 *d* (6.5)	3.82 *dd* (11.0, 8.3), 4.26 *dd* (11.0, 3.3)	0.88 *d* (6.1), 3.84 *dd* (11.0, 8.3), 4.25 *dd* (11.0, 4.0)	3.82 *dd* (11.0, 8.2), 3.84 *dd* (11.0, 8.3), 4.25 *dd* (11.0, 4.0)	0.88 *d* (6.2)	0.88 *d* (6.2), 3.85 *dd* (11.0, 8.3), 4.26 *dd* (11.0, 3.5)	0.88 *d* (6.1)	0.88 *d* (6.1), 3.85 *dd* (11.0, 8.3), 4.26 *dd* (11.0, 3.5)	0.88 *d* (6.1) 0.89 *d* (5.5)	0.89 *d* (6.1)	0.92 *d* (6.2)
18	4.11 *d* (11.3) 4.18 *d* (11.3)	0.76	0.79, 4.12 *d* (11.5), 4.18 *d* (11.5)	0.79	4.11 *d* (11.6) 4.18 *d* (11.6)	0.79	4.10 *d* (11.5) 4.18 *d* (11.5)	0.79	4.11 *d* (11.5), 4.15 *d* (11.5) 4.19 *d* (11.5), 4.20 *d* (11.5)	4.15 *d* (11.7) 4.19 *d* (11.7)	4.16 *d* (11.7) 4.19 *d* (11.7)
19	1.58	1.58	1.59 *d* (1.0)	1.59 *d* (1.0)	1.58 *d* (1.0)	1.58 *d* (1.0)	1.58 *br s*	1.58	1.58	1.59	1.59
20	0.97	0.99	0.99, 1.01	1.01	0.99	0.99, 1.01	0.99	0.99, 1.01	1.00, 1.01	1.00	1.01
2′, 3′	2.62	2.62	2.63	2.63	2.63	2.63	2.63	2.63	2.63	2.63	2.62
OMe	3.68, 3.69	3.69	3.69	3.70	3.69	3.70	3.69	3.70	3.68	3.68	3.68
2″					2.07 *s*	2.07	2.30 *t* (7.5)	2.30 *t* (7.5)			
3″–23″							1.25	1.25			
24″							0.88 *d* (6.6)	0.88 *d* (6.6)			

***15** has H-13 at δ2.39 *sextet* (6.7).



	R^1	R^2	R^3	
1	H	H	L	
2	J	H	L	
3	H	J	L	
4	K	H	L	
5	H	K	L	
6	H	K	M	mix. I
7	K	H	M	
8	H	K	N	mix. II
9	K	H	N	
10	H	K	P	mix. III
11	K	H	P	
12	H	K	Q	mix. IV
13	H	K	S	
14	H	K	T	
15	H	K	U	and **8** (mix. V)
16	K	H	U	
17	H	K	V	mix. VI
18	K	H	V	
19	H	K	W (Z = M)	mix. VII
20	K	H	W (Z = M)	
21	H	K	W (Z = N)	mix. VIII
22	K	H	W (Z = N)	
23	H	K	W (Z = X)	
24	H	K	X	
25	H	K	Y	



doublet at δ0.88 and C-20 methyl singlet at δ0.99 in the C-18 succinates. The configurations at the chiral centers are assumed to be the same as those found in previous kolavanes [1–5]; the relative configurations are supported by the similarity between the ^{1}H NMR spectra of kolavenol (**1**) and **2**–**25**, and their having the same absolute configuration is likely in view of the closeness of the optical rotations noted above for **2** and **3** to that of the acetate of kolavenol (**1**) (−45.7°) [1].

The ^{13}C NMR spectra of **2** and **3** (Table 2) support the

Table 2. ^{13}C NMR chemical shifts (δ, $CDCl_3$) for compounds **2**, **3** and **5**

C	2	3	5
1	26.6	27.2	27.3
2	17.7	19.4	19.6
3	120.5	120.5	120.6
4	144.0	143.5	143.4
5	38.0	41.8	41.8
6	36.0	30.1	30.4
7	22.4	27.1	27.3
8	40.7	36.0	36.1
9	37.7	37.9	38.0
10	46.2	46.8	46.9
11	32.7	31.8	32.2
12	36.6	36.7	36.9
13	140.2	140.1	139.8
14	122.7	122.4	123.4
15	59.2*	58.7	59.1
16	16.5	16.6	16.4
17	66.7	16.4	16.6
18	19.7	67.2	67.6
19	17.9	17.6	17.7
20	19.1	19.3	19.4
1′	172.5	172.4	172.2
2′	29.2*	29.0	28.8
3′	29.5*	29.2	29.2
4′	not seen*	176.2†	172.5
OMe			51.6

*The peaks for carbons near the hydroxyl and carboxyl groups in **2** were broadened as follows, probably due to a paramagnetic impurity: C-2′, broad; C-15 and C-3′, very broad; C-4′, too broad to observe.

