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AMOENOLIDE A AND RELATED ACETYLATED LABDANE DITERPENES FROM *AMPHIACHYRIS AMOENA*[1]

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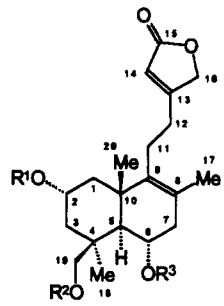
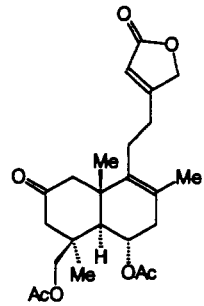
ABSTRACT.—Four new labdane diterpenes, amoenolide A [**1**] and its 19-acetate [**2**], 6-acetate [**3**], and 6,19-diacetate [**4**], were isolated from the above-ground parts of *Amphiachyris amoena* and their structures established by spectral and chemical methods. High-field ^{1}H- and ^{13}C-nmr assignments were made for each compound using 1D and 2D nmr techniques including ^{1}H single-frequency spin-coupling and nOe difference experiments, one-bond and long-range carbon-hydrogen correlations, and the INADEQUATE carbon-carbon connectivity experiment. The absolute stereochemistry was established by the Horeau partial-resolution chemical method with amoenolide A and racemic 2-phenylbutyric anhydride, and by the cd exciton splitting physical method on the synthetic amoenolide A 2-acetate 6,19-dibenzoate [**9**].

The annual plant, *Amphiachyris amoena* (Shinners) Solbrig (Compositae), one of the two species of the North American genus *Amphiachyris* (1), has had little phytochemical study, with only two terpenes isolated previously, one a linear monoterpene diacetate and the other a monocyclic sesquiterpene hydrocarbon (2). This report is on the isolation, structure elucidation (including absolute stereochemistry), and ^{1}H- and ^{13}C-nmr spectral assignments for four new labdane diterpene lactones and their derivatives. The parent diterpene, a triol, was given the name amoenolide A [**1**] and is the major diterpene of the above-ground parts of the plant. The other three compounds are the two monoacetates, **2** and **3**, and the 6,19-diacetate, **4**. The compounds were obtained from an EtOH extract residue which was divided into fractions of differing polarities by the





	R^1	R^2	R^3
1	H	H	H
2	H	Ac	H
3	H	H	Ac
4	H	Ac	Ac
5	Ac	Ac	Ac
7	Ac	H	H
8	Ac	Ac	H
9	Ac	Bz	Bz

6

[1]Taken from the Ph.D. dissertation of Dónal P. O'Mathúna which was accepted by the Graduate School, The Ohio State University, in August 1988.

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use of immiscible solvent pairs (3). The aqueous MeOH fraction was passed through a Sephadex LH-20 column to separate the terpenes from the phenolics and the terpene mixture was chromatographed on Si gel to give the reported compounds.

RESULTS AND DISCUSSION

Acetylation of the four isolated compounds **1–4** gave the same triacetate, **5**, thus showing the carbon skeleton, oxygenation pattern and stereochemistry to be the same for each. The spectral studies commenced on amoenolide A 6,19-diacetate [**4**] for which the fabms with MH^+ at *m/z* 435 supported the molecular formula, $C_{24}H_{34}O_7$, while eims showed only a fragment peak at *m/z* 315 ($MH^+ - 2HOAc$). The ^1H- and ^{13}C-nmr spectra revealed three methyl groups and an α,β-unsaturated γ-lactone with the diagnostic peaks at δ 5.87 (H-14) and 4.74 ppm (H-16, s) (4). The ir peak at 1790 cm^{-1} and the end absorption in the uv region are additional features of the lactone system (4). The 2D nmr experiments COSY (5) and CH-correlation (6–8), and the 1D ^1H-homonuclear decoupling revealed four coupled units: C-1 through C-3, C-5 through C-7, C-11 and C-12 to the lactone unit, and the isolated methylene at C-19.

The heteronuclear long-range (2- and 3-bond) nmr coupling experiment (COLOC) (9) allowed these subunits to be connected and the quaternary carbons to be assigned. For example, the quaternary carbon at 39.14 ppm was placed at C-4 because of coupling to H_3-18 at 1.23 ppm, and H-5 at 1.70 ppm. The H_3-18 signal was also coupled to C-5 at 54.27 ppm, as was H-3β (1.95 ppm). In this way, two-proton coupled units were connected to give the sequence C-1 through C-7. The quaternary carbon at 43.12 ppm (C-10) was coupled to H-1β (2.15 ppm), H_3-20 (1.10 ppm) and H-5. The H_3-20 signal also coupled to the olefinic quaternary carbon at 138.16 ppm (C-9) while the olefinic methyl (1.58 ppm) was coupled to the same carbon (C-9) and to the other olefinic carbon at 126.15 ppm (C-8), as well as to the methylene carbon at 40.80 ppm (C-7). These results supported the construction of the decalin system with placement of the hydroxymethyl group (methylene protons at 4.07 and 4.13 ppm as doublets later to be assigned as H_2-19) at C-4, and attachment of the lactone-bearing side-chain at C-9 to complete a normal labdane skeleton. Support for the C-19 methylene at C-4 was obtained from the COLOC experiments on amoenolide A [**1**], which showed 3-bond coupling from H_2-19 (3.86 and 4.51 ppm) to C-18 (32.36 ppm). Of the required eight double-bond equivalents, five were accounted for by the lactone and the two acetates, and the remaining three were satisfied by the bicyclic ring system and the olefinic group. The COLOC experiment also located one acetate group. The H_2-19 signal was coupled to the carbonyl carbon at 171.1 ppm as was the acetate methyl at 2.04 ppm; thus assigning that acetate to C-19. The second acetate was placed at C-6 because acetylation of diacetate **4** to triacetate **5** caused the carbinyl proton at 3.94 ppm (H-2) to be shifted downfield to 4.99 ppm, while the other at 5.23 ppm (H-6) remained unaffected. The C-11 resonance was distinguished from C-12 by homonuclear decoupling; irradiation at H-14 (5.87 ppm) sharpened the H-12 pattern and converted the H-16 doublet to a singlet.

