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NEW LABDANE DITERPENE GLYCOSIDES 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 diterpene glycosides, amoenolide A 19-β-D-glucopyranoside [**1**], and amoenolides E [**3**], F [**4**], G [**6**], and H [**8**] were isolated from the above-ground parts of *Amphiachyris amoena*. Amoenolide F is a 19-β-D-glucopyranoside, while the others are 6-α-L-arabinopyranosides. Their structures were established by chemical and spectral methods, especially high-field nmr techniques, and complete ^{1}H- and ^{13}C-nmr assignments are given.

The above-ground parts of the Composite, *Amphiachyris amoena* (Shinners) Solbrig have yielded a number of hydroxylated labdane diterpenes, most of which contain the ethyl α,β-unsaturated γ-lactone system. They have been named amoenolides with their alphabetical designation given roughly in the order of isolation, except when they are simple derivatives of a common structure. Studies on nine amoenolides have been published (1,2) and this report is on five new glycosides possessing labdane diterpenes as aglycones.

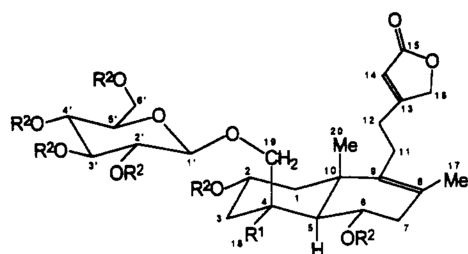
RESULTS AND DISCUSSION

Amoenolide A 19-β-D-glucopyranoside [**1**] was obtained as an amorphous residue from the *n*-BuOH-soluble partition fraction after extensive chromatography. The fabms supported the molecular formula of $C_{26}H_{40}O_{10}$, and the ^{1}H- and ^{13}C-nmr spectra were similar to those of the amoenolide A-type compounds (1). Its very polar nature and the additional peaks in the ^{1}H- and ^{13}C-nmr spectra located in the oxygenated regions pointed to a glycosidic structure, and extensive 1D and 2D nmr studies supported this assertion.[3] A direct comparison of the ^{13}C-nmr spectrum to that of methyl β-D-glucopyranoside showed the additional six carbon peaks of the glycoside to be essentially the same. Homonuclear ^{1}H-decoupling revealed the glucosyl protons as a single coupled unit with coupling constants consistent with axial positions in a chair conformation. Furthermore, the anomeric doublet (H-1′) at 4.89 ppm when irradiated in a nOe difference experiment (3) showed relaxation to H-3′ (5%) and H-5′ (9%) thus establishing the β-configuration for the glycosyl oxygen. A small (1%) but significant relaxation was also observed to one C-19 proton (3.66 ppm). This, and other nOe results, supported the glucosyl attachment to C-19; for example, irradiation at H-19 (3.66 ppm) showed 6% relaxation to H-1′, and irradiation at H_3-18 (1.72 ppm) gave 2% enhancement of H-1′. The ^{1}H- and ^{13}C-nmr assignments for amoenolide A 19-β-D-glucopyranoside [**1**] are given in Tables 1 and 2, respectively. When the ^{13}C-nmr values were compared, C-19 (76.97 ppm) of the glucoside was located 9.2 ppm downfield from C-19 in amoenolide A, a shift typically observed on glycosylation (4,5). Also, a 3-bond coupling from C-1′ (105.66 ppm) to H-19 (3.66 ppm) was observed in the COLOC (2- to 4-bond CH-coupling) nmr experiment. The lack of a downfield shift for H_2-19 in the ^{1}H-nmr

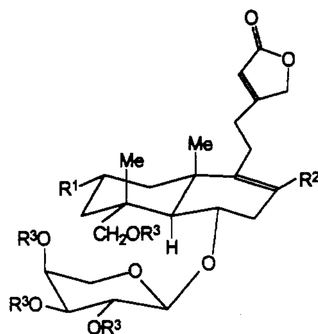
[1]Taken in part from the Ph.D. Dissertation of Dónal P. O'Mathúna as accepted by the Graduate School, The Ohio State University, in August, 1988.

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

[3]A summary of the results of these studies is available from the senior author.



1 R^1=Me, R^2=H
2 R^1=Me, R^2=Ac
4 R^1=CH_2OH, R^2=H
5 R^1=CH_2OAc, R^2=Ac



3 R^1=OH, R^2=Me, R^3=H
6 R^1=R^3=H, R^2=CH_2OH
7 R^1=H, R^2=CH_2OAc, R^3=Ac

spectrum and deshielding of 0.76 ppm and 0.45 ppm for carbinyl protons H-2 and H-6 in the peracetate **2** gave further support for glucosylation at C-19. Acid hydrolysis of glucoside **1** afforded a sugar with positive optical rotation for D-stereochemistry and, when co-chromatographed with the common aldohexoses on tlc, migrated with the same R_f and developed the same color with the detecting reagent as glucose.

Amoenolide E [**3**], also from the *n*-BuOH-soluble partition fraction, has the formula $C_{25}H_{38}O_9$ as supported by fabms. Comparison of its ^{1}H- and ^{13}C-nmr spectra with those of amoenolide A 19-glucoside [**1**] showed the two to be closely related. The difference of CH_2O in the molecular formula from that of glycoside **1** was indicative of a pentose replacing glucose as the sugar unit, and the ^{1}H-nmr decoupling studies revealed the six-proton spin system of a pentose.[3] The sugar component was established as α-L-arabinopyranosyl as follows: The anomeric proton (H-1′, 4.91 ppm) must be axial because the one-bond coupling ($^1J_{CH}$) to C-1′ (106.21 ppm), as determined from a fully ^{1}H-coupled ^{13}C-nmr spectrum, was 157 Hz, a value closer to the reported 160 MHz for α-arabinose than 168.9 Hz for β-arabinose. Anomeric axial protons of pyranoses show consistently lower values by about 10 Hz than anomeric equatorial protons (6,7). In a nOe experiment, irradiation of H-1′ (4.91 ppm) gave 8% relaxation to H-3′ (4.20 ppm) and to one H-5′ (3.84 ppm); a result requiring the H-3′ and H-5′ protons to be axial and the pyranose ring to be in the $^4C_1$ chair conformation. The size of the coupling constants (J=8.2 and 3.8 Hz) for the axial H-3′ was in accord with H-2′ being trans diaxial and H-4′ cis equatorial. Thus, the relative stereochemistry of the four asymmetric centers for the glycosyl unit corresponds to arabinose. Acid hydrolysis of amoenolide E [**3**] gave a sugar that on tlc migrated with arabinose and developed the same color as arabinose with the spray reagent. Its positive optical rotation places it in the Rosanoff L-series. In addition, comparison of the glycosyl component in the ^{1}H- and ^{13}C-nmr spectra of amoenolide E [**3**] with methyl α-L-arabinopyranoside showed them to be nearly identical.