†The 4′ carbonyl may overlap the 1′ carbonyl at δ172.4, and the peak at δ176.2 may be due to a trace of acetic acid.

proposed structures. The largest differences are of course in the shifts for C-17 and C-18, with the 'in between' carbons C-5–C-8 showing quite large differences as well, in the expected directions.

The EIMS of **2** (Scheme 1; all molecular formulas of fragment ions shown were verified by HRMS) contained a very weak $[M]^+$ peak at *m/z* 406 accompanied by stronger peaks at *m/z* 391 $[M-Me]^+$, 388 $[M-H_2O]^+$ and 288 $[M-118]^+$ ($C_{20}H_{32}O$). That the ion at *m/z* 118 ($C_4H_6O_4$) corresponding to succinic acid is strongly supported by an intense peak at *m/z* 101 ($C_4H_5O_3$), shifted to *m/z* 115 ($C_5H_7O_3$) in the methyl ester **4**. This latter peak, and the peak for loss of 132 mass units ($HOOCCH_2CH_2COOMe$) from M, were of major importance in deducing the molecular formulas of methyl alkyl succinates **4**–**22**. The observation of daughter fragments at *m/z* 307 ($C_{18}H_{27}O_4$) $[M-C_6H_{11}O]^+$ and *m/z* 189 ($C_{14}H_{21}$) $[M-(C_4H_6O_4+C_6H_{11}O)]^+$, derived either directly from M and *m/z* 288, respectively, or via initial loss of H_2O, upheld the presence of a $-CH_2CH_2CMe=CHCH_2OH$ side chain as in kolavenol (**1**), ruling out the possibility of succinic acid being attached at C-15 of the C-9 side chain and accounting for the last oxygen atom. Vanclevic acid B (**3**) displayed a mass spectrum (EI) which differed only quantitatively from that of vanclevic acid A (**2**), as expected for the proposed structure.

Methylation of **2** and **3** with methyl iodide [6] gave the corresponding methyl esters, **4** and **5**, respectively [**5**: ^1H NMR, OMe at δ3.69; $[\alpha]_D^{25}$ −65.1° ($CHCl_3$; *c*7.3); MS: *m/z* 420 (M^+)], identical with those obtained from the Silica gel column chromatography of the methylated product of the acid mixture.

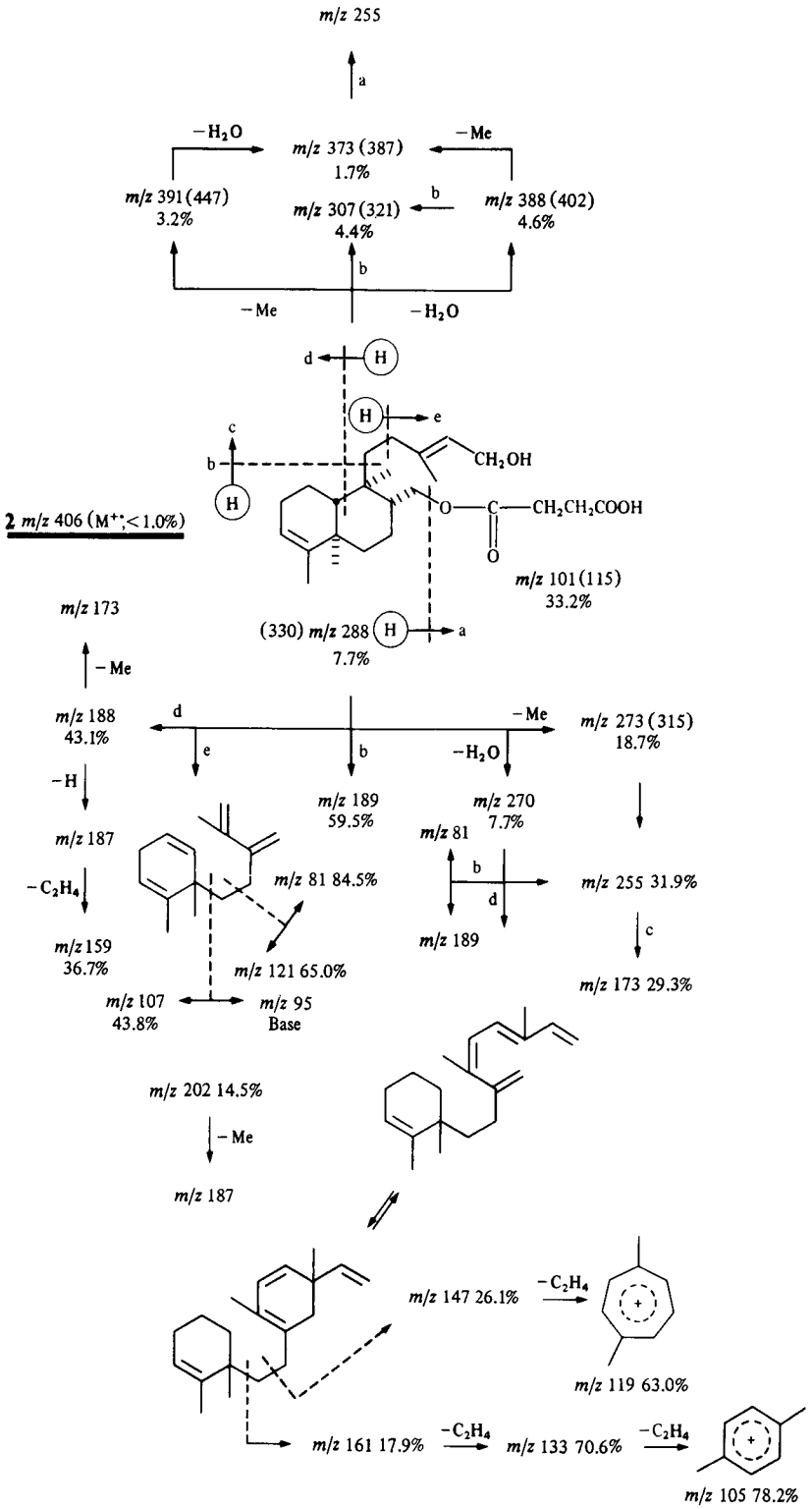
Oxidation of **5** with manganese dioxide gave a product from which two isomeric aldehydes, the E-(**24**, major component) and Z-(**25**, minor component) isomers, and a very small amount of dimerized aldehyde (**23**, E-isomer) were separated by silica gel preparative TLC. The IR (neat) spectra of these aldehydes (**23**–**25**), which lacked hydroxyl bands, showed, in addition to ester carbonyl bands, α,β-unsaturated C=O bands between 1670 and 1680 cm^{-1}, confirmed by ^1H NMR (Table 1) and mass spectra. The EIMS of **24**, which was very similar to that of **25**, displayed the same fragmentation pattern as that of **5** but some of the peaks, including M^+ (*m/z* 418) and *m/z* 286 (M-132), were shifted to lower mass number by two, confirming the conversion of **5** to **24**. The dimeric nature of **23** was immediately apparent from its EIMS, which exhibited a barely discernible M^+ (*m/z* 806) peak and a peak at *m/z* 674 corresponding to $[M-132]^+$. A group of peaks at *m/z* 404, 403 and 402, clearly suggested that **25** contained two succinyloxy kolavane units as shown in Scheme 2. The kolavane units then give rise to the principal fragments at *m/z* 287, 286, 271, 270, 255, 202, 189, 187, 173, 159, 145, 133, 132, 119, 115, 105, 95 and 81 by a breakdown pattern similar to that of **5** and **24**. The ^1H NMR spectrum of **23** (Table 1) also showed it to consist of a unit like **24** attached to a unit like **5**.

