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AMOENOLIDE K AND AMOENOLIDE K 19-ACETATE, TWO GRINDELANE PEROXIDES FROM *AMPHIACHYRIS AMOENA*. ISOLATION, STRUCTURE DETERMINATION, AND PREPARATION OF AMOENOLIDE K FROM AMOENOLIDE A BY PHOTOCHEMICAL OXYGENATION[1]

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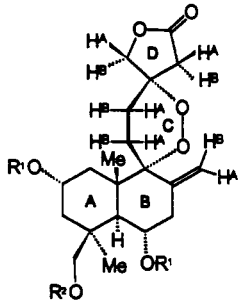
ABSTRACT.—Two new 9,13-diepoxy labdane diterpenes, amoenolide K [**1**] and its 19-acetate [**2**], were isolated from the aerial parts of the composite *Amphiachyris amoena* and their structures were established by spectral methods, especially high-field nmr spectroscopy. Amoenolide K [**1**] was prepared from amoenolide A [**3**] by singlet oxygen addition via the ene reaction. A study was made of the ene reaction products with amoenolide K triacetate [**5**] which showed them to arise from stereospecific oxygen addition to the 8,9-double bond, with the exception of the sterically hindered β-side at C-9, for which no products were isolated.

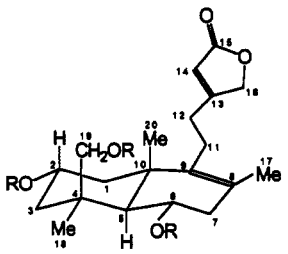
The annual composite, *Amphiachyris amoena* (Shinners) Solbrig has yielded 14 new labdanes (1–3) and two new chettaphane diterpenes (4). We report herein the isolation and characterization of two new grindelane peroxides, amoenolide K [**1**] and amoenolide K 19-acetate [**2**] from the same source, and photochemical oxygenation studies with amoenolide A [**3**] and its triacetate [**4**] (1). The latter study provided the grindelane peroxide ring system, as well as a number of interesting oxygenation products, which could be explained by the initial direction of oxygen addition by the ene reaction.

RESULTS AND DISCUSSION

Amoenolide K [**1**], mp 168–169°, and its 19-acetate [**2**], mp 159–160°, were isolated from the reported MeOH-soluble partition fraction (1) after repeated chromatography and have the respective formulas $C_{20}H_{30}O_7$ and $C_{22}H_{32}O_8$, as supported by fabms. Both gave the same triacetate [**5**], indicating an identical core structure with three acylable hydroxyls. The ^{1}H-nmr spectrum (Table 1) of amoenolide K [**1**], taken in



1 $R^1=R^2=H$
2 $R^1=H$, $R^2=Ac$
5 $R^1=R^2=Ac$



3 R=H
4 R=Ac

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

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TABLE 1. ^{1}H-Nmr Data for Compounds **1**, **2**, and **5–11**.[a]

