Five New Labdane Diterpenes from Amphiachyris amoena

Dónal P. O'Mathúna, and Raymond W. Doskotch

J. Nat. Prod., **1994**, 57 (10), 1382-1390• DOI: 10.1021/np50112a007 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 10, pp. 1382-1390, October 1994

FIVE NEW LABDANE DITERPENES FROM *AMPHIACHYRIS AMOENA*[1]

DÓNAL P. O'MATHÚNA[2] and RAYMOND W. DOSKOTCH*

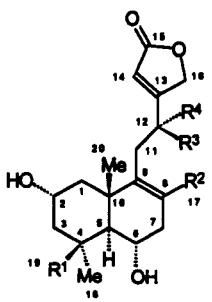
Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210-1291

ABSTRACT.—Five new labdane diterpenes, amoenolide A 19-aldehyde [**2**], and amoenolides B [**3**], C [**4**], D [**5**], and J [**6**] were isolated from the above-ground parts of *Amphiachyris amoena*, and their structures determined by spectral methods, in particular high-field nmr, and selective chemical studies. Amoenolide J [**6**] had its carbon skeleton established by the INADEQUATE ^{13}C-^{13}C connectivity nmr experiment.

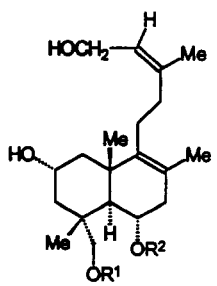
In a previous paper, we reported the isolation and structure elucidation of the labdane triol amoenolide A [**1**] and three of its acetates from the above-ground parts of *Amphiachyris amoena* (Shinners) Solbrig, family Asteraceae (1). In this paper, we describe five further new labdanes from the same source.

RESULTS AND DISCUSSION

Amoenolide A 19-aldehyde [**2**] was characterized by comparison of its spectral data with those of amoenolide A [**1**]. The fabms supported the molecular formula $C_{20}H_{28}O_5$ (mol wt 348), which is two hydrogens fewer than amoenolide A. The ^1H-nmr spectrum in pyridine-d_5 showed a peak at 10.42 ppm (Table 1) and the ^{13}C-nmr spectrum had an absorption at 206.57 ppm (Table 2), which under SFORD conditions was a doublet. This suggested the presence of an aldehyde function which was supported by the ir peak at 1735 cm^{-1}. The ^1H-nmr spectrum showed the same or similar patterns observed for amoenolide A [**1**] such as those for the carbinyl protons H-2 and H-6, the α,β-unsaturated γ-lactone protons, the H_2-1 and H_2-3 protons, and the three methyl groups. The H-19 carbinyl protons, however, were absent, thereby placing the aldehyde at C-

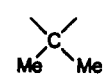


	R^1	R^2	R^3	R^4
1	CH_2OH	Me	H	H
2	CHO	Me	H	H
3	Me	CH_2OH	H	H
4	Me	Me	H	H
5	CH_2OH	Me	H	OH



	R^1	R^2
6	H	H

7



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

[2]Current address, Mount Carmel College of Nursing, Columbus, OH 43222.

TABLE 1. ^{1}H-Nmr Assignments for Compounds **2–7**[a].