Structures of **6**–**9**.

The acetates **6** and **7** were easily characterized by NMR spectral comparisons with **4**, **5** and geranyl acetate. Compound **8** was revealed by ^1H NMR to also be a C-15 ester of a fatty acid, but one with a much longer chain. The chain length was established as 24 (lignoceric acid) by mass spectrometry: the M^+ (*m/z* 770) peak was not visible in the EIMS, but peaks at *m/z* 638 [M − methyl succinate]$^+$, 623 $[638-Me]^+$, 449 [C-9 side chain]$^+$, 403 $[M-OCO(CH_2)_{22}Me]^+$, 402 $[M-HOOC(CH_2)_{22}Me]^+$, 368 $[HOOC(CH_2)_{22}Me$ (lignoceric acid)$]^+$ and 321 [M − C-9 side chain]$^+$ made the fatty acid chain length clear and confirmed its location. Other fragmentation peaks were similar to those of **5**. The EIMS of the mixture II containing **8** and **9** was very similar to that of **8**; the ^1H NMR spectrum of **9** (Table 1) revealed its structure.

Structures of **10** *and* **11**

The ^1H NMR parameters of **10** are close to those of **6** except in the region of the C-9 side chain, where **10** has absorptions for C-14–C-16 indicating the oxidized and double bond-shifted side chain shown. The much weaker peaks at δ0.79, 1.01, 4.97 and 5.09 in the TLC-homogeneous sample indicate that **10** is accompanied by the corresponding C-17 succinate **11**. No M^+ peak (*m/z* 436) was observed in the EIMS of the mixture III containing **10** and **11** but the diagnostic peaks following the loss of 131 and 132 mass units from M^+, corresponding to *m/z* 305

