Full Papers

cis-Clerodane Diterpene Lactones from *Amphiachyris dracunculoides*. 2[†]

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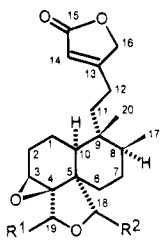
Nine *cis*-clerodane lactones, amphiacrolides E (**1**), F (**2**), G (**3**), H (**4**), I (**5**), O (**6**), and P (**7**) and amphiacric acids A (**9**) and B (**8**) were isolated from the ethanolic extract of the aerial parts of *Amphiachyris dracunculoides*, and their structures were established by physical methods. High-field 1D and 2D NMR techniques were used to make complete assignments for the ^{1}H- and ^{13}C-NMR spectra. Amphiacrolides O (**6**) and P (**7**) are α-L-arabinopyranosyl ester glycosides of the aglycon amphiacric acids B (**8**) and A (**9**), respectively. Amphiacrolides G (**3**), H (**4**), and O (**6**) were reported earlier from *Gutierrezia texana*; the others are new natural products.

Our study of *Amphiachyris dracunculoides* (DC.) Nutt. (Compositae) has already recorded the isolation and structure elucidation of diterpene lactones named amphiacrolides A, B, C, and D belonging to the *cis*-clerodane series.[1] We report herein nine compounds of the same class; five are named amphiacrolides E–I (**1**–**5**) with alphabetical designation reflecting generally their increased polarity. Two are glycosidic esters, amphiacrolides O (**6**) and P (**7**) formed from α-L-arabinopyranose and diterpenic acids, amphiacric acid B (**8**) and A (**9**), respectively. All of these compounds have the ethyl butenolide side chain as evidenced by the characteristic NMR peaks and the MS fragments. The differences are in the substituents at carbons 3, 4, 6, 18, and 19. Amphiacrolides G (**3**), H (**4**), and O (**6**) were reported from *Gutierrezia texana*.[2]

Amphiacrolide E (**1**) and I (**5**) with respective molecular formulas $C_{22}H_{32}O_6$ and $C_{21}H_{30}O_6$ as determined by HRMS are treated together because they differ only in one being the ethyl and the other the methyl derivative of the identical diterpenoid. They were characterized



1 R1= ···OEt, R2= ···OH
5 R1= ···OMe, R2= ···OH
10 R1= ···OEt, R2= ···OAc
11 R1= ···OMe, R2= ···OAc
12 R1= ···OEt, R2= =O
13 R1= OH, R2= OH
14 R1= OH, R2= ···OMe
15 R1= ···OAc, R2= ···OMe

by extensive spectral studies including 1D and 2D NMR, by comparison of data to previously reported related compounds, especially amphiacrolide D,[1] and by preparation of derivatives. Acetylation produced the acetates **10** and **11**, respectively, and CrO_3 oxidation of amphiacrolide E (**1**) gave the lactone **12**. The four oxygens remaining, after assignment of two for the α,β-unsaturated γ-lactone unit, are hydroxyl and three ethers. The ^{1}H,^{1}H-COSY experiment revealed four ^{1}H-coupled units for amphiacrolide I (**5**): the ethyl butenolide unit, the six-spin protons of C-1, -2, -3, and -10, the protons of C-6, -7, -8, and -17, and the two-spin double doublet of a carbinyl proton with a D_2O exchangeable hydroxide. Amphiacrolide E (**1**) had an additional five-spin system seen in the ^{1}H-NMR spectrum as an A_3MX pattern for the ethyl group. Arranging these units afforded, without stereochemistry, the clerodane unit of **1**, with an epoxy and a C-18, -19 ether. The last two carbons would bear an alkoxyl and hydroxyl. This arrangement accommodated the one-proton singlet on an oxygen-bearing carbon and the double doublets of the hydroxymethine.

Spacial assignment of the epoxide was based on comparison of the chemical shift of H-10 in amphiacrolides E (**1**) and I (**5**) to those in amphiacrolides C, D, and related compounds.[1] Amphiacrolide D with the firmly established β-faced epoxide has the α-faced H-10 at δ 1.64 ppm, which is little different from that of H-10 (1.67 ppm) in amphiacrolide C with an olefinic group instead of the epoxide in the same position. Amphiacrolides E (**1**) and I (**5**) show H-10 at δ 1.67 and 1.69 ppm, respectively, and these must have the β-faced epoxide. An α-faced epoxide would cause a significant downfield shift for the *syn* axial H-10.[3,4] (Two prepared compounds of the labdane series with 8,9-epoxides show H-5, a similarly γ-positioned proton, to be shifted ~0.5 ppm downfield in the *syn* epoxide from that in the *anti* epoxide, which was essentially the same in the compound with an 8,9-olefin.) In addition, when the epoxide oxygen is *syn* to an axial hydrogen in the γ-position, the carbon bearing that hydrogen is shielded by 3.5–6 ppm.[5] (The C-5 carbon shifts are at 47.41 ppm for the *syn* and at 55.50 ppm for the *anti*, or a shielding of 8 ppm for 2,3-epoxy steroids, with the related olefin at 54.10 ppm.) In amphiacrolides E (**1**) and I (**5**), C-10 is located at δ

[†] Part 1: ref 1. Taken in part from the Ph.D. dissertation of F. M. Harraz as accepted by the Graduate School, The Ohio State University in Aug 1984.

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37.98 and 37.76 ppm and in amphiacrolide C and D at 38.5 and 38.1 ppm, respectively. This lack of shift fits the β-epoxide.

The substituents on C-18 and C-19 were positioned from the NOE experiments with amphiacrolide I (**5**) at 500 MHz, with only the relevant results given here. Irradiation at H-3 (3.57 ppm) caused relaxation to the one-proton singlet at 4.88 ppm (H-19) by 2% and to the methoxyl at 3.40 ppm by 2%. Irradiation at H-19 showed relaxation back to H-3 by 2%, to the one-proton hydroxyl doublet by 1%, as well as a multiplet at 1.45 ppm (H-6α) by 2%. This located the hydroxymethine at C-18 and both H-18 and H-19 on the β-face of the molecule. Furthermore, irradiation of H-18 (5.52 ppm) showed relaxation back to H-19 by 1%, to H-6α by 2%, to H-7α (1.84 ppm) by 5%, and to HO-18 (3.28 ppm) by 5%. Additional NOE experiments identified neighboring protons of the methyl groups, from which, by CH-correlation and COLOC (two- to four-bond CH-coupling) experiments, the ^{1}H- and ^{13}C-NMR spectra were assigned.

Preparation of acetates **10** and **11** of amphiacrolides E (**1**) and I (**5**), respectively, and the CrO_3 oxidation of amphiacrolide E (**1**) to the lactone **12** confirmed the presence of the hydroxyl. With structures established for amphiacrolides E (**1**) and I (**5**), it appeared they were simple addition products of EtOH and MeOH to a diterpene bearing aldehyde groups at C-18 and C-19 to give an extended hemiacetal group. Both EtOH and MeOH were used in the extraction and separation of the plant constituents. Attempts at obtaining the dialdehyde or the hydration product **13** under a variety of conditions were unsuccessful.

Efforts to convert amphiacrolides E (**1**) and I (**5**) to the diterpene diol by $NaBH_4$ reduction were also unsuccessful; however, an interesting transformation was observed. A compound, more polar (TLC) than the starting materials, was isolated from the reaction mixtures and was characterized by extensive spectral studies to be the position isomer **14** of amphiacrolide I (**5**). The relevant evidence for that structure was obtained from the NMR studies. The downfield region of the ^{1}H-NMR spectrum showed a D_2O exchangeable one-proton doublet at δ 2.96 ppm coupled to a one-proton doublet at 5.21 ppm, which when irradiated in a NOE experiment showed relaxation to H-3 (3.55 ppm) of 2%. Irradiation of H-3 enhanced the proton at 5.21 ppm by 3% and the OH at 2.96 ppm by 1%, thus placing these substituents at C-19. Irradiation of the methoxyl (3.38 ppm) enhanced a one-proton singlet at 4.99 ppm by 7%, the H_2-16 (4.725 and 4.730 ppm) by 2%, H-14 (5.80 ppm) by 3%, and H-19 by 2%. This requires placement of the methoxyl and the singlet at 4.99 ppm at C-18. Irradiation at H-18 (4.99 ppm) showed relaxation to H-6α (1.47 ppm) and H-11 (1.40 ppm) together at 12%, to H-7α (1.81 ppm) at 5%, and to the methoxyl at 8%, thereby requiring H-18 on the β-face and the methoxyl on the α-face. The stereochemical disposition about C-19 would require H-19 be placed α from the methoxyl irradiation, except that irradiation of H-19 showed relaxation to H-6α (1.47 ppm) of 4% and also irradiation of HO-19 caused enhancement of the methoxyl by 3%. This evidence supports a stereochemical mixture at C-19 not unlike anomeric isomers in simple sugars. The acetate **15** of compound **14**, however, was not a mixture of C-19 epimers because irradiation at the methoxyl enhanced the acetate (2.03 ppm) by 2% and the irradiation of H-18 (5.04 ppm) enhanced H-19 (6.10 ppm) by 2%, as well as other positions consistent with H-18 and H-19 being located on the β-side.

