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cis-CLERODANE DITERPENE LACTONES FROM *AMPHIACHYRIS DRACUNCULOIDES*[1]

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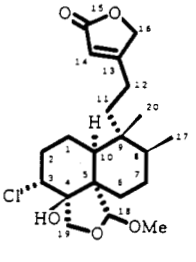
ABSTRACT.—Four *cis-ent*-neoclerodane lactones, amphiacrolide A [**2**], amphiacrolide B [**3**], amphiacrolide C [**4**] and amphiacrolide D [**5**] were isolated from the aerial parts of *Amphiachyris dracunculoides*, and their structures established by physical and chemical methods. High field ^{1}H- and ^{13}C-nmr assignments were made for each compound and some of their derivatives by using one- and two-dimensional nmr techniques including nOe difference, COSY, and CH-correlation of one-bond and multiple bond (COLOC) relationships. A chemical correlation of the amphiacrolides to gutierolide [**1**], a compound with absolute stereochemistry determined by X-ray analysis, established the stereochemistry for the group. Consequently, a compound having reported properties identical with amphiacrolide D [**5**] must have its structure revised to contain the β-faced epoxide. Amphiacrolide A [**2**] had not been reported previously.

Amphiachyris dracunculoides (DC.) Nutt. (Compositae) is a North American annual with use as a folk remedy in the treatment of coughs and colds in the southwestern part of the United States (1). The plant has had several botanical names, with *Gutierrezia dracunculoides* (DC.) Blake previously used in a report from this laboratory on the isolation of gutierolide [**1**], a chloro-containing diterpene (2). Subsequently, a taxonomic revision has resulted in the current designation (3). Three labdane diterpenes are also re-



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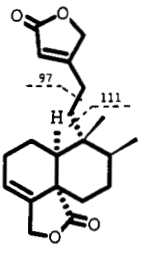

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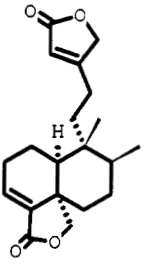

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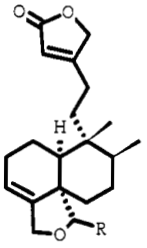

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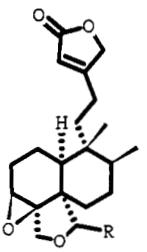
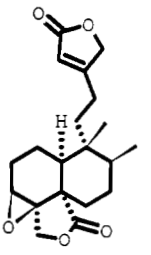
10 R=OAc
11 R=OMe



5 R=OH
14 R=OAc



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[1]Taken in part from the Ph.D. dissertation of Fathalla M. Harraz, accepted by the Graduate School, The Ohio State University, in August 1984.

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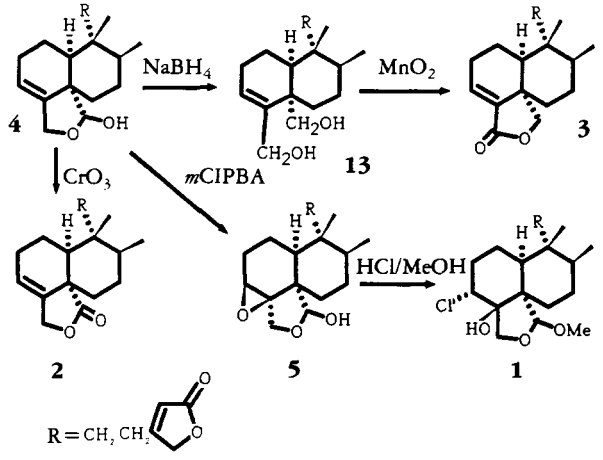
corded in the literature under the old plant name (4). This report concerns the 5α,10α-*cis*-clerodane lactones, amphiacrolides A [**2**], B [**3**], C [**4**], and D [**5**], their isolation and structure elucidation by spectral and chemical methods, and their chemical correlation to gutierolide [**1**] (Scheme 1), whose absolute stereochemical structure was established by X-ray crystallography. In addition, complete spectral assignments were made for the carbons and protons from high field nmr studies. A publication appeared after this work was completed on the last three compounds isolated from a different source (5); however, our results are in places at variance with that study.



SCHEME 1. Chemical interrelationship of gutierolide [**1**] and amphiacrolides A [**2**], B [**3**], C [**4**], and D [**5**].

The air-dried and powdered above-ground parts of *A. dracunculoides* on percolation with EtOH yielded a residue that, after partitioning between a series of solvent pairs and careful chromatography, afforded the crystalline diterpene lactones. Amphiacrolide A [**2**], mp 84–85°, has the molecular formula $C_{20}H_{26}O_4$ as supported by hrms and elemental analysis. The ir spectrum indicated an α,β-unsaturated γ-lactone with an α-hydrogen with bands at 1752 and 1785 cm^{-1} (6) supported by a positive reaction with Kedde's reagent (7) and another lactone carbonyl at 1767 cm^{-1}. The ms with peaks at *m/z* 97, 98, and 111 confirmed the presence of a two-carbon side chain with a butenolide unit as shown in structure **2** (8). With five of the eight double-bond equivalents required by the formula accounted for and an additional one from an olefinic double bond as indicated by the ^1H- and ^{13}C-nmr spectra (Tables 1 and 2) (and formation of epoxide **6**); the remaining two equivalents were assigned to a bicyclic ring system.

^1H-nmr decoupling and a COSY spectrum (9) at 500 MHz revealed coupled units such as: (a) H-14, H_2-16, H_2-11, and H_2-12 of the butenolide with the two methylenes; (b) H-10, H_2-1, H_2-2, and H-3; (c) H_2-6, H_2-7, H-8, and Me-17; and (d) H_2-19 with long range coupling to H-3 and H_2-2. The use of two different solvents ($CDCl_3$ and pyridine-d_5) was of value in clarifying overlapping spin patterns. The spin-coupled units were easily accommodated by the clerodane skeleton, a ring system present in the previously known gutierolide [**1**]. A 2D nmr CH-correlation experiment identified the protons on the carbons and thereby the methylene protons (10–12), while a long-range CH-correlation study (2- to 4-bond coupling) located the unprotonated carbons (13) and confirmed the location of many of the protonated carbons. For example, H-14 (5.82 ppm) is coupled to C-15 (174.6 ppm), C-13 (172.1 ppm), and C-12

TABLE 1. ^{1}H-nmr Data for Compounds **1–5**.[a]