Proton(s)	Compound										
	1[b]	**2**	**2**[b]	**5**	**5**[b]	**6**	**7**	**8**	**9**	**10**	**11**
H-1	1.42 α dd (11.4,11.4) 1.83 β ddd (11.9,4.0,2.3)	1.44 α dd (11.4,11.4) 1.85 β ddd (11.7,3.9,2.3)	1.43 α dd (11.6,11.6) 1.85 β ddd (11.9,4.0,2.2)	1.52 α dd (11.6,11.6) 1.90 β hm	1.61 α dd (11.6,11.6) 1.99 β hm	1.60 α dd (11.6,11.6) 1.85 β ddd (11.7,4.2,2.1)	1.32 α dd (11.4,11.4) 2.32 β ddd (11.6,3.6,2.0)	1.30 α dd (11.8,11.8) 2.33 β ddd (11.8,3.7,2.2)	1.55 α dd (11.4,11.4) 1.88 β dd (11.4,4.1,1.9)	1.37 α dd (12.0,12.0) 1.75 β ddd (12.2,4.0,2.2)	1.67 α dd (11.8,11.8) 1.83 β ddd (12.9,4.2,2.2)
H-2	3.71 ddddd (11.5,11.5,4.0, 4.0,3.9)	3.84 dddd (12.5,12.5, 3.8,3.8)	3.75 hm	4.91 hm [dddd] (11.7,11.7, 4.2,4.2)	4.93 dddd (11.8,11.8, 4.2,4.2)	4.95 dddd (11.8,11.8 4.2,4.2)	4.99 dddd (11.9,11.9 4.0,4.0)	4.97 dddd (11.9,11.9 41,4.1)	5.05 hm [dddd] (11.6,11.6, 4.5,4.4)	4.94 dddd (11.9,11.9, 4,2,4.2)	4.95 dddd (118,11.8, 4.2,4.2)
H-3	0.97 α dd (12.9,12.0) 1.89 β ddd (13.0,3.9,2.1)	1.08 α dd (12.5,12.5) 1.95 hm [ddd] (13.1, 3.9,2.3)	0.94 α dd (12.2,12.2) 1.94 β hm	1.24 α dd (12.8,12.8) 1.90 β hm	1.23 α dd (12.7,12.7) 1.91 β ddd (13.2,4.0,2.2)	1.25 α dd (12.8,12.8) 1.94 β hm	1.18 α dd (12.5,12.5) 2.04 β hm	1.17 α dd (12.6,12.6) 2.03 β hm	1.20 α hm [dd] (11,11) 2.03 β hm [ddd] (11, 4.5,2.0)	1.20 α dd (12.7,12.7) 1.95 β hm [ddd] (12.5, 4.0,2.2)	1.25 α dd (12.6,12.6) 1.93 β ddd (13.2,3.8,2.1)
H-5	1.98 d (11.0)	2.07 d (9.7)	2.06 d (12.1)	2.39 d (11.7)	2.39 d (11.8)	2.44 d (11.7)	1.63 d (8.7)	1.68 d (8.9)	1.61 d (11.3)	2.12 d (10.3)	2.25 d (11.8)
H-6	3.89 dddd (11.1,11.1, 5.4,4.6)	3.84 hm	3.80 hm	5.05 ddd (11.2,11.2, 5.4)	5.04 ddd (11.6,10.8, 5.3)	5.03 ddd (11.6,10.6, 5.2)	5.54 hm [dd] (8.3,6.1)	5.46 hm [dd] (8.9,5.8)	5.08 ddd (11.1,9.7,4.7)	5.13 ddd (10.3,7.6,4.8)	5.03 ddd (11.3,11.3, 5.2)
H-7	2.53 α dd (11.4,11.4) 2.39 β dd (11.8,4.5)	2.56 α dd (11.8,10.8) 2.52 β dd (12.0,5.2)	2.57 α br dd (11.3,11.3) 2.45 β dd (11.9,4.8)	2.50 α dd (11.2,11.2) 2.62 β dd (12.1,5.2)	2.49 α br dd (11.3,11.3) 2.56 β dd (11.9,5.2)	2.43 α dd (11.6,11.6) 2.61 β dd (12.4,5.2)	1.73 α d (15.5) 2.84 β dd (15.7,6.4)	1.80 α d (15.4) 2.41 β dd (15.6,6.2)	1.70 α dd (14.0,9.6) 2.66 β dd (14.1,4.7)	1.95 α dd (16.4,4.7) 2.51 β dd (16.4,7.6)	2.43 α hm [dd] (11.9, 11.9) 2.62 β dd (12.3,5.1)
H-11	2.00 (2H) m	1.96 (2H) m	2.02 (2H) hm	1.90 A hm 1.99 B ddd (14.0,5.0,2.5)	2.10 A hm 2.04 B hm	2.10 (2H) hm	5.53 t (7.5)	5.48 t (7.6)	6.26 d (15.8)	6.28 d (15.8)	2.18 A ddd (13.2,7.8,3.2) 2.05 B hm
H-12	2.31 A ddd (13.0,9.1,9.1) 1.94 B ddd (13.2,3.7,3.7)	2.32 A ddd (13.1,12.3,6.5) 1.79 B ddd (13.4,4.5,2.9)	2.31 A ddd (13.2,11.4,6.9) 1.94 B hm [ddd] (13.4, 3.7,3.7)	2.32 A ddd (13.4,13.4,5.2) 1.79 B ddd (13.3,4.7,2.3)	2.32 A m 1.97 B hm	2.54 (2H) m	3.59 (2H) d (7.3)	3.64 (2H) m	6.58 d (15.8)	6.62 d (15.9)	2.43 A hm 1.86 B hm
H-14	2.73 A d (18.2) 2.21 B d (18.2)	2.48 d (18.4) 2.46 d (18.4)	2.73 A d (18.3) 2.21 A d (18.3)	2.49 d (18.4) 2.44 d (18.4)	2.76 A d (18.2) 2.21 B br d (18.2)	5.87 m (5 pk) (1.6)	5.86 m (5 pk) (1.6)	5.81 m (5 pk) (1.6)	5.98 m (3 pk) (1.9)	5.98 m (3 pk) (1.4)	4.48 br s $\omega_{1/2}$=4.7 Hz
H-16	4.29 A d (10.0) 4.87 B d (10.0)	4.17 d (10.2) 4.94 d (10.2)	4.30 A d (10.0) 4.87 B d (10.1)	4.18 d (10.2) 4.94 d (10.2)	4.31 A d (10.2) 4.87 B d (10.8)	4.75 (2H) d (1.8)	4.79 (2H) d (1.3)	4.76 (2H) d (0.9)	4.91 dd (16.4,1.2) 4.96 dd (16.4,1.2)	4.95 dd (16.3,1.2) 5.00 dd (16.5,1.2)	4.14 A d (9.0) 4.06 B d (9.0)

TABLE 1. Continued.