The transformation of both amphiacrolides E and I to the same methoxy derivative **14** provides a method for selectively protecting C-18 or C-19 and may be of use in synthesis. Unfortunately, lack of starting material prevented further study of these alcohol addition products. The $NaBH_4$ treatment generated the alkaline conditions, which in MeOH most likely caused the formation of the C-18 methoxy product **14**.

Amphiacrolide F (**2**), mp 150.0−150.5 °C, has molecular formula $C_{20}H_{26}O_4$ as supported by HRMS and requires eight double-bond equivalents. The MS, IR, and NMR indicated an α,β-unsaturated γ-lactone with an ethyl side chain and accounted for three double-bond equivalents and two oxygens. The strong IR absorption at 1755 cm^{-1}, lack of hydroxyl absorption, an additional peak in the ^{13}C-NMR in the ester-lactone carbonyl region (169.81 ppm), and the one-proton double-doublet in the ^{1}H-NMR at 4.30 ppm supported a lactone function. From the ^{1}H,^{1}H-COSY and CH-correlation experiments the 4.30 ppm proton was shown to be part of a four-spin system $CHCH_2CH$ in which the other methine is coupled to a methyl doublet. These results established the partial structure from C-6 through C-8 to C-17 of ring B, with the lactone oxygen of C-6. The same experiments supported the six-spin system from C-3 through C-1 to C-10 of ring A, where C-3 is the olefin carbon (δ_C 136.26 and δ_H 6.81 ppm). The lactone carbon was placed at C-19 because the two quaternary methyls were located in the aliphatic region of the ^{1}H-NMR spectrum. All of these units were accommodated on a normal clerodane skeleton, with the stereochemistry established by NOE experiments, which also identified the substituents on the α- and β-faces of the molecule. Only the pertinent results are given. Irradiation of Me-17 (0.80 ppm) caused a 6% relaxation to H-8 (1.42 ppm) and a 3% relaxation to the methyl at 0.73 ppm, thereby identifying it as Me-20. Irradiation at H-6 (4.30 ppm) caused relaxation to H-7 (1.85 ppm) at 4%, to H-8 (1.42 ppm) at 6%, and to Me-18 (1.22 ppm) at 4%, thus locating these units on the α-face of the molecule. Irradiation of Me-18 showed relaxation to H-1α at 8%, to H-6 at 14%, and to H-10 (1.70 ppm) at 6% to complete the α-face substituents. The stereochemical disposition is shown in the structure, and the ^{1}H- and ^{13}C-NMR assignments from the 2D NMR experiments are found in Tables 1 and 2, respectively. A reported compound with essentially identical properties as amphiacrolide F (**2**) was obtained by oxidation of the corresponding 6α,19-dihydroxy-*cis*-clerodane.[2] Our results would require the 6α-oxo stereochemistry of both be revised to 6β-oxo.

A compound possessing the enantiomeric structure with amphiacrolide F (**2**) from a Brazilian composite *Symphiopappus itatiayensis* has the same mp, a positive specific rotation, and essentially the same cd curve but negative.[6] Comparison of the peaks given for the ^{1}H-NMR spectrum shows close identity to those of amphiacrolide F, as do the ^{13}C-NMR peaks. Although not all were assigned, the following revisions are required: C-3 and C-4 must be reversed, unassigned peaks at 30.5 (t)

Table 1. ^{1}H-NMR Data for Compounds **1**–**3**, **5**, **11**, and **14**–**17**[a]

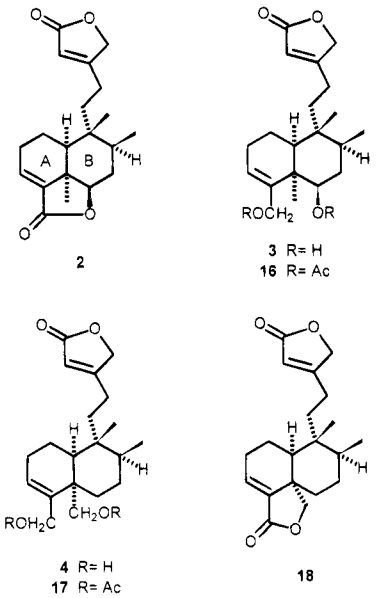
	compd								
proton	**1**	**2**	**3**	**5**	**11**	**14**	**15**	**16**	**17**
H-1	1.38 hm	1.90 (2H) m	2.01 α dddd (16.1, 9.6, 8.5, 6.5)	1.39 hm	1.38 hm	1.37 hm	1.35–1.45 (2H) hm	2.04 α hm	1.93 α dddd (16.3, 10.6, 10.6, 7.1)
	1.43 hm		1.68 β hm	1.43 hm	1.48 hm	1.43 hm		1.75 β hm	1.75 β hm
H-2	2.10 α dddd (14.6, 2.3, 2.3, 2.3)	2.35 (2H) m	2.14 hm	2.11 α dddd (14.8, 2.9, 2.7, 2.7)	2.16 α dddd (14.6, 2.9, 2.9, 2.5)	2.11 α dddd (14.7, 2.9, 2.5, 2.5)	2.13 α dddd (15.1, 2.8, 2.8, 2.8)	2.17 m	2.18 m
	1.64 β hm		2.17 hm	1.63 β dddd (14.7, 12.3, 5.1, 1.2)	1.63 β hm	1.60 β ddd (13.2, 12.8, 5.1)	1.66 β hm	2.25 hm	2.22 m
H-3	3.57 brs, $\omega_{1/2}$ = 4.5 Hz	6.81 t (3.6)	5.59 dd (3.5, 3.5)	3.57 brs, $\omega_{1/2}$ = 4.4 Hz	3.59 brs, $\omega_{1/2}$ = 4.3 Hz	3.55 brs, $\omega_{1/2}$ = 4.5 Hz	3.47 brs, $\omega_{1/2}$ = 4.5 Hz	5.69 dd (3.5, 3.5)	5.89 dd (3.4, 3.4)
H-6	1.45 α hm	4.30 dd (10.8, 7.0)	3.42 dd (11.9, 3.8)	1.45 α hm	1.58 α hm	1.47 α hm	1.52 α hm	4.62 dd (11.7, 3.9)	1.41 α hm
	1.81 β hm			1.84 β hm	1.89 β hm	1.80 β hm	1.83 β hm		1.89 β dd (11.0, 2.6)
H-7	1.84 α hm	1.85 α ddd (13.7, 6.9, 2.0)	1.59 α hm	1.84 α hm	1.84 α hm	1.81 α hm	1.81 α hm	1.56 α hm	1.37 (2H) hm
	1.35 β hm	1.24 β ddd (13.1, 13.1, 10.9)	1.71 β hm	1.36 β hm	1.37 β hm	1.35 β hm	1.41 β hm	1.67 β ddd (10.8, 10.8, 10.8)	
H-8	1.50 hm	1.42 ddq (13.0, 6.8, 2.0)	1.58 hm	1.50 hm	1.53 hm	1.50 hm	1.49 hm	1.72 hm	1.44 m
H-10	1.67 hm	1.70 dd (6.3, 1.4)	1.30 brd (6.5)	1.69 hm	1.46 hm	1.46 dd (12.4, 3.4)	1.68 dd (12.4, 3.8)	1.44 dd (6.7, 1.0)	1.67 d (6.3)
H-11	1.36 hm	1.50 ddd (14.8, 12.1, 4.9)	1.51 m	1.37 hm	1.29 ddd (13.8, 12.0, 5.0)	1.40 hm	1.40 hm	1.54 ddd (14.6, 12.1, 5.2)	1.54 ddd (14.0, 12.3, 4.8)
	1.70 hm	1.72 ddd (14.5, 11.8, 5.3)	1.70 m	1.71 ddd (13.2, 13.2, 3.9)	1.51 m	1.57 hm	1.58 hm	1.74 hm	1.76 hm
H-12	2.32 ddd (15.8, 12.4, 4.1)	2.28 (2H) ddd (12.1, 12.1, 4.6)	2.22 m	2.32 ddd (15.5, 12.6, 4.4)	2.20 m	2.31 m	2.32 m	2.25 (2H) hm	2.28 (2H) m
	2.45 ddd (15.7, 12.6, 3.2)		2.25 m	2.45 ddd (15.9, 12.7, 3.1)	2.26 m	2.38 m	2.39 m		
H-14	5.79 m (5 pk) (1.2)	5.82 m (5 pk) (1.6)	5.83 m (5 pk) (1.4)	5.79 m (5 pk) (1.6)	5.79 m (5 pk) (1.6)	5.80 m (5 pk) (1.6)	5.81 m (5 pk) (1.6)	5.83 m (5 pk) (1.6)	5.84 m (5 pk) (1.5)
H-16	4.72 (2H) d (1.3)	4.73 (2H) d (1.6)	4.74 (2H) d (1.5)	4.712 d (15.5), 4.716 d (15.5)	4.68 (2H) d (1.6)	4.725 dd (17.5, 1.7), 4.730 dd (17.5, 1.7)	4.72 (2H) d (1.5)	4.73 (2H) d (1.7)	4.75 (2H) d (1.6)
H-17	0.96 d (7.2)	0.80 d (6.8)	0.80 d (6.6)	0.97 d (7.2)	0.91 d (6.9)	0.95 d (7.1)	0.95 d (7.1)	0.81 d (6.3)	0.79 d (6.6)
H-18	5.49 d (2.4)	1.22 s	1.31 s	5.52 d (4.3)	6.38 s	4.99 s	5.04 s	1.14 s	3.87 d (11.1)
									3.93 d (11.1)
H-19	4.98 s		4.09 d (12.2)	4.88 s	4.94 s	5.21 d (8.3)	6.10 s	4.59 dddd (13.0, 1.2, 1.2, 1.2)	4.61 (2H) d (0.6)
			4.30 d (12.2)					4.84 dddd (12.8, 1.0, 1.0, 1.0)	
H-20	0.85 s	0.73 s	0.82 s	0.85 s	0.84 s	0.84 s	0.85 s	0.86 s	0.82 s
Misc	3.47 ($OC\mathit{H}_A H_B Me$) dq (9.7, 7.1), 3.79 ($OCH_A C\mathit{H}_B Me$) dq (9.7, 7.1), 1.18 ($OCH_A H_B \mathit{Me}$) t (7.1), 3.22 (OH) d (3.3)			3.40 (OMe) s, 3.28 (OH) brs (4.3)	3.36 (OMe) s, 2.09 (Ac) s	3.38 (OMe) s, 2.96 (OH) d (8.7)	3.32 (OMe) s, 2.08 (Ac) s	2.05 (Ac-6) s, 2.06 (Ac) s	2.05 (Ac-18) s, 2.06 (Ac-19) s