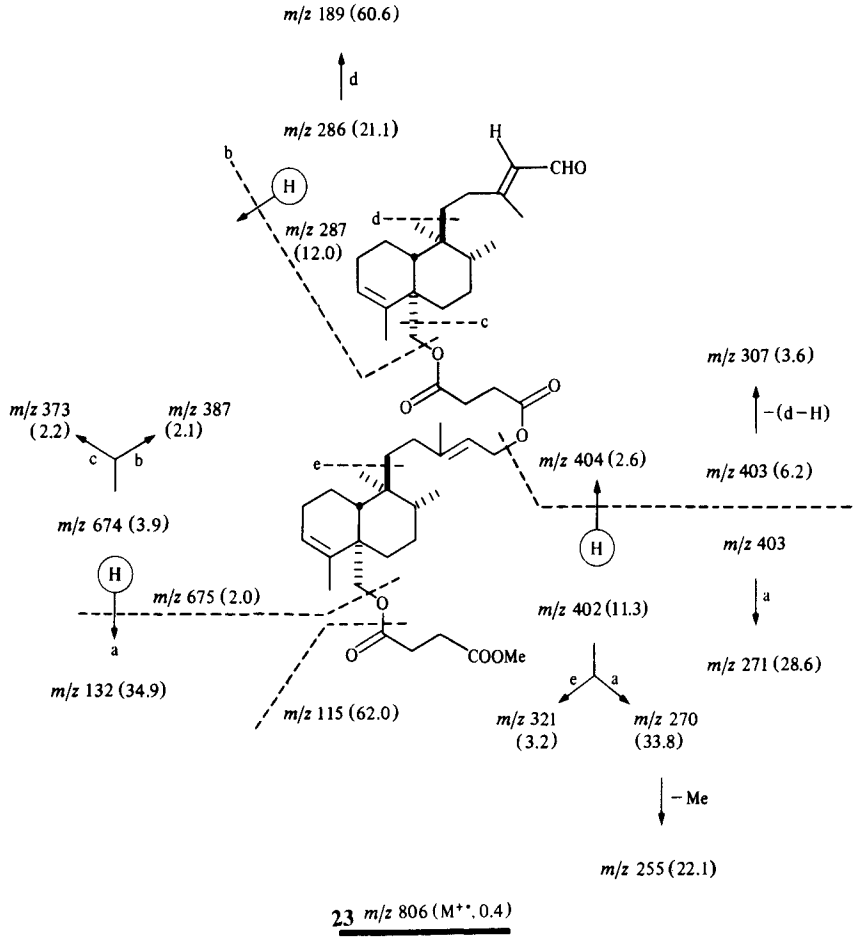


Scheme 1. Diagnostic EIMS fragments of **2**. Figures in parentheses represent analogous ions in the EIMS of a mixture of **6** and **7**.

and 304, respectively, characterized the nature of the C-9 side chain as depicted in Scheme 3.

Structures of **12** *and* **13**

The ^{1}H NMR spectrum of mixture IV showed it to contain **12** and **13**. Compound **12** has an extra methyl succinate grouping at C-15, as shown by its extra methoxyl peak, twice the usual absorption for succinate CH_2's, and absorption for the C-15 CH_2 indicating a C-15 ester. From its absorption for the C-12 CH_2 and C-16 methyl group and lack of absorption for H-14 and H-15, **13** is the chain-shortened methyl ketone shown. It was also detected as a minor byproduct of the oxidation of **5** to



Scheme 2. Some diagnostic fragments in the EIMS of **23** (relative intensities in parentheses).

24 mentioned above. The EIMS of the mixture fully supported these structures. The M^+ and pertinent peaks related to **13** were sorted out and rationalized as shown in Scheme 4; some of these ions come from **12** as well. The M^+ peak (*m/z* 534) for **12** was barely seen but was confirmed from the *m/z* 402 $[M-132]^+$ peak. The second succinate moiety was seen clearly from *m/z* 287 (*m/z* 402–115) and 270 (*m/z* 402–132) peaks. The C-9 side chain containing the succinate moiety was seen as a peak at *m/z* 213 and as a loss of 213 from M^+, and *m/z* 402 at *m/z* 321 and *m/z* 189, respectively.