Proton	Compound									
	1[b]	**1**[c]	**2**[b]	**2**[c]	**3**[b]	**3**[c]	**4**[b]	**4**[c]	**5**[b]	**5**[c]
H-1	1.78α dddd (13.0, 3.6, 3.6, 2.5) 1.52β hm	1.66 α dddd (13.6, 3.7, 3.7, 3.7) 1.43β hdq (13.3, 13.3, 13.3, 3.1)	1.78α hm 1.63β dddd (14.0, 10.2 10.2, 5.1)	1.60α hm 1.46β dddd (13.0, 11.3, 11.3, 5.2)	1.92α brd (13.9) 1.73β dddd (13.3, 13.3, 11.3, 5.7)	1.69α brd (13) 1.53β dddd (13.2, 13.2, 11.5, 5.6)	1.78 α m (11.9, 4.3, 2.1) 1.63β hm	1.75α brdm (12.8, 2.5, 2.5, 2.5) 1.62β hm	1.35α hm 1.46β hm	1.35α brd (13.1) 1.55β dq (13.0, 13.0, 13.0, 3.7)
H-2	1.63α dq (13.0, 13.0, 13,0, 3.2) 2.17β dddd (13.0, 4.8, 3.4, 3.4)	1.75α dq (13.0, 13.0, 13.0, 3.3) 2.12β dddd (13.3, 4.8, 3.5, 3.5)	1.98α hm 2.12β br dm (18.0, 3.7, 3.7, 3.7, 3.7)	1.85α hm 1.94β brd (17.9, 3.6, 3.6, 3.6, 3.6)	2.12α dddd (20.2, 11.1, 6.3, 2.8) 2.39β dddd (20.1, 5.7, 4.4, 1.4)	1.95α hm 2.17β dddd (20.2, 5.6, 4.1, 1.3)	2.00α brdm (16.8) 2.06β brd (16.8)	1.95–2.08 (2H) hm	1.64α hdt (11, 11, 5) 2.07β brd (14.5)	1.70α hdt (14.0, 14.0, 4.0) 2.04β brd (13.0)
H-3	3.97 dd (12.7, 5.0)	4.26 dd (12.8, 5.0)	5.77 brs $\omega_{1/2}=9.2$	5.62 brs $\omega_{1/2}=8.5$	6.80 dd (t) (3.5, 3.5)	6.82 dd (t) (3.5, 3.5)	5.61 brs $\omega_{1/2}=8$	5.57 brs $\omega_{1/2}=8.4$	3.26 s	3.36 s
H-6	1.99α dt (14.3, 4.8, 4.8, 0.9) 1.50 hm	2.28α hm 1.58β hddd (14.3, 10.6, 5.9)	1.85α dddd (13.9, 5.4, 2.0, 0.8) 1.79β hm	1.75α ddd (14.5, 6.3, 3.1) 1.62β ddd (14.2, 12.3, 5.8)	1.55α hm 1.62β hm	1.45 (2H) hm	1.51α hm 1.58β ddd (13.5, 13.5, 4.3)	1.63 (2H) hm	1.49α hm 1.84β hm	1.71α brd (14.1) 2.03β hm
H-7	1.70α hm 1.43β m	1.79α hm 1.35β m	1.92α hm (12.9, 12.9, 5.9, 5.9) 1.33β ddt (13.8, 5.8, 5.8, 2.7)	1.85α hm 1.19β ddt (13.9, 6.2, 6.2, 2.8)	1.95α tt (14.2, 11.9, 5.7, 5.7) 1.43β dddd (14.5, 3.4, 3.4, 3.2)	1.93α hm 1.20β dddd (14.4, 3.2, 3.2, 3.2)	1.91α tt (13.8, 13.8, 4.9, 4.9) 1.31β dddd (10.7, 3.6, 3.6, 3.6)	2.00α htt (13.0, 13.0, 5.5, 5.5) 1.26β dq (14.1, 3.6, 3.6, 3.6)	1.86α hm 1.35β hm	1.94α htt (13.7, 13.7, 5.2, 5.2) 1.27β m (13.4, 3.2)
H-8	1.53 hm	1.54 hm	2.00 hm	2.05 ddq (6.3, 6.3, 6.3)	1.62 hm	1.45 hm	1.52 hm	1.50 m	1.49 hm	1.48 brq (7.0)
H-10	1.46 hm	1.58 hm	1.71 dd (11.7, 2.7)	1.71 dd (11.8, 3.5)	1.39 dd (13.1, 2.6)	1.30 dd (12.9, 2.3)	1.67 d (13.6)	1.97 dd (12.8, 3.5)	1.64 hdd (11, 3)	1.96 hdd (12.9, 3)
H-11	1.87 dt (13.4, 13.4, 4.6) 1.48 hm	1.88 dt (13.6, 13.6, 4.2) 1.44 hm	2.45 hm 1.37 hm	2.40 hm 1.38 m	1.51 (2H) dd (9.8, 7.2)	1.45 (2H) hm	1.85 dt (12.9, 12.9, 3.4) 1.37 dt (13.2, 13.2, 4.9)	2.01 hddd (13.0, 13.0, 4.1) 1.42 ddd (13.1, 13.1, 4.7)	1.74 dt (13.1, 13.1, 3.8) 1.40 dt 13.3, 13.3, 4.8)	1.84 dt (13.1, 13.1, 3.8) 1.43 dt 13.2, 13.2, 4.6)

TABLE 1. Continued.

Proton	Compound									
	1[b]	1[c]	2[b]	2[c]	3[b]	3[c]	4[b]	4[c]	5[b]	5[c]
H-12	2.41 ddd (16.0, 12.7, 4.0) 2.32 ddd (16.0, 12.6, 3.8)	2.41 ddd (16.3, 12.8, 3.7) 2.28 hm	2.45 hm 2.38 hm	2.36 m 2.34 m	2.36 m 2.33 m	2.27 m 2.25 m	2.52 ddd (15.5, 12.2, 2.5) 2.28 ddd (16.3, 12.9, 4.4)	2.61 ddd (15.7, 12.2, 3.1) 2.26 ddd (16.2, 13.0, 4.3)	2.47 ddd (16.1, 12.9, 3.0) 2.30 ddd (15.9, 12.7, 4.5)	2.52 ddd (15.9, 12.8, 3.1) 2.30 ddd (16.2, 12.9, 4.0)
H-14	5.84 m (1.6) $\omega_{1/2}=4.2$	6.01 m (1.6) $\omega_{1/2}=4.9$	5.82 (1.5) $\omega_{1/2}=4$	5.99 m (1.3) $\omega_{1/2}=4.8$	5.81 m (tt) (1.6)	6.03 m (tt) (1.6)	5.76 m (1.5) $\omega_{1/2}=4.8$	5.95 m (1.3) $\omega_{1/2}=4.8$	5.78 brs $\omega_{1/2}=5$	5.97 m (1.4) $\omega_{1/2}=3.8$
H-16[d]	4.75 (2H) d (1.6)	4.78 dd (17.3, 1.4) 4.77 dd (17.3, 1.4)	4.81 dd (17.6, 1.7) 4.74 dd (17.6, 1.7)	4.83 dd (17.4, 1.4) 4.79 dd (17.4, 1.7)	4.73 (2H) d (1.8)	4.84 dd (17.5, 1.6) 4.82 dd (17.5, 1.8)	4.69 (2H) s	4.78 (2H) d (1.4)	4.72 (2H) d (1.2)	4.78 (2H) d (1.6)
Me-17	0.99 d (7.2)	0.92 d (7.1)	0.96 d (7.2)	0.86 d (7.1)	1.07 d (7.4)	0.94 d (7.5)	1.04 d (7.4)	1.02 d (7.4)	0.97 d (7.2)	0.92 d (7.2)
H-18	5.03 s	5.25 s			3.74α dd (8.0, 1.8) 4.56β d (8.0)	3.80α dd (8.1, 1.6) 4.73β d (8.1)	5.45 s	5.80 s	5.52 d (2.4)	5.92 s
H-19	4.30α d (10.6) 3.95β d (10.6)	4.54α d (10.2) 4.22β d (10.2)	4.48α ddd (10.8, 1, 0.8) 4.81β dddd (10.9,2.8, 2.8, 2.1)	4.45α d (10.8) 4.79β dddd (10.9, 2.8, 2.8, 2.0)			4.22α dddd (11.4, 1.1, 1.1, 1.1) 4.34β dddd (11.4, 2.9, 2.9, 2.3)	4.39α dddd (11.3, 1.8, 1.8, 1.4) 4.48β dddd (11.3, 2.9, 2.9, 2.4)	3.96α d (9.7) 3.82β d (9.7)	4.21α d (9.5) 4.04β d (9.5)
Me-20	0.92 s	0.81 s	0.95 s	0.83 s	0.98 s	0.83 s	0.92 s	0.88 s	0.84 s	0.79 s
Others	3.38 s (OMe) 4.10 s (OH)	3.32 (OMe)					3.92 brs (OH)		4.24 (OH)	

[a]Taken at 500 MHz unless stated otherwise in text, with data point resolution of 0.3 Hz and chemical shifts in ppm as referenced to TMS with residual solvent peaks taken as internal standard: $CHCl_3$ at 7.26 ppm and the upfield peak of pyridine-d_4 at 7.19 ppm, Stereochemical designations α and β following the chemical shift refer to 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; it refers to separation values solely for characterization and may not be the true *J* as in non-first-order patterns. Half-height width (ω) in Hz.

[b]In $CDCl_3$.

[c]In pyridine-d_5.

[d]H_2-16 and H-14 form an ABX or A_2X system as indicated by dd or d, respectively, for the H-16 pattern.

TABLE 2. ^{13}C-nmr Data for Compounds **1–5**.[a]

Carbon	1	multiplicity	1[b]	2	2[b]	3	3[b]	4[c]	4[b]	5	5[b]
C-1	26.7	t	26.8	23.6	23.7	22.2	22.2	23.0	23.6	18.3	18.8
C-2	33.9	t	34.1	25.2	25.2	26.8	26.8	26.3	26.8	27.1	27.5
C-3	62.8	d	64.9	123.2	123.0	136.1	135.8	119.4	119.0	60.2	60.1
C-4	83.3	s	83.5	136.7	137.1	134.7	135.4	141.0	142.9	67.2	67.7
C-5	56.7	s	57.1	46.6	46.6	43.2	43.3	49.0	49.7	45.5	45.9
C-6	17.1	t	17.6	25.4	25.5	25.1	25.2	23.7	24.5	19.8	20.5
C-7	26.4	t	26.6	25.8	26.0	25.3	25.4	25.9	26.4	25.6	25.9
C-8	36.7	d	36.4	34.4	34.4	35.3	35.7	37.1	37.3	35.7	35.6
C-9	39.4	s	39.4	38.2	38.2	38.4	38.3	37.9	38.2	37.7	37.8
C-10	40.6	d	40.8	37.4	37.6	44.9	44.5	38.5	39.2	38.1	38.6
C-11	41.5	t	41.2	40.0	39.9	39.6	39.4	39.9	40.3	39.3	39.4
C-12	23.6	t	23.5	24.2	24.1	24.0	23.9	23.8	24.2	23.9	24.0
C-13	170.4	s	171.8	172.1	172.8	170.5	171.8	172.8	173.4	172.9	173.0
C-14	115.5	d	115.1	115.1	115.0	115.3	115.0	114.1	114.6	114.5	114.7
C-15	173.9	s	174.2	174.6	174.4	173.8	174.2	174.8	174.5	175.1	174.3
C-16	73.2	t	73.3	73.5	73.5	73.1	73.4	73.4	73.5	73.7	73.5
C-17	17.5	q	17.3	17.0	16.9	17.9	17.8	17.7	17.9	17.1	17.0
C-18	110.7	d	111.3	178.7 s	178.6 s	76.5 t	76.5 t	100.9	101.6	102.7	103.0
C-19	76.1	t	76.2	69.4	69.3	170.4 s	170.4 s	67.2	67.5	66.9	67.0
C-20	23.1	q	22.4	22.0	21.4	22.3	22.1	22.4	22.4	22.2	22.0
	55.5 MeO	q	55.2								

[a]Taken at 67.9 HMz in $CDCl_3$ with multiplicities determined by SFORD unless stated otherwise. Multiplicities when different from those in column are given after the chemical shift in ppm and referenced to TMS with reference peak of solvent taken at 77.2 (center) for $CDCl_3$ and 123.5 (center) for upfield carbon of pyridine-d_5. Abbreviations are: s = singlet, d = doublet, t = triplet, and q = quartet.