Proton	Compound								
	2	**2**[b]	**3**	**3**[b]	**4**	**4**[b]	**5**[b]	**6**[b,d]	**7**[c]
H-1	1.14α dd (11.9,11.9) 2.08β ddd (11.6,3.9,2.2)	1.45α dd (11,11) 2.36β hm	1.19α dd (11.4,11.4) 2.10β ddd (11.5,3.5,2.4)	1.52α dd (11.4,11.4) 2.43β ddd (11.4,2.4,2.4)	1.13α dd (11.5,11.5) 2.07β ddd (11.4,3.7,2.4)	1.44α dd (11.4,11.4) 2.36β hm	1.59α dd (11.2,11.2) 2.49β hm	1.65α dd (11.5,11.5) 2.58β dd (11.8,3.4,1.5)	0.90α hm [dd] (11.6,11.6) 2.18β hm [ddd] (11.8,4.5,2.0)
H-2	3.95 dddd (11.5,11.5,4.3, 4.3)	4.35 m (5 pk)	3.95 dddd (11.4,11.4,4.0, 4.0)	4.28 dddd (11.3,11.3,3.9, 3.9)	3.90 dddd (11.4,11.4,4.1, 4.1)	4.22 ddddd (11.3,11.3,3.9, 3.9)	4.26 dddd (11.5,11.5,3.9, 3.9)	4.27 hm	4.09 hm
H-3	1.14α dd (11.9,11.9) 2.44β hm [ddd] (13.2,4.5,2.1)	1.44α dd (12,12) 2.93β brd (12.7)	1.20α dd (12.2,12.2) 1.77β ddd (12.6,4.0,2.3)	1.62α dd (13.6,13.6) 2.11β ddd (12.8,3.5,1.8)	1.18α dd (12.5,12.5) 1.73β ddd (12.6,4.1,2.3)	1.58α ht [dd] (12.5,12.5) 2.06β ddd (12.6,3.9,2.1)	1.40α dd (12.5,12.5) 2.35β ddd (12.0,3.8,2.2)	1.98α hm [dd] (11.9,11.9) 2.13β hm [ddd] (10.6,4.0,2.0)	1.29α hm [dd] (11.8,11.8) 1.62β hm [ddd] (11.7,4.4,1.7)
H-5	1.60 d (11.1)	1.85 d (11.4)	1.30 d (10.5)	1.63 d (10.0)	1.26 d (11.0)	1.52 d (11.0)	1.55 d (11.0)	1.97 d (12)	1.27 d (11)
H-6	4.37 ddd (11.9,10.1,5.9)	4.71 hm [ddd] (11.2,9.5,6.0)	4.16 ddd (10.6,8.3,6.5)	4.48 ddd (9.9,9.9,6.5)	4.11 ddd (10.9,9.0,6.4)	4.36 ddd (10.9,9.2,6.4)	4.65 ddd (10.4,10.4,6.5)	4.28 hm	4.08 hm
H-7	2.18α dd (16.7,9.8) 2.42β hm	2.46α dd (16.8,9.6) 2.58β dd (16.9,5.7)	2.19α dd (17.3,8.2) 2.71β dd (17.3,6.3)	2.77α dd (17.3,9.2) 3.20β dd (17.3,6.2)	2.04α dd (16.7,9.8) 2.46β dd (17.4,9.8)	2.39α dd (17.2,9.1) 2.58β dd (17.2,6.3)	2.40α dd (17.4,10.0) 2.61β dd (17.2,6.1)	2.40α dd (17.2,8.3) 2.62β dd (17.2,6.5)	2.59α dd (18.7,9.5) 2.02β hm
H-11	2.16 hm 2.34 hm	2.17 m 2.29 m	2.27 m 2.45 m	2.30 m 2.53 m	2.18 m 2.32 m	2.20 m 2.28 m	2.45 hm [dd] (14,2.5) 2.74 dd (14.3,10.5)	2.13 m 2.23 m	1.90 m 2.17 m
H-12	2.42 (2H) hm	2.36 (2H) m	2.47 (2H) m	2.50 (2H) m	2.42 (2H) m	2.35 (2H) m	5.10 hm	2.25 (2H) m	2.05 (2H) m
H-14	5.87 m (5 pk) (1.5)	5.98 m (5 pk) (1.3)	5.88 m (5 pk) (1.5)	5.92 brs $\omega_{1/2}$ 4.8 Hz	5.86 m (5 pk) (1.6)	5.95 m (5 pk) (1.3)	6.23 ddd (1.7,1.7,1.7)	5.72 t q (6.7,1.1)	5.40 t q (7.2,1.1)
H-16	4.74 (2H) d (1.5)	4.71 dd (17.3,1.4) 4.75 dd (17.3,1.4)	4.75 (2H) d (1.6)	4.66 dd (17.4,1.5) 4.68 dd (17.4,1.5)	4.74 (2H) d (1.7)	4.70 dd (17.4,1.6) 4.74 dd (17.3,1.6)	5.09 s	1.77 s	1.78 s

TABLE 1. Continued.

Proton	Compound								
	2	2[b]	3	3[b]	4	4[b]	5[b]	6[b,d]	7[c]
H-17	1.63 s	1.58 s	4.07 d (11.5) 4.10 d (11.6)	4.33 d (12.0) 4.41 d (12.0)	1.59 s	1.55 s	1.87 s	1.64 s	1.64 s
H-18	1.43 s	1.73 s	1.25 s	1.64 s	1.24 s	1.61 s	1.66 s	3.78 d (10.6) 4.14 d (10.6)	2.94 d (11.7) 3.57 d (11.7)
H-19	9.97 s	10.42 d[c] (2.4)	1.15 s	1.37 s	1.12 s	1.33 s	3.85 d (10.4) 4.50 d (10.5)	1.32 s	1.13 s
H-20	0.90 s	1.03 s	1.11 s	1.19 s	1.05 s	1.14 s	1.36 s	1.16 s	0.91 s

[a]Taken at 500 MHz in $CDCl_3$ or as stated otherwise with data point resolution of 0.3 Hz and chemical shifts (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 square parentheses.

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

[c]From "*W*-coupling" to H-3β as shown by homonuclear decoupling.

[d]H-15 (2H) at 4.54 (d) and 4.57 (d) ppm (J=12.9 Hz, the AB of an ABX system).

[e]At 270 MHz with H-15 (2H) at 4.12 hm and acetonide methyls at 1.29 s and 1.35 s.

TABLE 2. ^{13}C-Nmr Data for Compounds **2–7**.[a]

Carbon	Compounds							
	2[b]	multiplicity	**2**	**3**	**4**	**5**	**6**	**7**[b,c]
C-1	45.72	t	46.53	47.18	47.35	48.50	47.28	46.49
C-2	64.11	d	63.20	64.13	64.12	63.70	64.21	65.41
C-3	44.82	t	45.26	54.14	54.13	49.76	48.04	44.66
C-4	50.36	s	50.84	35.69	35.68	41.25	40.29	38.76
C-5	57.57	d	57.28	56.59	56.66	56.61	52.68	56.50
C-6	67.40	d	66.30	67.34	67.15	67.90	66.48	66.19
C-7	45.23	t	45.80	41.72	45.89	45.08	44.84	38.93
C-8	127.07	s	126.72	131.77	126.41	129.81	124.88	124.53
C-9	137.20	s	137.98	141.35	139.13	136.25	140.33	139.07
C-10	43.57	s	43.59	43.65	43.47	43.84	43.18	40.52
C-11	25.78	t	25.73	25.13	25.67	35.72	27.26	26.94
C-12	29.29	t	29.16	30.62	29.34	69.30d	33.18	33.11
C-13	169.86	s	171.23	171.19	171.30	175.58	137.75	140.41
C-14	115.44	d	114.87	114.84	114.86	114.04	126.34	124.01
C-15	173.99	s	174.21	174.19	174.19	174.19	58.68t	58.85t
C-16	73.08	t	73.19	73.18	73.18	71.62	23.32q	23.49q
C-17	19.40	q	19.20	62.16t	19.17	20.94	19.27	19.52
C-18	28.40	q	28.33	37.31	37.35	32.34	74.68t	74.59t
C-19	207.12	d	206.57	23.40q	23.34q	67.72t	19.27q	20.13q
C-20	22.39	q	21.64	22.55	22.53	23.09	22.65	21.35

[a]Taken at 67.9 MHz in pyridine-d_5 or as stated otherwise with multiplicities determined by SFORD. Each chemical shift (δ) in ppm was referenced to TMS with the reference peak of upfield solvent taken as 123.5 ppm (center). Data point resolution of 0.7 Hz.