Extensive 1D and 2D nmr experiments, not detailed here, showed the aglycone part of amoenolide E [**3**] to have the same ^{1}H-coupled units as amoenolide A (1), but there were significant chemical shift differences. These differences were shown to be a result of an epimeric change at C-4 and the glycosyl attachment of the C-6 hydroxyl. For example, irradiation at H_3-20 (1.21 ppm) in a nOe experiment gave 4% relaxation to H_3-19 (1.28 ppm), 8% to H-2 (4.37 ppm), 9% to H-6 (4.29 ppm), 4% to H-1β, and 3% to H-7β. These definied the β-face of the molecule. The hydroxymethyl protons were located when H_3-19 (1.28 ppm) was irradiated to give 3% relaxation to each of these protons (4.60 and 3.58 ppm), as well as to H-2 (4%), H-6 (6%), and H-1′ (2%). The

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

Proton	Compound							
	1	**2**[b,c]	**3**	**4**	**5**[b,d]	**6**	**7**[b,e]	**8**
H-1 . .	1.43α dd (11.3,11.3) 2.35β hm	1.29α dd (11.6,11.6) 2.05β hm	1.53α dd (11.4,11.4) 2.41β ddd (11.7,3.3,1.7)	1.47α dd (11.5,11.5) 2.41β hm	1.29α dd (11.5,11.5) 2.05β hm	1.26α ddd (12.5,12.5,3.1) 1.74β br d (12.5)	2.4–1.2 (2H) hm	1.44α ddd (12.6,12.6,3.2) 1.57β br d (12.0)
H-2 . .	4.19 hm	4.95 dddd (11.8,11.8,3.9,3.9)	4.37 dddd (11.5,11.5,4.2,4.2)	4.28 dddd (11.7,11.7,4.0,4.0)	4.98 hm	1.52α ddddd (13.7,3.4,3.4,3.4,3.4) 1.68β ddddd (13.5,13.5,13.5, 3.0,3.0)	2.4–1.2 (2H) hm	1.434α ddddd (13.7,3.2,3.2,3.2,3.2) 1.65β ddddd (12.6,12.6,12.6, 3.1,3.1)
H-3 . .	1.50α dd (12.5,12.5) 2.17β hm	1.06α dd (12.6,12.6) 2.05β hm	2.33α dd (12.0,12.0) 2.07β ddd (12.6,4.0,1.8)	1.86α dd (12.5,12.5) 2.44β hm	1.36α dd (12.5,12.5) 2.05β hm	2.00α ddd (13.3,13.3,4.1) 1.37β br d (13.2)	2.4–1.2 (2H) hm	1.99α ddd (13.5,13.5,4.3) 1.30β br d (13.4)
H-5 . .	1.58 d (11.0)	1.69 d (11.8)	2.37 d (10.4)	2.00 d (11.1)	1.97 d (11.3)	2.27 d (9.7)	1.69 d (10.9)	2.08 d (10.0)
H-6 . .	4.64 ddd (10.2,10.2,6.2)	5.19 ddd (12.0,8.6,6.6)	4.29 ddd (10.1,6.9,6.9)	4.62 ddd (10.3,10.3,6.3)	5.20 hm	4.33 hm [ddd] (9.9,6.7,6.7)	3.93 ddd (10.8,9.5,6.0)	4.11 ddd (10.1,10.1,3.7)
H-7 . .	2.37α hm 2.47β dd (17.2,5.8)	2.05α hm 2.54β dd (17.5,6.5)	2.69α dd (17.6,7.5) 2.90β dd (17.6,6.0)	2.35α dd (17.4,6.0) 2.44β dd (17.3,10.7)	2.05α hm 2.54β dd (17.5,6.4)	3.05α dd (17.5,7.0) 3.32β dd (17.5,5.9)	2.33α dd (17.6,8.7) 2.69β dd (17.6,5.9)	2.28α dd (12.0,10.9) 3.01β dd (12.7,3.7)
H-11 .	2.15 hm 2.25 m	2.18 m 2.32 hm	2.26 (2H) m	2.16 ddd (12.8,11.6,5.6) 2.25 m	2.18 m 2.30 m	2.25 m 2.47 m	2.4–1.2 (2H) hm	1.54 hm 1.90 m
H-12 .	2.36 (2H) hm	2.40 (2H) hm	2.22 (2H) m	2.25 (2H) hm	2.40 (2H) hm	2.40 (2H) m	2.45 (2H) m	2.41 ddd (16.6,11.5,4.6) 2.75 ddd (16.6,11.0,5.3)
H-14 .	5.95 s ($\omega_{1/2}$ 5.6)	5.88 m (5pk) (1.2)	5.93 m (5pk) (1.7)	5.95 s ($\omega_{1/2}$ 4.2)	5.87 m (5pk) (1.8)	5.98 m (5pk) (1.4)	5.87 m (5pk) (1.5)	6.01 m (5pk) (1.4)
H-16 .	4.70 d (17.4) 4.74 d (17.4)	4.75 (2H) d (1.6)	4.67 dd (17.4,1.7) 4.71 dd (17.4,1.7)	4.70 dd (17.4,1.6) 4.74 dd (17.4,1.6)	4.75 (2H) d (1.7)	4.76 (2H) d (1.6)	4.75 (2H) d (1.7)	4.74 dd (17.4,1.6) 4.82 dd (17.4,1.6)
H-17 .	1.54 s	1.58 s	1.51 s	1.52 s	1.58 s	4.26 d (11.9) 4.39 d (12.2)	4.49 d (12.1) 4.52 d (12.1)	1.40 s

TABLE 1. Continued.