Proton(s)	Compound										
	1[b]	2	2[b]	5	5[b]	6	7	8	9	10	11
H-17	5.20 A s	5.27 A s $\omega_{1/2}$=3.2 Hz	5.21 A br s $\omega_{1/2}$=4.0 Hz	5.33 A d (1.2)	5.31 A d (1.2)	5.17 A d (1.3)	1.45 s	1.48 s	1.15 s	1.15 s	5.04 A d (1.7)
	4.91 B s	4.86 B s $\omega_{1/2}$=2.7 Hz	4.93 B br s $\omega_{1/2}$=3.2 Hz	4.89 B s	5.05 B br s $\omega_{1/2}$=2.8 Hz	4.75 B hm					4.93 B br s $\omega_{1/2}$=3.1 Hz
H-18	1.28 s	1.32 s	1.32 s	1.19 s	1.21 s	1.21 s	1.00 s	1.00 s	1.23 s	1.09 s	1.20 s
H-19	3.51 dd (10.7,4.2)	4.16 d (11.0)	4.09 d (10.6)	4.06 d (11.5)	4.09 (2H) s	4.07 d (11.5)	4.14 d (11.4)	4.13 d (11.5)	3.97 d (11.6)	4.11 d (11.5)	4.06 d (11.5)
	3.87 dd (10.5,4.3)	4.23 d (11.0)	4.36 d (10.8)	4.08 d (11.5)		4.10 d (11.5)	4.27 d (11.5)	4.26 d (11.5)	4.12 d (11.6)	4.15 d (11.5)	4.09 d (11.5)
H-20	0.93 s	0.90 s	0.91 s	0.99 s	1.01 s	0.99 s	1.31 s	1.33 s	1.18 s	1.32 s	0.94 s
Misc.	3.50 (2-OH) hd	2.08 (Ac) s	2.00 (Ac) s	2.04 (Ac) s	1.97 (Ac) s	2.030 (Ac) s	2.03 (Ac) s	2.02 (Ac) s	2.03 (Ac) s	2.00 (Ac) s	2.03 (Ac) s
	3.82 (6-OH) d (4.7)			2.05 (Ac) s	1.98 (Ac) s	2.033 (Ac) s	2.05 (Ac) s	2.04 (Ac) s	2.04 (Ac) s	2.061 (Ac) s	2.03 (Ac) s
	4.03 (19-OH) dd (4.5,4.5)			2.06 (Ac) s	2.04 (Ac) s	2.06 (Ac) s	2.09 (Ac) s	2.08 (Ac) s	2.07 (Ac) s	2.062 (Ac) s	2.05 (Ac) s
						1.80 (9-OH)					3.05 (OH-14) br 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 Me_4Si with residual solvent peak ($CHCl_3$) as internal standard at 7.26 ppm. Stereochemical designations α and β following the chemical shift values refer to the proton below and above the plane, respectively, of the illustrated drawing. The spin coupling value (*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 or nOe studies and are reported after the hm designation in brackets.

[b]In Me_2CO-d_6 with reference to Me_2CO-d_5 at 2.04 ppm.

Me_2CO-d_6 because of insolubility in $CHCl_3$ and instability in MeOH and pyridine, showed multiplicity patterns in the carbinyl region at 3.71 and 3.83 ppm similar to those observed for H-2 and H-6 in amoenolide A [**3**]. Also, D_2O exchange removed three broadened downfield patterns for the three hydroxyls and the hydroxyl coupling from the carbinyl patterns. Extensive 1D and 2D nmr studies[3] established the complete stereochemical structure of **1** beginning with $^1H,^1H$-COSY and CH-correlation experiments. These revealed six ^1H-coupled units, three of which, when combined, formed a bicyclic system forming rings A and B, akin to those of amoenolide A [**3**], except that an exocyclic olefin replaces the C-8 to C-9 endocyclic double bond. For example, the H-2 carbinyl proton (3.71 ppm) was coupled to the C-2 (0.97 and 1.89 ppm) and C-1 (1.42 and 1.83 ppm) methylenes, of which the 1.42 ppm proton was "*W* -coupled" to Me-20 at 0.93 ppm. The Me-18 (1.28 ppm) signal was also "*W*-coupled" to one H-19 (3.87 ppm) of the C-19 hydroxymethylene, which makes up the second proton-coupled unit. The third unit involved the H-6 carbinyl proton (3.89 ppm) coupled to H-5 (1.93 ppm) and to the C-7 methylene (2.39 and 2.53 ppm) of which the latter proton was allylically coupled to two olefinic protons (4.91 and 5.20 ppm) at C-17. The long-range CH-correlation (COLOC) experiment identified the four quaternary carbons (Table 2) of the A/B ring system and confirmed the arrangement of the proton coupled units. The key interactions were: Me-18 (1.28 ppm) coupled to C-2 (48.58 ppm), C-4 (41.36 ppm), C-5 (51.07 ppm), and C-19 (67.80 ppm); Me-20 coupled to C-5 (51.07 ppm), C-9 (86.64 ppm), and C-10 (45.68 ppm); H-7 (2.39 ppm) coupled to C-5 (51.07 ppm), C-8 (145.21 ppm), and C-17 (114.72 ppm); H-1 (1.42 ppm) coupled to C-10 (45.68 ppm) and C-20 (19.34 ppm); and H-17 (5.21 ppm) coupled to C-7 (44.27 ppm) and C-9 (86.64 ppm).