The stereochemical assignments of amoenolide A 6,19-diacetate [**4**] were made by nOe difference spectroscopy (10,11), although the coupling constants for several of the proton patterns were strong evidence for spatial designation. For example, H-2 has two large (J=11.4 Hz) and two small (J=3.8 Hz) coupling constants, which support an axial orientation, as do the values for H-6 (J=11.9, 8.8, and 6.4 Hz) and for H-5 (J=11.9 Hz). The nOe studies as summarized in Figure 1 arranged the substituents on the top and bottom face of the decalin system and supported a trans ring junction, as well as confirming the axial positions for H-2, H-5, and H-6.

Oxidation of diacetate **4** with Jones' reagent (12) produced the 2-ketone **6** which was extensively analyzed by 1D and 2D nmr methods. The results are given in Tables 1 and

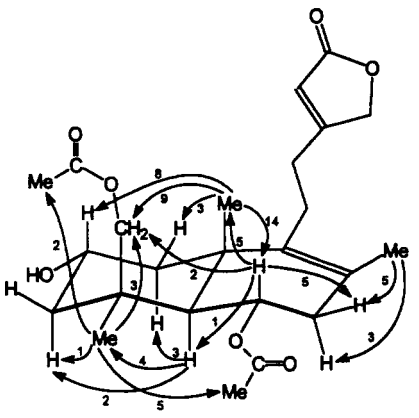

FIGURE 1. Percent nOe Enhancements by Difference Spectroscopy for Amoenolide A 6,19-diacetate [**4**].

2, and were used to substantiate the assignments made for the starting material. The same 1D nmr (^{1}H-, ^{13}C-, and nOe) and 2D nmr (COSY, CH-correlation, and COLOC) studies were also performed with amoenolide A 19-acetate [**2**], the 6-acetate, **3**, and the 2,6,19-triacetate, **5**. Unambiguous ^{13}C-nmr assignments for the two quaternary carbons, C-13 and C-15, of the lactone ring could not be made from the studies performed, but these were accomplished by measurement of the transverse relaxation time (T_1) by the use of the inversion-recovery method (13). Because this relaxation is accomplished through the neighboring protons, C-13 with five protons at the α-positions would relax more rapidly than C-15 with only one. Amoenolide A [**1**], which has no acetate carbonyls to obscure the relevant region, was chosen for the experiment at 125 MHz and showed times of 15.4 and 2.8 sec for carbons at 174.22 and 171.37 ppm, respectively. This clearly identified the former as C-15 and the latter as C-13. An examination of the SFORD ^{13}C-nmr spectra reported in this paper always showed the C-15 peak as a doublet with apparent J=3.0–3.5 Hz when the off-resonance irradiation position was set at δ_H −3.0 ppm at 270 MHz. The fully ^{1}H-coupled ^{13}C-nmr spectrum showed J_{CH}=9.1 Hz, a two-bond coupling to H-14; while C-13 appeared as a tight multiplet, $\omega_{1/2}$ 17 Hz.

In addition, the INADEQUATE carbon-carbon connectivity experiment (14,15) with amoenolide A [**1**] revealed the complete carbon network except for three bonds: C-4 to C-18, C-8 to C-9, and C-12 to C-13. This experiment, nonetheless, supported the assignments made earlier and unambiguously differentiated C-1 from C-3, C-8 from C-9, and C-11 from C-12.

Proof of the absolute stereochemistry was investigated by two procedures, the Horeau partial resolution chemical method (16) and the circular dichroic (cd) exciton-splitting physical method (17). In the chemical method, racemic 2-phenylbutyric anhydride was reacted with amoenolide A [**1**] to completely acylate the three hydroxyls, but only the 6-hydroxyl is on an asymmetric carbon, with the bulkiness rather different for the three substituents at C-6. Thus, a kinetic partial resolution of the racemic reagent would be expected, relative to the other hydroxyls, although spatial hindrance from the β-faced 19-hydroxymethyl and 20-methyl could have some effect on acylation of the 2-hydroxyl. The recovered 2-phenylbutyric acid showed a negative specific rotation, and, when corrected for the excess reagent required to insure complete acylation, gave an optical yield of 30%. This result supports the *S*-configuration at C-6 and the absolute stereochemistry of amoenolide A [**1**] and its acetates as given in the drawings.