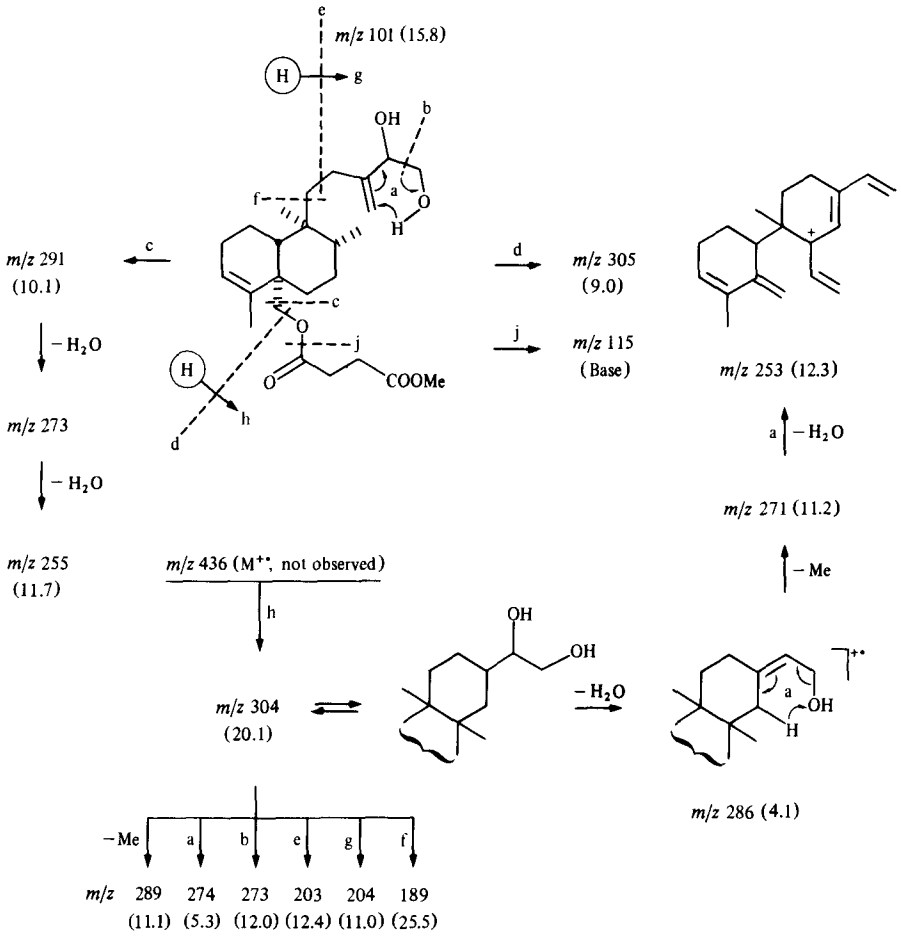
Structures of **14–16**

From their ^{1}H NMR spectra, **14–16** have lost C-15 through oxidation and are methyl esters of C-14 acids as shown. The H-16 vinyl protons of **14** absorb considerably further downfield than those of **10** because of conjugation with the C-14 carbonyl group. The doublets for the C-methy group of **15** and **16** are further downfield than the C-17 methyl groups doublets in compounds such as **15** because the former are β to a carbonyl group. The EIMS of the mixture V containing **14–16** gave an M^+ at *m/z* 436 and peaks at *m/z* 404 $[M-MeOH]^+$, 305 $[M-131]^+$, 304 $[M-132]^+$, 289 $[304-Me]^+$, 257 $[289-MeOH]^+$ for the major isomeric components **15** and **16** but no M^+ peak at *m/z* 434 for the minor component **14** was observed. It was, however, deduced from peaks at *m/z* 303 $[M-131]^+$, 302 $[M-132]^+$, 271 $[302-OMe]^+$, 203 $[302-H_2C=CCH_2COOMe].^+$ Loss of the C-9 side chain, $-CH_2CH_2\overset{\overset{\|}{CH_2}}{C}COOMe$ from *m/z* 302 in **14** and $-CH_2CH_2CMeCOOMe$ from *m/z* 304 in **15** and **16**, gave an intense peak at *m/z* 189.

Structures of **17–22**

The integrations of the ^{1}H NMR spectra of mixtures VI–VIII indicated them each to be mixtures of two dimeric components containing a C-17 succinate and the corresponding C-18 succinate. All of the groupings present were very well modelled by the monomeric compounds described above.

The mixture VI containing **17** and **18** did not exhibit an M^+ peak at *m/z* 808 but showed a peak at *m/z* 790 $[M-H_2O]^+$ and yielded a mass spectrum similar to that of **23**, which contains fewer hydrogen atoms, permitting quick identification. The fragments that retain the $-CH_2CH_2CMe{=}CHCH_2OH$ with or without loss of water were appropriately shifted as required by the substitution of a hydroxyl for a keto group at C-15 in **17** and **18**. These include *m/z* 659 $[790-131]$, 658 $[790-132]^+$, 405 and 270, corresponding to fragments of **23** at *m/z* 675, 674, 403 and 286, respectively.



Scheme 3. Pertinent fragment ions of **10** in the EIMS of the mixture of **10** and **11** (relative intensities in parentheses).

The overall fragmentation pattern in the EIMS of the mixture of **19** and **20** (VII) was very similar below *m/z* 405 to that of the mixture of **6** and **7** (I) but the peaks in the high mass range characterized its dimeric nature. Thus, the M^+ peak at *m/z* 850 and the peaks at *m/z* 790 [M −HOAc]$^+$, 718 [M−132]$^+$, 658 [790−132]$^+$ or [718 −HOAc]$^+$ and a pair of peaks at *m/z* 403 and *m/z* 387 adding up to the equivalent mass of the *m/z* 790 fragment clearly suggested that **19** and **20** consist of two units of **5** and **6** and **4** and **7**, respectively, connected by an ester linkage as shown.

The EIMS of the mixture VIII containing **21** and **22**, which did not display an M^+ peak at *m/z* 1158, was very similar to those of **8**, II and VII. As in VII, the heaviest peak, at *m/z* 790, corresponded to a loss of 368 mu [$HOOC(CH_2)_{22}Me$] from M^+, followed by a peak at *m/z* 658 [790−132]$^+$. As in **8** and II, the loss of 368 mass units gave a strong peak. These observations clearly suggested that **21** and **22**, like **19** and **20**, were dimers esterified with $Me(CH_2)_{22}COOH$ at C-15.

The EIMS of the majority of C-17 and C-18 succinyloxy kolavanes discussed above exhibited a base peak at *m/z* 95; possible formulation is shown in Scheme 5.