The stereochemical disposition of the substituents on the A/B rings was established from nOe studies by the difference method at 270 MHz with **2**. Nmr experiments gave the same proton-coupled units and arrangement as reported for **1**. C-19 contained the acetate group as shown by the downfield shift of H_2-19 from 3.51 and 3.87 ppm in **1** to 4.09 and 4.36 ppm in **2** and the long-range CH-coupling of H_2-19 to the acetate carbonyl at 171.13 ppm, which in turn was coupled to the acetate methyl at 2.00 ppm from the inverse COLOC (HMBC) experiment (5). From the nOe results, the following irradiations identified the β-faced substituents: Me-20 (0.91 ppm) showed relaxation to H-2 (3.75 ppm) at 4%, H-6 (3.80 ppm) at 13%, H-17B (4.93 ppm) at 2% and H-19 (4.36 ppm) at 10%; H-17A (5.21 ppm) relaxed to H-7 (2.45 ppm) at 5% and H-17B (4.93 ppm) at 24%; H-1 (1.85 ppm) relaxed to H-2 (3.75 ppm) at 7%, Me-20 (0.91 ppm) at 2% and the other H-2 (1.43 ppm) at 18%; and H-7 (2.45 ppm) relaxed to H-6 (3.80 ppm) at 7%, H-17A (5.21 ppm) at 7% and the other H-7 (2.57 ppm) at 16%. The α-faced substituents were likewise identified: Me-18 (1.32 ppm) relaxed to H-3 (0.94 ppm) at 5%, H-5 (2.06 ppm) at 15% and H-19 (4.09 ppm) at 4%; H-1 (1.43 ppm) relaxed to H-3 (0.94 ppm) and H-5 (2.06 ppm) both at 3% and to the other H-1 (1.85 ppm) at 17%; and H-7 (2.57 ppm) relaxed to H-5 (2.06 ppm) at 6% and to the other H-7 (2.45 ppm) at 17%. The nOe results also indicated that H-19 at 4.36 ppm resided over ring A because of its interaction with Me-20, while the H-19 at 4.09 ppm on the other hand relaxed to Me-18 at 2%.

With the A/B ring system established for **1** and its acetate **2** as a *trans*-decalin, the remainder of the structure required accommodation of six carbons, four oxygens, and three double-bond equivalents. The three remaining proton coupled units—a four-spin

[3]The references for the specified nmr experiments performed in this study can be found in O'Mathúna and Doskotch (1).

TABLE 2. ^{13}C-Nmr Data for Compounds **1**, **2** and **5–11**.[a]

Carbon	Compound											
	1[b]	Multiplicity	**2**	**2**[b]	**5**	**5**[b]	**6**	**7**	**8**	**9**	**10**	**11**
C-1	43.11	t	41.87	42.79	38.03	38.52	38.02	45.88	45.92	43.76	42.48	37.65
C-2	64.20	d	64.55	63.93	67.72	72.80	68.01	67.87	67.75	67.52	66.90	68.00
C-3	48.58	t	46.69	46.99	42.79	43.31	42.88	42.14	42.21	43.04	42.45	42.86
C-4	41.36	s	39.37	39.84	38.94	39.47	38.88	38.87	38.83	39.26	38.64	38.87
C-5	51.07	d	50.80	51.31	47.86	48.55	48.37	50.51	49.95	55.50	47.41	49.21
C-6	71.43	d	70.92	70.64	72.40	74.29	72.26	68.31	68.31	68.85	67.81	72.05
C-7	44.27	t	44.01	44.93	38.94	39.81	39.18	37.21	44.02	41.78	36.78	39.38
C-8	145.21	s	142.89	144.75	141.49	142.78	143.39	82.81	71.33	66.94	64.53	144.74
C-9	86.64	s	85.86	86.63	85.82	86.50	78.78	152.14	156.63	71.59	71.87	90.89
C-10	45.68	s	45.19	45.50	45.22	45.74	45.49	41.17	41.36	42.24	39.23	43.90
C-11	19.77	t	19.41	19.79	19.44	19.74	27.99	120.25 d	119.81 d	134.72 d	132.44 d	26.28
C-12	27.32	t	27.61	27.30	27.46	27.13	24.05	28.56	29.13	124.59 d	125.15 d	29.19
C-13	84.30	s	83.19	84.37	83.23	84.58	170.59 s	170.19[c]	170.41[c]	160.42	160.32	87.38
C-14	39.33	t	39.33	39.30	39.23	39.24	115.57 d	115.81 d	115.62 d	116.65 d	116.88 d	72.93 d
C-15	174.70	s	173.78	174.57	173.65	174.45	173.96	174.36	174.26	173.60	173.53	175.24
C-16	74.34	t	74.36	74.28	74.28	74.72	73.27	73.47	73.39	70.58	70.57	74.91
C-17	114.72	t	115.27	114.72	116.29	116.44	114.37	26.94 q	31.38 q	19.46 q	19.57 q	112.23
C-18	32.32	q	31.63	32.10	31.19	31.45	31.41	27.70	27.93	31.33	29.31	30.97
C-19	67.80	t	68.01	67.67	68.01	72.80	67.84	67.52	67.55	67.35	67.52	67.86
C-20	19.34	q	19.29	19.53	18.84	18.97	18.95	26.54	27.62	18.14	20.14	19.79
*Me*CO		q	21.29	20.76	20.89	20.63	20.95	20.94	20.98	20.87	20.94	20.87
*Me*CO		q			21.49	21.20	21.53	21.46	21.49	21.47	21.46	21.50
*Me*CO		q			21.86	21.70	21.91	21.80	21.82	21.78	21.76	21.84
Me*C*O		s	171.27	171.13	169.94	170.06	170.00	170.27	170.16	169.77	170.42	169.94
Me*C*O		s			170.53	170.40	170.54	170.60	170.56[c]	170.32	170.48	170.65
Me*C*O		s			171.07	171.01	171.16	171.13[c]	171.07	171.03	171.00	171.13