[b]In $CDCl_3$ with center of solvent peak set at 77.2 ppm.

[c]The acetonide carbons were at 24.93 (q), 26.43 (q), and 100.26 (s).

19. Formation of a diacetate supported a diol, and accounted for the remaining two oxygens.

Extensive 2D nmr studies (^{1}H,^{1}H-COSY, CH-correlation and COLOC, the long-range heteronuclear correlation) along with homonuclear decoupling and nuclear Overhauser effect (nOe) studies not detailed here,[3] allowed complete assignment of the nmr spectra (Tables 1 and 2) and stereochemical placement of the substituents on the labdane skeleton. For example, in the nOe difference experiment, irradiation of the aldehydic proton (10.42 ppm) showed relaxation to Me-18 (3%, 1.73 ppm), Me-20 (3%, 1.03 ppm), H-2 (1%, 4.35 ppm), and H-6 (8%, 4.71 ppm), thus supporting their location on the β-face of the molecule. The reduction of amoenolide A 19-aldehyde [**2**] with $NaBH_4$ gave amoenolide A [**1**], thereby confirming the spectrally derived structure and establishing its absolute stereochemistry (1).

Amoenolide B [**3**], with the formula $C_{20}H_{30}O_5$ supported by fabms, is an isomer of amoenolide A [**1**] as revealed by comparison of their ^{1}H-nmr spectra (Table 1). The patterns are essentially the same, but the chemical shifts differ, with the largest changes occurring for H-6, H_2-7, and the hydroxymethyl protons. Extensive 1D and 2D nmr studies (1) showed that the C-19 hydroxyl of amoenolide A [**1**] was located at C-17 in amoenolide B [**3**]. Support for this assignment was obtained from nOe difference studies (2,3) in which irradiation of H-7α (2.77 ppm) gave a 2% enhancement of the hydroxymethyl proton at 4.33 ppm and irradiation of H-7β (3.20 ppm) enhanced the other hydroxymethyl proton at 4.41 ppm by the same amount. Also, a COLOC nmr

[3]A detailed summary can be obtained from the senior author for compounds of this report.

experiment showed long-range coupling between the hydroxymethyl protons to C-8 (131.77 ppm) for a two-bond interaction and to C-9 (141.35 ppm) for a three-bond interaction, as well as to C-7 (41.72 ppm). The methyl groups were assigned by nOe experiments. For example, the methyl group at 1.37 ppm was placed at C-19 because its protons became enhanced by irradiation of the other two methyls, 7% from Me-18 (1.64 ppm) and 10% from Me-20 (1.19 ppm). Furthermore, the chemical shift values in the ^{13}C-nmr spectrum confirmed these assignments. The axial C-19 (23.40 ppm) and C-20 (22.55 ppm) have a number of γ-gauche interactions which shift them to higher fields while the equatorial C-18 (37.31 ppm) has none (4). The ^{1}H- and ^{13}C-nmr spectral assignments from these studies are found in Tables 1 and 2, respectively.

Amoenolide C [**4**], a slightly $CHCl_3$-soluble crystalline lactone, mp 176–177°, has the molecular formula $C_{20}H_{30}O_4$ as supported by fabms. The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2, respectively) indicated it to be closely related also to amoenolide A [**1**]. The formula for amoenolide C [**4**] contained one fewer oxygen as shown by the loss of the AB quartet for the C-19 protons of amoenolide A and its replacement by a singlet revealing a fourth methyl group. Detailed 1D- and 2D-nmr techniques previously described (1) allowed for complete assignment of the nmr spectra, and the nOe studies supported the stereochemical ordering. Comparison of the ^{13}C-nmr chemical shift changes observed for loss of the 19-hydroxyl from amoenolide A showed the α-carbon (C-19) to be shifted upfield 44 ppm, the β-carbon (C-4) shifted upfield 6 ppm, while the γ-carbons were moved downfield by 5 ppm for both C-3 and C-18, with little alteration for C-5. This compares well with the empirical data for this change which is an upfield shift of up to 48 ppm for the α-carbon, up to 10 ppm for the β-carbon and a downfield shift of up to 5 ppm for the γ-carbon (4). These results established amoenolide C [**4**] to be 19-deoxyamoenolide A.

Amoenolide D [**5**], mp 192–193°, was isolated from the EtOAc-soluble terpene fraction and has the fabms-supported formula $C_{20}H_{30}O_6$ which is one more oxygen than amoenolide A [**1**]. The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2, respectively) indicated that this oxygen, as a hydroxyl, was located in the side-chain (C-11 or C-12). Extensive 1D- and 2D nmr studies (1) established the stereochemical structure as that of amoenolide A [**1**] with the hydroxyl located at C-12. For example, in the amoenolides (1) with the ethyl side-chain, H-14 is a tight five-peak multiplet (J ca. 1.5 Hz) due to allylic coupling from the C-12 and C-16 protons, but for amoenolide D [**5**] H-14 is a simpler ddd pattern (J=1.7 Hz) from three rather than four protons. In addition, the C-11 protons exhibited the split AB quartet pattern of an ABX spin system, rather than the ddd (split triplet) for each H-11 of a non-first-order ABCD pattern. On the other hand, the chemical shift positions and the coupling patterns for the bicyclic component are changed little, except for those positions facing the side-chain (e.g., Me-17 and Me-20).