[a] Taken at 500 MHz in $CDCl_3$ or stated otherwise with data point resolution of 0.3 Hz and chemical shift (δ) in ppm as referenced to TMS with residual solvent peak ($CHCl_3$) taken as internal standard at 7.26 ppm. Stereochemical designations α and β following the chemical shift refer to the proton below and above the plane, respectively, of the illustrated drawing. Spin-coupled patterns are designated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broadened, and h = hidden or overlapped. The spin coupling (J) is given in parentheses in Hz and refers to separation values solely for characterization and may not be the true J as in non-first-order patterns. Some hidden patterns were clarified by homonuclear decoupling and NOE studies and are reported after the hm designation in brackets.

Table 2. ^{13}C-NMR Data for Compounds **1**–**3**, **5**, **11**, and **14**–**17**[a]

			compd							
carbon	**1**[b]	multiplicity	**2**	**3**	**5**[c]	**11**	**14**	**15**	**16**	**17**
C-1	18.10	t	17.73	17.21	18.05	17.73	17.93	17.83	17.03	17.19
C-2	26.65	t	24.64	23.75	26.62	26.28	26.56	26.41	23.59	23.93
C-3	57.40	d	136.26	129.53	57.23	56.92	57.10	57.46	129.73	132.99
C-4	69.55	s	133.45	141.53	69.61	68.57	70.80	68.90	136.29	134.14
C-5	46.25	s	39.56	42.65	46.01	44.99	46.17	46.12	41.40	40.14
C-6	20.13	t	85.06 d	79.93 d	20.00	20.50	20.09	20.13	80.79 d	30.81
C-7	25.53	t	34.83	37.68	25.42	25.38	25.59	25.61	33.70	27.99
C-8	35.48	d	32.14	36.20	35.68	32.13	34.51	34.36	36.06	37.20
C-9	37.70	s	39.16	40.20	37.52	37.84	37.72	37.78	40.27	40.14
C-10	37.98	d	41.51	45.77	37.76	39.63	38.32	38.03	45.46	38.98
C-11	39.46	t	35.16	35.26	39.28	38.23	38.76	38.72	35.27	35.36
C-12	23.80	t	22.17	22.16	23.77	23.57	23.61	23.60	22.15	22.14
C-13	172.29	s	170.53	171.21	172.63	170.27[d]	171.69	171.38	170.73	170.89
C-14	114.89	d	115.27	115.19	114.48	115.52	114.84	115.00	115.30	115.42
C-15	174.69	s	174.03	174.34	174.88	173.85	174.23	174.03	174.04	174.04
C-16	73.47	t	73.16	73.27	73.55	73.06	73.39	73.31	73.17	73.16
C-17	16.94	q	15.31	15.71	16.98	16.34	16.85	16.79	15.59	15.96
C-18	103.47	d	30.63 q	31.28	103.16	101.07	109.59	109.98	29.71 q	72.17 t
C-19	103.82	d	169.81 s	67.81 t	104.64	104.64	98.37	96.63	67.03 t	66.39 t
C-20	21.92	q	16.33	17.88	22.12	21.58	21.73	21.52	17.77	17.54
Misc	64.98	t			56.10 q	56.67 q	55.36 q	55.29 q	21.37 q	21.19 q
	OCH_2Me				OMe	OMe	OMe	OMe	*Me*CO-6	*Me*CO-18
	15.33	q				20.58		21.18	21.27	21.24
	OCH_2*Me*					*Me*CO		*Me*CO	*Me*CO-19	*Me*CO-19
						170.04[d] s		170.42 s	170.62 s	170.97 s
						Me*CO*		Me*CO*	Me*CO*-6	Me*CO*-18
									170.89 s	170.79 s
									Me*CO*-19	Me*CO*-19

[a] Taken in $CDCl_3$ at 67.9 Mz unless stated otherwise with multiplicities determined by SFORD and chemical shifts (in ppm) relative to TMS using the solvent peak (center) as reference, 77.2 for $CDCl_3$ and 123.5 for pyr-d_5. Multiplicities when different from those in column are given after the chemical shift. Abbreviations are as follows: s = singlet, d = doublet, t = triplet, and q = quartet. [b] At 75 MHz. [c] At 125 MHz. [d] May be interchanged.



2

3 R= H
16 R= Ac

4 R= H
17 R= Ac

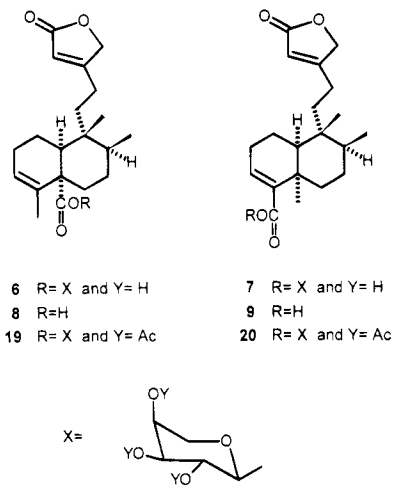
18

and 17.6 (q) ppm require their multiplicities reversed, and C-5 and C-9 peaks at 30.4 and 30.0 ppm are undoubtedly misprints.

Amphiacrolide G (**3**), mp 144–145 °C, has molecular formula $C_{20}H_{30}O_4$ as supported by elemental and spectral analyses and possesses the ethyl butenolide side chain. The two remaining oxygens are hydroxyl as evidenced from preparation of the diacetate **16**. An olefinic group (^{13}C-NMR) with one proton (^{1}H-NMR) and a bicyclic system account for the remaining three degrees of unsaturation. Homonuclear decoupling and ^{1}H,^{1}H-COSY experiments revealed the two proton-coupled spin systems of rings A and B as those present in amphiacrolide F (**2**). Extensive 2D NMR experiments and NOE studies supported the structure for amphiacrolide G (**3**) as the diol of amphiacrolide F (**2**) where the lactone carbonyl is reduced to the alcohol. Oxidation of amphiacrolide G (**3**) with activated MnO_2 formed amphiacrolide F (**2**) in good yield and established the stereochemical structure for the parent alcohol. Complete ^{1}H- and ^{13}C-NMR spectral assignments are given in Tables 1 and 2, respectively, for the diol **3** and its diacetate **16**. The individual acetate groups in the latter derivative were identified from the COLOC experiment.