[b]In pyridine-d_5.

[c]At 125 MHz.

(14.2 ppm), and H-3 (5.77 ppm) is coupled to C-1 (23.6 ppm), C-2 (25.2 ppm), C-5 (46.6 ppm), and C-19 (69.4 ppm). Additional results are in the Experimental. The shift assignments for C-13 and C-15 were based on the longitudinal relaxation (T_1) of these carbons as determined for amphiacrolide C [**4**] and on the long-range coupling of H_2-12 to C-13, but not to C-15. The methylene proton assignments were made from extensive nOe studies by the difference method (14,15), of which a select number are given in Figure 1, as well as from the coupling constants of the proton multiplets: e.g.,

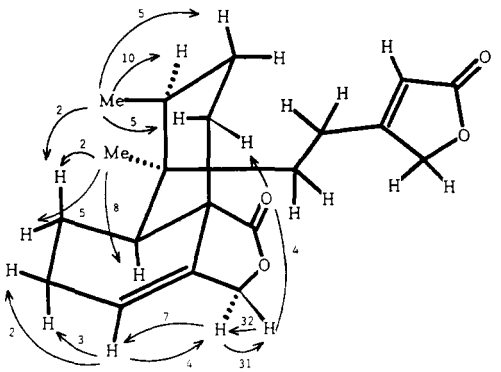
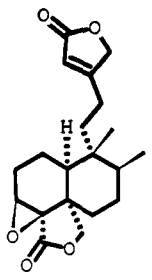


FIGURE 1. NOe enhancements (in percent) by difference spectroscopy for amphiacrolide A [**2**] at 270 MHz in $CDCl_3$.

the H-1 multiplet at 1.63 ppm with J values of 14.0, 10.2, 10.2, and 5.1 Hz must be an axial proton (β-oriented) with three large coupling constants for a geminal and two trans diaxial couplings and a smaller axial-equatorial coupling. In several cases, a clear multiplet pattern was obtained from nOe difference spectra, whereas in the normal spectrum the pattern was overlapped and hidden; e.g., the isolated H-2 pattern, a broadened doublet as 2.12 ppm with a band width of 33 Hz was placed in the β-position because the other H-2 pattern (1.98 ppm), also a broadened doublet but with a 42 Hz width as revealed by the nOe experiment, must be α to reflect the larger trans diaxial coupling in the latter versus a smaller equatorial-axial coupling in the former. The nOe enhancements from the olefinic proton (H-3) to both H_2-2 are essentially the same; thus, this method leaves them unassigned.

The epoxide **6** was prepared showing the spectral features recorded in the Experimental. The oxirane was positioned β from nOe difference studies; thus, H-3 relaxed to H-19α which relaxed to H-19β, which in turn relaxed to H-6α.

Amphiacrolide B [**3**], mp 189–191°, with the same molecular formula, $C_{20}H_{26}O_4$, as amphiacrolide A, based on hrms and elemental analysis, also had the same functional groups as indicated by the spectral data. However, one significant difference was the downfield position (6.80 ppm) for the olefinic proton H-3 (5.76 ppm for amphiacrolide A), suggesting a deshielding environment, namely a neighboring carbonyl. Epoxidation to compound **7** confirmed the presence of the olefinic group, and preparation of the pyrazoline **8** supported a second α,β-unsaturated lactone (16). This evidence and the presence of an AB quartet (3.74 and 4.56 ppm, $J = 8.0$, and W-coupling of 1.8 Hz to H-6β for the former) assigned H_2-18 supported the lactone carbonyl at C-19. The β stereochemistry of the epoxide was assigned on the basis of a weak (0.5%), but real, nOe enhancement of H-3 (3.57 ppm) upon irradiation of H-18α (4.03 ppm), which also enhanced H-10 (1.15 ppm) by 6%; these results place the interacting protons on the α face. Furthermore, irradiation of H-3 enhanced H-2α (1.69 ppm) more (4%) than H-2β (2.28 ppm, 2%); H-2α is identified from irradiation



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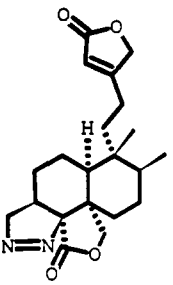

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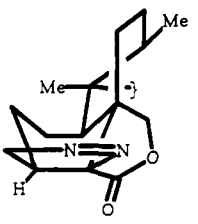
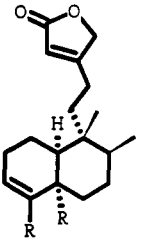


9



12 R=CHO
13 R=CH_2OH

of H-10. These values and others that established the stereotopic designations are given in the Experimental. The β epoxide also conforms with the diazo position in the pyrazoline **8**, which is in agreement with a β-side approach of the reagent.

Results of extensive nOe studies with pyrazoline **8** could only be accommodated if the conformations of rings A and B are both boat, as shown in representation **9**. Relevant to placing the azomethylene on the β face, the following results are important: irradiation of H-18α (4.30 ppm) enhanced H-1α (1.55 ppm, 5%), H-3 (2.23 ppm, 5%), and H-10 (1.43 ppm, 4%); irradiation of the α-side proton of the azomethylene enhanced H-3 (10%) and the geminal β-side proton (4.60 ppm, 25%); while irradiation of the β-side proton of the azomethylene, in addition to enhancing the geminal proton (29%), gave a 5% increase for H-2β and a 1% increase for H-2α. With irradiation of H-2β the Me-20 was enhanced 4%, thereby completing a connection from the α face (H-18α) to the β face (H-2β and Me-20) of the molecule. Additional results are found in the Experimental. Of note is the high field position for H-2β (1.09 ppm) and Me-20 (0.64) due to shielding by the β-oriented diazo group. With the aid of homonuclear decoupling, COSY, and CH-correlation, including long-range coupling (2- to 4-bond), assignments for ^{1}H- and ^{13}C-nmr spectra were made as given in Tables 3 and 4, respectively.

The compound deoxymarrubialactone from *Chaiturus* (or *Leonurus*) *marrubiastrum* Reichb. is reported to have the same structure (no stereochemistry given) as amphiacrolide B [**3**] (17). Its physical data (mp, ^{1}H nmr, ir, and ms), except specific rotation, which was of opposite sign, and the fact that two other compounds, marrubiastrol and aldehydomarrubialactone from the same source, have established stereochemistry, the latter by X-ray analysis (18), leads us to suggest that deoxymarrubialactone is the (−) enantiomer of amphiacrolide B.