The 2D nmr experiment for detecting two- and three-bond heteronuclear coupling (COLOC) (5) showed three-bond interactions between both H-11 protons and C-10, an effect not consistent with a hydroxyl at C-11. Furthermore, the fully ^{1}H-coupled ^{13}C-nmr spectrum showed C-14 (114.04 ppm) as a ddt pattern with J_{CH}=180, 3, and 3 Hz, representing one-bond coupling to H-14, three-bond to H-12, and three-bond to each H-16, respectively. On the other hand, amoenolide A [**1**] showed C-14 (114.81 ppm) as a dtt with J_{CH}=179, 3 and 3 Hz. A similar comparison of the C-16 patterns showed one fewer proton interaction in amoenolide D [**5**], whereas C-8, C-9, and C-10 were not significantly different. The fully ^{1}H-coupled ^{13}C-nmr spectrum also confirmed the assignments for C-13 and C-15. The signal at 175.58 ppm was a broad singlet ($\omega_{1/2}$=14 Hz) characteristic of C-13, while the signal at 174.19 ppm was a doublet (J=9 Hz)

characteristic of C-15, and is opposite to the chemical shift relationship observed for the lactones without the C-12 hydroxyl (1).

The absolute configuration of C-12 was established by the Horeau method (6). Acylation of amoenolide D [**5**] with *rac*-2-phenylbutyric anhydride resulted in the recovered acid having a specific rotation of $-6.6°$ after correction for excess anhydride and unresolved acid formation from the hydroxyls at C-2 and C-19, which are considerably less hindered and not expected to show much, if any, selectivity. The partially resolved acid is due to the two more hindered alcohols at C-6 and C-12. The contribution of the C-6 hydroxyl to the partial resolution was obtained from the Horeau method on amoenolide A [**1**] (1), for which the recovered 2-phenylbutyric acid gave a specific rotation of $-23.1°$, after correction. Therefore, the C-12 hydroxyl contribution to the partial resolution would be the difference between the two values, or a specific rotation of $+16.5°$. The relative bulkiness of the substituents about C-12 to yield (+)-2-phenylbutyric acid corresponds to an *R*-configuration. Thus, amoenolide D is 12(*R*)-hydroxyamoenolide A [**5**].

Amoenolide J [**6**] was isolated from the MeOH-soluble partition fraction and has the molecular formula $C_{20}H_{34}O_4$, as supported by fabms. Its structure was established from spectral data, especially 1D- and 2D nmr, details of which are not reported here, and their comparison to those of the other amoenolides. The ir and nmr spectra lacked peaks for the unsaturated lactone system, and having two fewer double-bond equivalents than amoenolide A [**1**] supported the loss of the lactone carbonyl and ring. Acetylation gave a tetraacetate. Thus, the four oxygens are hydroxyls, two primary and two secondary as shown from the multiplicities from the SFORD ^{13}C-nmr experiment for the hydroxyl-bearing carbons. The ^{1}H-nmr spectrum (Table 1) of the tetraacetate in which the carbinyl protons are unobscured revealed three of the four patterns to be the same as those for amoenolide A [**1**] triacetate (1), and consistent with the decalin system of the other amoenolides. The fourth, a primary acetate, showed the carbinyl protons as a split AB quartet at 4.52 and 4.56 ppm, or the AB part of an ABX pattern. The X-part was an olefinic proton (5.34 ppm), a triplet of quartets, where the quartet component is from an olefinic methyl (1.79 ppm). These patterns support the side-chain olefinic unit (C-13 to C-16), and the stereochemical disposition was established by nOe difference spectroscopy with amoenolide J [**6**]. For example, irradiation of the olefinic methyl (Me-16, 1.77 ppm) showed a 15% enhancement for the olefinic proton (H-14, 5.72 ppm), while irradiation of H-14 enhanced both Me-16 (7%) and the carbinyl protons at 4.54 and 4.57 ppm (H_2-15, 2%). Carbinyl proton irradiation (H_2-15) gave a 14% increase for H-14 and, in addition, enhanced the H_2-12 protons (2.25 ppm) by 7%. These experiments established the C-13, C-14 double-bond stereochemistry as *E*, where the hydroxymethyl group is *trans* to the olefinic methyl.

Although amoenolide J [**6**] gave nmr data in accord with the *trans*-decalin system of amoenolide A [**1**], the nOe results required the placement of the hydroxymethyl at C-18, instead of C-19. For example, irradiation of the methyl at 1.32 ppm enhanced the hydroxymethylene protons at 3.78 and 4.14 ppm by 2% and 3%, respectively, and the aliphatic methyl at 1.16 ppm by 8%, as well as H-2 and H-6 by 14% (combined). Irradiation of the 1.16 ppm methyl enhanced the 1.32 ppm methyl by 8%, and H-2 and H-6 by 22% (combined), while irradiation of the hydroxymethyl proton at 4.14 ppm increased the methyl signal at 1.32 ppm by 4%. These data require that the hydroxymethyl be at C-18, the 1.32 ppm methyl at C-19, and the 1.16 ppm methyl at C-20. The trans ring junction was established by the nOe enhancements of H-3α (1.98 ppm, 1%) and H-5 (1.97 ppm, 4%) when H-1α (1.65 ppm) was irradiated. Other irradiations and enhancements clearly defined the remaining groups on the α- and the β-faces of the molecule, which allowed for assignment of the ^{1}H- and ^{13}C-nmr spectra (Tables 1 and

2). Also, since an adequate amount of amoenolide J [**6**] was available, the carbon-carbon connectivity (INADEQUATE) nmr experiment (7) was performed which confirmed the carbon skeleton, the ^{13}C-nmr assignments, and resolved any ambiguities concerning the quaternary carbons.

