Full Papers

cis-Clerodane Diterpene Lactones from *Amphiachyris dracunculoides*. 3[†]

Fathalla M. Harraz[‡] and Raymond W. Doskotch*

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210-1291

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Five *cis*-clerodane lactones, amphiacrolides J (**1**), K (**2**), L (**3**), M (**4**), and N (**5**), were isolated from the aerial parts of the composite *Amphiachyris dracunculoides*. The first four are new compounds, and the fifth was previously reported from another source. All compounds were assigned stereochemical structures based upon spectral and chemical data, including high-field 1D and 2D NMR for complete assignment of ^{1}H- and ^{13}C-NMR spectra. Amphiacrolide N (**5**) was converted to amphiacrolide J (**1**) by treatment with MeOH and TFA, and amphiacrolide L (**3**) was prepared from amphiacrolide B (**9**) by CrO_3 oxidation to ketone **10** followed by its reduction with $NaBH_4$.

The annual composite *Amphiachyris dracunculoides* (DC.) Nutt. (Asteraceae) of the southwestern United States has been already recorded to yield 14 *cis*-clerodane lactones from the aerial parts.[1,2] This report is on five such diterpenes, amphiacrolides J (**1**), K (**2**), L (**3**), M (**4**), and N (**5**). All are new natural products, except amphiacrolide N (**5**), which was reported from another source,[3] but with limited physical data. All of these compounds possess the ethyl butenolide side chain as supported by characteristic MS fragments (*m/z* 111, 98, and 97) and ^{1}H- and ^{13}C-NMR peaks. The NMR assignments (Tables 1 and 2) for **1**−**5** were made from highfield and extensive 1D (homonuclear decoupling, and NOE difference) and 2D [^{1}H,^{1}H-COSY, NOESY and one-bond and long-range CH-correlation (COLOC)] studies.[4]

Results and Discussion

Amphiacrolides J (**1**), mp 148−150 °C, and N (**5**), a colorless gum, are treated together because they showed very similar NMR spectra, and the latter compound was converted to the former by MeOH and trifluoroacetic acid (TFA). Their molecular formulas as supported by HRMS are, respectively, $C_{21}H_{32}O_6$ and $C_{20}H_{30}O_6$. Amphiacrolide N (**5**) has two acylable hydroxyls and forms the diacetate **6**, thereby leaving two oxygens unassigned. The ^{13}C-NMR peaks at δ 110.74 and 104.00 ppm for amphiacrolide N and J, respectively, are consistent with acetal and hemiacetal carbons, and the double-bond equivalents from the molecular formulas require a three-ring system in addition to the butenolide. The third ring on a clerodane skeleton, the ring system common to the other diterpenes from *A. dracunculoides*, is created between C-18 and C-19 by an oxygen bridge. The acetal/hemiacetal carbon was identified as C-18 by NOE (see below). Because the diacetate **6** still showed hydroxyl absorption in the IR spectrum, and the ^{13}C-NMR spectra of amphiacrolide J and N showed quaternary carbons at 84.42 and 85.06 ppm, the last oxygen must be a tertiary hydroxyl.

Table 1. ^{1}H-NMR Data for Compounds **1**−**5**, **10**, and **12**[a]