Amphiacrolide C [**4**], mp 145–147°, $C_{20}H_{28}O_4$ as supported by hrms and elemental analysis, contains a butenolactone, olefinic groups, a secondary hydroxyl (a D_2O exchangeable one-proton doublet in the ^{1}H-nmr spectrum), and two methyl groups, one

TABLE 3. ^{1}H-nmr Data for Compounds **6–8**, **10**, **11**, **13**, and **14**.[a]

Proton	Compound						
	6	**7**	**8**	**10**	**11**	**13**	**14**
H-1	1.35–1.56 hm	1.52 hm	1.55–1.60 hm	1.91α hm 1.68β hm	1.80α brdd (10.0, 3.3) 1.65β	1.91α dddd (15.2, 9.6, 9.6, 7.0) 1.75β m	1.40α hm 1.50β
H-2	1.71α hm 2.19β dddd (14.8, 2.9, 2.9, 2.4)	1.69α m 5 pk (8) 2.28β dddd (15.1, 3.0, 3.0, 1.9)	1.55α hm 1.09β m	2.02α m 2.13β brd (18)	2.01α brdd (17.8) 20.7β brd (17)	2.22 hm 2.18 hm	1.66α hdt (13, 13, 4, 1) 2.14β dddd (14.6, 2.7, 2.6, 2.6)
H-3	3.33 brs $\omega_{1/2}=4.7$	3.57 dd (1.3, 1.3)	2.23 hm	5.65 brs $\omega_{1/2}=9.5$	5.57 brs $\omega_{1/2}=8.5$	5.85 dd (t) (3.8, 3.8)	3.31 brs $\omega_{1/2}=4.5$
H-6	1.84α hm 2.02β m	1.50α hm 1.85β m	1.80 hm 2.76 β m 5 pk	1.63 hm	1.57 m	1.24α hm 2.24β hm	1.60α hm 1.89β dt (12.5, 12.5, 6.3)
H-7	1.79α hm 1.33β hm	1.87α dddd (12.9, 12.9, 4.8, 4.8) 1.45β hm	1.60 hm 1.80 hm	1.89α hm 1.37β dddd (14.2, 4.2, 4.2, 3.7)	1.90α dddd (13.9, 12.7, 5.3, 5.3) 1.37β dddd (14.3, 4.1, 3.7, 3.7)	12.6α hm 1.44β hm	1.83α hm 1.37β hm
H-8	2.44 m	1.50 hm	1.47 m	1.64 hm	1.55 hm	1.44 hm	1.55 hm
H-10	1.57 m	1.15 dd (9.6, 5.6)	1.43 dd (7.0, 7.0)	1.55 dd (13.1, 2.1)	1.68 hd (10.5)	1.53 hd (6.9)	1.37 hm
H-11	1.81 hm 1.43 ddd (14.0, 11.7, 4.8)	1.53 hm 1.45 hm	1.60 hm (2H)	1.62 hddd (13.8, 11.7, 5.1) 1.30 ddd (13.5, 11.8, 5.3)	1.76 ddd (12.8, 12.8, 4.3) 1.41 ddd (13.1, 13.1, 4.4)	1.74 hm 1.52 hm	1.55 hm 1.25 m
H-12	2.48 m 2.43 m	2.32 m 5 pk (8.0)	2.27 hm	2.33 m 2.28 m	2.51 dddd (16.0, 12.7, 4.3, 1.3) 2.31 dddd (16.8, 12.5, 4.3, 1.3)	2.29 m	2.26 m 2.18 m
H-14	5.82 m (1.6)	5.85 m (1.6)	5.83 m (1.5)	5.78 m (1.5)	5.80 m (1.6)	5.84 m (1.6)	5.78 m (1.6) $\omega_{1/2}=4$
H-16	4.77 dd (17.4, 1.7) 4.74 dd (17.4, 1.7)	4.75 d (1.6)	4.74 d (1.0)	4.71 dd (17.3, 1.6) 4.67 dd (17.3, 1.6)	4.73 dd (17.3, 1.7) 4.71 dd (17.3, 1.7)	4.74 d (1.7)	4.69 (2H) d (1.4)
Me-17	0.85 d (6.9)	0.98 d (7.1)	0.86 d (6.8)	1.03 d (7.2)	1.06 d (7.4)	0.79 d (7.8)	0.91 d (7.0)
H-18		4.03α dd (8.8, 1.3) 4.65β d (8.8)	4.03α d (8.6) 4.16β d	6.39 s	4.92 s	3.63 d (9.8) 3.30 d (9.9)	6.41 s
H-19	3.92α d (10.5) 4.41β d (10.5)			4.24α dddd (11.5, 1.5, 1.5, 1.5) 4.42β dddd (11.5, 3.0, 3.0, 2.3)	4.16α dddd (11.6, 1.6, 1.6, 1.6) 4.39β dddd (11.6, 3.1, 3.1, 2.3)	4.25 brd (11.4) 4.00 d (11.6)	3.88α d (9.8) 3.92β d (9.8)
Me-20	0.83 s	0.90 s	0.64 s	0.95 s	0.94 s	0.79 s	0.84 s
Misc.			4.39α dd (17.2, 6.8) 4.60β dd (17.3, 1.3) (azomethylene)	2.00 s (Ac)	3.22 s (MeO)	3.00 br (OH)	2.08 s (Ac)

[a]Taken at 500 MHz in $CDCl_3$ under the conditions and with designations as stated in Table 1.

of which is secondary from the spectral data. Preparation of an acetate **10** and a methyl derivative **11**, the latter with acidic MeOH, substantiated the presence of a hemiacetal. The proton on the hydroxyl-bearing carbon collapsed from a doublet to a singlet in the

TABLE 4. ^{13}C-nmr Data for Compounds **6–8**, **10**, **11**, **13**, and **14**.[a]

Carbon	Compound							
	6	multiplicity	**7**[b]	**8**	**10**	**11**	**13**	**14**[b]
C-1 . .	17.3	t	18.3	20.1	23.4	23.3	17.6	17.9
C-2 . .	25.4	t	26.4	25.5	26.4	26.4	24.0	26.7
C-3 . .	60.7	d	58.8	33.7	120.2	118.8	131.6	60.3
C-4 . .	63.0	s	60.6	96.6	139.7	141.6	140.1	65.9
C-5 . .	42.5	s	38.5	44.4	48.4	49.3	41.9	44.5
C-6 . .	21.0	t	20.1	34.0	24.1	24.5	31.4	20.2
C-7 . .	25.3	t	24.2	27.2	26.1	26.4	28.2	25.3
C-8 . .	30.1	d	34.1	36.0	33.6	36.8	37.3	32.1
C-9 . .	37.7	s	37.7	39.7	38.4	38.2	40.2	37.9
C-10 . .	37.8	d	45.5	38.3	41.5	38.9	41.1	39.9
C-11 . .	37.5	t	38.8	35.6	39.2	39.6	35.2	38.2
C-12 . .	23.5	t	23.6	22.7	24.1	24.0	22.2	23.6
C-13 . .	171.5	s	169.8	170.1	170.62	172.1	171.2	170.4
C-14 . .	115.1	d	115.5	115.6	115.4	114.6	115.3	115.5
C-15 . .	174.3	s	173.5	173.8	174.0	174.2	174.2	173.9
C-16 . .	73.4	t	72.9	73.1	73.1	73.5	73.3	73.1
C-17 . .	16.1	q	16.5	16.0	17.5	18.0	16.1	16.4
C-18 . .	179.1	s	76.6 t	79.0 t	100.3 d	107.9 d	75.5 t	101.9 d
C-19 . .	67.0	t	173.1 s	172.1 s	68.7	67.6	65.4	67.7
C-20 . .	19.8	q	21.2	19.5	21.8	22.5	17.6	20.7
				82.9 t (CH_2N_2)	21.6 q (*Me*CO) 170.57 s (Me*C*O)	54.7 q (MeO)		21.5 q (*Me*CO) 170.0 s (Me*C*O)

[a]Taken at 67.9 MHz in $CDCl_3$ unless stated otherwise with conditions and abbreviations as given in Table 2.

[b]Taken at 125 MHz.

^{1}H-nmr spectrum with D_2O exchange, suggesting the α carbon must be quaternary. Amphiacrolide C [**4**] was converted to amphiacrolide A [**2**] by CrO_3 oxidation, supporting a clerodane structure with the hemiacetal carbon at C-18. A by-product of the oxidation was the dialdehyde **12**. Detailed nmr studies, including 2D techniques, were used to make complete assignments for the ^{1}H-nmr (Table 1) and ^{13}C-nmr (Table 2) spectra of amphiacrolide C. In addition, T_1 (spin-lattice relaxation) measurements were made by the inversion-recovery method to assign the quaternary carbons C-13 and C-15 because the literature was ambiguous (18–21). The results follow the principle that C-13, with a total of five protons on neighboring α carbons, would be expected to relax faster by the predominant dipole-dipole mechanism than C-15, which has only one α proton (23). The carbon at 172.8 ppm gave a T_1 of 6.5 sec, and the one at 174.8 ppm 27 sec, indicating the former to be C-13 and the latter C-15.

The nOe difference studies in $CDCl_3$ and in pyridine-d_5 showed the hemiacetal proton H-18 of amphiacrolide C [**4**] to be relaxing to H-6α, H-10, and one or both H_2-11 and H-14, thus requiring an equilibrium mixture of both C-18 epimers to be present in solution (not unlike the anomeric mixtures of reducing sugars). By the same method, H-18 in both the acetate **10** and methyl ether **11** derivatives was assigned the β position from strong cross-relaxation to H-7α, although in the acetate **10** a weak cross-relaxation (~1%) was observed to H-10, as well as in reverse, and could be accommodated by a twist of the relevant rings. The stereochemistry at C-18 in the compounds is opposite to that in gutierolide [**1**] as determined by X-ray crystallography and by nOe

studies (H-18 cross-relaxed 3% by H-10); thus the nature of ring A appears to be the determinant. In this connection, the acetate **14** of amphiacrolide D [**5**] has H-18 positioned β as for amphiacrolide C acetate [**10**]. Spectral assignments for the amphiacrolide C derivatives **10** and **11**, as well as the $NaBH_4$ reduction product **13**, as determined by the 2D nmr studies, are given in Tables 3 and 4.