A compound was isolated from a subfraction chromatographed with hexane/Me_2CO as solvent, which after detailed spectral analysis was found to be amoenolide J 6,18-acetonide [**7**], the acetone adduct[4] of amoenolide J [**6**]. This artifact substantiates the equatorial disposition of the two hydroxyls required for its formation and confirmed the placement of the hydroxymethyl at C-18. A diacetate derivative was also prepared from the acetonide **7**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The instruments used and conditions under which measurements were made, the initial handling of the plant extract, and the first column chromatographic separations are detailed in Ref. (1). The 2D INADEQUATE nmr experiment was performed with a 1/4J delay of 5.5 msec (7).

Amoenolide A 19-aldehyde [**2**].—Fraction No. 9 (4.0 g) from the first column separation of the terpenes from the MeOH-H_2O (9:1) solubles was adsorbed into 10 g of Si gel 60 from $CHCl_3$ and added to a 90-g column of Si gel 60 poured in hexane. Elution was with mixtures of hexane-Me_2CO (4:1), (3:1), (2:1), (1:1), (1:2), and (1:3) and effluent fractions analyzed by tlc using the column solvent to give ten pooled fractions. The seventh fraction (1.19 g) that was eluted by the 1:1 mixture gave 56 mg of crystalline aldehyde **2** from that solvent. The mother liquor residue was separated on a Merck RP-8 reversed-phase column by elution with MeOH-H_2O (1:9), (1:4), (3:7), (2:3), (1:1), and (3:2). The 3:7 eluted fraction (353 mg) crystallized from hexane-Me_2CO (1:1) to give 312 mg of amoenolide A 19-aldehyde [**2**] (7.8×10^{-3}% of dried plant) as colorless needles: mp 149–150°, $[\alpha]^{25.5}D$ +47° (c=0.3, MeOH); ir (KBr) ν max 1785, 1755, 1735, 1715 (C=O), 1635 (C=C), 1450, 1045 (C-O) cm^{-1}; uv (MeOH) λ (end abs) 205 nm (log ϵ 4.19); fabms (glycerol) m/z 349 (MH^+, 0.3%); eims m/z 330.1800 (M^+-H_2O, 2, $C_{20}H_{26}O_4$ requires 330.1832), 312 (M^+-2H_2O, 1), 119 (32), 98 (36), and 41 (100). The ^{1}H- and ^{13}C-nmr data are given in Tables 1 and 2, respectively.

Amoenolide A 19-aldehyde 2,6-diacetate.—Amoenolide A 19-aldehyde [**2**] (5 mg) was acetylated with Ac_2O/pyridine and worked up at previously described (1). The diacetate as a heavy oil had the following ^{1}H-nmr spectrum ($CDCl_3$, 250 MHz) δ 9.72 (1H, s, H-19), 5.87 (1H, br s, H-14), 5.54 (1H, ddd, J=12, 9, and 6 Hz, H-6), 4.96 (1H, dddd, J=12, 12, 4, and 4 Hz, H-2), 4.74 (1H, d, J=2 Hz, H-16), 2.59 (1H, dd, J=17 and 6 Hz, H-17β), 2.09 (3H, s, Ac), 2.04 (3H, s, Ac), 1.87 (1H, d, J=12 Hz, H-5), 1.61 (2H, s, H_2-17), 1.28 (2H, s, H_2-18), and 1.00 (2H, s, H_2-20).

Reduction of amoenolide A 19-aldehyde [**2**].—Amoenolide A 19-aldehyde [**2**] (15 mg) in 1 ml of MeOH at 0° was treated incrementally, while stirring, with $NaBH_4$ (22 mg) over 4 h until all of the starting material was gone as revealed by tlc with $CHCl_3$-MeOH-H_2O (16:3:1, lower phase). Addition of 1 ml 5% aqueous NH_4OH and 20 ml H_2O followed by $CHCl_3$ extraction (3×20 ml) gave from the $CHCl_3$ extract, after the removal of solvent, 15 mg of residue that crystallized from $CHCl_3$-MeOH (1:1). The crystals showed physical data (mp, $[\alpha]D$, ir, ^{1}H-nmr and tlc mobility) identical with amoenolide A [**1**].

PREPARATION AND SEPARATION OF EtOAc SOLUBLES.—The H_2O phase of the $CHCl_3/H_2O$ partitioning of the 955 g EtOH extract (1) was extracted in sequence with an equal volume of EtOAc (3×) and of n-BuOH (3×) to give, after evaporation of solvents, 46 g and 128 g of residues, respectively. The EtOAc solubles were chromatographed on Sephadex LH-20 in MeOH and monitored by tlc with the lower phase of $CHCl_3$-MeOH-H_2O (17:2:1). The fractions containing the terpenoids (blue and purple zones with p-anisaldehyde spray reagent) were combined to give 16.6 g of residue. This residue was adsorbed onto 60 g of Si gel 60 and added as a powder to a 600-g column of Si gel 60 poured in $CHCl_3$. The column was eluted with mixtures of MeOH-$CHCl_3$ (1:199), (1:99), (1:49), (1:9), and (1:4). Effluent fractions were analyzed by tlc using column solvents to give 16 pooled fractions designated fractions 1 through 16.