proton	1	2	3	4	5	10	12
H-1	1.75 α dddd (13.5, 3.7, 3.7, 3.7)	2.02 α hm (12.6, 5.9)	2.27 α hm [br dd] (12.6, 5.9)	1.67 α hm	1.76 α dddd (13.6, 3.4, 3.4, 3.4)	2.62 α dd (17.5, 5.5)	2.09 α hm [dd] (12.5, 2.0)
	1.47 β hm	1.17 β ddd (13.0, 6.0, 0.9)	1.78 β dd (13.2, 13.2, 10.1)	1.50 β hm	1.49 β hm	2.67 β dd (17.5, 13.4)	2.86 β dd (15.0, 12.8)
H-2	1.30 α dddd (12.7, 12.7, 12.7, 3.2)	4.44 ddd (4.4, 4.4, 1.1)	4.36 dddd (9.4, 6.6, 6.3, 2.5)	3.93 m [ddd] (10.5, 7.9, 5.0)	1.30 α dddd (12.5, 12.5, 12.5, 2.6)		
	1.92 β hm				1.91 β hm		
H-3	3.72 dd (12.0, 5.1)	6.36 d (5.7)	6.69 d (1.7)	3.36 s	3.72 dd (11.8, 5.1)	6.56 s	3.31 s
H-6	1.95 α hm	1.11 α ddd (13.3, 13.3, 4.7)	1.58 α hm [ddd] (14.4, 3.9, 3.9)	1.50 α hm	1.97 α hm	1.69 α hm	1.69 α hm
	1.46 β hm	2.00 β ddd (13.6, 2.6, 2.6)	1.73 β hm [dddd] (13.9, 13.9, 3.5, 1.8)	1.82 β hm	1.51 β hm	1.75 β hm	2.10 β hm
H-7	1.71 α hm	1.54 α hm	1.96 α dddd (14.1, 14.1, 4.6, 4.6)	1.85 α hm	1.82 α dddd (13.5, 11.6, 5.7, 5.7)	2.05 α dddd (14.3, 12.8, 5.2, 5.2)	1.93 α dddd (13.7, 13.7, 5.3, 5.3)
	1.41 β hm	1.42 β hm	1.50 β hm	1.37 β hm	1.41 β hm	1.57 β dddd (14.6, 3.3, 3.3, 2.7)	1.50 β brd (13)
H-8	1.52 hm	1.45 hm	1.66 hm	1.50 hm	1.54 hm	1.72 hm	1.58 m [ddq] (7.2, 4.6, 4.6)
H-10	1.39 dd (13.3, 3.4)	1.48 hm [dd] (9.5, 6.0)	1.62 brd (14.9)	1.78 hm	1.36 (dd (13.5, 2.9)	2.15 dd (13.3, 5.4)	2.39 dd (15.4, 1.5)
H-11	1.45 hm	1.33 m	1.48 hm	1.40 ddd (13.2, 13.2, 4.8)	1.44 hm	1.53 (2H) m	1.74 hm
	1.89 hm [ddd] (13.2, 13.2, 4.3)	1.40 hm	1.52 hm	1.72 ddd (13.3, 13.3, 3.6)	1.94 hm [ddd] (12.4, 12.4, 4.4)		1.43 ddd (13.3, 13.3, 4.9)
H-12	2.32 ddd (15.6, 12.9, 4.2)	2.31 (2H) t (8.6)	2.35 m	2.29 ddd (16.1, 12.3, 3.9)	2.31 ddd (16.4, 12.3, 4.0)	2.37 (2H) dd (10.9, 5.8)	2.30 ddd (16.1, 12.7, 4.2)
	2.41 ddd (15.8, 13.1, 4.2)		2.37 m	2.45 ddd (16.5, 12.5, 2.6)	2.41 ddd (16.4, 12.3, 4.0)		2.44 ddd (16.6, 13.2, 2.6)
H-14	5.84 m (5 pk) (1.5)	5.80 m (5 pk) (1.1)	5.83 m (5 pk) (1.7)	5.78 brs	5.84 m (5 pk) (1.4)	5.81 m (5 pk) (1.6)	5.80 m (5 pk) (1)
H-16	4.76 (2H) d (1.2)	4.72 (2H) d (1.3)	4.75 (2H) d (1.7)	4.73 (2H) d (1)	4.75 dd (17.6, 1.6) 4.77 dd (17.6, 1.6)	4.72 (2H) d (1.6)	4.74 (2H) d (1.4)
H-17	1.01 d (7.2)	0.81 d (6.4)	1.11 d (7.4)	0.98 d (7.2)	1.01 d (7.1)	1.13 d (7.5)	1.03 d (7.2)
H-18	4.99 s	3.29 α d (7.2) 2.88 β d (7.2)	3.79 α dd (8.1, 1.6) 4.56 β d (8.1)	5.49 s	5.48 s	4.00 α dd (8.4, 0.9) 4.70 β d (8.4)	5.54 s
H-19	4.34 α d (10.6) 3.86 β d (10.6)	4.37 (2H) d (1.2)[b]		3.98 α d (10.0) 3.79 β d (10.0)	4.27 α d (10.5) 3.97 β d (10.5)		4.06 α d (10.2) 3.85 β d (10.5)
H-20	0.93 s	0.45 s	1.02 s	0.87 s	0.92 s	0.99 s	0.88 s
Misc	3.39 s (MeO) 2.50 brs (HO-3) 3.90 s (HO-4)		2.27 d (OH) (6.7)	4.44 brs (HO-18) 2.38 d (HO-2)	5.48 s (HO-18) 4.16 s (HO) 3.03 s (HO)		4.81 brs (HO-18)

[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. [b] In one experiment, H$_2$-19 appeared as a dd ($J = 6.1$, 1 Hz) and HO-19 as d ($J = 6.1$ Hz) at δ 1.62; and D$_2$O removed the 6.1 Hz coupling.