[a]Taken at 67.9 MHz in $CDCl_3$ or as stated otherwise with multiplicities determined by SFORD. The chemical shifts (δ) in ppm were referenced to Me_4Si with the reference peak of solvent taken as 77.2 ppm (center). Data point resolution of 0.7 Hz.

[b]In Me_2CO-d_6 with solvent peak referenced at 29.8 ppm.

[c]May be interchanged.

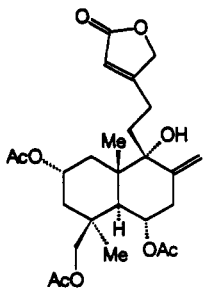
dimethylene and two isolated methylenes—were easily placed into a double spiro system at C-9 to conform with the labdane skeleton and the isoprene order of the side-chain. Compound **1** lacked the characteristic ^{1}H- and ^{13}C-nmr peaks and patterns for the α,β-unsaturated γ-lactone (butenolide) group (1), but did show one carbon peak at 174.70 ppm, as well as a strong ir peak at 1790 cm^{-1} in support of a saturated lactone. The last two oxygens had to be ether oxygens and were designated as epidioxides forming the endoperoxide six-membered ring C. This was supported by the instability of the compounds and a positive starch iodide test. Stereospecific assignments for the spiro protons were made from nOe studies at 270 MHz on **2**, with the pertinent results as follows: irradiation of H-17B (4.93 ppm) showed relaxation to H-11 (2.02 ppm) at 3% and H-12A (2.31 ppm) at 4%. This and a NOESY experiment that showed interaction between Me-20 and H_2-11 required that the dimethylene group be placed on the β-side (equatorial). In turn, irradiation at H-12A gave relaxation to H-17B and H-14A (2.73 ppm) each at 5% and H-12B (1.94 ppm) at 16%, while irradiation at H-14A produced relaxation to H-16A (4.30 ppm) at 2% and H-14B (2.21 ppm) at 29%. Furthermore, irradiation at H-16B showed relaxation to H-16A at 27% and to H-1α (1.43 ppm) at 3%. The latter proton (H-1α) in a reciprocal irradiation relaxed to H-16B at 4%, but not to H-14B; thereby requiring a conformation for ring D in which H-14A and H-16A are quasi axial and the oxymethylene unit of the lactone to be positioned on the ring A side. Differentiation between H_2-14 and H_2-16 was made from their ^{1}H-nmr chemical shifts and J values (6).

The nOe studies with **2** allowed complete assignment of the ^{1}H-nmr spectrum which, with the 2D nmr experiments (CH-correlation and HMBC), allowed for a complete ^{13}C-nmr assignment. Comparison of these results with the nmr data from **1** and its triacetate [**3**] along with their 2D nmr studies established the assignment of their ^{1}H- and ^{13}C-nmr spectra as found in Tables 1 and 2, respectively.

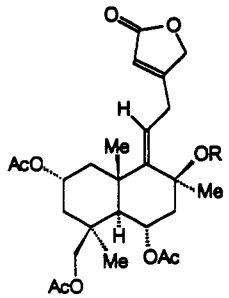
The grindelane peroxide structure of **1** as determined from the spectral data suggested it could originate from **3** by a two-step sequence involving first, singlet oxygen addition via the less-hindered α-side to the 8,9-double bond by the ene reaction (7,8) to give the 9-hydroperoxide and the 8,17-double bond, followed by a regioselective Michael-type addition of the hydroperoxide to the 13,14-double bond. Indeed, this reaction was accomplished with methylene blue as sensitizer, but only in a 14% yield along with other intractable products. The yield was raised to 40% when amoenolide A triacetate [**4**] was used. This transformation did establish the absolute stereochemistry for **1** and **2**, since the stereochemistry of **3** is known (1). Evidence for the hydroperoxide intermediate was obtained from the photo-oxygenation of amoenolide A triacetate [**4**], in the presence of thiourea as reducing agent which gave alcohol **6** in a yield of 12%. The yield of amoenolide K triacetate [**5**] dropped by half to 21%, thus supporting the precursor-product relation between the hydroperoxide and the endoperoxide **5**.