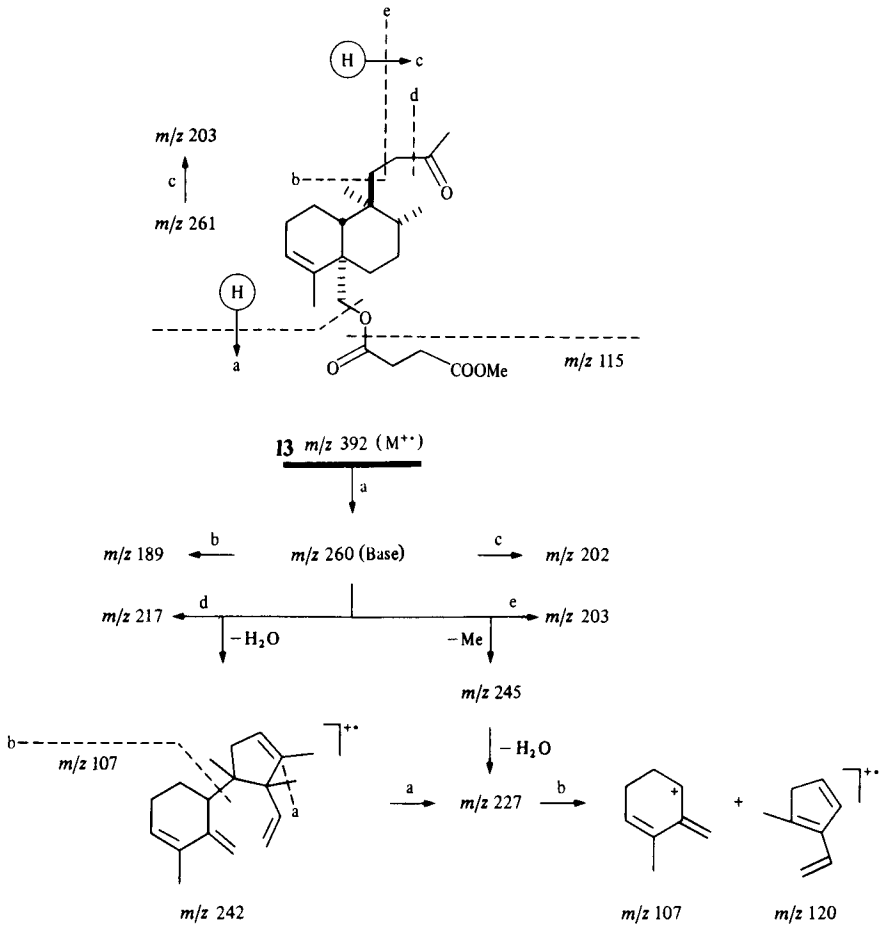
EXPERIMENTAL

Plant material. The plant collections were done in Utah, Wayne County, 12.9 miles north of Hanksville in September 1985 (SPM 3090) and in Arizona (Coconino County, 11.5 miles south of Page during September 1986, SPM 3948A). Specimens were deposited in the Herbarium at the University of Arizona, Tucson. All plant material was air-dried, ground to 3 mm particle size and stored at 5° prior to extraction.

Extraction and separation of the acidic fraction. The ground aerial parts of *V. stylosa* were extracted exhaustively with CH_2Cl_2 and the solvent-free CH_2Cl_2 extract was triturated with Et_2O, left in the refrigerator overnight and filtered. The Et_2O-soluble filtrate was washed with 5% aq. Na_2CO_3 until the washings were nearly colourless. The emulsions formed during washings were broken by centrifugation. The combined washings were acidifed to pH 4 with 25% aq. HCl and the liberated acids were taken up in Et_2O and dried (Na_2SO_4). Removal of Et_2O under vacuum gave the crude acid mixture.

In the case of the Utah collection, the acid mixture was first methylated [6], and chromatographed on silica gel to isolate compounds **5–16**, **19–22**, **26** and **27**. Vanclevic acid B (**3**) and **28** were isolated directly from the acid mixture. In the case of the Arizona collection, the acid mixture was first chromatographed on silica gel, which resulted in the isolation of vanclevic acids A (**2**) and B (**3**), and the resulting combined fractions were then methylated followed by silica gel CC to isolate compounds **5–9**, **17**, **18**, **26** and **27**. The isolation of all these compounds was performed qualitatively.

Isolation of vanclevic acid B (**3**). A small portion of the acid mixture (6.0 g) was chromatographed on silica gel (150 g packed in CH_2Cl_2) eluting the column with CH_2Cl_2–EtOAc–AcOH



Scheme 4. Some pertinent fragment ions from **13** in the EIMS of a mixture of **12** and **13**.

[90:10:1 (and 2)] and CH_2Cl_2–MeOH (1:1). Fractions eluted with CH_2Cl_2–EtOAc–AcOH (45:5:1) contained **3** and a small amount of **28**. The latter (**28**) separated out as yellow crystals (mp 262–263°) when treated with Et_2O, identified by direct comparison of its ^1H NMR parameters with those of an authentic sample. From the Et_2O-soluble fraction, **3** was separated by silica gel prep. TLC [CH_2Cl_2–EtOAc–AcOH (90:10:7), single development] and purified by further prep. TLC (same solvent system).