The ^{1}H,^{1}H-COSY and the CH-correlation experiments provided the following proton-coupled unit for **1**: the ethyl butenolide, H-10 to H$_2$-1 through to H-3, H$_2$-6 through to H$_3$-17, and H-18 to H$_2$-19 by the long-range "W-coupling" between H-18 and H-19α. The last observation, when taken with the results from the NOE by difference studies at 270 MHz allowed stereochemical placement of the protons on a *cis*-clerodane ring system. The key results that supported the assignments for amphiacrolide J (**1**) are as follows. Irradiation at δ 4.99 ppm (H-18) showed relaxation to 1.39 ppm (H-10) of 1% and to 3.38 ppm (MeO) of 8%. This designated H-18 to the stereochemical α-position, while irradiation of the methoxyl showed relaxation to 3.38 ppm (H-19β) and to a D$_2$O-exchangeable proton at 3.90 ppm, both of 1%.
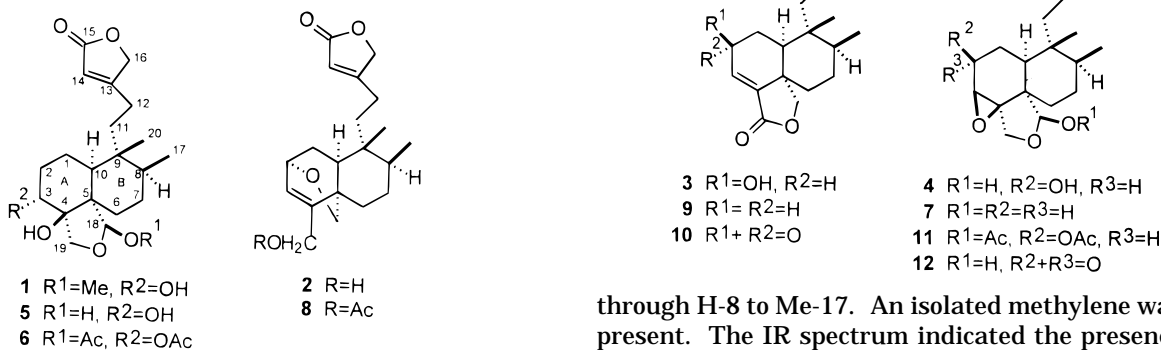
The latter result located one of the hydroxyls at C-4, and when the doublet at H-19α (4.34 ppm) was irradiated, relaxation occurred to H-10 of 3%, to H-2α (1.30 ppm) of 8%, and to H-19β of 27%. This last irradiation also showed a negative NOE to H-2β (1.92 ppm) of 1%, which results from collinearity of H-19α, H-2α, and H-2β and supports the chair conformation of ring A in which C-19 is axial and C-18 equatorially disposed. Thus, irradiation of the axial H-3 (3.72 ppm) identified H-1β (1.47 ppm) and H-2β (1.92 ppm) from relaxation of 4% for each as well as HO-3 (2.50 ppm) of 4% and HO-4 (3.90 ppm) of 2%. Irradiation of Me-20 (0.93 ppm) showed relaxation to H-1α (1.75 ppm) of 11%, in agreement with ring B, also existing in a chair conformation, where Me-20 is equatorially and the ethyl

Table 2. ^{13}C-NMR Data for Compounds **1−5**, **10**, and **12**[a]

				compound				
carbon	**1**	multiplicity	**2**	**3**[b]	**4**	**5**	**10**	**12**
C-1	25.08	t	29.86	32.69	28.08	25.15	38.42	35.15
C-2	29.62	t	67.12 d	68.75 d	70.41 d	30.50	198.31 s	207.68 s
C-3	72.60	d	128.47	136.71	63.02	72.58	127.56	61.10
C-4	84.42	s	146.39	136.73	71.82	85.06	153.64	76.97
C-5	55.12	s	39.07	43.82	45.06	54.68	44.40	46.08
C-6	17.18	t	28.03	25.03	19.71	17.28	24.61	20.05
C-7	26.52	t	28.89	25.50	25.57	26.24	24.79	25.55
C-8	36.68	d	36.88	35.08	35.65	36.85	35.00	35.45
C-9	39.35	s	38.80	38.62	37.68	39.18	38.21	38.36
C-10	40.66	d	41.83	45.54	37.05	40.29	45.51	40.93
C-11	41.47	t	34.12	39.38	39.11	41.43	39.08	38.66
C-12	23.66	t	22.24	24.00	23.91	23.67	23.80	23.74
C-13	170.68	s	170.84	170.22	172.68	171.54	170.16	171.97
C-14	115.40	d	115.30	115.51	114.66	115.03	115.43	114.90
C-15	174.04	s	174.14	173.91	175.15	174.75	173.91	174.87
C-16	73.24	t	73.16	73.21	73.76	73.54	73.19	73.67
C-17	17.48	q	15.73	17.81	17.04	17.58	17.90	16.91
C-18	110.74	d	75.31 t	76.35 t	102.27	104.00	76.16 t	101.85
C-19	75.74	t	61.44 t	169.75 s	66.24	74.90	168.60 s	65.39
C-20	23.31	q	15.76	22.40	22.35	23.74	21.86	21.87
misc	55.57 (MeO)	q						

[a] Values were taken in CDCl$_3$ at 67.9 MHz with multiplicities determined by SFORD unless stated otherwise and chemical shifts (δ) in ppm relative to TMS using the solvent peak (center) as reference, 77.2 for CDCl$_3$. Multiplicities, when different from those in the column, are given after the chemical shift. Abbreviations are as follows: s = singlet, d = doublet, t = triplet, and q = quartet. [b] Value determined at 125 MHz.

butenolide side chain is axially disposed. Similar irradiation of the axial Me-17 identified the β-face protons of C-6 and C-7.

1 R^1=Me, R^2=OH
5 R^1=H, R^2=OH
6 R^1=Ac, R^2=OAc

2 R=H
8 R=Ac

3 R^1=OH, R^2=H
9 R^1= R^2=H
10 R^1+ R^2=O

4 R^1=H, R^2=OH, R^3=H
7 R^1=R^2=R^3=H
11 R^1=Ac, R^2=OAc, R^3=H
12 R^1=H, R^2+R^3=O

Amphiacrolide N (**5**) was also subjected to detailed NOE by difference studies at 500 MHz for its ^{1}H-NMR assignments and to confirm those of amphiacrolide J (**1**). The ^{1}H-NMR results are found in Table 1, and from the CH-correlations, the ^{13}C-NMR assignments are in Table 2. It appears that amphiacrolides J and N could be derived from amphiacrolide D (**7**)[1] by epoxide opening to give the 3,4-trans diol followed by methylation of HO-18 in the case of amphiacrolide J. The absolute stereochemistry of amphiacrolide D is known; therefore, that of amphiacrolide J and N would be as illustrated in the drawings. The limited data in the literature[3] for the compound with the structure of amphiacrolide N (**5**) are expanded by this study and also require some revision for the ^{13}C-NMR spectrum. The C-1 and C-6 assignments should be reversed, and the C-19 value of δ 66.7 ppm is probably a misprint and should be 75.1 ppm.