Amphiacrolide D [**5**], mp 118–120°, with formula $C_{20}H_{28}O_5$ from hrms and elemental analysis, has one more oxygen than amphiacrolide C [**4**]. Analysis of the ^1H and ^{13}C-nmr data suggested it to be an epoxide of amphiacrolide C. Acetylation gave acetate **14** with the stereochemical designation of α for the acetate group as established by nOe difference studies, specifically the relaxation enhancement for H-18 to H-7α of 3%. Also, the appreciable upfield shift of H-10 from 1.64 ppm to 1.37 ppm upon acetylation must result from the diamagnetic anisotropic shielding of the ester carbonyl and could only occur with the acetate placed α. The parent alcohol **5** gave nOe results that support a mixture of both C-18 epimers (in solution) as was observed for amphiacrolide C. From nOe studies with amphiacrolide D [**5**] and its acetate **14** the oxirane ring was positioned on the β face; enhancements were shown for the sequence H-3 to H-19α to H-19β to H-6α. Oxidation of amphiacrolide D [**5**] with Jones' reagent gave amphiacrolide A epoxide [**6**], thereby confirming the amphiacrolide ring system. Complete nmr spectral assignments, as given in Tables 3 and 4, were made from detailed 2D (COSY, CH-correlation, and COLOC) studies.

The four amphiacrolides reported here were chemically correlated to gutierolide [**1**] as shown in Scheme 1. As already stated, amphiacrolide C [**4**] was oxidized with CrO_3 to amphiacrolide A [**2**], while epoxidation with *m*-chloroperoxybenzoic acid gave amphiacrolide D [**5**], which, when treated with methanolic HCl, formed gutierolide [**1**]. The diol **13**, a reduction product of amphiacrolide C [**4**], upon oxidation with MnO_2 or Jones' reagent, afforded amphiacrolide B [**3**]. In this way, the amphiacrolides have their absolute stereochemistry assigned and are *cis-ent*-neoclerodanes.

Amphiacrolides B [**3**], C [**4**], and D [**5**] have been reported previously from *Gutierrezia texana* (DC.) T. and G. var *texana* (5). However, the structure assignment for amphiacrolide D [**5**] differs from that published, in which the epoxide is placed on the α face. Our nOe results and the chemical transformation to gutierolide [**1**] would require a revision to the β-faced epoxide. The hemiacetal containing amphiacrolides C [**4**] and D [**5**], from the chemical correlation, should have the hydroxyl placed β, yet the nOe data from the acetates **10** and **14** and the methyl derivative **11** support the α position. The nOe studies with the parent hemiacetals show the existence in solution of both α and β isomers. Thus, the X-ray results (5) of a crystalline hemiacetal (our amphiacrolide C) would be of the least soluble isomer (α as it turned out) and need not remain the same in derivatives, e.g., gutierolide [**1**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected and were taken on a Thomas-Hoover unimelt or a Fisher-Johns hot stage apparatus. Nmr spectra were determined in the stated solvent with reference to TMS as internal standard or residual solvent peak ($CHCl_3$ taken as 7.26 ppm for ^1H and $CDCl_3$ center peak at 77.2 for ^{13}C) on the following instruments: Bruker HX-90E, WP-80, WM-300, AM-500, IBM AF-270, and GE-Nicolet NT-500, with chemical shifts (δ) in ppm and coupling constants (*J*) in Hz. Ir spectra were recorded on a Beckman 4320 instrument and uv spectra on a Beckman 5260 instrument. Ms were obtained on DuPont 21-491 and Kratos MS-30, DS-55 mass spectrometers. Optical rotations were measured on a Perkin-Elmer 241 photoelectric polarimeter, and the cd spectra on a Jasco IF-500 instrument. Si gel G and Si gel for cc were from E. Merck. Solvents were reagent grade and distilled before use.

The pulse programs for the 2D nmr experiments were those provided by Bruker Instruments, COSY.AU for ^1H shift correlation, XHCORRD.AU for CH-correlation with homonuclear ^1H decoup-

ling, COLOC.AU for long-range (2- to 4-bond) CH-correlation with polarization delay (D2 0.06 sec) set for 8 Hz coupling, and NOEMULT.AU for nOe difference.[3]

PLANT MATERIAL.—The aerial parts of *A. dracunculoides* were collected in September 1968, and October 1981, 3 mi south of Harper, Kansas. Voucher specimens are on file in the College of Pharmacy, Ohio State University. The plant was authenticated by Professor R.L. McGregor, University of Kansas and confirmed by Professor M.A. Lane, University of Colorado.

EXTRACTION AND INITIAL FRACTIONATION.—The air-dried and powdered *A. dracunculoides* (4.0 kg) was extracted to exhaustion by percolation at room temperature with 95% EtOH (55 liters), and the extract was evaporated under reduced pressure at 36° to give 635 g of residue. A sample (415 g) of the crude residue was partitioned between H_2O (2 liters) and $CHCl_3$ (4 × 2 liters), and the combined $CHCl_3$ extract on removal of solvent at reduced pressure gave 327 g of residue, which was divided between 2 liters of 10% aqueous MeOH and 3 × 2 liters of hexane. Reduced pressure evaporation gave 48 g of hexane solubles, while the MeOH solubles were redissolved in MeOH-H_2O (7:3) (2 liters) and successively extracted with the following solvents (3 × 2 liters for each): hexane, $CHCl_3$-hexane (1:4), and $CHCl_3$-hexane (1:1). Removal of solvent gave from hexane 3.6 g, $CHCl_3$-hexane (1:4) 55 g (fraction F1), $CHCl_3$-hexane (1:1) 157 g (fraction F2), and MeOH-H_2O (7:3) 52 g (fraction F3) of residue.

TLC ANALYSIS.—Si gel G plates of 0.25 mm were used with development by 5% MeOH in $CHCl_3$ or as given otherwise and visualization by spraying with *p*-anisaldehyde–H_2SO_4–95% EtOH (1:1:18) and heating at 110° to give yellow to brown and blue to violet colored zones. The Kedde reagent was also used for detection and gave pink to violet zones for butenolides (7).

CHROMATOGRAPHY OF FRACTION F1.—A column of Si gel 60, PF-254 (1.25 kg, 6.5 × 94.5 cm) in $CHCl_3$ was loaded with 22.7 g of fraction F1 in $CHCl_3$ and eluted with $CHCl_3$ and increasing amounts of MeOH in $CHCl_3$. Effluent fractions of 80 ml were analyzed by tlc after evaporation of solvent at reduced pressure. Similar fractions were combined and induced to yield the crystalline products as described below.

AMPHIACROLIDE A.—The pooled fraction (2.5 g) with R_f 0.70 [tlc, $CHCl_3$-EtOAc (3:1)] crystallized from $CHCl_3$-hexane to give 550 mg of amphiacrolide A as prisms: mp 84–85°; $[\alpha]_D$ +35.9° (c = 1.0, $CHCl_3$); cd (c 6 × 10^{-4}M, MeOH) $[\theta]_{205}$ −24,400, $[\theta]_{216}$ 0, $[\theta]_{230}$ +33,000, $[\theta]_{255}$ 0; ir ($CHCl_3$) ν max 3020 (olefinic H), 1785 and 1752 (α,β-unsaturated γ-lactone with α-H), 1767 (γ-lactone), 1642 cm^{-1} (C=C); uv (MeOH) λ_{end} 210 mm (log ε 4.23); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; nOe ($CDCl_3$, 270 MHz) results are in Figure 1. Long-range CH-correlation ($CDCl_3$, 67.9 MHz) gave H_2-6 (1.81 ppm) coupling to C-18 (178.7 ppm); H-19α (4.48) to C-3 (123.2), C-4 (136.7), and C-18 (178.7), Me-17 (0.947) to C-7 (25.8), C-8 (34.4), and C-9 (38.2), Me-20 (0.954) to C-9 (38.2), C-10 (37.4), and C-11 (40.0); eims *m/z* 330.1867 (5%, $[M]^+$, $C_{20}H_{26}O_4$ requires 330.1832), 286.1960 (6, M-CO_2 requires 286.1933), 233 (0.4), 219.1429 (0.5, M-$C_6H_7O_2$ requires 219.1385), 173 (90), 111 (18), 98.0411 (100), ($C_5H_6O_2$ requires 98.0368), 97 (4). Calcd for $C_{20}H_{26}O_4$, C 72.70, H 7.93; found C 72.31, H 8.05.