To confirm this result by the cd method an amoenolide A derivative with two

TABLE 1. ^{1}H-Nmr Data for Compounds **1–8**.[a]

Proton	Compound									
	1[b]	**2**	**2**[b]	**3**	**4**	**5**	**5**[b]	**6**	**7**	**8**
H-1	1.49α dd (t) (11.4, 11.4) 2.42β hm	1.16α dd (t) (11.6, 11.6) 2.12β hm	1.52α dd (t) (11.5, 11.5) 2.42β	1.20α dd (t) (11.5, 11.5) 2.13β dd (12.4, 3.5)	1.19α dd (t) (11.6, 11.6) 2.15β ddd (11.7, 3.7, 2.1)	1.26α dd (t) (11.4, 11.4) 2.02β hm	1.43α dd (t) (11.6, 11.6) 2.32β hm [ddd] (11.6, 3.7, 2.1)	2.24α d (15.5) 2.52β d (15.4)	1.23α dd (12.2, 12.2) 2.05β ddd (11.4, 3.6, 2.2)	1.24α dd (11.5, 11.5) 2.08β hm [ddd] (11, 4.5, 2.2)
H-2	4.27 dddd (11.4, 11.4, 3.9, 3.9)	3.91 dddd (11.5, 11.5, 4.0, 4.0)	4.37 hm	3.92 dddd (11.5, 11.5, 3.9, 3.9)	3.94 dddd (11.4, 11.4, 3.8, 3.8)	4.99 dddd (11.7, 11.7, 4.0, 4.0)	5.25 dddd (11.8, 11.8, 4.0, 4.0)		4.93 dddd (11.8, 11.8, 4.0, 4.0)	4.98 dddd (11.8, 11.8, 4.1, 4.1)
H-3	1.54α dd (t) (12.1, 12.1) 2.35 hm	1.10α dd (t) (12.9, 12.9) 1.96β ddd (13.2, 3.9, 2.1)	1.45α dd (t) (12.4, 12.4) 2.55β hm	1.01α dd (t) (12.4, 12.4) 2.13β dd (12.4, 3.5)	1.10α dd (t) (12.5, 12.5) 1.95β ddd (13.2, 3.6, 1.9)	1.17α dd (t) (12.8, 12.8)	1.28α dd (t) (12.5, 12.5) 2.20β hm [ddd] (13.1, 3.8, 1.7)	2.16α d (15.2) 2.69β d (15.2)	1.23α dd (12.9, 12.2) 1.73β ddd (13.2, 4.0, 2.0)	1.18α dd (12.6, 12.6) 2.02β ddd (10.9, 4.1, 2.1)
H-5	1.66 d (11.1)	1.45 d (11.2)	1.74 d (11.4)	1.69 d (11.9)	1.70 d (11.9)	1.72 d (11.8)	1.79 d (11.9)	2.04 hd (11)	1.41 d (11.0)	1.47 d (11.2)
H-6	4.61 ddd (10.3, 10.3, 6.3)	4.15 ddd (10.9, 9.8, 6.1)	4.39 hm	5.25 ddd (11.8, 8.5, 6.7)	5.23 ddd (11.9, 8.8, 6.4)	5.23 ddd (11.8, 8.7, 6.5)	5.45 ddd (11.9, 8.8, 6.5)	5.18 ddd (11.9, 9.0, 6.2)	4.27 ddd (10.5, 10.5, 5.9)	4.16 ddd (11.1, 9.6, 6.0)
H-7	2.40α hm [dd] (17.4, 10.2)	2.11α hm [dd] (17.0, 9.4) 2.39β dd (17.5, 6.1)	2.42α hm [dd] (16.9, 9.4) 2.56β hm [dd] (17.2, 6.2)	2.01α dd (17.6, 8.8)	2.01α dd (17.4, 8.5) 2.57β dd (17.3, 6.4)	2.00α hm [dd] (17.6, 8.7) 2.58β dd (17.3, 6.4)	2.01α hm [dd] (17.0, 8.7) 2.57β dd (17.2, 6.4)	2.03α hdd (17.4, 8.3) 2.62β dd (17.3, 6.2)	2.12α dd (17.1, 10.4) 2.32β dd (17.6, 5.6)	2.11 hm [dd] (17, 9.5) 2.40 hm [dd] (17.0, 7.1)
H-11	2.20 m 2.30 m	2.17 m 2.33 m	2.18 m 2.27 m	2.20 m 2.35 m	2.20 m 2.35 m	2.18 m 2.33 m	2.18 m 2.26 m	2.15 m 2.28 m	2.14 m 2.32 m	2.16 m 2.32 m
H-12	2.35 (2H) m	2.43 (2H) m	2.34 (2H) m	2.43 (2H) m	2.43 (2H) m	2.40 (2H) m	2.43 (2H) m	2.41 (2H) m	2.42 (2H) m (8.2)	2.40 (2H) m
H-14	5.97 m (5 pk) (1.5)	5.87 m (5 pk) (1.3)	5.98 m (5 pk) (1.5)	5.88 m (5 pk) (1.4)	5.87 m (5 pk) (1.3)	5.87 m (5 pk) (1.3)	5.99 m (5 pk) (1.4)	5.86 m (5 pk) (1.5)	5.87 m (5 pk) (1.5)	5.86 m (5 pk) (1.6)
H-16	4.71 dd (17.3, 1.7) 4.75 dd (17.3, 1.5)	4.74 d (1.5)	4.71 dd (17.4, 1.7) 4.75 dd (17.4, 1.7)	4.75 d (1.7)	4.75 d (1.7)	4.75 s	4.71 dd (17.4, 1.7) 4.75 dd (17.4, 1.7)	4.73 d (1.6)	4.75 d (1.6)	4.74 d (1.7)
H-17	1.57 s	1.61 s	1.54 s	1.58 s	1.58 s	1.58 s	1.50 s	1.60 s	1.62 s	1.61 s
H-18	1.74 s	1.32 s	1.76 s	1.22 s	1.23 s	1.22 s	1.35 s	1.25 s	1.34 s	1.31 s
H-19	3.84 d (10.4) 4.49 d (10.5)	4.17 d (11.1) 4.38 d (11.2)	4.74 d (10.5) 4.86 d (10.7)	3.56 d (10.8) 3.69 d (10.8)	4.07 d (11.4) 4.13 d (11.4)	4.07 d (11.5) 4.18 d (11.5)	4.31 d (11.4) 4.43 d (11.4)	4.03 d (11.3) 4.11 d (11.3)	3.47 d (10.2) 4.23 d (10.4)	4.15 d (11.4) 4.45 d (11.3)
H-20	1.27 s	1.06 s	1.16 s	1.10 s	1.10 s	1.15 s	1.12 s	1.20 s	1.18 s	1.10 s
Misc	5.59 br s 6-OH 5.89 br s 2-OH 6.83 br s 19-OH	2.09 s 19-Ac	2.01 s 19-Ac	2.07 s 6-Ac	2.08 s 6-Ac 2.04 s 19-Ac	2.03 s Ac 2.04 s Ac 2.07 s Ac	2.01 s Ac 2.06 s Ac 2.13 s Ac	2.04 s Ac 2.09 s Ac	2.08 s Ac	2.03 s 2-Ac 2.10 s 19-Ac