Amphiacrolide H (**4**), mp 145–146 °C, with molecular formula $C_{20}H_{30}O_4$ as indicated from elemental and spectral analyses, is isomeric with amphiacrolide G (**3**) and yields a diacetate **16**. The ^{1}H-NMR spectrum shows only two methyl groups—a singlet and a doublet—as compared to three methyls for amphiacrolide G, suggesting the 6-hydroxyl of the latter is now on one of the quaternary methyls, either Me-18 or Me-20. Detailed NMR studies supported the 18-hydroxyl structure, and its transformation to amphiacrolide B (**18**)[1] by oxidation with Jones' reagent or MnO_2 confirmed the location of the hydroxyls and its complete absolute stereochemistry. Amphiacrolide H is also identical with the diol produced on $NaBH_4$ reduction of amphiacrolide C.[1] The ^{1}H- and ^{13}C-NMR spectral assignments from the 1D and 2D NMR studies were the same as those reported for the prepared compound.[1]

Amphiacrolide O (**6**), with molecular formula $C_{25}H_{36}O_8$ as obtained from FABMS, gave fragment ions for an ethyl butenolide side chain and a pentose unit. This suggested a diterpene pentoside and accounted for seven of the eight oxygens. The ^{1}H- and ^{13}C-NMR spectra supported these units and also showed three methyls, two quaternary and one secondary, and a second olefinic



unit with one proton and a carboxylic acid or ester carbonyl (δ_c 176.38 ppm). The IR spectrum with a double intensity peak at 1750 cm^{-1} was in agreement with an acid–ester carbonyl. Acetylation produced the neutral triacetate **19**, and hydrolysis by acid or base gave the same aglycon, a carboxylic acid named amphiacric acid B (**8**), which was also shown to be present in the plant extract. Assuming the clerodane skeleton, a feature of the already characterized compounds from this plant, and because one of the quaternary methyls was olefinic (δ_H 1.61 ppm in $CDCl_3$ and 1.86 ppm in pyridine-d_5), the glycosidic ester must be at C-18 or C-20. Irradiation of the nonolefinic methyl (δ_H 0.84 ppm) in an NOE experiment at 270 MHz in pyridine-d_5 showed relaxation to H-1β of 6%, to H_2-11 of 4%, and to H_2-12 of 4% and clearly identified the irradiated methyl as C-20. Also, the COLOC experiment with the triacetate **19** exhibited a three-bond coupling from H-6β (δ 1.66 ppm) to the carbonyl (δ_c 175.32 ppm) of the glycosyl ester.

The sugar of amphiacrolide O (**6**) was indicated to be arabinose from the NMR experiments in pyridine-d_5. ^1H,^1H-COSY revealed the six-proton coupled sequence from H-1′, and the large coupling constants supported four of these to be axial (H-1′, H-2′, H-3′, and one H-5′) in a pyranose chair conformation. The NOE experiments confirmed this arrangement. For example, irradiation of the H-5′ at 3.80 ppm showed relaxation to H-1′ of 13%, H-3′ of 3%, H-4′ of 8%, and the other H-5′ of 30%, thereby placing all but the last on one face of the pyranose ring. H-4′ as a broadened singlet ($\omega_{1/2}$ = 5 Hz) would be equatorial with three small values. The relative configurations of the asymmetric centers corresponded to that of α-arabinopyranose. The sugar isolated after acid hydrolysis of amphiacrolide O migrated with authentic arabinose on TLC and gave a positive specific rotation supportive of the L enantiomeric series. Furthermore, direct comparison of the ^{13}C-NMR spectra of amphiacrolide O (**6**) with methyl α- and β-arabinopyranosides confirmed the identification.[7] The ^1H- and ^{13}C-NMR assignments for amphiachloride O (**6**) and its triacetate **19** were made from 1D and 2D NMR studies, not reported here, and are given in Tables 3 and 4, respectively. The results are consistent with a chair conformation for ring B in which H_3-20 is axial and the ethyl butenolide unit is equatorial, as illustrated for amphiacric acid A (**9**) in Figure 1. This conformation

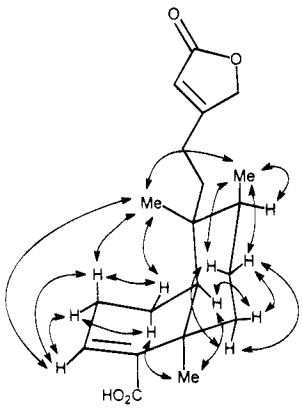


Figure 1. Selected NOESY correlations for amphiacric acid A (**9**) at 500 MHz.

is the chair form other than the one observed for gutierolide whose structure is supported by X-ray analysis.[8]

Amphiacrolide P (**7**) with the same molecular formula as amphiacrolide O (**6**) was shown to be a position isomer of the latter where the acyl arabinoside unit is located at C-19. The same series of spectral and chemical experiments were performed as given for amphiacrolide O but are not detailed here, except for several key observations. For example, in the ^1H-NMR spectrum of amphiacrolide P (**7**) the olefinic methyl peak is replaced by an aliphatic methyl (1.23 ppm), which showed a NOE enhancement of 2% when H-10 was irradiated, thus requiring a methyl at C-18. A similar irradiation at H-3 (6.81 ppm) in pyridine-d_5 gave a 1% enhancement of the anomeric proton (6.26 ppm), thereby locating the acyl glycoside carbonyl at C-19. Additional support for the C-18 and C-19 assignments came from the 2D NMR COLOC studies; H-3 (6.78 ppm) showed three-bond coupling to C-19 (165.93 ppm) and C-5 (36.60 ppm), and H_3-18 showed three-bond coupling to C-4 (137.42 ppm) and C-10 (45.52 ppm) and two-bond coupling to C-5 (36.60 ppm). The other NMR results were essentially the same as observed for amphiacrolide O (**6**), with the same conformation, and allowed the spectral assignments (Tables 3 and 4) to be made. Preparation of triacetate **20** and hydrolysis of amphiacrolide P (**7**) to give amphiacric acid A (**9**) and L-arabinose confirmed the proposed structure.

Amphiacric acid A (**9**), a hydrolysis product of amphiacrolide P and also a plant constituent, was characterized by detailed 1D and 2D NMR studies which allowed for complete assignment of the ^1H- and ^{13}C-NMR spectra as given in Tables 3 and 4, respectively. The NOESY results for rings A and B are given in Figure 1 and show the conformation of the *cis*-decalin system observed for the two arabinosides and their aglycons.

Our study is at variance with assignments made in the ^{13}C-NMR spectra for those given in the literature[2] and which we name amphiacrolide G (**3**) at C-2, C-7, C-11, and C-12, amphiacrolide H (**4**) at C-2, C-6, and C-7, and amphiacrolide O (**6**) at C-6, C-7, C-2′, and C-4′. Also, the 6α-hydroxyl must be revised to 6β-hydroxyl for the amphiacrolide G equivalent, in keeping with earlier documented stereochemical reversal at C-6 for the amphiacrolide F equivalent.

Table 3. ^{1}H-NMR Data for Compounds **6**–**9**, **19**, and **20**[a]