EPOXIDATION OF AMPHIACROLIDE A [2].—Amphracrolide A (27 mg) in $CHCl_3$ (1 ml) was treated with *m*-chloroperoxybenzoic acid (28 mg) for 15 h at 0°, then 12 h at room temperature. Addition of $CHCl_3$ (6 ml) and extraction with 5% aqueous $NaHCO_3$ and H_2O gave a residue after evaporation of $CHCl_3$. The residue was purified on a Si gel 60 (6 g) column with EtOAc-hexane (2:3) to give 22 mg of epoxide **6** as a heavy oil: $[\alpha]_D$ +58.1° (c = 2.2, $CHCl_3$): ir ($CHCl_3$) ν max 1785 and 1755 (α,β-unsaturated γ-lactone), 1643 (C=C), 923 and 843 cm^{-1} (epoxide); ^{1}H nmr see Table 3; ^{13}C nmr see Table 4; nOe ($CDCl_3$, 270 MHz) irradiation at 3.33 (H-3) observed 1.71 (5, H-2α), 2.19 (5, H-2β), 3.92 (5, H-19α), and 4.41 (−0.9, H-19β for collinearity of H-3, H-19α, and H-19β); irradiation at 3.92 (H-19α) observed 3.33 (8, H-3) and 4.41 (30, H-19β), irradiation at 4.41 (H-19β) observed 1.84 (4, H-6α), 3.92 (32, H-19α), and 3.33 (−0.8, H-3 for collinearity of H-19β, H-19α and H-3); cims (isobutane) *m/z* $[MH]^+$ 347 (98%), 111 (100); eims *m/z* $[M]^+$ 346 (1%), 249 (1), 235 (12), 111 (100), 98 (29), 97 (9).

AMPHIACROLIDE B [3].—The pooled column fraction with R_f 0.33 [tlc, EtOAc-hexane (3:2)] crystallized from $CHCl_3$/hexane to give 340 mg of amphiacrolide B: mp 189–191°; $[\alpha]_D$ +39.9° (c = 2.0, $CHCl_3$); cd (c = 2.9 × 10^{-4}M, MeOH) $[\theta]_{215}$ +21,300, $[\theta]_{234}$ 0, $[\theta]_{248}$ −13,400; ir ($CHCl_3$) ν max 1787 and 1755 (α,β-unsaturated γ-lactone with α-H), 1643 cm^{-1} (C=C); uv (MeOH) λ max 221 nm (log ε 4.32); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; nOe ($CDCl_3$, 270 MHz) irradiation at 3.74 (H-18α) observed 1.39 (5, H-10), 1.51 (2, H_2-11), 2.33–2.35 (1, H_2-12), 4.56 (21, H-18β), 5.81 (1, H-14),

[3]Detailed summaries of the nOe and 2D nmr experiments for the compounds are available from the senior author.

6.80 (1, H-3); irradiation at 4.56 (H-18β) observed 1.51 (7, H_2-11), 1.55 (2, H-6α), 1.95 (6, H-7α), 3.74 (27, H-18α). Long-range CH-correlation ($CDCl_3$, 67.9 MHz) gave Me-20 (0.83 ppm) coupling to C-8 (35.7 ppm), C-9 (38.3), C-10 (44.5), and C-11 (39.4); H-3 (6.82) to C-5 (43.3) and C-19 (170.4); H-2β (2.17) to C-3 (135.8) and C-4 (135.4); H_2-12 (2.25–2.27) to C-13 (171.8) and C-14 (115.0). Cims (isobutane) *m/z* $[MH]^+$ 331 (64%), 53 (100); eims *m/z* $[M]^+$ 330.1870 (20%), ($C_{20}H_{26}O_4$ requires 330.1832), $[M-Me]^+$ 315 (3), $[M-H_2O]^+$ 312 (27), $[M-CH_2O]^+$ 300 (25), $[M-C_5H_5O_2]^+$ 233 (14), 219 (22), 111 (52), 98 (69), 97 (17). Calcd for $C_{20}H_{26}O_4$, C 72.70, H 7.93; found C 72.32, H 7.92.

EPOXIDATION OF AMPHIACROLIDE B [**3**].—Amphiacrolide B (45 mg) in $CHCl_3$ (2 ml) was treated with *m*-chloroperoxybenzoic acid (40 mg) at 0°. After 12 h at room temperature, 10 mg more of reagent was added, and after 12 h, $CHCl_3$ (6 ml) was added. The mixture was extracted successively with 5% aqueous $NaHCO_3$ and H_2O. The $CHCl_3$ residue was chromatographed on Si gel 60 (6 g) with EtOAc-hexane (1:1) to give first 8 mg of starting material and 18 mg of a residue which crystallized (16 mg) from $CHCl_3$/hexane to give epoxide **7**: mp 215–217°; $[\alpha]_D$ +46.6° (*c* = 0.3, $CHCl_3$); ir ($CHCl_3$) ν max 1792 and 1757 (lactone), 1640 cm^{-1} (C=C); ^1H nmr see Table 3; ^{13}C nmr see Table 4; nOe ($CDCl_3$, 270 MHz) irradiation at 1.15 (H-10) observed 0.90 (1, Me-20), 1.52 (3, H-1α and H-11), 1.69 (3, H-2α), 2.32 (5, H_2-12), and 4.03 (6, H-18α); irradiation at 4.03 (H-18α) observed 1.15 (6, H-10), 2.32 (1, H_2-12), 3.57 (0.5, H-3), 4.65 (21, H-18β), 5.85 (0.6, H-14), 1.84 (−0.5, H-6α for collinearity of H-18α, H-18β, and H-6α). Long-range CH-correlation ($CDCl_3$, 125 MHz for ^{13}C) gave Me-20 (0.90 ppm) coupling to C-8 (34.1 ppm), C-9 (37.7), and C-11 (38.8); H-2α (2.28) to C-4 (60.6). Inverse CH-correlation of long-range coupling ($CDCl_3$, 500 MHz for ^1H) H-10 (1.15) to C-5 (38.5), C-9 (37.7), C-11 (38.8), and C-18 (76.6); H-18α (4.03) to C-6 (20.1) and C-10 (45.5); H-18β (4.65) to C-4 (60.6), C-5 (38.5), C-6 (20.1), and C-19 (173.1). Cims (isobutane) *m/z* $[MH]^+$ 347 (5%), 91 (100); eims *m/z* $[M]^+$ 346 (4%), $[M-Me]^+$ 331 (3), 249 (8), 235 (9), 111 (47), 98 (57), 97 (22), 55 (100).

PYRAZOLINE **8** OF AMPHIACROLIDE B [**3**].—Amphiacrolide B (32 mg) in MeOH (1 ml) was treated with excess CH_2N_2 in Et_2O (2 ml) for 24 h at room temperature. The white solid left after evaporation of solvent crystallized from EtOAc/hexane to give 31 mg of pyrazoline **8** as prisms: mp 174–176°; $[\alpha]_D$ −75.7° (*c* = 1.6, $CHCl_3$); ir ($CHCl_3$) ν max 1787 and 1758 (lactone), 1643 cm^{-1} (C=C); ^1H nmr see Table 3; ^{13}C nmr see Table 4; nOe ($CDCl_3$, 270 MHz) irradiation at 0.64 ppm (Me-20) observed 0.86 ppm (5%, Me-17), 1.09 (9, H-2β), 1.60 (10, H_2-11), 1.80 (7, H-7β), 2.27 (2, H_2-12), 2.76 (2, H-6β); irradiation at 1.09 (H-2β) observed 0.64 (4, Me-20), 1.55 (21, H-2α), 4.60 (6, β-H of azomethylene); irradiation at 2.76 (H-6β) observed 0.64 (1, Me-20), 1.80 (34, H-6α and H-7β); irradiation at 4.16 (H-18β) observed 1.43 (2, H-10), 1.80 (5, H-6α), 4.30 (20, H-18α); irradiation at 4.30 (H-18α) observed 1.43 (4, H-10), 1.55 (5, H-1α), 2.23 (5, H-3), 4.16 (23, H-18β). Long-range CH-correlation ($CDCl_3$, 67.9 MHz) gave Me-20 (0.64 ppm) coupling to C-8 (36.0 ppm), C-9 (39.7), C-10 (38.3), and C-11 (35.6); H-10 (1.43) to C-2 (25.5), C-4 (96.6), C-5 (44.4), C-9 (39.7), and C-18 (79.0); H-18β (4.16) to C-4 (96.6) and C-19 (172.1); azomethylene β-H (4.60) to C-4 (96.6) and C-19 (172.1). Cims (isobutane) *m/z* $[MH]^+$ 373 (1%), $[MH-N_2]^+$ 345 (51), $[M-N_2]^+$ 344 (100); eims *m/z* $[M-N_2]^+$ 344 (100), 314 (10), 247 (6), 233 (11), 165 (11), 111 (23), 98 (26), 97 (9).