[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 nOe studies and are reported after the hm designation in brackets.

[b]In pyridine-d_5 with the peak of pyridine-d_4 set at 7.19 ppm.

TABLE 2. ^{13}C-Nmr Data for Compounds **1–8**.[a]

Carbon	Compound											
	1[b]	Multiplicity	$^1J_{CH}$	**2**	**2**[b]	**3**	**4**	**5**	**5**[b]	**6**	**7**	**8**
C-1	47.58	t	127.1	46.52	47.29	45.93	46.42	42.54	42.29	51.89	43.21	43.02
C-2	63.73	d	138.6	64.41	63.70	64.01	64.03	67.69	68.02	209.61 s	68.09	67.92
C-3	49.66	t	126.6	47.69	47.52	45.83	47.26	42.93	43.11	50.82	44.94	42.70
C-4	41.20	s		39.20	39.76	40.52	39.14	38.90	39.15	40.10	40.51	38.87
C-5	57.15	d	124.6	56.84	57.34	54.01	54.27	54.10	54.00	53.22	56.56	56.66
C-6	68.01	d	140.7	67.94	66.76	70.19	70.36	70.11	70.03	69.80	68.14	67.35
C-7	44.60	t	125.8	44.67	46.13	40.59	40.80	40.65	40.82	40.30	43.51	44.59
C-8	126.57	s		126.82	126.59	125.78	126.15	126.42	126.03	126.73	127.43	126.90
C-9	139.12	s		138.02	139.08	138.22	138.16	137.70	138.28	135.95	137.88	137.81
C-10	43.58	s		43.40	43.42	42.93	43.12	42.93	43.11	44.47	43.34	43.02
C-11	25.72	t	124.0	25.74	25.82	25.56	25.76	25.49	25.43	25.85	25.60	25.47
C-12	29.31	t	131.9	29.45	29.39	29.22	29.41	29.44	29.36	29.41	29.46	29.35
C-13	171.37	s		170.03	171.11	170.68[c]	169.95[c]	169.97	170.84	169.26	170.15	169.89
C-14	114.81	d	178.9	115.41	115.03	115.00	115.41	115.49	115.13	115.65	115.33	115.28
C-15	174.22	s		174.03	174.08	174.46	173.87	173.86	174.11	173.69	174.06	173.83
C-16	73.20	t	151.0	73.10	73.16	73.20	73.01	73.00	73.17	72.94	73.06	72.93
C-17	19.22	q	125.1	19.32	19.19	18.99	19.13	19.18	18.97	19.28	19.39	19.19
C-18	32.36	q	125.8	31.39	31.70	30.30	30.75	30.91	30.87	30.63	31.73	31.24
C-19	67.76	t	140.0	68.65	67.54	65.21	67.66	67.28	67.11	68.06	68.85	67.95
C-20	22.56	q	124.6	22.74	23.11	22.78	22.66	22.42	22.10	22.87	22.23	22.51
2-*Me*CO								21.49[d]	21.25[d,e]		21.47	21.37
6-*Me*CO						21.80	20.79	20.89[d]	20.68[d,e]	20.90		
19-*Me*CO				21.14	20.76		21.74	21.82[d]	21.64[d,e]	21.71		20.90
2-Me*C*O								170.44[c]	170.21[c]		170.75	170.36
6-Me*C*O						170.75[c]	170.25[c]	170.31[c]	170.24[c]	170.26		
19-Me*C*O				171.06	171.02		171.11	171.07[c]	170.84[c]	170.84		170.84

[a]Taken at 67.9 MHz in $CDCl_3$ or stated otherwise with multiplicities determined by SFORD. The chemical shift (δ) in ppm was referenced to TMS with reference peak of solvent taken as 77.2 ppm (center). Abbreviations are s=singlet, d=doublet, t=triplet and q=quartet. Data point resolution of 0.7 Hz and J values in Hz taken from fully ^{1}H-coupled spectrum.

[b]In pyridine-d_5 with upfield carbon (center) set at 123.5 ppm.

[c,d]May be interchanged in the same column.

[e]From CH-correlation acetate protons attached to methyl carbon are δ_H 2.01 to δ_C 21.25; 2.06 to 20.68; and 2.13 to 21.64 ppm.

distantly disposed hydroxyls is required. Of the isolated products, only the 6-acetate, **3**, would be useful, but its natural availability was limited. Consequently, the major isolate, amoenolide A [**1**] was acetylated with one equivalent of Ac_2O and the products separated by chromatography. In addition to the natural product acetates, two new acetates, amoenolide A 2-acetate [**7**] and the 2,19-diacetate **8** were obtained and characterized by the same spectral methods used for the natural products. The 2-acetate **7** was benzoylated and the cd curve of the dibenzoyl product **9** showed a Cotton effect curve with a positive maximum at 235 nm and a negative maximum at 220 nm, supporting a clockwise rotation for the benzoates (Figures 2 and 3) when viewed linearly (17). Thus, the cd exciton-splitting method confirmed the results of the Horeau procedure and hence the amoenolide A compounds have absolute stereochemistry as illustrated.