Fraction 11 (2.49 g) on 6 g of Si gel was added to a 65-g Si gel column packed in hexane and eluted by hexane-Me_2CO (3:1), (2:1), (1:1), (2:3), (1:2), (2:5), and (1:3) to give fractions 11-A to 11-J as monitored by tlc.

[4]It is probable that this compound was formed on the Si gel column, since the adsorbent had previously been regenerated by a process which involved treatment with dilute HCl. The adsorbent surface, although thoroughly washed with H_2O, may have retained enough acid to catalyze the addition.

Amoenolide B [**3**].—The hexane-Me_2CO (2:1)-eluted column fraction 11-D (293 mg) was separated on a 10-g column of Sephadex LH-20 with H_2O-*i*-PrOH (1:14) to give 194 mg of a mixture which was next chromatographed on 8 g of Si gel with the lower phases of $CHCl_3$-MeOH-H_2O (17:2:1), (16:3:1), and (15:4:1). The second eluting solvent gave 77 mg of a fraction that was chromatographed on 7 g of Si gel in $CHCl_3$ with elution by MeOH-$CHCl_3$ (1:99) to give 29 mg of amoenolide B [**3**] as a homogeneous heavy oil (7.3×10^{-4}% of dried plant): $[\alpha]^{23.5}D$ +38° (*c*=0.5, MeOH); ir (KBr) ν max 1780 and 1740 (C=O), 1630 (C=C), 1460, 1380, 1180 and 1040 (C-O) cm^{-1}; uv (MeOH) λ (end abs) 205 nm (log ϵ 4.20); fabms (on glycerol) *m/z* 351 (MH^+, 0.5), 333 (MH^+-H_2O, 0.6), 315 (MH^+-2H_2O, 1) and 297 (MH^+-3H_2O, 0.8); eims *m/z* 330 (1), 314.1905 (M^+-2H_2O, 2, $C_{20}H_{26}O_3$ requires 314.1833), 219 (28), 95 (50), and 55 (100). The ^{1}H- and ^{13}C-nmr spectra are given in Tables 1 and 2, respectively.

Amoenolide C [**4**].—The first Si gel column separation (1) of the MeOH-soluble terpenes gave fractions 7-8 (2.1 g combined) which were further separated by Si gel chromatography using solvent mixtures of hexane-Me_2CO (4:1) to (1:3) to give 19 fractions, 7A–7S. The fraction 7L (399 mg) eluted by the 2:1 solvent mixture deposited 55 mg of crystalline amoenolide C [**4**] and the mother liquor residue on reversed-phase chromatography with a Merck RP-8 column and mixtures of MeOH-H_2O from 1:5 to 5:3 afforded from hexane-Me_2CO another 112 mg of needle-like crystals of amoenolide C [**4**] (total yield 1.6×10^{-2}% of dried plant): mp 176–177°; $[\alpha]^{23.5}D$ +62° (*c*=0.5, MeOH); ir (KBr) ν max 1800 and 1740 (C=O), 1630 (C=C), 1430, 1190, 1050 (C-O), and 900 cm^{-1}; uv (MeOH) λ (end abs) 203 nm (log ϵ 4.28); fabms (glycerol) *m/z* 335 (MH^+, 1); eims *m/z* 316.1980 (M^+-H_2O, 6, $C_{20}H_{28}O_3$ requires 316.2039), 298 (M^+-2H_2O, 1), 283 (32), 119 (49) and 69 (100); ^{1}H- and ^{13}C-nmr spectra are given in Tables 1 and 2, respectively.

Amoenolide D [**5**].—The column fraction 11-E (219 mg) from the EtOAc-soluble terpenes eluted by hexane/Me_2CO was chromatographed on Si gel with mixtures of $CHCl_3$-MeOH-H_2O (lower phase) (17:2:1), (16:3:1), and (15:4:1). The material eluted by the second solvent crystallized from $CHCl_3$-MeOH (1:1) to give 30 mg (7.5×10^{-4}% of dried plant) of amoenolide D as needle-like crystals: mp 192–193°; $[\alpha]^{23.5}D$ +52° (*c*=0.5, MeOH); ir (KBr) ν max 1740 (C=O), 1640 (C=C), 1440, 1390, 1070, 1040, and 1030 (C-O); uv (MeOH) λ (end abs) 205 nm (log ϵ 4.20); fabms *m/z* 367 (MH^+, 0.2), 349 (MH^+-H_2O, 0.4), 331 (MH^+-2H_2O, 1); eims *m/z* 331.1698 ($MH-2H_2O$, 1, $C_{20}H_{27}O_4$ requires 331.1910), 315 (3), 119 (22), 91 (23), and 83 (100). The ^{1}H- and ^{13}C-nmr spectra are given in Tables 1 and 2, respectively.