Proton	Compound							
	1	2[b,c]	3	4	5[b,d]	6	7[b,e]	8
H-18 .	1.72 s	1.19 s	3.58 d (10.7) 4.60 d (10.7)	4.24 d (10.9) 4.37 d (10.9)	4.05 d (11.4) 4.27 d (11.2)	3.47 d (10.9) 4.43 d (10.8)	4.08 d (10.8) 4.10 d (10.8)	3.35 d (10.9) 4.49 d (10.9)
H-19 .	3.66 d (11.0) 4.79 d (10.9)	3.57 d (9.2) 3.73 d (9.2)	1.28 s	3.99 d (11.3) 4.72 d (11.5)	3.63 d (9.5) 3.91 d (9.6)	1.14 s	1.04 s	1.05 s
H-20 .	1.23 s	1.12 s	1.21 s	1.27 s	1.14 s	1.13 s	1.12 s	0.92 s
H-1′ .	4.89 d (7.8)	4.50 d (8.0)	4.91 d (6.4)	4.93 d (7.7)	4.48 d (8.0)	4.90 d (6.5)	4.63 d (6.2)	4.97 d (5.8)
H-2′ .	4.04 dd (8.2,8.2)	4.96 dd (9.6,8.0)	4.42 dd (8.2,6.6)	4.04 m	4.86 dd (9.5,8.0)	4.39 dd (7.7,6.1)	5.17 dd (8.7,6.2)	4.42 dd (7.5,5.9)
H-3′ .	4.21 dd (8.9,8.9)	5.20 dd (9.5,9.5)	4.20 dd (8.2,3.8)	4.15 m	5.18 dd (9.5,9.5)	4.18 dd (8.2,3.5)	5.08 dd (8.6,3.5)	4.25 dd (7.6,3.4)
H-4′ .	4.17 dd (8.9,8.9)	5.05 dd (9.7,9.7)	4.35 hm	4.17 m	5.03 dd (9.7,9.7)	4.32 hm	5.24 ddd (3.7,3.7,3.7)	4.38 ddd (4.5,3.4,2.2)
H-5′ .	3.96 m	3.69 ddd (10.0,5.0,2.3)	3.84 m 4.35 hm	3.91 ddd (9.2,5.4,2.4)	3.67 ddd (9.8,5.0,2.4)	3.75 dd (12.0,1.8) 4.27 dd (12.0,3.6)	3.63 dd (12.8,2.0) 4.02 dd (12.8,3.9)	3.75 dd (11.7,2.2) 4.30 dd (11.8,4.5)
H-6′ .	4.35 dd (11.7,5.4) 4.54 dd (11.6,1.7)	4.12 dd (12.2,2.2) 4.23 dd (12.2,5.0)		4.33 dd (11.9,5.4) 4.51 dd (11.9,2.3)	4.11 dd (12.3,2.3) 4.19 dd (12.3,5.0)			

[a]Taken at 500 MHz in pyridine-d_5 unless stated otherwise with data-point resolution of 0.3 Hz and chemical shifts (δ) in ppm as referenced to TMS with residual solvent peak (pyridine-d_4) upfield set at 7.19 ppm as internal standard. 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 a 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]Taken in $CDCl_3$ referenced at 7.26 ppm for residual $CHCl_3$.

[c]Acetates at 2.00, 2.02, 2.03 (2), 2.04, and 2.09 ppm.

[d]Taken at 270 MHz with acetates at 2.08, 2.06, 2.03, 2.02, 2.01, 2.00, and 1.98 ppm.

[e]Taken at 270 MHz with acetates at 2.14, 2.08, 2.06, 2.05, and 2.02 ppm.

TABLE 2. ^{13}C-Nmr Data for Compounds **1**, **3**, **4**, **6**, and **8**.[a]

Carbon	Compound					
	1	multiplicity	**3**	**4**	**6**	**8**
C-1	47.27	t	47.12	47.05	37.36	40.13
C-2	63.49	d	64.15	63.69	18.83 t	18.42 t
C-3	49.29	t	48.04	43.14	37.90	38.52
C-4	40.42	s	40.27	45.39	38.96	38.77
C-5	57.53	d	49.06	52.79	50.00	53.48
C-6	67.84	d	79.10	67.40	79.51	79.64
C-7	44.55	t	42.97	44.36	38.43	51.98
C-8	126.26	s	126.55	126.12	131.86	72.57
C-9	138.47	s	139.05	138.49	141.96	61.02 d
C-10	43.41	s	42.74	43.37	41.12	39.24
C-11	25.64	t	25.46	25.61	24.95	23.49
C-12	29.19	t	29.27	29.15	30.79	31.66
C-13	171.56	s	171.34	171.57	171.39	172.75
C-14	114.59	d	114.74	114.56	114.86	114.89
C-15	174.37	s	174.27	174.41	174.28	174.69
C-16	73.20	t	73.19	73.21	73.28	73.55
C-17	19.09	q	19.31	19.07	62.42 t	26.04
C-18	32.55	q	72.85 t	70.57 t	72.89 t	73.48 t
C-19	76.97	t	20.00 q	73.11 t	19.22 q	18.93
C-20	22.10	q	22.56	22.26	21.38	17.21
C-1′	105.66	d	106.21	105.60	106.04	105.51
C-2′	75.06	d	72.46	75.02	72.59	72.62
C-3′	78.29	d	74.58	78.23	74.57	74.34
C-4′	71.47	d	68.84	71.43	68.89	68.46
C-5′	78.20	d	66.13 t	78.36	66.13 t	65.55 t
C-6′	62.51	t		62.44		

[a]Taken at 67.9 MHz in pyridine-d_5 with multiplicities determined by SFORD. Chemical shifts (δ) in ppm were referenced to TMS with the upfield solvent peak taken as 123.5 ppm (center). Abbreviations are s=singlet, d=doublet, t=triplet, and q=quartet. Data point resolution was 0.7 Hz.

hydroxymethyl group, therefore, must be at C-18, and the arabinosyl unit on the decalin ring opposite to the lactone ring. That this attachment was at C-6 was supported by the 12% signal enhancement of H-6 (4.29 ppm) when H-1′ (4.91 ppm) was irradiated, and the lack of a downfield shift for H-6 when amoenolide E [**3**] was acetylated. Furthermore, C-6 was located at 79.10 ppm in the ^{13}C-nmr spectrum, while amoenolides lacking glycosyl groups at C-6 have values of 66.5–68.0 ppm (2). A downfield shift of 10–11 ppm has been reported for the α-carbon of secondary alcohols when the β-carbon has at least one equatorial proton (4). Thus, amoenolide E [**3**] is 4-*epi*-amoenolide A 6-α-L-arabinopyranoside.