AMPHIACROLIDE C [**4**].—The pooled fraction (1.8 g) with R_f 0.40 [tlc, EtOAc-hexane (3:2)] crystallized from EtOAc/hexane to give 1.4 gm of colorless prisms of amphiacrolide C: mp 145–147°; $[\alpha]_D$ −44.7° (*c* = 0.55, $CHCl_3$); cd (*c* = 6.0×10^{-4}M, MeOH) $[\theta]_{210}$ −30,500, $[\theta]_{230}$ 0, $[\theta]_{240}$ +1,700; ir ($CHCl_3$) ν max 3590 and 3280 (OH), 1785 and 1750 (lactone C=O), 1640 cm^{-1} (C=C); uv (MeOH) λ max 204 nm (log ε 4.22); ^1H nmr see Table 1; ^{13}C nmr see Table 2; nOe ($CDCl_3$, 270 MHz) irradiation at 4.22 (H-19α) observed 4.34 (22, H-19β) and 5.61 (7, H-3); irradiation at 4.34 (H-19β) observed 1.53 (2, H-6α) and 4.22 (26, H-19α); irradiation at 5.45 (H-18) observed 1.51 (4, H-6α), 1.67 (0.5, H-10), 1.85 (5, H-11), 3.92 (11, -OH), and 5.76 (1, H-14); nOe (pyridine-d_5, 270 MHz) irradiation at 4.39 (H-19α) observed 4.48 (28, H-19β), 5.57 (9, H-3); irradiation at 4.48 (H-19β) observed 1.63 (3, H-6α), 4.39 (29, H-19α); irradiation at 5.80 (H-18) observed 1.42 (1, H-11), 1.97 (2, H-10), 2.01 (10, H-11). Long-range CH-correlation ($CDCl_3$, 67.9 MHz) gave Me-20 (0.92 ppm) coupled to C-8 (37.2 ppm), C-9 (38.1), C-10 (38.9), and C-11 (40.2); Me-17 (1.04) to C-7 (26.1), C-8 (37.2), and C-9 (38.1); H_2-12 (2.28 and 2.52) to C-11 (40.2), C-13 (172.8), C-14 (114.6), and C-16 (73.6); H_2-19 (4.22 and 4.34) to C-3 (120.0) and C-4 (141.1); H-18 (5.45) to C-4 (141.1), C-5 (49.4), and C-6 (24.0). Cims (isobutane) *m/z* $[MH]^+$ 333 (4%), $[MH-H_2O]^+$ 315 (100); eims *m/z* $[M-17]^+$ 315.1959 (2%) ($C_{20}H_{27}O_3$ requires 315.1961), 314 (13), 285 (36), 111 (29), 98 (56), 97 (6). Calcd for $C_{20}H_{28}O_4$, C 71.89, H 8.60; found C 72.28, H 8.43.

AMPHIACROLIDE C ACETATE [**10**].—Amphiacrolide C (20 mg) was dissolved in pyridine and Ac_2O (0.5 ml each) and kept at ambient temperature for 24 h. The residue left after evaporation of solvent at reduced pressure was crystallized from toluene/hexane to give fine needles of acetate **10**: mp 124–125°; $[\alpha]_D$ −54.7° (*c* = 2.0, $CHCl_3$); ir ($CHCl_3$) ν max 1790 and 1755 (lactone and acetate C=O), 1643

(C=C), 1245 cm^{-1} (C-O); ^1H nmr see Table 3; ^{13}C nmr see Table 4; nOe ($CDCl_3$), 270 MHz irradiation at 6.39 (H-18) observed 1.30 (3, H-11), 1.62 and 1.63 (12, H-6α and H-11), 1.89 (8, H-7α). Long-range CH-correlation ($CDCl_3$, 67.9 MHz) gave H-19β (4.42) coupled to C-4 (139.7); H-3 (5.65) to C-1 (23.4) and C-5 (48.4); H-18 (6.39) to C-4 (139.7), C-19 (68.7), and Me*C*O (170.57); *Me*CO (2.00) to Me*C*O (170.57). Cims (isobutane) *m/z* $[MH-AcO]^+$ 332 (1%), $[M-AcO]^+$ 331 (3), $[MH-HOAc]^+$ 315 (54), $[AcOH_2]^+$ 61 (100); eims *m/z* 315.1996 (5) ($[M-AcO]^+$ requires 315.1961), $[M-HOAc]^+$ 314 (5), 111 (13), 105 (86), 98 (23), 91 (100).

METHYL AMPHIACROLIDE C [**11**].—Amphiacrolide C (6 mg) dissolved in MeOH (0.7 ml) was treated with 1 N HCl (0.1 ml) for 24 h at ambient temperature. Evaporation of solvent at reduced pressure left 5 mg of a white crystalline material which was recrystallized from EtOAc/hexane to give 4 mg of methyl derivative **11** as needles: mp 129–131°; $[\alpha]_D$ −64.9° (*c* = 1.61, $CHCl_3$); ir ($CHCl_3$) ν max 2840 (OMe), 1788 and 1753 (lactone C=O), 1640 (C=C), 1235 (C-O), 1095 cm^{-1} (O-C-O); ^1H nmr see Table 3; ^{13}C nmr see Table 4; nOe ($CDCl_3$, 270 MHz) irradiation at 3.22 (MeO) observed 4.16 (2, H-19α), 4.71 and 4.73 (2, H_2-16), 4.92 (7, H-18), 5.80 (3, H-14); irradiation at 4.16 (H-19α) observed 3.22 (1, MeO), 4.39 (32, H-19β), 5.57 (6, H-3); irradiation at 4.39 (H-19β) observed 4.16 (35, H-19α); eims *m/z* $[M-OMe]^+$ 315.1940 (5%) ($C_{20}H_{27}O_3$ requires 315.1961) 286.1946 (4, $C_{19}H_{26}O_2$) and 285.1895 (8, $C_{19}H_{25}O_2$).

OXIDATION OF AMPHIACROLIDE C [**4**] TO AMPHIACROLIDE A [**2**] AND DIALDEHYDE **12**.—Amphiacrolide C (50 mg) in Me_2CO (5 ml) at 0° was treated dropwise while shaking with Jones' reagent (22) to excess (10 drops) when an orange color persisted. After 5 min, the mixture was diluted with H_2O (20 ml) and mixed with 5% aqueous $NaHCO_3$ (20 ml), then extracted with Et_2O (60 ml). The Et_2O extract was washed with 5% aqueous $NaHCO_3$ (6 × 20 ml) and H_2O (8 × 20 ml), then evaporated to dryness to give a colorless oil (42 mg), which was separated on a Si gel 60 column (6 g, 40–63 μm) with EtOAc-hexane (3:7). The first eluted material (12 mg) was crystallized from $CHCl_3$/hexane to give 10 mg of amphiacrolide A [**2**] as colorless prisms: mp 84–85°, $[\alpha]_D$ +38.3° (*c* = 0.47, $CHCl_3$) and spectral data (ir, ^1H nmr, and ms) identical with an authentic sample.

The second eluted material (15 mg), a colorless oil, was dialdehyde **12**: $[\alpha]_D$ +10.7° (*c* = 0.35, $CHCl_3$); ir ($CHCl_3$) ν max 2720 (CHO), 1787 and 1753 (lactone C=O), 1715 (CHO), 1682 cm^{-1} (conj. C=O); ^1H-nmr ($CHCl_3$, 300 MHz) 10.12 (s, H-19), 9.24 (s, H-18), 7.03 (dd, *J* = 5.5 and 2.5 Hz, H-3), 5.82 (m, *J* = 1.6, H-14), 4.73 (d, *J* = 1.7, H_2-16), 0.97 (d, *J* = 6.7, Me-17), 0.93 ppm (s, Me-20); eims *m/z* $[M-CHO]^+$ 301 (7%), $[M-CH_2O]^+$ 300 (4), 189 (17), 165 (26), 111 (100), 98 (92), 97 (24).

$NaBH_4$ REDUCTION OF AMPHIACROLIDE C [**4**] TO DIOL **13**.—Amphiacrolide C (32 mg) in MeOH (5 ml) at 0° was treated with $NaBH_4$ (60 mg) for 1.5 h. Addition of H_2O (5 ml) and evaporation of MeOH at reduced pressure left needle-like crystals in aqueous medium. Extraction of the suspension with $CHCl_3$ (4 × 10 ml), washing of combined $CHCl_3$ extract with H_2O (5 × 6 ml), and evaporation of $CHCl_3$ left 30 mg of residue. Crystallization of residue from $CHCl_3$/hexane gave fine crystals as needles of diol **13**: mp 145–146°; $[\alpha]_D$ −30.7° (*c* = 0.55, MeOH); ir ($CHCl_3$) ν max 3610 and 3380 (OH), 1785 and 1753 (lactone C=O), 1640 (C=C), 1230 (C-O), 1035 cm^{-1} (OH); ^1H nmr see Table 3; ^{13}C nmr see Table 4; nOe ($CDCl_3$, 270 MHz) irradiation at 3.63 (H-18) observed 1.24 (1, H-6α), 1.53 (0.5, H-10), 1.91 (6, H-1α), 3.30 (17, H-18); irradiation at 4.00 (H-19) observed 4.25 (17, H-19), 5.85 (11, H-3); irradiation at 4.25 (H-19) observed 1.24 (5, H-6α), 4.00 (21, H-19); eims *m/z* $[M-OH]^+$ 317 (2%), $[M-H_2O]^+$ 316 (8), 301 (0.7), 286 (16), 205 (6), 187 (32), 175 (32), 173 (60), 111 (29), 98 (100), 97 (6).