HOREAU METHOD ON AMOENOLIDE D [**5**].—Amoenolide D (3 mg, 8 μmol) in 0.25 ml of a 12.5% solution of 2-phenylbutanoic anhydride (31 mg, 0.10 mmol) in anhydrous pyridine was kept at ambient temp for 48 h. H_2O (3 drops) was added and after 30 min the solvents were removed at reduced pressure. The residue was extracted with $CHCl_3$ (3×5 ml). The $CHCl_3$ phase was evaporated to dryness at reduced pressure to give amoenolide D 2,6,12,19-tetra-2-phenylbutyrate as a heavy oil (7 mg, 7 μmol): fabms [on "magic bullet" (8) dithiothreitol-dithioerythritol (3:1)] *m/z* 973.5270 (MNa^+, 0.3, $C_{60}H_{70}O_{10}Na$ requires 973.4867), 949 (MH^+, 0.01), 809 ($MNa^+-C_{10}H_{12}O_2$, 0.2), 623 ($MH^+-2C_{10}H_{11}O_2$) and 91 (100); ^{1}H nmr ($CDCl_3$, 250 MHz) 7.33–7.15 (5H, m, ArH), 5.84 (1H, br s, H-14), 5.61 (1H, br dd, *J*=11 and 11 Hz, H-12), 5.16 (1H, ddd, *J*=5, 5, and 13 Hz, H-6), 4.92 (1H, hm, H-2), 4.18 (1H, s, H-16), 3.38 (2H, m, H'$_2$-2), 1.61 (3H, s, Me) and 1.30 (3H, s, Me).

The aqueous phase was acidified with 10% HCl and extracted with $CHCl_3$ (3×5 ml). After washing with H_2O (2×20 ml), the $CHCl_3$ phase was evaporated to dryness at reduced pressure to give 13 mg (0.079 mmol) of 2-phenylbutyric acid $[\alpha]^{23.5}D$ −1.1° (*c*=0.6, MeOH), identical in mp and ^{1}H nmr with an authentic sample. Correction of the specific rotation for the excess reagent and the unresolved acid from acylation of the 6-OH and the 19-OH gave $[\alpha]D$ −6.6°. The Horeau method on amoenolide A [**1**] similarly corrected gives $[\alpha]D$ −23.1°. Thus, the partial resolution caused by the 12-OH contribution is the difference, or $[\alpha]D$ +16.5°. (+)-2-Phenylbutyric acid results from an asymmetric secondary alcohol that translates to a 12*R*-configuration for amoenolide D [**5**].

Amoenolide J [**6**].—Column fractions 10–12 from the first Si gel column of the MeOH solubles were combined (12.2 g) and separated on a Si gel column (258 g) with mixtures of hexane-Me_2CO (3:1), (2:1), (1:1), (1:2), and (1:3). The 2:1 eluted fraction (2.5 g) was next separated on Si gel (80 g) with lower phases of mixtures of $CHCl_3$-MeOH-H_2O (18:1:1), (17:2:1), and (16:3:1). The second solvent gave 1.39 g of amoenolide J [**6**] as a homogeneous oil. A similar series of column separations of the combined fractions 14–16 yielded additional material for a total of 3.25 g (0.48% of the dried plant) of amoenolide J [**6**]: $[\alpha]^{23.5}D$ +66° (*c*=0.4, MeOH); ir end abs, (KBr) ν max 1660 (C=C), 1450, 1380, and 1040 cm^{-1}; uv (MeOH) λ max 240 nm (log ϵ 3.51) and 202 nm (4.05); fabms (glycerol) *m/z* 339 (MH^+, 1); eims *m/z* 302.2252 (M^+-2H_2O, 3, $C_{26}H_{30}O_2$ requires 320.2247); cims (isobutane) *m/z* 339 (MH^+, 4), 321 (MH^+-H_2O, 5), 303 (MH^+-2H_2O, 44), 284 (MH^+-3H_2O, 100), and 267 (MH^+-4H_2O, 17). The ^{1}H- and ^{13}C-nmr spectra are given in Tables 1 and 2, respectively.

Amoenolide J tetraacetate.—Amoenolide J [**6**] (15 mg) was acetylated by Ac_2O/pyridine under the

conditions and workup as previously reported (1). The tetraacetate was a heavy oil (15 mg): $[\alpha]^{23.5}D$ +27° (c=0.7, MeOH); ir ($CHCl_3$) ν max 1740 (C=O), 1370, 1250 (acetate C-O), and 1030 (C-O) cm^{-1}; fabms ["magic bullet" (8)] *m/z* 507 (MH^+, 0.4), 447 (MH^+-AcOH, 0.7), 387 ($MH^+-2AcOH$, 0.8), 327 ($MH^+-3AcOH$, 3); eims *m/z* 476.2353 (M^+-2Me, 0.3, $C_{26}H_{36}O_8$ requires 476.2410), 43 (MeCO, 100); ^1H nmr (270 MHz, $CDCl_3$) 5.34 (1H, ddq, J=7.4, 7.4, and 1.3 Hz, H-14), 5.20 (1H, ddd, J=11.7, 8.7, and 6.6 Hz, H-6), 5.05 (1H, ddd, J=10.7, 10.7, 4.1, and 4.1 Hz, H-2), 4.56 (1H, dd, A of ABX, J=11.9 and 7.6 Hz, H-15a), 4.52 (1H, dd, B of ABX, J=11.9 and 7.6 Hz, H-15b), 4.10 (1H, d, A of ABq, J=10.8 Hz, H-18a), 3.82 (1H, d, B of ABq, J=10.8 Hz, H-18b), 2.52 (1H, dd, J=17.2 and 6.5 Hz, H-7β), 2.2–1.6 (7H, hm, H-1β, H-3β, H-7α, H_2-11, and H_2-12), 2.09 (3H, s, Ac), 2.05 (3H, s, Ac), 2.04 (3H, s, Ac), 2.03 (3H, s, Ac), 1.86 (1H, d, J=12 Hz, H-5), 1.79 (3H, br s, Me-16), 1.61 (3H, s, Me-17), 1.52 (1H, dd, J=12.3 and 12.3 Hz, H-3α), 1.34 (1H, dd, J=12.3 and 12.3 Hz, H-1α), 1.16 (3H, s, Me-19), and 0.99 (3H, s, Me-20).