Amphiacrolide K (**2**), mp 131−134 °C, has the molecular formula C$_{20}$H$_{28}$O$_4$ as supported by HRMS. The ^{1}H,^{1}H-COSY and CH-correlation experiments revealed two additional proton-coupled units besides the ethyl butenolide. They are the ring-A protons from H-10 to H$_2$-1 through H-3 to H$_2$-19 and the ring-B protons H$_2$-6

through H-8 to Me-17. An isolated methylene was also present. The IR spectrum indicated the presence of a hydroxyl, and the formation of monoacetate **8** with a downfield shift of 0.44 ppm for the methylene protons supported a primary hydroxyl in amphiacrolide K. The fourth oxygen must be an ether oxygen because the acetate **8** lacked both hydroxyl and carbonyl absorptions in the IR spectrum. The proton-coupled units were easily assembled on a clerodane skeleton containing an additional olefinic group (^{1}H- and ^{13}C-NMR data). The remaining degree of unsaturation beyond the three for the butenolide and the three in the decalin system was generated by forming a fourth ring containing an ether oxygen. The proton chemical shifts of δ 4.44 for a methine and 2.88 and 3.29 ppm for a methylene are consistent with those on ether-bearing carbons. Structure **2** with the C-2 to C-18 ether bridge, less stereochemistry, was supported by all the spectral data.

The stereochemical structure of amphiacrolide K (**2**) was established by NOE difference spectroscopy, and the relevant results are given in Figure 1. These experiments, in conjunction with the 2D one-bond and several-bond (COLOC) CH-correlation studies, allowed complete assignment of the ^{1}H- and ^{13}C-NMR spectra as given in Tables 1 and 2, respectively. The chosen absolute stereochemistry is that of the compounds from
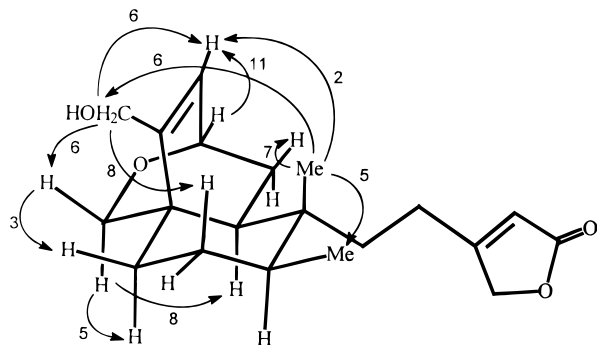
Figure 1. Selected NOE enhancements, in percent, from difference spectroscopy for amphiacrolide K (**2**) at 270 MHz in CDCl₃.

A. dracunculoids for which it was established.[1,2] An example of a *trans*-clerodane with a C-2 to C-18 ether link is brevifloralactone from *Salvia breviflora*[5] and *Salvia melissodora* (Labiatae).[6]

Amphiacrolide L (**3**), mp 182–184 °C, with a molecular formula $C_{20}H_{26}O_5$, as supported by HRMS, has eight degrees of unsaturation. The double intensity in the lactone carbonyl region of the IR spectrum suggested an additional lactone system. Hydroxyl absorption was also present. The ¹H,¹H-COSY and CH-correlation results completely ordered the substituents on the decalin system of the clerodane skeleton. The three major proton-coupled units were recognized as follows: the ethyl butenolide, the H-10 to H_2-1 through to H-3, and the Me-17 to H-8 through to H_2-6. In addition, long-range "W-coupling" was observed between a C-18 proton (3.79 ppm) and a C-6 proton (1.73 ppm). This result supported, indirectly, the second lactone at C-19 and was confirmed by NOE relaxation of the H-18 at 3.79 ppm to H-14 of 2%. Also, olefinic proton H-3 (6.69 ppm) showed five-bond homoallylic coupling to the other H-18 (4.56 ppm). Because long-range coupling occurs when coplanarity is present for those units,[7] the conformation of the *cis*-decalin system was established. Ring B would be in a chair form, in which the Me-20 is equatorial and the ethyl butenolide is axially disposed.

This conformation was supported by the NOE-difference experiments, with the results for the pertinent interactions as follows. The axial Me-17 (1.11 ppm), when irradiated, relaxed to H-1β (1.78 ppm) by 4%, to H-6β (1.73 ppm) and H-8 (1.66 ppm) by 7%, to H-7β (1.50) by 4%, and to Me-20 (1.02 ppm) by 2%; to identify the protons on the β-face of the molecule. The α-faced protons were located by two irradiations, first at H-2 (4.36 ppm) to show relaxation to H-1α (2.27 ppm) of 5%, to H-3 (6.69 ppm) of 10%, and to H-10 (1.62 ppm) of 4%; and second at H-18α (3.79 ppm) to give relaxation to H-10 of 4%. These results gave the stereochemical structure of amphiacrolide L as **3** and allowed complete assignment of the ¹H- and ¹³C-NMR spectra as given in Tables 1 and 2, respectively.