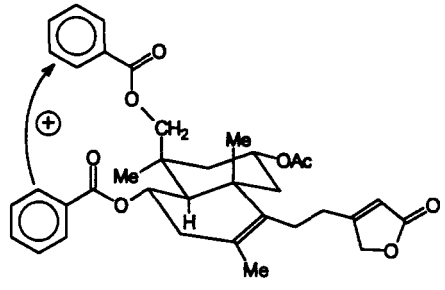

FIGURE 2. Drawing of Amoenolide A 2-acetate 6,19-dibenzoate [**9**] Showing the Absolute Stereochemistry that Would Result in a Positive Cotton Curve for the Dibenzoyl Exciton Splitting.

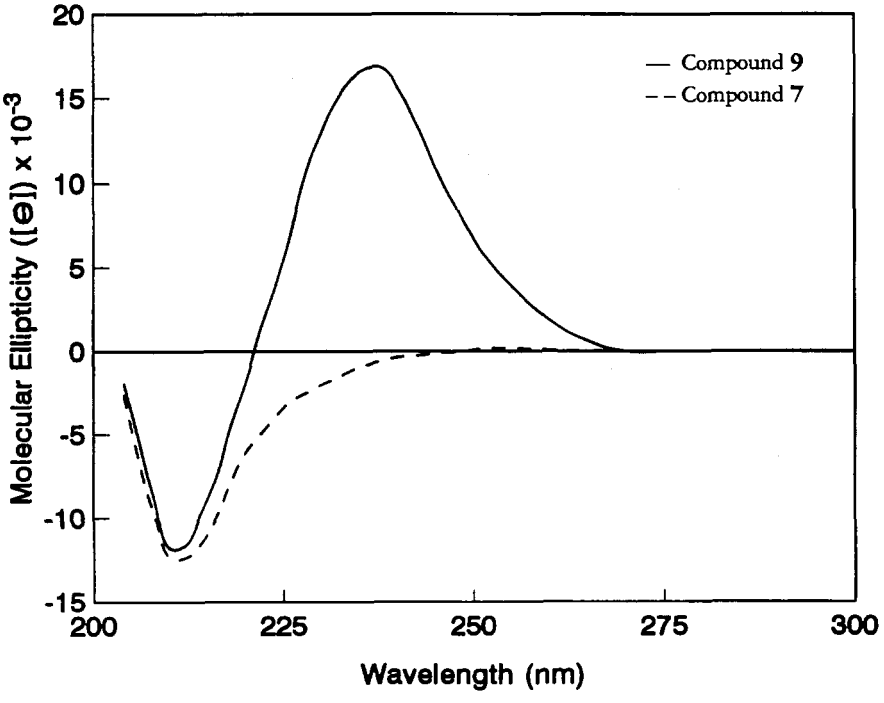

FIGURE 3. Circular Dichroic Curves for Amoenolide A 2-acetate [**7**] and Amoenolide A 2 acetate 6,19-dibenzoate [**9**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were measured on a Fisher-Johns melting-point apparatus and are uncorrected. The following instruments were used: uv, Beckman UV 5260 spectrophotometer; ir, Beckman IR 4230 spectrophotometer; optical rotations, Perkin-Elmer 241 polarimeter; cd, Jasco J-500A spectropolarimeter; ms, DuPont 21-491 and Kratos MS-30 mass spectrometers, the latter equipped with an Iontech fab gun; nmr, Bruker WM-300, AM-500, IBM AF-270 and GE-Nicolet NT-500. The fabms were obtained by dissolving the samples in a glycerol or "magic bullet" (18) matrix [dithiothreitol and dithioerythritol (3:1)] and bombarded by Xe gas accelerated at 8000 volts. Elemental analyses were performed at Galbraith Laboratories, Knoxville, TN.

The pulse programs for the nmr experiments were those provided by Bruker Instruments; COSY 16 for ^{1}H shift correlation; XHCORRD for CH-correlation with homonuclear ^{1}H decoupling; COLOC for long-range (2- to 4-bond) CH-correlation, with polarization delay D2=0.06 sec for J=8 Hz for routine analyses and D2=0.03 and 0.09 sec for J=5 and 17 Hz coupling for additional analyses; INADDEC for C-C connectivity; INVRECX for T_1 measurement; and NOEMULT for nOe difference experiments at 270 MHz.

EXTRACTION AND ISOLATION.—The above-ground parts of *Amphiachyris amoena* (Shinners) Solbrig were collected in Texas in August 1982, and identified by Professor Meredith A. Lane, University of Colorado, Boulder, Colorado, where a voucher specimen is on file. The dried and powdered plant material (4 kg) was extracted by percolation at ambient temperature with EtOH (50 liters) and the extract was evaporated at reduced pressure at 40° to give 995 g of residue. The residue was partitioned between equal volumes of $CHCl_3$ (3 times) and H_2O in an initial ratio of solvents to residue of less than 10:1. The $CHCl_3$ solubles (484 g) were partitioned between hexane (3 times) and MeOH-H_2O (9:1) to give 375 g of a MeOH-soluble extract which was chromatographed on Sephadex LH-20 (Pharmacia, Inc.) with MeOH in a ratio of 1:10 (sample to adsorbent). The combined terpene fraction (260 g) as revealed by tlc analysis (see below) was chromatographed first on Si gel PF 254 using a sample-to-adsorbent ratio of 1:40 and eluting solvents of $CHCl_3$ containing MeOH (0.5% to 40%). The diterpenes appeared in the following effluents: 6%, diacetate **4**; 10%, monoacetate **3**; 12%, monoacetate **2**; and 15%, triol **1**; for a total of 16 pooled fractions as monitored by tlc. Rechromatography of these fractions was on Si gel 60 in a sample to adsorbent ratio of 1:20 and elution by mixtures of hexane–Me_2CO (4:1, 3:1, 2:1, 1:1, and 1:2).