The photo-oxygenation byproducts reported below were all characterized by spectral methods, especially 1D (homonuclear decoupling and nOe) and 2D (COSY, NOESY, CH-correlation and COLOC) nmr studies with details not reported here. Only the relevant data for assigning the structures are given. Alcohol **6** has the formula $C_{26}H_{36}O_9$, as supported by fabms with rings A and B the same as for **5**, and the side-chain unit that of **3**. Positioning of the side-chain in the equatorial β position was supported by the nOe experiment in which Me-20 was irradiated and gave a 5% enhancement of the H_2-11 pattern at 1.99 ppm. Alcohol **6** is thus the reduced product of the corresponding C-9 hydroperoxide.

In addition to the formation of **5** on photo-oxygenation of **4**, a number of other products were indicated by tlc examination. Four of the most stable were isolated and



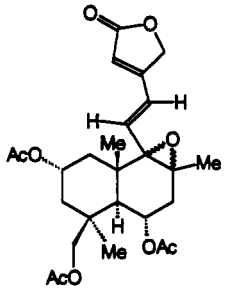
6



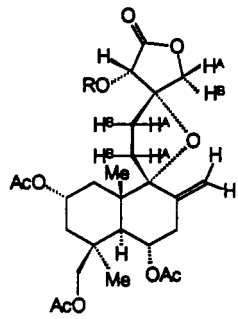
7 R=OH
8 R=H

characterized. One, an allylic hydroperoxide, was assigned structure **7**. The fabms supported the molecular formula $C_{26}H_{36}O_{10}$ with two oxygens more than in the starting material and no change in the degrees of unsaturation. A positive peroxide test (9) and the 1D and 2D nmr studies showed that the change in structure from the starting material involved C-8, C-9, and C-11. The olefinic methyl group was replaced by a tertiary methyl on an oxygen-bearing carbon and the endocyclic olefin became exocyclic. The compound was established as the ene reaction product in which the oxygen attacked C-8 from the β-side with a concerted C-9 to C-11 double bond formation and hydrogen transfer from C-11 to the oxygen. The nOe difference experiments established the stereochemistry thus: irradiation of H-7α (1.73 ppm) showed relaxation to Me-17 (1.45 ppm) at 2% and to H-7β (2.84 ppm) at 11%; irradiation at H-5 (1.63 ppm) gave relaxation to Me-17 at 4%, to H-1α (1.31 ppm) at 3%, to H-3α (1.18 ppm) at 2% and to Me-18 (0.99 ppm) at 3%; irradiation of Me-17 caused enhancement of H_2-12 (3.59 ppm) by 6%, of H-14 (5.86 ppm) by 2% and of H-7α by 3%; and irradiation of H-1β (2.32 ppm) gave relaxation to H-11 (5.54 ppm) at 13%. Additional irradiation supported the olefin substitution as drawn with the designation *Z*. Reduction of compound **7** with Ph_3P produced the corresponding alcohol **8**, which substantiated the presence of the hydroperoxide group.

The two isomeric lactones, compounds **9** and **10**, have the same molecular formula, $C_{26}H_{34}O_9$, as supported by fabms, and comparison of their nmr spectra showed them to have nearly identical ^{1}H-nmr patterns and the same ^{13}C-nmr multiplicities. The additional oxygen and one degree of unsaturation more than that in the starting material suggested they were not primary products of photo-oxygenation. Comparison of the ^{13}C-nmr spectra with that of the starting material showed the loss of the methylenes at C-11 and C-12, and the olefinic carbons at C-8 and C-9. There were present, however, two olefinic carbons as doublets (SFORD), and the carbons for the butenolide unit along with the typical protons of that unit in the ^{1}H-nmr spectrum. This and the strong uv absorption at about 260 nm supported the butenolide unit as being conjugated with the olefin formed at C-11 and C-12 as in several *Scutellaria* diterpenes (10,11). Rings A and B contained the same proton-coupled units as the starting material, the same number of double-bond equivalents and methyl groups, and an extra oxygen but no olefinic carbons. The presence of an epoxide group at C-8 and C-9 and its α or β orientation would satisfy the spectral data for compounds **9** and **10** except for specific stereochemical ordering. The *J* value of 15.8 Hz for the olefinic protons supported a trans substitution (12), and the nOe difference studies confirmed this and identified H-11 and H-12. For example, irradiation of the proton doublet at 6.25 ppm of epoxide **9** showed relaxation to H-1α (1.55 ppm) at 5%, to H_2-16 (4.92 ppm) at 7%, and to Me-17 (1.15 ppm) at 2%, thereby placing the irradiated proton at H-11. Irradiation at 6.58 ppm (H-12) caused



9 8,9-β-epoxide
10 8,9-α-epoxide



11 R=H
12 R=Ac

relaxation to H-14 (5.99 ppm) at 11%. Because relaxation was not observed to one olefinic proton when the other was irradiated, their orientation must be trans. Any ambiguity about the H-11 and H-12 assignments was eliminated by the long-range CH-correlation experiment (COLOC), which showed coupling of the 6.58 ppm (H-12) proton to C-14 (116.65 ppm) and C-16 (70.58 ppm), also H-14 (5.99 ppm) coupled to C-12 (124.59 ppm). Similar data were obtained for epoxide **10**.