Amoenolide F [**4**] with molecular formula $C_{26}H_{40}H_{11}$, as supported by fabms, was the third glycoside isolated from the *n*-BuOH partition fraction. The six carbons over the twenty required for the diterpene suggested a hexose, and comparison of the ^{1}H-nmr spectrum to that of glycoside **1** revealed a close relationship. The major spectral difference was the replacement of one methyl signal by two one-proton doublets. The preparation of heptaacetate **5** revealed an additional hydroxyl over glycoside **1** and explained the methyl loss with a replacement by a hydroxymethyl. Extensive 1D and 2D nmr studies, not reported here, revealed the coupled units to be the same as those of amoenolide A 19-β-D-glucopyranoside [**1**] with the addition of the two doublets of the hydroxymethyl on a quaternary carbon. Irradiation of the anomeric H-1′ (4.93 ppm) showed relaxations of 6% to H-3′ (4.15 ppm), 8% to H-5′ (3.91 ppm), and 4% to the

doublet of the aglycone at 3.99 ppm. The large coupling constants in the peracetate **5** revealed that protons H-1′ through H-5′ were all axial and with the nOe results require the sugar unit to be β-glucopyranosyl (and therefore D) in the $^4C_1$ chair conformation. Comparing the ^{13}C-nmr (Table 2) peaks of this unit with those of methyl β-D-glucopyranoside showed them to be nearly identical. Irradiation of the nonolefinic H_3-20 at 1.27 ppm gave relaxations of 2% to H-1β (2.41 ppm), 5% to H-2 (4.28 ppm), 7% to H-6 (4.62 ppm), 3% to one H-19 (4.72 ppm), and 1% to the other H-19 (3.99 ppm). Irradiation at 3.99 ppm (H-19) enhanced the anomeric H-1′ (4.93 ppm) by 5%, thereby placing the glucosyl unit at C-19. These results located the units on the β-face of the labdane ring.

The COLOC nmr results confirmed the glycosyl location, assigned the quaternary carbons, and located the hydroxymethyl group (C-18). For example, one H-19 (3.99 ppm) showed coupling to C-1′ (105.60 ppm), C-4 (45.39 ppm), and C-5 (52.79 ppm). The other H-19 (4.72 ppm) was coupled to C-18 (70.57 ppm), while both H-18 protons (4.72 and 4.37 ppm) were coupled to C-4 (45.39 ppm) and C-3 (43.14 ppm). Couplings from the methyl groups assigned C-8, C-9, and C-10. Thus, the spectral data support amoenolide F [**4**] as the 18-hydroxy derivative of amoenolide A 19-β-D-glucopyranoside [**1**].

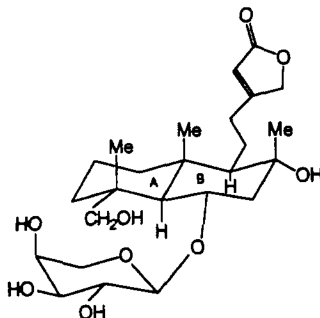
Amoenolide G [**6**] was obtained from the EtOAc partition fraction (1) of the EtOH extract residue after extensive chromatography, and has the same molecular formula as amoenolide E [**3**], $C_{25}H_{38}O_9$, as supported by fabms. Peracetylation produced a pentaacetate **7**. The ^{1}H- and ^{13}C-nmr studies (Tables 1 and 2) showed the presence of the α,β-unsaturated γ-lactone bearing an ethyl (dimethylene) side-chain as one coupled unit. The lactone peaks are at δ_H 5.98 (H-14) and 4.76 (H_2-16), and δ_C 174.28 (C-15), 171.39 (C-13), 114.86 (C-14), and 73.28 ppm (C-16). The ethyl group was revealed from the ^{1}H,^{1}H-COSY experiment in which allylic and homoallylic coupling was observed for H-14 and H-16 to H_2-12 (2.40 ppm). The H_2-12 signal was in turn coupled to H_2-11 (2.25 and 2.47 ppm). Another coupled unit was shown to be α-arabinopyranosyl from ^{1}H,^{1}H-COSY and nOe difference experiments. For example, starting with H-1′ (4.90 ppm) and following the coupling identified the sequence of protons to H_2-5′. Also, the nOe results showed H-1′ relaxes to H-3′ (4.18 ppm) by 3% and to one H-5′ (3.75 ppm) by 5%, thus placing these protons at axial positions and requiring the pyranose ring to be in the $^4C_1$ conformation. Examination of the J values revealed that H-4′ (4.32 ppm), although partially hidden, is a multiplet with three small coupling constants (3.6, 3.5, and 1.8 Hz) and must, therefore, be equatorially disposed. With the four asymmetric centers (C-1′, -2′, -3′, and -4′) established in relative stereochemical terms, the sugar must be α-arabinose and in the enantiomeric L-series (8). Paucity of starting material precluded hydrolysis of glycoside **6** for confirmation of this. The $^1J_{CH}$ coupling of 156.1 Hz for C-1 from the fully ^{1}H-coupled ^{13}C-nmr spectrum supported the axial H-1 position in the pyranose form (6,7). In unraveling the spectral patterns in the ^{1}H-nmr spectrum at 4.5–4.2 ppm the 2D nmr homonuclear J-resolved spectrum (9) and the CH-correlation experiment were utilized. Also, direct comparison of the ^{1}H- and ^{13}C-nmr spectra of the glycosyl unit to those of methyl β-D-arabinopyranoside and methyl α-L-arabinopyranoside showed the latter to be essentially the same. Thus, the lactone and sugar units of amoenolide G [**6**] are the same as for amoenolide E [**3**], but the central core has several differences as indicated by the major changes in the ^{1}H-nmr spectrum.