Tlc was employed to monitor the separations and was performed on Si gel G (Merck) with detection by spraying the developed plates with *p*-anisaldehyde-H_2SO_4-EtOH (5:5:90) and heating at 110°. The terpenes gave blue-purple-gray zones, and with the solvent system of $CHCl_3$-MeOH-H_2O (17:2:1, lower phase) the R_f values were as follows: amoenolide A [**1**] 0.14, 6-acetate [**3**] 0.21, 19-acetate [**2**] 0.39, and 6,19-diacetate [**4**] 0.54. The prepared compounds in the same system showed R_f values of: 2-acetate [**7**] 0.46, 2,19-diacetate [**8**] 0.74, and 2,6,19-triacetate [**5**] 0.90.

Amoenolide A [**1**].—From the first column, fraction No. 13 (4.2 g) was separated on a second column of Si gel. Elution with mixtures of hexane-Me_2CO gave, from the 1:1 mixture, 352 mg of amoenolide A [**1**] as needle-like crystals from the eluting solvent. First column fractions No. 9–12 and 14 when similarly chromatographed yielded a total of 4.12 gm (0.1% of dried plant) of amoenolide A: mp 193–195°; $[\alpha]^{23.5}D$ +78° (c=0.5, MeOH); ir (KBr) ν max 1800 and 1730 (lactone C=O), 1620 (C=C) and 1040 (C-O) cm^{-1}; uv (MeOH) λ (end abs) 202 nm (log ϵ 4.11); and eims m/z 330 ($M^+-H_2-H_2O$, 6), 314 (M^+-2H_2O, 32), 299 (56), 296 (M^+-3H_2O, 30), 183 (92), 171 (91) and 119 (100). *Anal.*, calcd for $C_{20}H_{30}O_5$, C 68.53, H 8.63; found C 68.56, H 8.57. The ^{1}H- and ^{13}C-nmr spectral data are given in Tables 1 and 2, respectively.

Amoenolide A 19-acetate [**2**].—Fraction No. 5 (3.5 g) from the first column, crystallized from $CHCl_3$-MeOH to give 1.27 g (0.03% of dried plant) of amoenolide A 19-acetate [**2**] as cubic crystals: mp 177–178°; $[\alpha]^{23.5}D$ +41° (c=0.5, MeOH); ir ($CHCl_3$) ν max 3480 (OH), 1790 and 1730 (C=O), 1235 and 1030 (C-O) cm^{-1}; uv (MeOH) λ (end abs) 203 nm (log ϵ 4.24); cd ($c=4.9\times10^{-5}$ M, MeOH) $[\theta]_{248}$ 0, $[\theta]_{211}$ −21,400 (min) and $[\theta]_{203}$ 0; fabms (glycerol) m/z 393 (MH^+, 0.2), 375 (MH^+-H_2O, 2), 357 (MH^+-2H_2O, 1); eims m/z 374.2065 (M^+-H_2O, 1, $C_{22}H_{30}O_5$ requires 374.2094), 332 (M^+-HOAc, 3), 314 ($M^+-H_2O-HOAc$, 5), 296 ($M^+-2H_2O-HOAc$, 5) and 43 (Ac, 100); and ^{1}H- and ^{13}C-nmr spectral data are given in Tables 1 and 2, respectively.

Amoenolide A 6-acetate [**3**].—First column fractions Nos. 7 and 8 (2.10 g) were chromatographed in Si gel with mixtures of hexane-Me_2CO to give 19 fractions as followed by tlc. Subfraction 9 (80 mg), eluted with the 3:1 mixture, was rechromatographed on Si gel with MeOH-$CHCl_3$ mixtures 1:99, 1:49, and 1:19. The 1:49 effluent yielded amoenolide A 6-acetate [**3**] as a homogeneous oil (36 mg) (9.0×10^{-4}% of the dried plant); $[\alpha]^{23.5}D$ +45° (c=0.5, MeOH); ir (KBr) ν max 1800 and 1730 (C=O), 1620 (C=C) and 1040 (C-O) cm^{-1}; uv (MeOH) λ (end abs) 202 nm (log ϵ 4.11); fabms (glycerol) m/z 393 (MH^+, 1); eims m/z 357 (MH^+-2H_2O, 0.2), 332 (M^+-HOAc, 0.5), 314.1778 ($M^+-H_2O-HOAc$, 1, $C_{20}H_{26}O_3$ requires 314.1883),

196 ($M^+-2H_2O-HOAc$, 1) and 43 (Ac, 100). The ^1H- and ^{13}C-nmr spectral data are given in Tables 1 and 2, respectively.

Amoenolide A 6,19-diacetate [**4**].—First column fraction No. 4 on chromatography on Si gel with hexane/Me_2CO mixtures gave, with the 2:1 system, amoenolide A 6,19-diacetate [**4**] as a homogeneous oil (3.1 g, 0.08% yield of the dried plant): $[\alpha]^{23.5}D$ +69° (*c*=0.5, MeOH); ir ($CHCl_3$) ν max 3480 (OH), 1790 and 1750 (C=O), 1640 (C=C), 1260 (acetate C-O) and 1040 (C-O) cm^{-1}; uv (MeOH) λ (end abs) 203 nm (log ϵ 4.21); fabms (glycerol), *m/z* 435 (MH^+, 0.2), 375 (MH^+-HOAc, 1), 357 ($MH^+-H_2O-HOAc$, 0.3), 315 ($MH^+-2HOAc$, 1); eims *m/z* 315 ($MH^+-2HOAc$, 1), 296.1720 ($M^+-H_2O-2HOAc$, 23, $C_{20}H_{24}O_2$ requires 296.1777) and 281 (100). The ^1H- and ^{13}C-nmr data are given in Table 1 and 2, respectively.