Because amphiacrolide L (**3**) appeared to be the 2β-hydroxy derivative of amphiacrolide B (**9**) and because the absolute stereochemistry of the latter compound is known,[1] preparation of amphiacrolide L from amphiacrolide B was carried out for confirmation. CrO₃ oxidation produced ketone **10**, which, on NaBH₄ reduction, introduced the hydride from the less hindered α-side to give amphiacrolide L (**3**). This established the absolute stereochemistry as drawn in **3**. Ketone **10** was sub-

jected to extensive 1D and 2D NMR studies, details of which are not given here, but the results are found in Tables 1 and 2.

The epimer at C-8 of amphiacrolide L has been isolated from *Solidago gigantea*,[8] and the *trans*-clerodane, articulin, epimeric at C-8 and C-9, is known from *Baccharis articulata*.[9] Both plants are of the Composite family.

Amphiacrolide M (**4**), mp 160–161 °C, has the molecular formula $C_{22}H_{28}O_6$ as determined from HRMS and elemental analysis. After examination of the ¹H- and ¹³C-NMR spectra and from extensive 1D and 2D studies, the details of which are not given here, amphiacrolide M (**4**) was established as a hydroxyl derivative of amphiacrolide D (**7**).[1] The molecular formula requires seven degrees of unsaturation, and the lack of olefinic carbons in the ¹³C-NMR spectrum, other than those for the butenolide, necessitates a four-ring system in the decalin portion. Preparation of the diacetate **11** supported two hydroxyls and together with the butenolide unit accounts for four of the six oxygens in the molecule. The other two must be ether oxygens, and the ¹H,¹H-COSY results with the CH-correlation data revealed the two major proton-coupled units: H-10 to H_2-1 through to H-3, and Me-17 to H-8 through to H_2-6. Furthermore, four-bond long-range coupling from H-3 to each unit of a methylene AB quartet (δ 3.79 and 3.98 ppm) was observed in the COSY experiment, along with "W-coupling" of one of the methylene protons (3.98 ppm) to a downfield one-proton singlet at 5.49 ppm. This revealed the hemiacetal ring system between C-18 and C-19, with the methylene at C-19. The fourth ring must be an epoxide between C-3 and C-4.

NOE-difference experiments showed relaxation of H-3 (3.36 ppm) to only one (3.98 ppm) H-19 methylene proton of 3%, and to H-2 (3.93 ppm) of 5%, placing these protons on one side, the α-side, when taking into evidence the irradiation of H-2, which relaxed to H-10 (1.78 ppm) by 3% and H-3 by 9%. Also, when H-19α and H-19β were irradiated separately only the former (3.98 ppm) showed relaxation (7%) to H-3, a result requiring a β-epoxide. Additional NOE data helped to identify the α- and β-face protons, which, when compared to the results for amphiacrolide D (**7**) gave the ¹H-NMR assignments for amphiacrolide M (Table 1). The ¹³C-NMR (Table 2) designations followed from the CH-correlation and COLOC studies.

To confirm the hydroxyl position at C-2, selective oxidation of amphiacrolide M (**4**) with MnO₂ formed ketone **12**, which showed the C-1 and C-10 protons in the ¹H-NMR spectrum as an AMX pattern (each proton is a double-doublet). Also, the expected downfield chemical shifts for these protons were observed. Complete spectral assignments were made for the ¹H- and ¹³C-NMR spectra (Tables 1 and 2, respectively) after detailed 1D and 2D NMR experiments—not given here—which supported the values for amphiacrolide M (**4**).

Experimental Section

General Experimental Procedures. The instruments used, conditions under which measurements were made, and the source of plant material, along with the handling of the plant extract, are given in Harraz *et al.*[1,2]

Isolation of Terpenoids. The solvent partition fraction F3 (MeOH–H$_2$O, 7:3, solubles),[1] 100 g, was separated on a 275-g Sephadex LH-20 (Pharmacia) column with MeOH in 20-g portions to give 36.3 g of terpenes (generally blue-purple zones on TLC with *p*-anisaldehyde or red zones with Kedde spray reagent). The terpenes were chromatographed on a Si gel (1.3 kg) column with CHCl$_3$ and CHCl$_3$–MeOH mixtures. The effluent fractions (75 mL) were monitored by TLC (Si gel, EtOAc–CHCl$_3$, 3:1) and dry weight analyses, then combined to give 11 pooled fractions designated F3-A through F3-K.

Isolation of Amphiacrolide J (1). Column fraction F3-D (1.6 g) was chromatographed on 65 g of Si gel with EtOAc–CHCl$_3$ (1:2) to give an 80-mg fraction R_f 0.15 on Si gel TLC (EtOAc–CHCl$_3$, 3:1), which was further cleaned up on a 7-g Si gel column with EtOAc–CHCl$_3$ (2:1) followed by crystallization from CHCl$_3$–hexane to give 40 mg of **1**.