Amoenolide J 6,18-acetonide [7].—The combined column fractions 14–16 (25.0 g) from the first Si gel column of the MeOH solubles were chromatographed on 500 g of Si gel with hexane-Me_2CO mixtures (3:1), (2:1), (1:1), (1:2), and (1:3) to give, after tlc monitoring, fractions 14A to 14I. Fraction 14F (4.2 g) eluted with the 1:2 mixture was separated on 100 g of Si gel with the lower phase of $CHCl_3$-MeOH-H_2O (18:1:1), (17:2:1), (16:3:1), and (15:4:1) to give from the first solvent 101 mg of acetonide **7** as a heavy oil: $[\alpha]^{23.5}D$ +11° (c=0.6, MeOH); ir ($CHCl_3$) ν max 3420 (OH), 1670 (C=C), 1390, 1220, 1030 (C-O), and 910 cm^{-1}; uv (MeOH) λ max (end abs) 202 nm (log ε 3.93); fabms (glycerol) *m/z* 379 (MH^+, 0.8), eims *m/z* 360.2603 (M^+-H_2O, 0.3, $C_{23}H_{26}O_3$ requires 360.2666), 302 ($M^+-H_2O-Me_2CO$, 8), 284 ($M^+-2H_2O-Me_2CO$, 5), 189 (100); and the ^1H- and ^{13}C-nmr spectra are in given Tables 1 and 2, respectively.

Amoenolide J 6,18-acetonide 2,15-diacetate.—Acetylation of acetonide **7** (20 mg) by Ac_2O/pyridine and subsequent workup was as previously described (1). The diacetate (24 mg) was a heavy oil: $[\alpha]^{23.5}D$ −13° (c=0.7, MeOH); ir ($CHCl_3$) ν max 1740 (C=O), 1460, 1390, 1250 (acetate C-O), 1070, and 1030 (C-O) cm^{-1}; fabms [magic bullet (8)] *m/z* 485 (MNa^+, 1%); eims *m/z* 386.2467 ($M^+-C_3H_8O_2$, 0.4, $C_{24}H_{36}O_4$ requires 386.2458), 149 (10), 131 (19), 109 (18) and 43 (100, Ac); ^1H nmr (270 MHz, $CDCl_3$) 5.33 (1H, ddq, J=7.3, 7.3, and 1.3 Hz, H-14), 5.23 (1H, dddd, J=11.7, 11.7, 4.4, and 4.4 Hz, H-2), 4.56 (1H, dd, A of ABX, J=12.4 and 7.4 Hz, H-15a), 4.51 (1H, dd, B of ABX, J=12.4 and 7.4 Hz, H-15b), 4.10 (1H, ddd, J=9.7, 9.7, and 3.5 Hz, H-6), 3.57 (1H, d, A of ABq, J=11.6 Hz, H-18a), 2.93 (1H, d, B of ABq, J=11.7 Hz, H-18b), 2.59 (1H, dd, J=18.8 and 9.4 Hz, H-7α), 2.2–1.9 (6H, hm, H-1β, H-7β, H_2-11 and H_2-12), 2.041 (3H, s, Ac), 2.037 (3H, s, Ac), 1.79 (3H, s, Me-16), 1.64 (3H, s, Me-17), 1.38 (1H, dd, J=11.7 and 11.7 Hz, H-3α), 1.35 (3H, s, Me of acetonide), 1.293 (1H, d, J=9.0 Hz, H-5), 1.290 (3H, s, Me of acetonide), 1.18 (3H, s, Me-19), 1.00 (1H, dd, J=11.7 and 11.7 Hz, H-1α), and 0.96 (3H, s, Me-20).

ACKNOWLEDGMENTS

We thank Dr. C.E. Cottrell for the nmr spectra at 500 MHz and the INADEQUATE experiment, and Mr. C.R. Weisenberger and Mr. D. Chang for the mass spectra obtained at The Ohio State University Chemical Instrument Center. The Ft-nmr spectra at 11.75 Tesla (500 MHz) were obtained at the Center using equipment funded in part by NIH Grant #1-S10 RR01458-01A1 (AM-500 only).

LITERATURE CITED

1. D.P. O'Mathúna and R.W. Doskotch, *J. Nat. Prod.*, **57**, 767 (1994).
2. D. Neuhaus, *J. Magn. Reson.*, **53**, 109 (1983).
3. M. Kinns and J.K.M. Sanders, *J. Magn. Reson.*, **56**, 518 (1984).
4. F.W. Wehrli, A.P. Marchand, and S. Wehrli, "Interpretation of Carbon-13 Nmr Spectra," John Wiley and Sons, New York, 1988, 2nd Ed., p. 52.
5. H. Kessler, C. Griesinger, J. Zarbock, and H.R. Loosli, *J. Magn. Reson.*, **57**, 331 (1984).
6. A. Horeau, in: "Stereochemistry, Fundamentals and Methods." Ed. by H.B. Kagan, Georg Thieme, Stuttgart, 1977, Vol. 3, pp. 51–94.
7. D. Piveteau, M.-A. Delsuc, and J.-Y. Lallemand, *J. Magn. Reson.*, **63**, 255 (1985).
8. J.L. Witten, M.H. Schaffer, M. O'Shea, J.C. Cook, M.E. Hemling, and K.L. Rinehart, Jr., *Biochem. Biophys. Res. Commun.*, **124**, 350 (1984).

Received 14 March 1994