AMPHIACROLIDE D [**5**].—The pooled chromatographic fraction following amphiacrolide C was purified on a Si gel 60 (56 g, 40–63 μm) column with EtOAc-$CHCl_3$ (1:1), and the Kedde-reacting effluent material was pooled and crystallized from EtOAc/hexane/C_6H_6 to give 370 mg of amphiacrolide D [**5**] as colorless prisms: mp 118–120°; $[\alpha]_D$ −15.1° (*c* = 0.86, $CHCl_3$); ir ($CHCl_3$) ν max 3590 and 3400 (OH), 3020 (epoxide), 1787 and 1750 (lactone C=O), 1640 (C=C), 1032 (COH), 1070–1172 (O-C-O), 930 (epoxide), 840 cm^{-1} (trisubstituted C=C); uv (MeOH) λ end 210 nm (log ε 4.15); cd (*c* = 5.7 × 10^{-4}M, MeOH) $[\theta]_{210}$ −6100, $[\theta]_{280}$ 0; ^1H nmr see Table 1; ^{13}C nmr see Table 2; nOe ($CHCl_3$, 270 MHz) irradiation at 3.26 (H-3) observed 1.64 (2, H-2α), 2.07 (2, H-2β), 3.96 (4, H-19α); irradiation at 3.82 (H-19β) observed 1.49 (2, H-6α), 3.96 (26, H-19α); irradiation at 3.96 (H-19α) observed 3.26 (8, H-3), 3.82 (28, H-19β), 4.24 (2, OH); irradiation at 4.24 (OH) observed 1.64 (1, H-10), 3.96 (1, H-19α), 5.52 (7, H-18), 5.78 (2, H-14); irradiation at 5.52 (H-18) observed 1.86 (5, H-7α), 4.24 (15, OH). Long-range CH-correlation ($CHCl_3$, 67.9 MHz) gave H-3 (3.26) coupled to C-1 (18.3), C-2 (27.1), and C-4 (67.2); H-18 (5.52) to C-4 (67.2) and C-19 (66.9). Eims *m/z* $[M-OH]^+$ 331.1956 (94%) ($C_{20}H_{27}O_4$ requires 331.1910), 302 (6), 301 (6), 285 (7), 219 (9), 165 (9), 111 (100), 98 (90). Calcd for $C_{20}H_{28}O_5$, C 68.96, H 8.04; found C 68.96, H 8.18.

AMPHIACROLIDE D ACETATE [**14**].—Amphiacrolide D (100 mg) in pyridine (0.5 ml) was treated

with Ac_2O (0.5 ml) at ambient temperature for 24 h. The residue (110 mg) remaining after evaporation at reduced pressure was crystallized from EtOAc/hexane to give 80 mg of acetate **14** as needle-like crystals: mp 118–120°; $[\alpha]D$ −20.6° (c = 0.74, $CHCl_3$); ir ($CHCl_3$) ν max 1787 and 1753 (α,β-unsaturated γ-lactone C=O), 1750 cm^{-1} (overlapped ester C=O); ^1H nmr see Table 3; ^{13}C nmr see Table 4. Long-range CH-correlation ($CDCl_3$, 125 MHz) gave Me of Ac (2.08 ppm) coupled to C=O of Ac (170.0); H-12's (2.18 and 2.26) to C-13 (170.4); H_2-16 (4.69) to C-13 (170.4) and C-15 (173.9); H-18 (6.41) to C=O of Ac (170.0). Eims m/z $[M-CH_2CO]^+$ 348.1996 (2%) ($C_{20}H_{28}O_5$ requires 348.1937), $[M-OAc]^+$ 331.1880 (7) ($C_{20}H_{27}O_4$ requires 331.1909), 111 (65), 98 (52), 97 (10), 43.0211 (100) (Ac requires 43.0184).

CrO_3 OXIDATION OF AMPHIACROLIDE D [**5**] TO DILACTONE **6**.—Amphiacrolide D (11 mg) in Me_2CO (1.5 ml) at 0° was treated with 4 drops of Jones reagent (22) for 6 min. H_2O (5 ml) and 5% $NaHCO_3$ (15 ml) were added, and the mixture was extracted with Et_2O (3 × 20 ml). The combined Et_2O extract was washed successively with 5% $NaHCO_3$ (3 × 15 ml) and H_2O (3 × 15 ml) and evaporated to dryness. The residue as an oil (9 mg), $[\alpha]D$ +59.4° (c = 0.88, $CHCl_3$) was found to be identical (ir, ^1H nmr, and ms) with the epoxide **6** of amphiacrolide A [**2**].

PREPARATION OF GUTIEROLIDE [**1**] FROM AMPHIACROLIDE D [**5**].—Amphiacrolide D (40 mg) in MeOH (1 ml) and 5 N HCl (1 ml) was kept at ambient temperature for 16 h. MeOH (4 ml) was added, and the crystalline product (30 mg) that formed was collected and washed with MeOH. Recrystallization from $CHCl_3$-MeOH (1:1) gave 28 mg of gutierolide [**1**] as colorless needles: mp 208–210° with spectral properties identical to those previously reported (2) and high-field nmr studies as follows: ^1H nmr see Table 1; ^{13}C nmr see Table 2; nOe ($CDCl_3$, 270 MHz) irradiation at 3.38 (MeO) observed 3.95 (1, H-19β), 4.10 (2, OH), 5.03 (5, H-18); irradiation at 4.10 (OH) observed 1.99 (6, H-6α), 3.38 (2, MeO), 3.95 (5, H-19β); irradiation at 4.30 (H-19α) observed 1.46 (4, H-10), 1.63 (6, H-2α), 3.95 (33, H-19α); nOe (pyridine-d_5, 500 MHz) irradiation at 4.22 (H-19β) observed 3.32 (2, MeO), 4.54 (20, H-19α); irradiation at 4.26 (H-3) observed 1.43 (3, H-1β), 1.58 (4, H-6β), 2.12 (4, H-2β); irradiation at 4.54 (H-19α) observed 1.58 (3, H-10), 1.75 (7, H-2α), 4.22 (34, H-19β); irradiation at 5.43 (H-18) observed 1.54 (6, H-8), 1.58 (3, H-10), 1.88 (9, H-11), 2.41 (3, H-12), 3.32 (9, MeO).

CONVERSION OF AMPHIACROLIDE C [**4**] TO AMPHIACROLIDE D [**5**].—Amphiacrolide C (25 mg) in $CHCl_3$ (1 ml) at 0° was treated with a solution of *m*-chloroperoxybenzoic acid (23 mg) in $CHCl_3$ (0.5 ml) for 12 h. Dilution with $CHCl_3$ (4 ml) and successive extraction with 5% $NaHSO_3$ (6 × 5 ml), 5% $NaHCO_3$ (6 × 5 ml), and H_2O (10 × 8 ml) left, after evaporation of solvent at reduced pressure, a residue (30 mg) that crystallized from $CHCl_3$/hexane as colorless prisms showing physical properties (mp, specific rotation, ir, ^1H nmr, and ms) identical with those of amphiacrolide D [**5**].

CONVERSION OF DIOL **13** TO AMPHIACROLIDE B [**3**] BY MnO_2.—Diol **13** (12 mg) in CH_2Cl_2 (2 ml) was passed into a column (1.5 g) of MnO_2(Winthrop)-diatomaceous earth (1:2) in CH_2Cl_2. After 3 h the column was washed with Me_2CO (30 ml), and the residue remaining on evaporation of solvent was crystallized from $CHCl_3$/hexane to give colorless needle-like crystals with physical properties (mp, specific rotation, ir, ^1H nmr, ms, and tlc) identical with those of amphiacrolide B [**3**].

CONVERSION OF DIOL **13** TO AMPHIACROLIDE B [**3**] BY CrO_3.—Diol **13** (50 mg) in Me_2CO (5 ml) at 0° was treated with 10 drops of Jones' reagent (22) while stirring. After 10 min, H_2O (20 ml) and 5% $NaHCO_3$ (20 ml) were added, and the mixture was extracted with Et_2O (6 × 20 ml). Evaporation of the Et_2O extract and crystallization of the oil (38 mg) from $CHCl_3$/hexane gave amphiacrolide B [**3**] as needle-like crystals with physical properties identical with those of an authentic sample.

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