Amoenolide A 2,6,19-triacetate [**5**].—Samples of amoenolide A [**1**] or its acetate derivatives [**2–4**] (10–20 mg) were treated for 20 h in 1 ml each of Ac_2O and pyridine. H_2O (1 ml) was added and the mixture evaporated to dryness under reduced pressure. The residue was chromatographed on 5 g of Si gel with $CHCl_3$ to give amoenolide A 2,6,19-triacetate [**5**] as a heavy oil: $[\alpha]^{23.5}D$ +45° (*c*=0.5, MeOH); ir ($CHCl_3$) ν max 1790 (lactone C=O), 1750 (ester C=O), 1250 (acetate C-O), 1260 and 1040 (C-O) cm^{-1}; uv (MeOH) λ (end abs) 203 nm (log ϵ 4.15); fabms (glycerol) *m/z* 477 (MH^+, 0.3), 417 (MH^+-HOAc, 0.6), 357 ($MH^+-2HOAc$, 1), 297 ($MH^+-3HOAc$, 2); eims *m/z* 314 ($M^+-HOAc-OAc-Ac$, 1), 296.1710 ($M-3HOAc$, 2, $C_{20}H_{24}O_2$ requires 296.1777) and 43 (Ac, 100). The ^1H- and ^{13}C-nmr spectral data are given in Tables 1 and 2, respectively.

2-Dehydroamoenolide 6,19-diacetate [**6**].—A 20 mg sample of amoenolide A 6,19-diacetate [**4**] in 1 ml of Me_2CO at 0° was treated dropwise with Jones' Reagent (12) until an orange color persisted. After 5 min, 5 ml each of H_2O and 5% aqueous $NaHCO_3$ were added and the mixture was extracted with 10 ml of Et_2O (3×). The combined Et_2O extract was extracted with 25 ml 5% aqueous $NaHCO_3$ followed by 25 ml of H_2O (5×) until the extract was neutral. The dried (anhydrous $MgSO_4$) Et_2O extract was evaporated to dryness to give 18 mg of the ketone **6** as a heavy oil: $[\alpha]^{23.5}D$ +81° (*c*=0.5, MeOH); ir ($CHCl_3$) ν max 1790 (lactone C=O), 1750 (ester C=O), 1720 (ketone C=O), 1250 (ester C-O) and 1040 (C-O) cm^{-1}; uv (MeOH) λ (end abs) 204 nm (log ϵ 4.17); fabms (glycerol) *m/z* 433 (MH^+, 0.6), 373 (MH^+-HOAc, 5), and 313 ($MH^+-2HOAc$, 1); eims *m/z* 313.2729 ($M^+-2HOAc$, 0.5, $C_{20}H_{25}O_3$ requires 313.1804), 149 (20), 57 (52), and 43 (100). The ^1H- and ^{13}C-nmr data are given in Tables 1 and 2.

HOREAU PROCEDURE WITH AMOENOLIDE A [**1**].—Amoenolide A (5 mg, 14 μmol) in 0.33 ml of a 12.5% solution of 2-phenylbutanoic anhydride (41 mg, 120 μmol) in pyridine was left for 43 h at room temperature; then 3 drops of H_2O were added and after 30 min the solvents were removed at reduced pressure. The residue was mixed with 5 ml each of $CHCl_3$ (3×). The combined $CHCl_3$ phase was taken to dryness at reduced pressure to give 11 mg (14 μmol) of 2,6,19-tri-2-phenylbutanoyl ester of amoenolide A; fabms (glycerol) *m/z* 811 (MNa^+, 0.7), 788 (M^+, 0.1, $C_{50}H_{60}O_8$), 625 ($MH^+-C_{10}H_{12}O_2$, 0.2), 461 ($MH^+-2C_{10}H_{12}O_2$, 2), 297 ($MH^+-3C_{10}H_{12}O_2$, 8) and 91 (100); ^1H nmr ($CDCl_3$, 250 MHz) δ 7.33–7.14 (m, ArH), 5.78 (H-14, br s), 4.65 and 4.57 (2H each, H_2-16 and H_2-19) and 3.35 ppm (H-2s, m).

The aqueous $NaHCO_3$ phase was acidified with 10% HCl and extracted with 5 ml $CHCl_3$ (3×). The combined $CHCl_3$ extract after washing with H_2O (2×20 ml) was evaporated to dryness to give 30 mg (183 μmol) of 2-phenylbutanoic acid, identical (mp and ^1H nmr) with an authentic sample. The $[\alpha]^{23.5}D$ −2.7° (*c*=0.5, MeOH) when corrected for excess (8.6×) reagent afforded a 30% optical yield. (−)-*R*-2-Phenylbutyric acid has $[\alpha]^{22}D$ −77.4° (*c*=10, EtOH).