	compd							
proton	**6**	**6**[b]	**7**	**7**[b]	**8**	**9**	**19**[c]	**20**[d]
H-1	1.83 α m	1.86 α hm	2.03 α m	1.90 α m	1.80 α hm	2.06 α dddd (15.3, 9.4, 9.4, 6.6)	1.75 α hm	2.05 α hm
	1.50 β hm	1.50 β m	1.77 β m	1.67 β dd (15.4, 9.2)	1.60 β hm	1.80 β m	1.49 β m	1.77 β dd (15.1, 9.0)
H-2	2.03 α brm	1.97 (2H) brs, $\omega_{1/2}$ = 15.4 Hz	2.24 α m	2.06 α ddd (20.7, 9.4, 3.2)	2.00 hm	2.26 α hm	2.04 α hm	2.25 α hm
	1.96 β br		2.33 β m	2.20 β hm	2.10 hm	2.39 β dddd (21.3, 9.1, 9.1, 4.2)	1.99 β hm	2.36 β dddd (21.3, 9.0, 9.0, 4.3)
H-3	5.57 d (4.9)	5.52 brs, $\omega_{1/2}$ = 8.5 Hz	6.78 dd (3.7, 3.7)	6.81 dd (3.6, 3.6)	5.63 brs, $\omega_{1/2}$ = 10.4 Hz	6.82 dd (3.8, 3.8)	5.57 m, $\omega_{1/2}$ = 11 Hz	6.80 dd (3.8, 3.8)
H-6	2.13 α ddd (14.0, 4.5, 4.5), 1.64 β hm	2.23 α ddd (13.9, 5.7, 5.1), 1.73 β hm	1.10 α m	1.14 α hm	1.97 α hm	1.10 α ddd (13.5, 13.5, 2.7), 2.75 β ddd (14.4, 2.2, 2.2)	2.06 α hm	1.10 α ddd (13.7, 13.7, 2.9), 2.66 β ddd (14.1, 3.0, 3.0)
			2.65 β hm	3.01 brd (13.6)	1.80 β hm		1.66 β m	
H-7	2.00 α hm	2.12 hm	1.27 α m	1.18 α hm	1.84 α hm	1.32 α dddd (13.3, 3.2, 3.2, 3.2), 1.18 β dddd (13.6, 13.6, 13.6, 2.0)	1.82 α m	1.29 α dddd (13.3, 3.2, 3.2, 3.2), 1.00 β dddd (13.4, 13.0, 11.2, 2.9)
	1.29 β dddd (13.9, 5.3, 4.4, 4.4)	1.24 m (6 pk)	1.10 β hm	1.27 β hm	1.31 β m		1.28 β m	
H-8	1.52 hm	1.56 dq (6.9, 5.2)	1.45 m	1.32 hm	1.54 m	1.47 ddq (11.1, 6.8, 3.5)	1.52 hm	1.45 ddq (11.7, 6.7, 3.1)
H-10	2.22 dd (11.2, 2.7)	2.48 dd (10.2, 3.5)	1.38 d (6.1)	1.34 hd [8.6]	2.21 dd (8.2, 4.3)	1.39 d (5.8)	2.23 dd (10.1, 3.6)	1.38 d (5.3))
H-11	1.56 hm	1.64 hm	1.52 m	1.37 hm	1.61 hm	1.54 m	1.55 hm	1.52 m
	1.63 hm	1.67 hm	1.72 hm	1.61 m	1.65 hm	1.74 m	1.61 hm	1.72 m
H-12	2.31 m	2.32 (2H) t (8.3)	2.27 (2H) hm	2.17 (2H) t (9.3)	2.36 m	2.29 (2H) m	2.32 m	2.27 m
	2.34 m				2.38 m		2.38 m	2.29 m
H-14	5.89 brs, $\omega_{1/2}$ = 4.5 Hz	6.05 brs, $\omega_{1/2}$ = 5.1 Hz	5.83 m (5 pk) (1.5)	6.01 brs, $\omega_{1/2}$ = 6.0 Hz	5.84 m (5 pk) (1.5)	5.85 m (5 pk) (1.4)	5.84 m (5 pk) (1.4)	5.83 m (5 pk) (1.5)
H-16	4.75 dd (17.4, 1.3), 4.78 dd (17.4, 1.3)	4.78 dd (17.3, 1.3), 4.86 dd (17.3, 1.0)	4.75 (2H) d (1.4)	4.82 (2H) s	4.73 dd (17.1, 1.4), 4.75 dd (17.1, 1.4)	4.75 d (1.5)	4.75 (2H) d (0.5)	4.74 (2H) d (1.6)
H-17	0.94 d (7.1)	0.86 d (7.0)	0.75 d (6.8)	0.60 d (6.4)	0.92 d (7.8)	0.78 d (6.8)	0.896 d (6.8)	0.75 d (6.8)
H-18			1.23 s	1.33 s		1.24 s		1.23 s
H-19	1.61 brs	1.86 brs			1.73 brs		1.60 brs	
H-20	0.91 s	0.84 s	0.80 s	0.70 s	0.91 s	0.83 s	0.90 s	0.77 s
H-1′	5.34 d (7.6)	6.10 d (7.1)	5.48 d (7.6)	6.26 d (6.9)			5.45 d (7.8)	5.72 d (6.7)
H-2′	3.78 dd (8.3, 8.3)	4.47 dd (7.7, 7.7)	3.86 dd (8.3, 8.3)	4.61 dd (8.0, 7.1)			5.25 dd (9.8, 7.8)	5.30 dd (8.6, 6.8)
H-3′	3.66 dd (8.9, 3.2)	4.16 dd (8.3, 3.0)	3.73 dd (8.9, 2.8)	4.26 dd (8.2, 3.2)			5.08 dd (9.9, 3.6)	5.14 dd (8.6, 3.5)
H-4′	3.99 brs, $\omega_{1/2}$ = 8 Hz	4.26 brs, $\omega_{1/2}$ = 5 Hz	3.98 hbrs	4.36 brs, $\omega_{1/2}$ = 8.5 Hz			5.32 brs, $\omega_{1/2}$ = 7.8 Hz	5.30 hddd [4.0, 3.5, 2.1]
H-5′ax	3.61 d (12.1)	3.80 brdd (13.0, 2.7)	3.67 d (12.1)	3.89 hdd [11.7, 1.5]			3.76 dd (13.4, 2.6)	3.77 dd (12.9, 2.1)
H-5′eq	3.91 dd (12.8, 2.0)	4.25 hdd [13.0, 3.3]	4.00 hdd [13, 2.2]	4.39 dd (11.8, 3.5)			3.94 dd (13.4, 1.5)	4.03 dd (12.9, 4.0)

[a] See Table 1 for conditions used and designations given in reporting the spectra. [b] Taken in pyridine-d_5 with reference to pyr-d_4 at 7.19 ppm (upfield peak). [c] Acetates at 1.98, 2.00, and 2.13 ppm. [d] Acetates at 2.020, 2.022, and 2.12 ppm.

Table 4. ^{13}C-NMR Data for Compounds **6**–**9**, **19**, and **20**[a]

	compd								
carbon	**6**	multiplicity	**6**[b]	**7**	**7**[b]	**8**[c]	**9**[c]	**19**[d]	**20**[e]
C-1	22.40	t	22.13	17.07	16.99	21.53	17.13	22.14	17.02
C-2	26.24	t	25.97	24.37	24.28	25.60	24.47	25.82	24.34
C-3	125.75	d	125.79	142.10	140.79	126.81	142.34	126.89	142.57
C-4	135.30	s	135.46	137.42	138.51	134.46	137.65	133.82	137.03
C-5	51.54	s	51.40	36.60	36.71	50.75	36.51	51.37	36.57
C-6	25.29	t	26.57	36.73	36.94	27.86	36.92	26.26	36.69
C-7	26.52	t	27.04	28.52	28.68	27.04	28.68	26.62	28.59
C-8	35.03	d	34.96	37.95	37.85	36.37	38.06	34.30	37.93
C-9	39.01	s	39.06	40.39	40.35	39.31	40.45	39.10	40.42
C-10	43.22	d	42.68	45.52	45.43	41.60	45.70	42.42	45.53
C-11	37.60	t	37.27	35.38	35.22	37.29	35.44	37.20	35.39
C-12	23.50	t	23.30	22.34	22.07	23.12	22.40	23.26	22.36
C-13	172.83	s	172.74	171.41	172.36	171.77	171.16	171.69	170.95
C-14	114.69	d	114.60	115.19	114.86	115.08	115.29	114.77	115.32
C-15	175.64	s	174.63	174.43	174.36	174.72	174.23	174.40	174.08
C-16	73.93	t	73.63	73.36	73.41	73.49	73.25	73.33	73.19
C-17	16.94	q	16.67	16.09	15.92	16.68	16.09	16.51	16.08
C-18	176.38	s	172.74	33.63 q	33.62 q	182.61	33.53 q	175.32	33.33 q
C-19	19.51	q	20.04	165.93 s	166.87 s	20.10	173.27 s	19.71	164.92 s
C-20	21.24	q	20.40	18.08	17.93	20.10	18.05	20.16	17.93
C-1′	95.21	d	96.53	94.63	96.23			93.22	91.90
C-2′	70.73	d	71.22	70.76	71.27			67.87	68.35
C-3′	73.35	d	74.47	73.42	74.29			70.24	69.88
C-4′	68.13	d	68.78	68.28	68.82			67.50	67.27
C-5′	66.94	t	67.46	66.90	67.36			65.00	63.78

[a] For conditions in collecting spectra and designations see Table 2. [b] Taken in pyridine-d_5. [c] Taken at 125 MHz. [d] *Me*CO at 20.69, 20.72, and 20.95 (3′) and Me*C*O at 169.16, 169.98, and 170.13 (3′)) ppm. [e] *Me*CO at 20.78, 20.80, and 21.03 and Me*C*O at 169.26, 169.99, and 170.24 ppm.