Amphiacrolide J (1): colorless crystals; mp 148–150 °C; [α]$_D$ +13.9° (*c* 1.2, CHCl$_3$); IR (CHCl$_3$) ν_{max} 3580 and 3500 (OH), 1784 and 1752 (lactone C=O), 1637 (C=C), 1475–1445, 1150, 1083 (MeO), and 1035 (OH) cm^{-1}; UV (MeOH) $\lambda_{end\ abs}$ 210 nm (log ϵ 4.15); HRMS *m/z* 349.2015 (2, M$^+$ – MeO, C$_{20}$H$_{29}$O$_5$ requires 349.2015), 210.1649 (100, M – OMe – CO – C$_6$H$_7$O$_2$, C$_{13}$H$_{22}$O$_6$ requires 210.1646), 192 (9), 111.0492 (55, C$_6$H$_7$O$_2$ requires 111.0446), 98.0406 (42, C$_5$H$_6$O$_2$ requires 98.0366) and 97 (24); ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Isolation of Amphiacrolide K (2). Column fraction F3-E (1.27 g) was separated on a 140 g Si gel column with EtOAc–CHCl$_3$ (2:1) to give 170 mg of essentially one-spot material on TLC (Si gel with EtOAc–CHCl$_3$, 3:1). Another column separation on 35 g of Si gel and EtOAc–CHCl$_3$ (3:1) gave 65 mg of amphiacrolide K that crystallized from EtOAc–hexane.

Amphiacrolide K (2): colorless crystals; mp 131–134 °C; [α]$_D$ –2.8° (*c* 0.35, CHCl$_3$); CD (5.8 × 10^{-4} M, MeOH) [θ]$_{205}$ +19 300, [θ]$_{228}$ 0, and [θ]$_{235}$ –900; IR (CHCl$_3$) ν_{max} 3610 and 3440 (OH), 1788 and 1755 (C=O, lactone), 1642 (C=C), 1173, 1037 (C-O), 995, 890 and 857 cm^{-1}; UV $\lambda_{end\ abs}$ 208 nm (log ϵ 4.24); HRMS *m/z* 332.1987 (0.1, M$^+$, C$_{20}$H$_{28}$O$_4$ requires 332.1986), 314.1881 (0.1, M – H$_2$O), 284.1784 (23, M – H$_2$O – CH$_2$O), 111.0470 (15, C$_6$H$_7$O$_2$) and 98.0379 (100, C$_5$H$_6$O$_2$); ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Amphiacrolide K Acetate (8). A 10-mg sample of amphiacrolide K was acetylated and purified as given for amphiacrolide N (**5**), except that the reaction time was 12 h, to give 6 mg of a colorless gum of acetate **8**: [α]$_D$ –12.4° (*c* 0.5, CHCl$_3$); IR (CHCl$_3$) ν_{max} 1788 and 1755 (double intensity) (C=O, lactone and acetate), 1642 (C=O), 1375, 1235–1210 (CO), and 1038 cm^{-1}; ^{1}H-NMR (CDCl$_3$, 500 MHz) δ 6.39 (1H, d, *J* = 5.7 Hz, H-3), 5.83 (1H, s, H-14), 4.81 (2H, s, H$_2$-19), 4.74 (2H, s, H$_2$-16), 4.45 (1H, t, *J* = 5.0 Hz, H-2), 3.32 (1H, d, *J* = 7.1 Hz, H-18α), 2.93 (1H, d, *J* = 7.1 Hz, H-18β), 2.33 (2H, t, *J* = 8.6 Hz, H$_2$-12), 1.96 (1H, br d, *J* = 14.4 Hz, H-6β), 1.34 (1H, m, H-11), 1.20 (2H, m, H-6α and H-11), 0.83 (3H, d, *J* = 6.1 Hz, Me-17), and 0.49 (3H, s, Me-20); HRMS *m/z* 332.1965 (1, M$^+$ – CH$_2$CO, C$_{20}$H$_{28}$O$_4$ requires 332.1988), 330.1884 (9, M – CH$_2$CO – H$_2$), 300.1723 (10, M – CH$_2$CO – H$_2$ – CH$_2$O), 284.1815 (89, M – AcOH – CH$_2$O), 111.0476 (31, C$_6$H$_7$O$_2$ requires

111.0446), 98.0355 (91, C$_5$H$_6$O$_2$ requires 98.0368), and 43.0328 (100, Ac requires 43.0184).

Isolation of Amphiacrolide L (3). The mother liquor residue (2.3 g) of column fraction F3-F (3.0 g), from which amphiacrolide H[2] was crystallized, was chromatographed on 135 g of Si gel with EtOAc–CHCl$_3$ (2:1). A 75-mg fraction was obtained that yielded 40 mg of crystalline amphiacrolide L (**3**) from EtOAc-hexane.