PARTIAL ACETYLATION OF AMOENOLIDE A [**1**].—Amoenolide A (1.05 g, 3 mmol) in 50 ml of anhydrous pyridine was mixed with 10 ml of 3% Ac_2O in anhydrous pyridine (1 equivalent) and stirred for 3 h. H_2O (10 ml) was added and the mixture was evaporated at reduced pressure to give 1.18 g of a crystalline mass that was absorbed onto 3 g of Si gel by dissolving in 5 ml of MeOH and removing the solvent at reduced pressure. The powdered mixture was added to a 35 g column of Si gel packed in hexane. The column was eluted with hexane-Me_2CO (4:1), (3:1), (2:1), and (1:1). Analysis by tlc of effluent fractions using column solvents gave from the (4:1) mixture 9 mg of the triacetate **5**, from the (2:1) mixture 307 mg of the 19-acetate **2**, and from the (1:1) mixture 464 mg of starting material **1** in addition to two new acetates which are described below.

Amoenolide A 2-acetate [**7**].—The hexane-Me_2CO (2:1) effluent from the partial acetylation reaction gave 140 mg of crystalline 2-acetate [**7**]: mp 156–158°; $[\alpha]^{23.5}D$ +37° (*c*=0.5, MeOH); ir ($CHCl_3$) ν max 1790 and 1760 (C=O), 1650 (C=C), 1390, 1260, and 1040 (C=O) cm^{-1}; uv (MeOH) λ (end abs) 205 nm (log ϵ 4.78); cd (*c*=4:1×10^{-4} M, MeOH) $[\theta]_{249}0$, $[\theta]_{211}-12{,}300$ (min) and $[\theta]_{203}0$; fabms (glycerol) *m/z* 393 (MH^+, 2), 375 (MH^+-H_2O, 1), 315 ($MH^+-H_2O-HOAc$, 10), and 297 ($M^+-H_2O-HOAc$, 2); eims *m/z* 314.1915 ($M^+-H_2O-HOAc$, 4, $C_{20}H_{26}O_3$ requires 314.1883), 296 ($M^+-2H_2O-HOAc$, 2) and 43 (100). The ^1H- and ^{13}C-nmr data are given in Tables 1 and 2, respectively.

Amoenolide A 2,19-diacetate [**8**].—The hexane-Me_2CO (3:1) effluent from the partial acetylation reaction gave 60 mg of the 2,19-diacetate **8**: mp 134–135°; $[\alpha]^{23.5}D$ +41° (*c*=0.2, MeOH); ir ν max 1790 and 1750 (C=O), 1650 (C=C), 1390, 1260, and 1040 (C=O) cm^{-1}; uv (MeOH) λ (end abs) 205 nm (log ε 4.32); eims *m/z* 374 (M^+-HOAc, 3), 314 ($M^+-2HOAc$, 7), 296 ($M^+-H_2O-2HOAc$, 5); *anal.* calcd for $C_{24}H_{34}O_7$, C 66.32, H 7.89; found C 64.16, H 7.85. The ^1H- and ^{13}C-nmr spectral data are given in Tables 1 and 2, respectively.

Amoenolide A 2-acetate 6,19-dibenzoate [**9**].—To a mixture of 40 mg (0.10 mmol) of amoenolide A 2-acetate [**7**] and 10 mg (0.08 mmol) of 4-$Me_2NC_5H_5N$ in 1 ml of anhydrous pyridine, 0.1 ml (1.1 mmol) of benzoyl chloride was added and stirred overnight at room temperature. Then, 2 ml of H_2O were added and the solvent removed at reduced pressure. The residue was dissolved in 5 ml of $CHCl_3$ and extracted with 5 ml of saturated aqueous $NaHCO_3$ and 5 ml of H_2O (3×). The $CHCl_3$ phase was taken to dryness at reduced pressure and the residue chromatographed on 7 g of Si gel and eluted with MePh-Me_2CO (99:1), (49:1), and (19:1). The second solvent gave benzoic acid and the third solvent gave 43.4 mg (0.07 mmol) of dibenzoate **9** as an amorphous solid: $[\alpha]^{23.5}D$ +62° (*c*=0.5, MeOH); ir ($CHCl_3$) ν max 1790, 1750, and 1730 (C=O), 1460, 1280, 1120, 1030 (C-O), and 710 (Ar-H) cm^{-1}; uv (MeOH) λ max 276 nm (log ε 3.44), 268 (3.55) and 222 (4.51); cd (*c* 1.6×10^{-5} M, MeOH) $[\theta]_{270}$ 0, $[\theta]_{237}$ +16,800 (max), $[\theta]_{223}$ 0, $[\theta]_{210}$ −12,300 (min) and $[\theta]_{204}$ 0, [lit. values (17) for dibenzoyl esters, first and second Cotton effect maxima at 235 and 220 nm]; fabms ("magic bullet") *m/z* 623 (MNa^+, 1), 479 ($MH^+-C_7H_6O_2$, 1), 419 ($MH^+-C_7H_6O_2-HOAc$, 1), 297 ($MH^+-2C_7H_6O_2-HOAc$, 6) and 105 (C_7H_5O, 100); ^1H nmr ($CDCl_3$, 270 MHz) δ 8.02 (2H, dm, *o*-ArH), 794 (2H, dm, *o*-ArH), 7.59–7.34 (6H, m, ArH), 5.90 (br s, H-14), 5.66 (ddd, *J*=12.8 and 7 Hz, H-6), 5.11 (dddd, *J*=12, 12, 3, and 3 Hz, H-2), 4.77 (d, *J*=2 Hz, H-16), 4.65 (A of ABq, *J*=11 Hz, H-19a), 4.23 (B of ABq, *J*=11 Hz, H-19b), 2.76 (dd, *J*=18 and 7 Hz, H-7β), 2.04 (s, Ac), 1.98 (d, *J*=12 Hz, H-5), 1.63 (s, Me-17), 1.41 and 1.31 (2 s, 2 Me).

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