Within the same bicyclic labdane skeleton of the amoenolides already discussed, two major ^{1}H-coupled units could be readily accommodated. One, a three-methylene component was located at C-1 to C-3 and the other the C-5 to C-7 component. Also, two sets of double doublets (AX patterns) were observed at 4.43 and 3.47 ppm (J=10.8 Hz)

and at 4.39 and 4.26 ppm (J=12.1 Hz) for two hydroxymethyl groups each located on a quaternary carbon and showing downfield shifts upon acetylation. The remaining protonated carbons were two nonolefinic methyl groups with close chemical shifts (1.14 and 1.13 ppm). Location of the hydroxymethyl with δ 4.39 and 4.26 ppm at C-17 was based on the former proton showing relaxation to H-7 (3.05 ppm) in a NOESY experiment, and the H-7 chemical shifts located downfield by about 0.4 ppm from those in amoenolide E [**3**], as expected if H-7 is β to a hydroxymethyl instead of a methyl. The other hydroxymethyl (4.43 and 3.47 ppm) was located as C-18 by nOe difference studies. Irradiation at 4.43 ppm gave 4% relaxation to H-5 (2.27 ppm), 20% to the other proton (3.47 ppm) of the hydroxymethyl, and 3% to the methyl at 1.14 ppm. Irradiation at 3.47 ppm gave 19% enhancement of the geminal proton (4.43 ppm), and 3% each of H-3β (1.37 ppm) and the methyl at 1.14 ppm. Irradiation at the 1.14 ppm methyl enhanced the hydroxymethyl protons 4.43 and 3.47 ppm by 2% and 3%, respectively, as well as H-6 (4.43 ppm) by 7%, H-2β (1.68 ppm) by 3%, and H-3β (1.37 ppm) by 2%. These results also located the β-face protons, as did irradiation of the other methyl group (1.13 ppm), which showed enhancements of H-6 (4.33 ppm) by 8%, H-2β (1.68 ppm) by 6%, H-1β by 2%, H-7β by 3%, and one H-11 (2.25 ppm) by 5%. Clearly, the substitution pattern for the bicyclic ring of amoenolide G [**6**] is that of the previous labdanes with the methyls at C-19 (1.14 ppm) and C-20 (1.13 ppm). The nOe results also differentiated H-1 from H-3 in the symmetrical trimethylene unit (C-1 through C-3) and confirmed the axial location of H-2β, which was also suggested by its coupling pattern formed from three large (J=13.5 Hz) and two small (J=3.0 Hz) coupling constants.

The 2D nmr CH-correlation experiment identified the geminal protons of the methylenes and the COLOC experiment identified the quaternary carbons and confirmed the placement of the substituents from the other experiments. The pertinent results are: three-bond coupling was observed from H_3-20 (1.13 ppm) to the olefinic carbon at 141.96 ppm placing it at C-9 and 2-bond coupling to the quaternary carbon at 41.12 ppm to locate it at C-10. Similarly, H_3-19 (1.14 ppm) showed 2-bond coupling to the quaternary carbon at 38.96 ppm to assign it to C-4. The other olefinic carbon (C-8, 131.86 ppm) was located by 2-bond couplings from both C-7 protons (3.32 and 3.05 ppm). Attachment of the α-arabinopyranosyl unit to the oxygen at C-6 was established from the H-1′ irradiated (4.90 ppm) nOe experiment which gave a 13% enhancement of H-6 (4.33 ppm), and from the 3-bond coupling of H-6 to C-1′ (106.04 ppm) in the COLOC experiment.

Amoenolide H [**8**] has nearly the same chromatographic properties as amoenolide G [**6**] and was separated from it by repeated chromatography. Its molecular formula $C_{25}H_{40}O_9$, as supported by fabms, is two hydrogens more than amoenolide G [**6**] and thus has one degree less of unsaturation. The ^{13}C-nmr spectrum showed only three peaks above



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110 ppm and which were of the lactone unit (olefin and carbonyl). Thus, the second double bond present in the other four glycosides of this report is absent. Extensive 1D and 2D nmr experiments were performed, details of which are not given, and led to the structure assignment. Comparison of the results with those of amoenolide G [**6**] showed the following similarities: there is present an α,β-unsaturated γ-lactone with an ethyl side-chain, an α-L-arabinopyranosyl unit and ring A with the same substitution. The extra methyl and lack of one hydroxymethyl along with no change in the proton coupled unit from H-5 to H-7, suggested the presence of a tertiary hydroxyl if two hydroxyls were to be located in the bicyclic system. Its location at C-8 rather than C-9 was consistent with the absence of a methyl doublet. The proton on C-9 was established from the H-11 patterns and the $^{1}H,^{1}H$-COSY experiment which showed that the H_2-11 and H_2-12 resonances were now part of a five-spin system. One unobscured H-11 (1.90 ppm) was a non-first-order pattern (9 peaks) rather than the expected split triplet (ddd) of a four-spin system, and upon double irradiation the narrow one-proton multiplet at 1.49 ppm collapsed to a somewhat broadened singlet, thereby identifying H-9. Its disposition to the α-face of the molecule was made by the 4% signal enhancement in a nOe experiment when H-5 (2.08 ppm) was irradiated, along with enhancements of H-7α (2.28 ppm) by 2% and one H-18 (4.49 ppm) by 1%.