Experimental Section

General Experimental Procedures. The instruments used and conditions under which measurements were made and the source of plant material along with the handling of the plant extract are given in ref 1.

Isolation of Terpenoids. Three solvent partition fractions F1, F2, and F3 were reported[1] and provided the following: fraction F1 [$CHCl_3$–hexane (1:4) solubles] chromatographed on Si gel gave amphiacrolide E (**1**) after elution of amphiacrolide D. Amphiacrolide F (**2**) was obtained from the same fraction but only from the 1981 plant collection. Fraction F2 [$CHCl_3$–hexane (1:1) solubles] on Si gel chromatography gave amphiacric acid A (**6**), amphiacrolide G (**3**) and H (**4**), and amphiacric acid B (**7**) after prior separation on Sephadex LH-20 (Pharmacia). Fraction F3 [MeOH–H_2O (7:3) solubles] after Sephadex LH-20 chromatography afforded amphiacrolide I and the glycosides amphiacrolide O (**9**) and P (**8**).

Chromatography on Sephadex LH-20. Fraction F2. In 9 g portions, fraction F2 was separated on a 180 g column of Sephadex LH-20 with MeOH. The effluent fractions combined after TLC analysis were pooled to give three fractions, a tarry forerun, the terpenoids, and the flavonoids, for a total recovery of 57% terpenoids and 10% flavonoids.

Fraction F3. In 20 g portions, fraction F3 was separated on a 275 g column of Sephadex LH-20 as given above to give a recovery of 56% terpenoids and 19% flavonoids.

Amphiacrolide E (1). From the chromatography of fraction F1[1] on Si gel a material with R_f 0.42 [TLC, EtOAc–hexane (3:2)] was rechromatographed on Si gel (56 g) with EtOAc–hexane (1:1) to give 100 mg of amphiacrolide E (**1**) as a homogeneous heavy oil: $[\alpha]_D$ −43.7° (c = 5.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3590 and 3410 (OH), 3005 (HC=C), 1787 and 1755 (C=O, lactone), 1640 (C=C), 1240 (CO), 960, and 850 cm^{-1} (epoxide); UV (MeOH) λ end abs. 210 nm (log ϵ 4.15); FABMS (*m*-nitrobenzyl alcohol) *m/z* 393 (4, MH^+), 375 (24, MH − H_2O), 347 (31, MH − EtOH), and 136 (100); EIMS *m/z* 346.2111 (0.9, $M^+ - CH_2O_2$, $C_{21}H_{30}O_4$ requires 346.2145), 346.1789 (0.05, M^+ − EtOH, $C_{20}H_{26}O_5$ requires 346.1798), 111 (46, $C_6H_7O_2$), 98 (37, $C_5H_6O_2$), and 41 (100, EtO); ^{1}H- and ^{13}C-NMR data given in Tables 1 and 2.

Amphiacrolide E Acetate (10). A mixture of amphiacrolide E (25 mg), pyridine (0.7 mL), and Ac_2O (0.7 mL) after 24 h at room temperature was evaporated to dryness at reduced pressure, and the residue was chromatographed on Si gel (6 g) with $CHCl_3$. A homogeneous fraction with R_f 0.66 on TLC [Si gel, $CHCl_3$–MeOH (19:1)] had the following properties: $[\alpha]_D$ +42.6° (c = 1.25, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3030 (epoxide), 1787, 1755 (lactone C=O), 1740 (shld, ester C=O), 1235 (CO), 940, and 820 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.35 (s, H-18), 5.78 (5pk m, J = 1.5 Hz, H-14), 5.04 (s, H-19), 4.69 (d, J = 1.6 Hz, H_2-16), 3.71 (dq, J = 9.7, 7.0 Hz, OCH_AH_BMe), 3.61 (brs, H-3), 3.46 (dq, J = 9.7, 7.0 Hz, OCH_AH_BMe), 2.09 (s, Ac), 1.15 (t, J = 7.1 Hz, OCH_2Me), 0.90 (d, J = 6.9 Hz, Me-17), and 0.84 (s, Me-20) ppm.

Dehydroamphiacrolide E (12). At 0 °C a solution of amphiacrolide E (**1**) (20 mg) in Me_2CO (1 mL) was added six drops of Jones' reagent[9] with shaking. After 6 min, H_2O (5 mL) was added, and the mixture was extracted with Et_2O (4 × 25 mL). The Et_2O extract was extracted with 5% aqueous $NaHCO_3$ (6 × 15 mL) and H_2O (5 × 20 mL) and then dried over anhydrous $MgSO_4$. The evaporation of Et_2O left a heavy oil (**12**): $[\alpha]_D$ +123.8° (c = 0.84, $CHCl_3$); IR ($CHCl_3$) 3020 (epoxide), 1780, 1750, and 1640 (α,β-unsaturated γ-lactone), 1220 (CO), 960, and 850 (epoxide) cm^{-1}; ^{1}H-NMR (90 MHz, $CDCl_3$) δ 5.82 (5 pk m, J = 1.6 Hz, H-14), 5.53 (s, H-19), 4.76 (d, J = 1.9 Hz, H_2-16), 4.2 − 3.5 (m, OCH_2Me), 3.69 (d, J = 1.9 Hz, H-3), 1.23 (t, J = 7.0 Hz, OCH_2Me), 0.84

(d, J = 6.7 Hz, Me-17), and 0.83 (s, Me−20); CIMS (isobutane) *m/z* 391 (16, MH^+) and 235 (100); EIMS *m/z* 390 (1, M^+), 345 (4, M − EtO), 235 (100), 111 (61), 98 (40), and 97 (16).

Treatment of Amphiacrolide E (1) and I (5) with $NaBH_4$. Amphiacrolide E (130 mg) in MeOH (5 mL) at 0 °C was treated with 13 mg of $NaBH_4$. After 1 h 15 mg of $NaBH_4$ was added and stirring continued at room temperature. TLC monitoring [$CHCl_3$−MeOH (19:1)] showed the starting material (R_f 0.55) gone in 2 days. Then H_2O (5 mL) was added and the MeOH removed by evaporation. The aqueous solution was extracted with $CHCl_3$ (4 × 10 mL) and the $CHCl_3$ extract washed with H_2O. The $CHCl_3$ residue was chromatographed on Si gel (7 g) with $CHCl_3$−MeOH (49:1) to give 26 mg of product **14** as a colorless gum: R_f 0.45; $[\alpha]^{21}_D$ +6.0° (c = 0.95, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3470 (OH), 1783 and 1750 (lactone C=O), 1638 (C=C), 1233, 1172, 1004, and 997 cm^{-1}; FABMS ("magic bullet") *m/z* 379 (1.4, MH^+), 361 (29, $MH^+ - H_2O$), and 91 (100); HRMS *m/z* 361.1969 (2, M^+ − OH, $C_{21}H_{29}O_5$ requires 361.2015), 347.1851 (7, M^+ − MeO, $C_{20}H_{27}O_5$ requires 347.1859), 161 (56), 111 (95, $C_6H_7O_2$), 98 (100, $C_5H_6O_2$); ^1H- and ^{13}C-NMR data in Tables 1 and 2, respectively.

A similar treatment of amphiacrolide I (**5**) gave product **14** in the same yield.

Isolation of Amphiacrolide I (5). Fraction F3 (**1**) (36.3 g) on Si gel (1.35 kg) chromatography with $CHCl_3$ and $CHCl_3$−MeOH mixtures gave a fraction with six spots on TLC [Kedde reagent, EtOAc−$CHCl_3$ (3:1)]. A 1.6 g sample in Si gel (65 g) chromatography (2×) with EtOAc−$CHCl_3$ (1:2) gave a total of 100 mg of amphiacrolide I (**5**) with R_f 0.40 in the TLC system.

Amphiacrolide I (5). A heavy gum: $[\alpha]_D$ +64.1° (c = 1.5, $CHCl_3$); IR ($CHCl_3$) 3590 and 3420 (OH), 1788 and 1755 (C=O), 1643 (C=C), 1240 (CO), and 1115 cm^{-1}: UV (MeOH) λ end abs 210 nm (log ϵ 4.15); FABMS (*m*-nitrobenzyl alcohol) *m/z* 379 (4, MH^+), 361 (44, MH − H_2O), 347 (39, MH − MeOH), 157 (100), and 136 (55); EIMS 346.1791 (1, M^+ − MeOH, $C_{20}H_{26}O_5$ requires 346.1802), 328.1661 (0.6, M^+ − MeOH − H_2O, $C_{20}H_{24}O_4$ requires 328.1646), 111.0466 (100, $C_6H_7O_2$ requires 111.0486), and 98 (77, $C_5H_6O_2$); ^1H- and ^{13}C-NMR data in Tables 1 and 2, respectively.