Methylation of **3**. Methylation of **3** with MeI [6] gave **5**, purified by silica gel prep. TLC [CH_2Cl_2–EtOAc–AcOH (140:10:7), two developments], identical to that obtained by silica gel CC of the methylated product of the acid mixture.

Oxidation of **3**. A mixture of **3** (1.0 g) and MnO_2 (10.0 g) in CCl_4 (70 ml) was vigorously stirred for 1 hr, filtered and solvent freed. Silica gel prep. TLC [*n*-hexane–Et_2O (3:5), single development] of the reaction product gave fractions A-D [A (nil), B (129 mg), C (524 mg) and D (40 mg)]. Fraction B (129 mg) contained **23** and **24** which were separated from one another by prep. TLC [*n*-hexane–Et_2O (1:1), 2 developments]. Fraction C (524 mg) contained **23** (major component) and **25** (minor). Silica gel prep. TLC [*n*-hexane–Et_2O(4:5), 3 developments] gave two fractions, C1 (pure **23**) and C2 (**25** + trace of **23**). Purification of C2 by prep. TLC [*n*-hexane–Et_2O (1:1), 3 developments] gave pure **25**.

Acetylation of **3**. Acetylation with $(CH_3CO)_2O$ and pyridine gave an acetate identical to natural **7** found in mixture I.

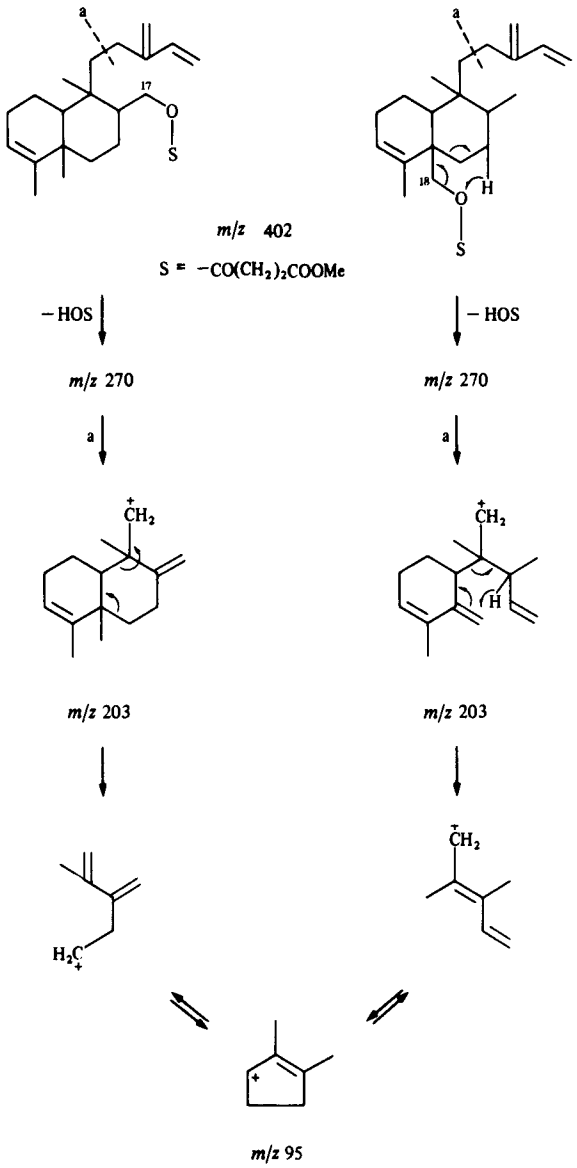
Isolation of compounds **6**–**16** *and* **19**–**22**. The methyl ester mixture (62 g) was chromatographed on silica gel (1800 g packed in *n*-hexane). Elution with *n*-hexane containing various concentrations of Et_2O gave fractions 4–30 (500 ml each; fractions 1–3, 1 l each, were discarded), from which compounds **6**–**16** and **19**–**22** were isolated.

Compounds **6** *and* **7** *(mixture I) and* **26**. These compounds were present in fraction 8 which was eluted with *n*-hexane–Et_2O (4:3). When fraction 8 was treated with Et_2O, **26** separated out as yellow needles, mp 151–152° (identical in all aspects with the literature data [7]), which were filtered off. Compounds **6** and **7** were isolated as a TLC homogeneous mixture I from the Et_2O-soluble portion by silica gel prep. TLC [*n*-hexane–Et_2O (1:1), single development].

Compounds **8** and **9** *(mixture II)*. Present in fraction 4 which was eluted with *n*-hexane–Et_2O (4:3). Silica gel prep. TLC [*n*-hexane–Et_2O (5:2), single development] gave a crude mixture of **8** and **9** which was purified by a second prep. TLC [*n*-hexane–Et_2O (30:7), 2 developments].