The three methyl groups, all singlets in the ^{1}H-nmr spectrum, were shown to be on the β-face of the molecule from nOe difference experiments at 270 MHz. Irradiation at 1.05 ppm (H_3-19) enhanced both H-18 signals (3.35 ppm) by 3% and (4.49 ppm) by 2%, H-6 (4.11 ppm) by 4% and the methyl at 0.92 ppm (assigned as H_3-20) by 5%. Irradiation of the methyl at 0.92 ppm (H_3-20) enhanced H-6 (4.11 ppm) by 7%, H-1β (1.57 ppm) and H-2β (1.65 ppm) together by 13%, H_3-19 (1.05 ppm) by 5% and the third methyl (1.40 ppm), assigned as H_3-17, by 7%. Irradiation of H_3-17 enhanced H-7β (3.01 ppm) by 4%, H-6 (4.11 ppm) by 5%, and H_3-20 (0.92 ppm) by 5%. Incidentally, the $^{1}H,^{1}H$-COSY experiment showed 4-bond long-range *W*-coupling of the 1.40 ppm methyl (H_3-17) to H-7α. The nOe studies also provided evidence for placing the α-L-arabinopyranosyl group at the C-6 oxygen. Irradiation at the anomeric proton (H-1′, 4.97 ppm) caused signal enhancements of H-6 (4.11 ppm) by 7%, H-18 (4.49 ppm) by 2%, H_3-19 (1.05 ppm) by 1%, and H-7β (3.01 ppm) by 3%. Also, the reciprocal irradiations of H-6, H-7, and H_3-19 caused H-1′ to be increased by 9, 3, and 1%, respectively.

The COLOC experiment assigned the quaternary carbons except C-13 and C-15 which came from the fully ^{1}H-coupled ^{13}C-nmr spectrum where C-15 appeared as a doublet with $^{2}J_{CH}=9.3$ Hz from coupling to H-14, while C-13 was a broadened singlet from coupling to five protons (H_2-12, H-14, and H_2-16). This method was used for assigning the C-13 and C-14 quaternary carbons in the other glycosides. In each case the downfield carbon (174 ppm) was a sharp doublet ($J=13$–16 Hz) while the other (171–172 ppm) was a broadened singlet ($\omega_{1/2}=13$–16 Hz) and hence were designated C-15 and C-13, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The instruments used and the conditions under which measurements were made are recorded by O'Mathúna and Doskotch (1).

CHROMATOGRAPHY OF *n*-BuOH SOLUBLES.—Preliminary fractionation procedures for *Amphiachyris amoena* have been reported (1). A 25-g sample of the *n*-BuOH-soluble fraction was chromatographed on 180 g of Sephadex LH-20 with MeOH. The effluent fractions were monitored by tlc using $CHCl_3$-MeOH-H_2O (17:2:1) as solvent and *p*-anisaldehyde as detecting reagent. The fractions giving the blue and purple zones, representing the terpenes, were pooled to give 8.9 g of residue. This material in MeOH was mixed with 15 g of Si gel and the solvent removed at reduced pressure. The resultant powder was added to a 250-g Si gel

60 column poured in $CHCl_3$-MeOH-H_2O [(17:2:1), lower phase] and eluted with solvents of composition (18:1:1), (17:2:1), (16:3:1), (15:4:1), and (14:5:1). Effluent fractions were pooled according to tlc results using the column solvent systems to give 16 fractions designed fractions A through P.

Amoenolide A 19-β-D-glucopyranoside [**1**].—Column fraction J (1.58 g) was separated on a reversed-phase RP-8 column by addition as a 50% solution in MeOH to a H_2O-equilibrated column and elution by the following solvent mixtures: MeCN-H_2O (1:39), (1:19), (1:9), (1:4), and (2:3). The (1:19) solvent system gave 1.33 g of a fraction detected by tlc with $CHCl_3$-MeOH-H_2O (15:4:1), that was separated on a Si gel column (37 g) with MeCN-Me_2CO-H_2O (39:1:1) and (38:2:1) as solvents. The latter solvent gave 611 mg (7.5×10^{-2}% of dried plant) of amoenolide A 19-glucoside [**1**] as an amorphous gum: $[\alpha]^{23.5}D$ +26° (c=0.5, MeOH); ir (KBr) ν max 1790 and 1750 (C=O), 1640 (C=C), 1440, 1390, 1080, and 1040 (C-O), and 900 cm^{-1}; uv (MeOH) λ (end abs.) 205 nm (log ϵ 4.23); fabms (glycerol) m/z 535.2302 (1, MNa^+, $C_{26}H_{40}O_{10}Na$ requires 535.2518), 459 (1, MH^+-$3H_2O$), 333.1941 (2, M-$C_6H_{11}O_6$, $C_{20}H_{29}O_4$ requires 333.2067), 315 (13, M-$C_6H_{11}O_6$-H_2O), 159 (19), 135 (19), and 93 (100); ^1H- and ^{13}C-nmr spectral data shown in Tables 1 and 2, respectively.

Amoenolide A 19-β-D-glucopyranoside peracetate [**2**].—A 15-mg sample of glucoside **1** was acetylated under conditions previously described (1). The amorphous product (15 mg) showed: $[\alpha]^{23.5}D$ +25° (c=0.5, MeOH); ir ($CHCl_3$) ν max 1790 and 1760 (C=O), 1370, 1230 (Ac, C-O), and 1040 (C-O) cm^{-1}; fabms ["magic bullet" (10,11)] m/z 787 (2, MNa^+), 705 (0.2, MH^+−HOAc), 645 (0.6, MH^+−2HOAc), 331 [38, $C_6H_7O_5(Ac)_4$] and 169 (100); ^1H-nmr spectrum shown in Table 1.

Hydrolysis of amoenolide A 19-β-D-glucopyranoside [**1**].—Glucoside **1** (21 mg) in 2 ml each of MeOH and 10% HCl was stirred at 50° for 96 h. MeOH was removed at reduced pressure and 20 ml of H_2O added, followed by extraction with EtOAc (20 ml×3). The aqueous phase was passed through an anionic-exchange column (1.1×12.5 cm) in the OH^- form, and the effluent evaporated at reduced pressure to leave a white solid (5 mg): $[\alpha]^{23.5}D$ +73° (c=0.3, H_2O) [lit. (12)+112°→+53°]. Si gel tlc with $CHCl_3$-MeOH-H_2O (19:9:2, lower phase) as solvent and p-anisaldehyde spray reagent gave a spot with R_f 0.13 (gray color) that corresponded to glucose. Galactose showed R_f 0.22 (green-yellow) and mannose showed R_f 0.25 (green).