Amphiacrolide L (3): prismatic crystals; mp 182–184 °C; [α]$_D$ –46.3° (*c* 1.6, CHCl$_3$); IR (CHCl$_3$) ν_{max} 3600 and 3460 (OH), 1785–1750 (C=O, lactones), 1688 and 1642 (C=C), 1455, 1192, 1010, and 892 cm^{-1}; UV (MeOH) λ_{max} 210 nm (log ϵ 4.38); CD (5.78 × 10^{-4}M, MeOH) [θ]$_{200}$ +28 000, [θ]$_{214}$ 0, and [θ]$_{250}$ –18 300; HRMS *m/z* 346.1813 (5, M$^+$, C$_{20}$H$_{26}$O$_5$ requires 346.1781), 331.1529 (11, M – CH$_3$), 328.1713 (6, M – H$_2$O), and 111.0450 (100, C$_6$H$_7$O$_2$); ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Oxidation of Amphiacrolide B (9) with CrO$_3$. Amphiacrolide B (50 mg) in 2 mL of pyridine was treated portionwise with CrO$_3$ (270 mg) while being stirred at room temperature. After 48 h, 10 mL of PhMe was added and the mixture filtered. The residue that was obtained after evaporation of the filtrate under reduced pressure was separated on 6.5 g of Si gel with CHCl$_3$ to give 17 mg of ketone **10** as a homogeneous oil: [α]$_D$ +10.6° (*c* 2.1, CHCl$_3$); IR (CHCl$_3$) ν_{max} 1777 and 1755 (C=O, lactones), 1685 and 1640 (C=C), 1330, 1190, and 1023 cm^{-1}; HRMS *m/z* 344.1615 (12, M$^+$, C$_{20}$H$_{24}$O$_5$ requires 344.1606), 247.1339 (35, M – C$_5$H$_5$O$_2$), 233.1173 (25, M-C$_6$H$_7$O$_2$), 111.0390 (54, C$_6$H$_7$O$_2$), and 98.0369 (100, C$_5$H$_6$O$_2$); ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Reduction of 2-Ketoamphiacrolide B (10). Ketone **10** (15 mg) in 2 mL of MeOH at 0 °C was treated with 1.6 mg of NaBH$_4$ for 30 min while being stirred. TLC on Si gel with EtOAc–CHCl$_3$ (3:1) or MeOH–CHCl$_3$ (1:19) indicated one product. The reaction residue chromatographed on 1 g of Si gel with MeOH–CHCl$_3$ (1:19) gave 12 mg of pure product that crystallized from EtOAc–hexane to give 10 mg of colorless needles, mp 182–184 °C, and showed the same IR, ^{1}H-NMR, and MS spectra, specific rotation, and TLC mobility as amphiacrolide L (**3**).

Isolation of Amphiacrolide M (4) and N (5). Column fraction F3-H (2.3 g) was chromatographed on 135 g of Si gel with EtOAc–CHCl$_3$ (2:1) to give two fractions, R_f 0.14 (1.3 g) and R_f 0.10 (0.44 g), on Si gel TLC with EtOAc–CHCl$_3$ (3:1). The R_f 0.14 material crystallized from CHCl$_3$–hexane to give 500 mg of amphiacrolide M (**4**). Another 60 mg was obtained by similarly chromatographing fraction F3-I.

The R_f 0.10 material was rechromatographed on 100 g of Si gel with the same solvent system to give 180 mg of amphiacrolide N (**5**).

Amphiacrolide M (4): white crystals; mp 160–161 °C; [α]$_D$ –56.5° (*c* 1.0, CHCl$_3$); IR (CHCl$_3$) ν_{max} 3590 and 3410 (OH), 1788 and 1755 (C=O, lactone), 1642 (C=C), 1028 (C-O) and 878 cm^{-1}; UV (MeOH λ_{max} 210 nm (log ϵ 4.25); CD (5.49 × 10^{-5} M, MeOH) [θ]$_{208}$ –10 738, [θ]$_{240}$ +2000; HRMS *m/z* 365.1958 (0.7, MH$^+$, C$_{20}$H$_{28}$O$_6$ requires 364.1912), 347.1833 (4, M – OH), 253.1489 (1.5, M – C$_6$H$_7$O$_2$), 111.0413 (91, C$_6$H$_7$O$_2$), 98.0383 (65, C$_5$H$_6$O$_2$), and 41.0389 (100, C$_3$H$_5$). Anal. Calcd for

$C_{20}H_{28}O_6$: C, 65.94, H, 7.69. Found: C, 65.49; H, 7.79. ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Amphiacrolide M Diacetate (11). Amphiacrolide M (20 mg) was acetylated with 0.5 mL each of Ac_2O and pyridine for 24 h at room temperature. The residue, after evaporation of the reaction mixture with N_2, was crystallized from $CHCl_3$–hexane to give 20 mg of colorless needles of diacetate **11**: mp 145–147 °C; $[\alpha]_D$ –48.1° (*c* 1.9, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1788 and 1755 (C=O, lactone), 1740 (C=O, esters), 1642 (C=C), 1377, 1243–1210 (CO), 1005, and 943 cm^{-1}; ^{1}H-NMR (CDCl$_3$, 500 MHz) δ 6.39 (1H, s, H-18), 5.80 (1H, 5 pk m, H-14), 5.11 (1H, ddd, J = 10.9, 5.8, and 1.0 Hz, H-2), 4.698 (1H, d A of ABq, J = 17 Hz, H-16), 4.696 (1H, d B of ABq, J = 17 Hz, H-16), 3.92 (2H, s, H$_2$-19), 3.46 (1H, s, H-3), 2.26 (1H, m, H-12), 2.18 (1H, m, H-12), 2.13 (3H, s, Ac), 2.11 (3H, s, Ac), 0.93 (3H, d, J = 7.0 Hz, Me-17), and 0.86 (3H, s, Me-20); MS m/z 388 (6, M$^+$ − AcOH), 346 (18, M − AcOH − CH$_2$CO), 328 (31, M − 2AcOH), 217 (27, M − 2AcOH − C$_6$H$_7$O$_2$), 111 (94, C$_6$H$_7$O$_2$), 98 (100, C$_5$H$_6$O$_2$), and 60 (92, AcOH).