Compounds **10** *and* **11** *(mixture III)*. These compounds were present in fraction 30 which was eluted with Et_2O (100%). Fraction 30 was dissolved in Et_2O, pptd with petrol and the mother liquor, when submitted to silica gel prep. TLC [Et_2O–EtOAc (6:1), single development], gave three fractions (A–C). Further prep. TLC of fraction B [CH_2Cl_2–EtOAc–AcOH (20:5:2), single development] gave a TLC homogeneous mixture (III) of **10** and **11**.

Compounds **12** *and* **13** *(mixture IV) and* **19** *and* **20** *(mixture VII)*. These were present in fractions 9–12 which were eluted with *n*-



Scheme 5.

hexane–Et_2O (4:3 and 1:1). The combined fractions 9–12 (5.9 g) were chromatographed on silica gel (240 g packed in *n*-hexane). Elution with *n*-hexane–Et_2O (2:1) gave 20 fractions. Fractions 11–15 containing **12** and **13** and **19** and **20** when submitted to prep. TLC [petrol–EtOAc (5:2), single development] gave fractions A–D. TLC homogeneous mixtures IV containing **12** and **13**, and VII containing **19** and **20**, were isolated by repetitive prep. TLC of fractions C and B, respectively, using the same solvent system.

Compounds **8** *and* **8, 14–16** (*mixture V*). These were present in fraction 7 which was eluted with *n*-hexane–Et_2O (4:3). Silica gel prep. TLC [*n*-hexane–Et_2O (2:1), 2 developments] of this fraction gave three fractions A–C. Fraction A was TLC homogeneous and identified as **8**. Fraction B, on repeated prep. TLC [*n*-hexane–Et_2O (1:1 and 1:2)] gave a TLC homogeneous mixture V which contained **14–16** and a small amount of **8**.

Compounds **21** *and* **22** (*mixture VIII*). These were present in fraction 6 which was eluted with *n*-hexane–Et_2O (4:3). Silica gel prep. TLC of this fraction [*n*-hexane–Et_2O (3:1)] gave two fractions A and B. From fraction B, **21** and **22** were isolated as a TLC homogeneous mixture VIII by further prep. TLC [*n*-hexane–Et_2O (1:1)].

Compound **27**. This was present in fractions 17–23 which were eluted with *n*-hexane–Et_2O (1:1). When the combined fractions, after freeing from solvent, were treated with Et_2O, **27** separated out as canary yellow needles which were filtered off and crystallized from Me_2CO, mp 165–166°. Its ^1H NMR and MS parameters were in accord with literature data [7].

Isolation of vanclevic acid A(**2**). The acid mixture (149 g) from *V. stylosa* from the Arizona collection was subjected to silica gel CC (2 kg packed in CH_2Cl_2). Elution with CH_2Cl_2–EtOAc–AcOH [120:5:1, three 1 l fractions, discarded] followed by CH_2Cl_2–EtOAc–AcOH (120:10:1) gave fractions 4–23 (500 ml). TLC showed vanclevic acids A (**2**) and B (**3**) in fractions 5–23 and **3** in fraction 4 (23.6 g) as major spots. Silica gel prep. TLC [CH_2Cl_2–EtOAc–AcOH (45:6:2)] of a portion of these fractions gave pure **2** and **3** identical to that obtained from the Utah collection.

Isolation of compounds **5–9, 17, 18, 26** *and* **27**. The remaining fraction 4 (23 g) was submitted to two-funnel partition between C_6H_6–MeOH–H_2O (8:5:1, 250 ml each phase). The combined lower phases, after freeing from solvent, were dissolved in Me_2CO and decolorized. The decolorized material (16.1 g) was methylated with MeI [6] and the methylated product (16.0 g) was submitted to Silica gel CC (400 g packed in *n*-hexane). Elution with *n*-hexane–Et_2O (2:1) gave fractions 1–57 [1 (1l), 2 (500 ml) and 3–57 (100 ml)] from which compounds **5**, **6**,**7** (mixture I), **8**, **9** (mixture II) and **17**, **18** (mixture VI) were isolated by silica gel prep. TLC, developing with various proportions of *n*-hexane–Et_2O. Except for concns of the components in mixtures I [**6** (75%) and **7** (25%) and II [**8** (95%) and **9** (5%)], their ^{1}H NMR parameters were similar to those obtained from the Utah collection. From the combined upper phases **26** and **27** were isolated. We have not described the isolation of **5**, mixtures I and II, **26** and **27**.

Compound **17** *and* **18** (*mixture VI*). These were present in fractions 14–29 (2.25 g) which were eluted with *n*-hexane–Et_2O (4:3). A portion of this fraction when submitted to silica gel prep. TLC [*n*-hexane–$Et_2$0 (1:2), two developments] gave two fractions A and B. Compounds **17** and **18** were obtained as a TLC homogeneous mixture VI from the latter fraction by prep. TLC [*n*-hexane–Et_2O (1:3), 2 developments].

The physical and spectral [^{1}H NMR (Bruker WM-250), ^{13}C NMR (Bruker WH-90) and MS (Varian MAT 311A)] parameters of the compounds above are described in the text.

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