Amphiacrolide I Acetate (11). A 10 mg sample of alcohol **5** was acetylated as given for amphiacrolide E to yield a heavy oil: $[\alpha]^{19}_D$ +55° (c = 0.04, MeOH); IR ($CHCl_3$) ν_{max} 1790, 1755 (C=O), 1650 (C=C), 1250 (CO), and 960 cm^{-1}; UV (MeOH) λ end abs 210 nm (log ϵ 4.12); HRMS *m/z* 389.1917 (1, M − OMe, $C_{22}H_{29}O_6$ requires 389.1964), 361.1988 (9, M − OAc, $C_{21}H_{29}O_5$ requires 361.2015), 329 (3, M − OMe − OAc), 372 (16), 111 (32), 98 (31), and 43 (Ac); with ^1H- and ^{13}C-NMR data in Tables 1 and 2, respectively.

Acetylation of Compound 14. A 20 mg sample of compound **14** was acetylated as described for amphiacrolide E acetate (**10**) to give, after passing through a small Si gel column with $CHCl_3$, the acetate **15** as a colorless gum: HRMS *m/z* 361.1987 (8, M^+ − AcO, $C_{21}H_{29}O_5$ requires 361.2015), 360 (1, M^+ − AcOH), 329.1709 (2, M^+ − AcO − MeOH, $C_{20}H_{25}O_4$ requires 329.1753), 111 (30, $C_6H_7O_2$), 98 (34, $C_5H_6O_2$), and 43 (100, Ac); ^1H- and ^{13}C-NMR data in Tables 1 and 2.

Isolation of Amphiacrolide F (2). The $CHCl_3$−hexane (1:4) partition fraction (30 g) was chromatographed on Si gel (550 g) with $CHCl_3$ and monitored by TLC [EtOAc−hexane (2:3)] to give a 1.2 g fraction. This fraction was separated further on a Si gel column (90 g) with EtOAc−hexane (2:3) to give 50 mg of a residue that after another separation on Si gel (6 g) with EtOAc−hexane (1:1) crystallized to give 40 mg of amphiacrolide F (**2**).

Amphiacrolide F (2). A crystalline product: mp 150.0−150.5 °C; $[\alpha]_D$ −30.6° (c = 1.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1785, 1755 (lactone C=O), 1640 (C=C) and 1220 (CO) cm^{-1}; UV (MeOH) λ end abs 210 nm (log ϵ 4.34); CD (3.03×10^{-5} M, MeOH) $[\theta]_{247}$ +19 100 and $[\theta]_{210}$ −21 100); EIMS *m/z* 330.1861 (38, M^+, $C_{20}H_{26}O_4$ requires 330.1832), 315 (48, M − CH_3), 312 (29), 286.197 (3, M − CO_2, $C_{19}H_{26}O_2$ requires 286.1934, 121 (100), 111 (28), 98 (25), and 97 (13); ^1H- and ^{13}C-NMR in Tables 1 and 2, respectively.

Isolation of Amphiacrolide G (3). The terpenoid fraction (36.5 g) from the Sephadex LH-20 separation of the $CHCl_3$−hexane (1:1) solvent partition fraction[1] was chromatographed on Si gel (1.3 kg) and eluted with $CHCl_3$ and mixtures of MeOH in $CHCl_3$. Eight pooled fractions were formed from TLC analysis of effluent fractions. The fourth fraction (12.5 g) was separated first on a Si gel column (558 g) with EtOAc−$CHCl_3$ (3:1), and then the 3.7 g residue was chromatographed on Si gel (144 g) with EtOAc−$CHCl_3$ (2:1) to give 275 mg of material, which crystallized from EtOAc−$CHCl_3$ as prisms (210 mg) of amphiacrolide G (**3**).

Amphiacrolide G (3). A crystalline solid: mp 144−145 °C; $[\alpha]_D$ −22.8° (c = 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3620 and 3430 (OH), 1785, 1753 (lactone C=O), 1640 (C=O), 1030, 1007, and 855 cm^{-1}; UV (MeOH) λ end abs 208 nm (log ϵ 4.28); CD (5.99×10^{-4} M, MeOH) $[\theta]_{210}$ +2200 and $[\theta]_{200}$ −3800; EIMS *m/z* 316.2017 (8.5, M^+ − H_2O, $C_{20}H_{28}O_3$ requires 316.2038), 302 (6, M − H_2O − Me), 301.1821 (23, M − H_2 − CH_2OH, $C_{19}H_{25}O_3$ requires 301.1838), 205 (14, M − H_2O − $C_6H_7O_2$), 111 (45, $C_6H_7O_2$) and 98 (100, $C_5H_6O_2$). Anal. Calcd for $C_{20}H_{30}H_4$: C, 71.82; H, 9.04. Found: C, 70.39; H, 8.87. The ^1H- and ^{13}C-NMR spectral data are in Tables 1 and 2, respectively.

Amphiacrolide G Diacetate (16). Amphiacrolide G (**3**) (40 mg), pyridine (0.4 mL), and Ac_2O (0.4 mL) were reacted at room temperature for 24 h. The residue (48 mg) was chromatographed on Si gel (7 g) with $CHCl_3$ and the effluent oil crystallized from EtOAc−hexane to give diacetate **16** (40 mg): mp 147−148 °C; $[\alpha]_D$ −26.8° (c = 2.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1790 and 1755 (lactone C=O), 1735 (acetate C=O), 1643 (C=C) 1255 (acetate CO), 1210 and 1025 cm^{-1}; EIMS *m/z* 358 (2, M^+ − AcOH), 298 (36, M − 2AcOH), 187 (100, M − 2AcOH − $C_6H_7O_2$), 111 (18, $C_6H_7O_2$), and 98 (50, $C_5H_6O_2$); ^1H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Conversion of Amphiacrolide G (3) to Amphiacrolide F (2). Amphiacrolide G (20 mg) in CH_2Cl_2 (2 mL) was passed into a column of MnO_2−diatomaceous earth (1:2) (1.8 g) poured in CH_2Cl_2. After 3.5 h the column was washed with Me_2CO and the effluent residue was crystallized from EtOAc−$CHCl_3$−hexane to give 14 mg of white prisms, mp 150−151 °C, $[\alpha]_D$ −29.8°, identical (TLC, IR, UV, MS, ^1H- and ^{13}C-NMR) with amphiacrolide F (**2**).

Isolation of Amphiacrolide H (4). The terpenoid fraction (2.9 g) following the one that gave amphi-

acrolide G (**3**) from the Sephadex LH-20 separation showed one major zone on TLC [EtOAc–$CHCl_3$ (3:1)] and yielded prismatic crystals of amphiacrolide H (**4**) (600 mg) from EtOAc–hexane. Additional material (250 mg) was obtained by chromatography of the mother liquor residue (2.3 g) on Si gel (140 g) with EtOAc–hexane (1:1) followed by crystallization.

Amphiacrolide H (4). A crystalline product: mp 145–146 °C; $[\alpha]_D$ −29.9° (c = 1.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3610 and 3400 (OH), 1788, 1755 (lactone C=O), 1642 (C=C), 1235 (CO), and 1037 cm^{-1}; UV (MeOH) λ end abs 208 (log ϵ 4.32); HRMS *m/z* 286.1951 (14, $M^+ - H_2O - CH_2O$, $C_{19}H_{26}O_2$ requires 286.1943); MS *m/z* 316 (8, $M^+ - H_2O$), 286 (22), 111 (40), and 98 (100). Anal. Calcd for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04. Found: C, 70.41; H, 8.97%. The ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Amphiacrolide H Diacetate (17). Amphiacrolide H (**4**) (40 mg) was acetylated and chromatographed as given for amphiacrolide G (**3**) to yield the acetate **17** as an oil (43 mg): $[\alpha]_D$ −40.6° (c = 4.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1788, 1755 (lactone C=O), 1740 (acetate C=O), 1642 (C=C), and 1250 (CO) cm^{-1}; MS 358 (1, M^+ – AcOH), 298 (31, M^+ – 2AcOH), 285 (81, M – AcOH – $AcOCH_2$), 111 (23), 105 (100), and 98 (40); ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Isolation of Amphiacrolide O (6). The terpenoid material (36.3 g) from the Sephadex LH-20 column separation of the MeOH–H_2O (7:3) solubles of the solvent partition fraction was chromatographed in Si gel (1.3 kg) with $CHCl_3$ and $CHCl_3$–MeOH mixtures. The pooled polar fraction (2.3 g) with three spots, R_f 0.80, 0.60, and 0.13, on TLC [EtOAc–$CHCl_3$ (3:1)] was rechromatographed on Si gel (135 g) to give amphiacrolide O (**6**) (1.28 g), R_f 0.60, as a homogeneous colorless gum.