Amoenolide E [**3**].—Column fraction H (210 mg) was chromatographed on a RP-8 column with mixtures of MeCN-H_2O (1:39), (1:19), (1:9), (1:4), and (2:3). The (1:9) solvent solution gave 94 mg of a fraction that separated on 8 g of Si gel with MeCN-Me_2CO-H_2O (38:2:1) to give 63 mg (7.9×10^{-3}% of dried plant) of amoenolide E [**3**] as an oil: $[\alpha]^{23.5}D$ +33° (c=0.5, MeOH); ir (neat) ν max 3380 (OH), 1780 and 1740 (C=O), 1630 (C=C), 1440, 1380, 1130, and 1040 (C-O) cm^{-1}; uv (MeOH) λ (end abs.) 204 nm (log ϵ 4.17); fabms (glycerol) m/z 483.2605 (2, MH^+, $C_{25}H_{39}H_9$ requires 483.2594), 333 (1, MH^+−$C_5H_{10}O_5$) and 93 (100); ^1H- and ^{13}C-nmr spectra shown in Tables 1 and 2, respectively. The fully ^1H-coupled ^{13}C-nmr spectrum showed $^1J_{CH}$=156.5 Hz for C-1′ (106.21 ppm), $^2J_{CH}$=9.1 Hz for 174.27 ppm (C-15, doublet), and $^2J_{CH}$=3.8 Hz for 171.34 ppm (C-13, 5 pk multiplet).

Hydrolysis of amoenolide E [**3**].—A sample of amoenolide E [**3**] (28 mg) was hydrolyzed and processed as reported for glucoside **1** except that the hydrolysis was performed at room temperature. The sugar [3 mg, $[\alpha]^{23.5}D$ +80° (c=0.3, H_2O), lit. (13) $[\alpha]^{20}D$ +105° (c=3.0, H_2O)] on tlc analysis had the same R_f and color development as arabinose. The standard sugars gave the following R_f values and colors: arabinose 0.37 (yellow-green), lyxose 0.37 (green), ribose 0.45 (green-gray), and xylose 0.37 (blue-gray).

Amoenolide F [**4**].—Column fraction N (958 mg) was separated on a RP-8 column with the same solvent mixtures used for amoenolide E. The (1:4) mixture gave 472 mg of a residue that were chromatographed on Si gel (36 g) with MeCN-Me_2CO-H_2O (38:2:1), (36:4:1) and (36:6:1) and monitored by tlc on Si gel with $CHCl_3$-MeOH-H_2O (15:4:1, lower phase). The material (366 mg) eluted with mixture (36:4:1) was rechromatographed on Si gel (7 g) with the lower phases of $CHCl_3$-MeOH-H_2O (18:1:1), (17:2:1) and (16:3:1). The (17:2:1) solvent gave 255 mg (3.2×10^{-3}% of dried plant) of amoenolide F as a heavy oil: $[\alpha]^{23.5}D$ +45° (c=0.5, MeOH); ir (KBr) ν max 1780 and 1740 (C=O), 1640 (C=C), 1440, 1390, and 1080 and 1040 (C-O) cm^{-1}; uv (MeOH) λ (end abs.) 204 nm (log ϵ 4.52); fabms ["magic bullet" (10,11)] m/z 551 (7, MNa^+), 529.2666 (4, MH^+, $C_{26}H_{41}O_{11}$ requires 529.2649), 511 (1, MH^+−H_2O), 475 (1, MH^+−$3H_2O$), 367 (2), 349 (13, MH^+−$C_6H_{12}O_6$), 119 (69) and 85 (100); ^1H- and ^{13}C-nmr spectra shown in Tables 1 and 2, respectively. The fully ^1H-coupled and ^{13}C-nmr spectrum showed $^1J_{CH}$=158.4 Hz for C-1 (105.60 ppm), $^2J_{CH}$=9.0 Hz for 174.41 (C-15, doublet) and a broadened singlet $\omega_{1/2}$=16 Hz for the peak at 171.56 ppm (C-13).

Amoenolide F peracetate [**5**].—A 10-mg sample of amoenolide F [**4**] was acetylated according to the procedure in Ref. (1). The peracetate (12 mg) was a heavy oil: $[\alpha]^{23.5}D$ +20° (c=0.5, MeOH); ir ($CHCl_3$) ν max 1790 and 1760 (C=O), 1370, 1220 (C-O, Ac) and 1440 (C-O) cm^{-1}; fabms ("magic bullet") m/z 845 (0.8, MNa^+), 763 (0.2, MH^+−AcOH), 703 (0.1, MH^+−2AcOH), 643 (0.7, MH^+−3AcOH), 331 [27, $C_6H_7O_5(Ac)_4$], 169 (100) and 109 (60); ^1H-nmr spectrum shown in Table 1.

ISOLATION OF AMOENOLIDES G [**6**] AND H [**8**].—The Si gel column fraction XIII (1.7 g) from the EtOAc-soluble partition fraction (2) was chromatographed on a RP-8 column with mixtures of MeCN-H_2O (1:39), (1:19), (1:9), (1:4), and (2:3). The fractions eluted by the (1:4) mixture were combined (270 mg) based on tlc analysis on Si gel with $CHCl_3$-MeOH-H_2O [(15:4:1), lower phase], and chromatographed over 8 g of Si gel 60 with mixtures of MeCOEt-EtOAc-H_2O (38:2:1), (36:4:1), and (34:6:1). The (36:4:1) solvent mixture gave 125 mg of material that was further separated on a RP-8 column with MeOH-H_2O (1:9), (1:4), (3:7), (2:3), (1:1), and (3:2). Analysis by tlc on RP-18 plates with EtOH-H_2O (1:1) showed two spots in the MeOH-H_2O (3:7) eluted fraction (96 mg), which in previous tlc systems had the same R_f. Chromatography on a RP-18 column with EtOH-H_2O (1:9), (1:4), (3:7), (2:3), (1:1), and (3:2) gave from the (3:7) solvent a partially separated mixture, which after a number of passes through the column afforded 29 mg (7.2 $\times 10^{-4}$% of dried plant) of amoenolide G [**6**] and 36 mg (9.0×10^{-4}% of dried plant) of amoenolide H [**8**], both as heavy oils.