The stereochemical positioning of the epoxide was based on the observation that an upfield chemical shift change of 3.5–6 ppm, relative to the starting olefin, occurs for the homoallylic carbon bearing an axial hydrogen in a six-membered ring, when the epoxide is cis to the axial hydrogen (13). In epoxides **9** and **10**, C-5 is homoallylic with an axial hydrogen. Their C-5 chemical shifts are 55.50 and 47.41 ppm, respectively, with that of the starting olefin **4** at 54.10 ppm. The upfield difference of 6.7 ppm for compound **10** places the epoxide in the α-position, while the other isomer, compound **9**, shows a small downfield shift. Furthermore, shielding is observed in the ^{1}H-nmr spectrum for protons above and below the plane of the epoxide ring, except for those close to the oxygen atom, in which case deshielding occurs (14). Deshielding was reported for H-5 in the 8,9-epoxides of other diterpenes (15), and would therefore be expected for H-5 in compound **10**. The value of 2.12 ppm for H-5 is downfield and deshielded in epoxide **10**, as compared to 1.61 ppm for epoxide **9**. This further supports the α-epoxide assignment for compound **10**.

The epoxides **9** and **10** were likely produced from the corresponding 8-hydroperoxides, one of which, compound **7**, was identified and could be the precursor of epoxide **9**. One of the oxygens of the hydroperoxide unit would attack olefinic C-9 to form the epoxide, while the other oxygen would abstract H-12 to split off water and form a double bond at C-11 and C-12.

Compound **11**, the fifth product of photo-oxygenation of **4** has the molecular formula $C_{26}H_{36}O_{10}$, as supported by fabms, and incorporates both atoms of molecular oxygen. Its ^{1}H- and ^{13}C-nmr spectra are similar to those of **5** except for the signals associated with rings C and D. For example, C-14 has only one proton (4.48 ppm) instead of two (2.44 and 2.49 ppm) and is coupled to a D_2O-exchangeable proton. The presence of a hydroxyl at C-14 was supported by preparation of acetate **17** with four acetate groups. In the ^{1}H-nmr spectrum, this showed a downfield shift for H-14 from 4.48 ppm to 5.79 ppm. The other oxygen must form a tetrahydrofuran ring with C-9, C-11, C-12, and C-13. Extensive 1D and 2D nmr studies supported structure **11**. The key elements of those studies included nOe difference experiments. Irradiation at H-1α (1.67 ppm) showed relaxation to H-14 (4.48 ppm) at 4%—which located the hydroxy group to the ring-A side—to H-3α (1.25 ppm) at 4%, to H-5 (2.25 ppm) at 5% and to H-1β (1.83

ppm) at 15%. Irradiation of the olefinic proton at 5.04 ppm caused enhancement of the other olefinic proton (4.93 ppm) by 18%, and H-6 (5.03 ppm) and H-7β (2.62 ppm) together by 8%, and assigned H-17A and H-17B. Irradiation of H-17B (4.93 ppm) located H-11A (2.18 ppm) by a 12% enhancement; and irradiation of H-14 (4.48 ppm) caused relaxation to H-1α (1.67 ppm) by 2% and one H-16 (4.14 ppm) by 4%. The latter proton, H-16, on irradiation enhanced H-12A (2.43 ppm) by 2%, thereby locating the irradiated proton in the A-position. These results established the lactone carbonyl on the ring-A side and specifically located the protons of the spiro system.

Because the absolute stereochemistry of **3** is known (1), the absolute configuration of C-14 must be *S*. Confirmation of this was obtained from the Horeau esterification method of partial resolution with α-phenylbutanoic anhydride (16). The procedure gave phenylbutyric acid with a specific rotation of +2.5°, which, on the basis of assignment of relative bulkiness of substituent at C-14, corresponds to an *S*- configuration.

The hydroxygrindelane **11** would appear to form from the C-9 β-hydroperoxide of the ene reaction with the hydroperoxide cleaving between the oxygens; one adding to C-13 to form the tetrahydrofuran ring and the other as the hydroxyl adding in a stereospecific and probably a concerted manner to C-14. This addition results in the lactone ring being disposed in the opposite manner to that in **1**.

Peroxy compounds are widely distributed among the various classes of natural products but fewer than fifteen are diterpenoids (17). Compounds **1** and **2** appear to be the first 9,13-diepoxy labdane or grindelane-type diterpenoids and most likely arise from oxygenation of **3** and its 19-acetate, the two most abundant terpenes in the plant. Considering the relative instability of these peroxides, the actual yields in the plant are higher than those we report.

EXPERIMENTAL

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

PLANT MATERIAL.—As reported previously (1).

EXTRACTION AND ISOLATION.—As reported previously (1).