2-Dehydroamphiacrolide M (12). Amphiacrolide M (20 mg) in 2 mL of CH_2Cl_2 was passed into a 2-g column of MnO_2 (Winthrop)−Celite (1:2) packed in CH_2Cl_2. After 4 h, the column was eluted with Me_2CO (40 mL). The residue was chromatographed on two columns of Si gel (7 g each) first with EtOAc−CHCl$_3$ (1:1) then with MeOH−CHCl$_3$ (1:19) to give ketone **12** (11 mg) as a colorless oil: R_f 0.49 on TLC (Si gel with EtOAc−CHCl$_3$, 3:1, Kedde reagent); $[\alpha]_D$ −22.4° (*c* 0.6, CHCl$_3$); IR (CHCl$_3$) ν_{max} 3590 and 3400 (OH), 1790 and 1756 (C=O, lactone), 1723 (C=O, ketone), 1648 (C=C), 1035, 1022, 945, and 852 cm^{-1}; FABMS ("magic bullet" in glycerol) m/z 363.1806 (19, MH$^+$, C$_{20}$H$_{27}$O$_6$ requires 363.1808), 345.1682 (24, MH − H$_2$O, C$_{20}$H$_{25}$O$_5$ requires 345.1702), and 85 (100); HRMS m/z 344.1582 (5, M$^+$ − H$_2$O, C$_{20}$H$_{24}$O$_5$ requires 344.1623), 111.0516 (83, C$_6$H$_7$O$_2$), 98.0401 (83, C$_5$H$_6$O$_2$), and 41.0424 (100, C$_3$H$_5$); ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Amphiacrolide N (5): colorless gum; $[\alpha]_D$ −7.3° (*c* 3.5, CHCl$_3$); IR (CHCl$_3$) ν_{max} 3580 and 3440 (OH), 1785 and 1750 (lactone), 1637 (C=C), 1455, 1170, 1064, and 888 cm^{-1}; UV (MeOH) $\lambda_{end\ abs}$ 210 nm (log ϵ 4.24); CIMS (isobutane) m/z 349 (44, MH$^+$ − H$_2$O) and 111 (100); MS m/z 348 (1, M$^+$ − H$_2$O), and 111 (100); HRMS m/z 335.1871 (4, MH$^+$ − HO − CH$_3$, C$_{19}$H$_{27}$O$_5$ requires 335.1859), 307 (8), 261 (21), 210.651 (15, M − OH − C$_6$H$_7$O$_2$, C$_{13}$H$_{22}$O$_2$ requires 210.1620), 111.0446 (100, C$_6$H$_7$O$_2$ requires 111.0446), and 98.0388 (53, C$_5$H$_6$O$_2$ requires 98.0368); ^{1}H- and ^{13}C-NMR data are in Tables 1 and 2, respectively.

Amphiacrolide N 2,18-Diacetate (6). Amphiacrolide N (12 mg) was reacted with 0.3 mL of Ac_2O and 0.3 mL of pyridine for 24 h at room temperature. The reaction mixture was evaporated at reduced pressure, and the residue (20 mg) was chromatographed on 7 g of Si gel with CHCl$_3$ to give 15 mg of diacetate **6** as an oil: R_f 0.50 on TLC with Si gel and EtOAc−CHCl$_3$ (3:1); $[\alpha]_D$ +16.7° (*c* 1.6, CHCl$_3$); IR (CHCl$_3$) ν_{max} 3580 and 3500 (OH), 1788 and 1753 (C=O, lactone), 1740 (C=O, acetate), 1643 (C=C), and 1245−1210 (COC) cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 6.57 (1H, s, H-18), 5.91 (1H, 5 pk m, J = 1.2 Hz, H-14), 4.97 (1H, dd, J = 12.1 and 5.3 Hz, H-3), 4.84 (1H, dd, J = 17.6 and 1.6 Hz, H-16A), 4.78 (1H, dd, J = 17.5 and 1.6 Hz, H-16B), 4.39 (1H, d, J = 10.7 Hz, H-19α), 4.03 (1H, d, J = 10.7 Hz, H-19β), 2.40 (2H, t, J = 8.6 Hz, H$_2$-12), 2.11 (3H, s, Ac), 2.06 (3H, s, Ac), 1.94 (1H, s, HO-4, lost with D$_2$O), 1.53 (1H, m, H-8), 1.30 (1H, dddd, J = 13.0, 13.0, 13.0, and 3.3 Hz, H-2α), 1.03 (3H, d, J = 7.2 Hz, Me-17), and 0.98 (3H, s, Me-20); CIMS (isobutane) m/z 451 (0.4, MH$^+$) and 61 (100, AcOH$_2^+$); EIMS m/z 435 (1, M$^+$ − Me), 390 (2, M − AcOH), 111 (57), 98 (50), 97 (45), 60 (65, AcOH) and 55 (100).

Conversion of Amphiacrolide N (5) to Amphiacrolide J (1). A 15-mg sample of amphiacrolide N (**5**) in 1 mL of MeOH was treated with three drops of TFA for 24 h at room temperature. After evaporation of the reaction mixture under reduced pressure, the residue (18 mg) was chromatographed on 7 g of Si gel with 2% MeOH in CHCl$_3$ to give 7 mg of amphiacrolide J (**1**), identical (TLC, $[\alpha]_D$, IR, ^{1}H-NMR, and HRMS) with the isolated natural product.

References and Notes

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