Amphiacrolide O (6). A colorless gum: $[\alpha]_D$ −24.5° (c = 2.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3440 (OH), 1787, 1750 (lactone C=O), 1640 (C=C), 1235 (CO), 1077 and 1035 cm^{-1}; UV (MeOH) λ end abs 208 nm (log ϵ 4.14); CD (6.47×10^{-5} M, MeOH) $[\theta]_{225}$ −25 400; FABMS (glycerol) *m/z* 487 (35, MNa^+) and 503 (2, MK^+) when spiked with KCl; EIMS *m/z* 331 (0.2, $M^+ - C_5H_9O_4$), 315 (0.2), 133 (17, $C_5H_9O_4$), 111 (24), and 98 (100); ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Amphiacrolide O Triacetate (19). Amphiacrolide O (**6**) (50 mg) was acetylated and the product chromatographed as described for amphiacrolide G (**3**) to give acetate **19** as a colorless oil (57 mg): $[\alpha]_D$ −24.5° (c = 1.6, $CHCl_3$) ν_{max} 1787 (very intense, C=O), 1643 (C=C), 1253 and 1220 (CO) cm^{-1}; EIMS *m/z* 332 (2, $MH^+ - C_{11}H_{15}O_7$), 259 (57, $C_{11}H_{15}O_7^+$) 173 (55), 156 (49), 149 (20), 139 (54), 121 (24), 111 (26), 105 (59), and 98 (100); ^{1}H- and ^{13}C-NMR data given in Tables 3 and 4.

Hydrolysis of Amphiacrolide O (6). (A) With NaOH. Amphiacrolide O (**6**) (120 mg) in EtOH (10 mL) and 0.5 N NaOH (5 mL) was stirred at room temperature for 48 h and then neutralized with 0.5 N H_2SO_4 and concentrated at reduced pressure to 5 mL. The aqueous solution was extracted with $CHCl_3$ (6 × 20 mL), and the $CHCl_3$ extract after washing with H_2O and drying (anhydrous Na_2SO_4) was evaporated to give a yellow oil (55 mg) which was chromatographed in Si gel with 2% MeOH in $CHCl_3$. Amphiacric acid B (**8**) was eluted as a colorless gum (48 mg): $[\alpha]_D$ −30.6° (c = 2.2, $CHCl_3$); UV (MeOH) λ end abs 210 nm (log ϵ 4.19); IR ($CHCl_3$) ν_{max} 3500–2500 (COOH), 1785 and 1753 (lactone C=O), 1693 (COOH), and 1640 cm^{-1}; EIMS *m/z* 332.1950 (3, $M^+ - C_{20}H_{28}O_4$ requires 332.1988), 288 (6, M – CO_2), 173 (54), 111 (25), and 98 (100); ^{1}H- and ^{13}C-NMR data in Tables 3 and 4.

(B) With HCl. Amphiacrolide O (50 mg) in dioxane (1 mL) and 5 N HCl (1 mL) was reacted for 24 h at room temperature and then concentrated to 1 mL at reduced pressure and extracted with $CHCl_3$ (3 × 2 mL). The $CHCl_3$ extract after separation on a Si gel (7 g) column gave 30 mg of amphiacric acid B (**8**) as a colorless gum.

The aqueous phase was examined by TLC against arabinose, ribose, and xylose in two solvent systems: MeCN–CS_2–H_2O (17:1:2) and $CHCl_3$–MeOH–H_2O (19:9:2). The R_f values (0.31 and 0.25, respectively) and the color with the spray reagent for the sugar of the hydrolysate were the same as for arabinose. Evaporation of the aqueous extract to dryness left a gun (14 mg) with $[\alpha]_D$ +136° (c = 1.4, H_2O) (lit.[10] L-arabinose $[\alpha]_D$ +173° → $[\alpha]_D$ +105.1° (c = 3, H_2O).

Isolation of Amphiacrolide P (7). The Si gel column that afforded amphiacrolide O gave a fraction (6.4 g) following it which showed one major, R_f 0.10, and several minor zones on the [EtOAc–$CHCl_3$ (3:1)]. Chromatography of this material on Si gel (360 g) with EtOAc–$CHCl_3$ (1:1, 2:1, 3:1) and EtOAc gave amphiacrolide P (**7**) as a homogeneous amorphous fraction (0.34 g).

Amphiacrolide P (7). An amorphous gum: $[\alpha]_D$ −21.9° (c = 2.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3580 and 3450 (OH), 1790, 1755 and 1733 (lactone and ester C=O), 1642 (C=C), 1230–1210 (CO), 1085 and 1040 cm^{-1}; UV (MeOH) λ end abs 210 nm (log ϵ 4.26); CD (6.47×10^{-5} M, MeOH) $[\theta]_{213}$ +12 400, $[\theta]_{245}$ +13 500; FABMS (glycerol) *m/z* 487 (6, MNa^+) and 503 (2, MK^+) when spiked with KCl; HRMS *m/z* 446.2333 (0.7, $M^+ - H_2O$, $C_{35}H_{34}O_7$ requires 446.2304), 428.2158 (0.2, M – $2H_2O$), 332.2002 (5, $C_{20}H_{28}O_4$ requires 332.1988), 111 (43), and 98 (35); ^{1}H- and ^{13}C-NMR data are in Tables 3 and 4, respectively.

Amphiacrolide P Triacetate (20). Amphiacrolide P (45 mg) was acetylated and the product chromatographed as described for amphiacrolide G (**3**) to give acetate **20** as an amorphous solid: $[\alpha]_D$ −32.6° (c = 5.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1785 and 1755 (ester and lactone C=O), 1640 (C=C) and 1230–1210 (CO) cm^{-1}; EIMS *m/z* 548 (0.7, $M^+ - CH_2CO$), 331 (2, M – $C_{11}H_{15}O_7$), 314 (39), 299 (52), 259 (19, $C_{11}H_{15}O_7$) 216 (34), 174 (40), 156 (72), 97 (61), and 68 (100); ^{1}H- and ^{13}C-NMR data are in Tables 3 and 4, respectively.

Hydrolysis of Amphiacrolide P (7). (A) With NaOH. Amphiacrolide P (160 mg) was hydrolyzed and worked up as described for amphiacrolide O (**6**), except that hydrolysis was for 3 days, to give amphiacric acid A (**9**), mp 180–181 °C, with $[\alpha]_D$, IR, ^{1}H-NMR, MS, and TLC behavior identical with an authentic sample.

(B) With HCl. Amphiacrolide P (50 mg) was hydrolyzed as given for amphiacrolide O and the $CHCl_3$ extract of the hydrolysate gave a residue that crystallized from hexane–$CHCl_3$ to give 32 mg of amphiacric acid A. The aqueous phase yielded a gum that on TLC showed R_f, color formation, and $[\alpha]_D$ identical with L-arabinose.

Isolation of Amphiacric Acid A (9). A 30 g sample of the $CHCl_3$–hexane (1:1) solubles from the solvent partition fractionation was chromatographed on 1.3 kg of Si gel with $CHCl_3$ and $CHCl_3$–MeOH mixtures. The effluent fractions were monitored by TLC on Si gel and 4% MeOH in $CHCl_3$ as solvent system. The fraction (2.5 g) with R_f 0.26 and 0.43 Kedde reagent positive material was rechromatographed on Si gel with EtOAc–hexane (2:3). The first eluted compound, amphiacric acid A (**9**) (50 mg), crystallized from $CHCl_3$–hexane as colorless rosettes of needles, mp 180–181 °C.

Amphiacric Acid A (9). A crystalline product: mp 180–181 °C, $[\alpha]_D$ −60.5° (c = 0.9, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3520–2600 (OH), 1790 and 1756 (lactone C=O), 1690 (acid C=O) and 1643 (C=C) cm^{-1}; UV (MeOH) λ end abs 208 nm (log ϵ 4.30); CD (3.0×10^{-5} M, MeOH) $[\theta]_{205}$ +5300, $[\theta]_{215}$ 0 and $[\theta]_{235}$ −14 900; EIMS *m/z* 332.2006 (5, M^+, $C_{20}H_{28}O_4$ requires 332.1987), 301 (4, $M - CH_3$), 299 (72, $M - CH_3 - H_2O$), 111 (66, $C_6H_7O_2$), 98 (46, $C_5H_6O_2$), 97 (5, $C_5H_5O_2$), and 41 (100); and ^1H- and ^{13}C-NMR data in Tables 3 and 4, respectively.

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