Amoenolide G [**6**].—The heavy oil had the following properties: $[\alpha]^{23.5}$D +40° (c=0.5, MeOH); ir (KBr) ν max 1780 and 1740 (C=O), 1630 (C=C), 1440, 1390, 1080, and 1050 cm^{-1}; uv (MeOH) λ (end abs.) 204 nm (log ϵ 4.23); fabms (glycerol) *m/z* 505 (1, MNa^+), 483 (2, MH^+), 333 (2, $MH^+-C_5H_{10}O_5$) and 315 (4, $MH^+-H_2O-C_5H_{10}O_5$); eims *m/z* 348.1916 (0.5, $M^+-C_5H_{10}O_4$, $C_{20}H_{28}O_5$ requires 348.1936), 330.1844 (1, $M^+-H_2O-C_5H_{10}O_4$, $C_{20}H_{26}O_4$ requires 330.1831), 149.0466 (11, $C_5H_9O_5$ requires 149.0450) and 43 (100); ^{1}H- and ^{13}C-nmr data shown in Tables 1 and 2, respectively. The fully ^{1}H-coupled ^{13}C-nmr spectrum gave $^1J_{CH}$=156.1 Hz for C-1′ (106.04 ppm), $^2J_{CH}$=9.1 Hz for the peak at 174.28 ppm (C-15, doublet), and a broadened singlet $\omega_{1/2}$=13 Hz for the peak at 171.39 ppm (C-13).

Amoenolide G peracetate [**7**].—Amoenolide G [**6**] (4 mg) was acetylated under the conditions given in Ref. (1) to give a heavy oil (5 mg) of the acetate **7**: $[\alpha]^{23.5}$D +27° (c=0.4, MeOH); ir ($CHCl_3$) ν max 1790 and 1750 (C=O), 1640 (C=C), 1380, 1240 (Ac, C-O), and 1040 (C-O) cm^{-1}; fabms [dithiothreitol-dithioerythritol (5:1) (10,11)] *m/z* 715 (0.4, MNa^+), 259 [3, $C_5H_6O_4Ac^+_3$] and 57 (100); ^{1}H-nmr data shown in Table 1.

Amoenolide H [**8**].—The heavy oil had the following properties: $[\alpha]^{23.5}$D +31° (c=0.5, MeOH); ir (neat) ν max 3480, 1780, and 1730 (C=O), 1630 (C=C), 1450, 1340, 1300, 1130, 1130, 1040, and 1050 cm^{-1}; uv (MeOH) λ (end abs.) 206 nm (log ϵ 4.17); fabms (glycerol) *m/z* 485.2755 (3, MH^+, $C_{25}H_{41}O_9$ requires 485.2751), 467 (2, MH^+-H_2O) 449 (1, MH^+-2H_2O) and 335 (6, $MH^+-C_5H_{10}O_5$); eims *m/z* 307 (1), 286.1977 (1, $M^+-C_5H_{10}O_5-CH_2OH-H_2O$, $C_{19}H_{26}O_2$ requires 286.1934), 189 (2), 179 (2), 165 (3), 149 (8), 123 (6), 109 (8), 95 (12), 84 (100), 71 (20), and 56 (87); ^{1}H- and ^{13}C-nmr data shown in Tables 1 and 2, respectively. The fully ^{1}H-coupled ^{13}C-nmr spectrum showed $^1J_{CH}$=157.6 Hz for C-1′ (105.51 ppm), $^2J_{CH}$=9.3 Hz for peak at 174.69 ppm (C-15, doublet), and a broadened singlet $\omega_{1/2}$=13.8 Hz for the peak at 172.75 ppm (C-13).

Methyl α-L-*arabinopyranoside*.—The sample was prepared from L-arabinose (Difco) via the triacetyl β-L-arabinopyranosyl bromide (14) which was treated with Ag_2CO_3 in MeOH followed by deacetylation with $Ba(OMe)_2$ (15). The ^{1}H- and ^{13}C-nmr spectra were assigned by decoupling, nOe difference, ^{1}H,^{1}H-COSY, and CH-correlation studies: ^{1}H nmr (270 MHz, pyridine-d_5) δ 4.54 (d, J=6.9 Hz, H-1), 4.41 (dd, J=8.6 and 7.0 Hz, H-2), 4.30 (ddd, J=3.0, 2.7, and 2.7 Hz, H-4), 4.29 (dd, J=12.8 and 2.8 Hz, H-5eq), 4.15 (dd, J=8.6 and 3.3 Hz, H-3), 3.73 (dd, J=12.9 and 2.5 Hz, H-5ax) and 3.57 (s, OMe); ^{13}C nmr (67.9 MHz, pyridine-d_5) δ 106.00 (d, C-1), 74.46 (d, C-3), 72.30 (d, C-2), 69.29 (d, C-4), 66.71 (t, C-5), and 56.34 ppm (q, OMe).

Methyl β-D-*arabinopyranoside*.—The commercial sample (Sigma) had the spectra assigned as given for the α-anomer: ^{1}H nmr (270 MHz, pyridine-d_5) δ 5.14 (d, J=3.4 Hz, H-1), 4.60 (dd, J=9.4 and 3.4 Hz, H-2), 4.44 (dd, J=9.4 and 3.4 Hz, H-3), 4.35 (ddd, J=3.0, 2.2, and 2.2 Hz, H-4), 4.00 (2H, d, J=2.1 Hz, H_2-5), and 3.40 (s, OMe); ^{13}C nmr (67.9 MHz, pyridine-d_5) δ 102.12 (d, C-1), 70.89 (d, C-3), 70.53 (d, C-2), 70.13 (d, C-4), 63.93 (t, C-5), and 55.33 ppm (q, OMe).

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