Amoenolide K [**1**].—Pooled Si gel column fractions 14–16 (1) (25 g) from the MeOH solubles, after separation on Sephadex LH-20 were chromatographed on 500 g of Si gel packed in hexane and eluted with hexane-Me_2CO (3:1, 2:1, 1:1, 1:2, 1:3). A fraction (4.2 g) eluted by the (1:2) solvent mixture, as monitored by tlc using the column solvent system, was then chromatographed on 100 g of Si gel in $CHCl_3$ and eluted with the lower phases of $CHCl_3$-MeOH-H_2O (18:1:1, 17:2:1, 16:3:1, 15:4:1). The (16:3:1) solvent system gave a fraction (435 mg) that was separated on a reversed-phase RP-8 column (500 g) equilibrated with H_2O and eluted with MeOH-H_2O (1:9, 1:4, 3:7). The (1:4) solvent eluted 280 mg of material that crystallized from hexane-Me_2CO (1:2) to give 152 mg (3.8×10^{-3}% of dry wt) of amoenolide K [**1**]: mp 168–169°; $[\alpha]^{23.5}D$ −50° (*c*=0.5, MeOH); ir (KBr) ν max 1790 (lactone C=O), 1390, 1180, 1040 (C-O) cm^{-1}; uv (MeOH) λ (end absorption) 202 nm (log ε 3.29); fabms (glycerol) *m/z* 383.2077 (0.4, MH^+, $C_{20}H_{31}O_7$ requires 383.2070), 365 (1, MH^+-H_2O), 347 (1, MH^+-2H_2O), 329 (0.5, MH^+-3H_2O), 277 (4), 185 (55), 93 (100), 75 (42), 57 (36); ^1H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

Amoenolide K 19-acetate [**2**].—Fraction 9 (1) (4.0 g) from the MeOH solubles Si gel column, after prior separation on Sephadex LH-20, was chromatographed on 100 g of Si gel with hexane-Me_2CO (4:1, 3:1, 2:1, 1:1, 1:2, 1:3). The material (426 mg) eluted with hexane-Me_2CO (1:1) crystallized from the same solvent to give 48 mg of acetate **2**. The mother liquor residue when separated on a reversed-phase RP-8 column equilibrated with H_2O and eluted with MeOH-H_2O (1:9, 1:4, 3:7, 2:3, 1:1, 3:2) gave from the (3:7) mixture, after crystallization, an additional 109 mg of acetate **2** as needles (total yield 3.3×10^{-3}% of dry wt): mp 159–160°; $[\alpha]^{23.5}D$ −166° (*c*=0.5, MeOH); ir ($CHCl_3$) ν max 3420 (OH), 1790 (C=O), 1730 (C=O), 1710 (C=O), 1610 (C=C), 1380, 1230 (acetate C-O), 1040 (C-O) cm^{-1}; uv (MeOH) λ (end absorption) 202 nm (log ε 3.31); fabms (glycerol) *m/z* 425.2181 (1, MH^+, $C_{22}H_{33}O_8$ requires 425.2175); eims *m/z* 406 (0.2, MH^+-H_2O), 345 (1), 332 (1), 208 (11), 179 (21), 135 (6), 121 (11), 97 (18), 79 (14), 43 (100, CH_3CO). ^1H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

Amoenolide K triacetate [**5**].—Acetylation of amoenolide K [**1**] (10 mg) and the 19-acetate [**2**] (39 mg) was performed separately as described for amoenolide A [**3**] (1) to give the same crystalline triacetate [**5**] from hexane-Me_2CO (1:1): mp 165–166°; $[\alpha]^{23.5}D$ −92° (*c*=0.4, MeOH); ir ($CHCl_3$) ν max 1790 (lactone C=O), 1740 (acetate C=O), 1370, 1260 (acetate C-O), 1040 (C-O) cm^{-1}; fabms (glycerol) *m/z* 509.2366 (1.0, MH^+, $C_{26}H_{37}O_{10}$ requires 509.2387), 449 (1, MH^+−HOAc), 389 (1, MH^+−2HOAc), 329 (2, MH^+−3HOAc), 185 (68), 93 (100); ^1H- and ^{13}C-nmr data in Tables 1 and 2, respectively.

PHOTO-OXYGENATION OF **3**.—Amoenolide A [**3**] (100 mg) and methylene blue (10 mg) were dissolved in 7 ml of absolute EtOH in a 20-ml reaction vessel (18) and irradiated by a DWY 120V 650W quartz-halogen lamp for 1 h while O_2 was bubbled through it. The apparatus was placed into a silver-lined large Dewar flask and H_2O cooled to 11±1°. The residue, after evaporation of EtOH, was passed through a Si gel (5 g) column eluted with $CHCl_3$ and $CHCl_3$-MeOH (49:1) to remove the dye. The eluted residue was chromatographed on Si gel (6 g, 230–400 mesh) with $CHCl_3$ and $CHCl_3$-MeOH (499:1, 199:1). The last solvent gave a fraction [tlc, $CHCl_3$-MeOH-H_2O (16:3:1) lower phase, and *p*-anisaldehyde spray reagent (1)] that crystallized from hexane-Me_2CO (1:1) to give 15 mg (14%) of **1** identical (mp, ir, ^1H-nmr, specific rotation, and tlc mobility) to the natural product.

PHOTO-OXYGENATION OF **4** WITH THIOUREA.—The photo-oxygenation was performed as described above for **3** with **4** (145 mg), methylene blue (10 mg), and thiourea (115 mg) in 7 ml of absolute EtOH irradiated for 1 h. The brown oil residue (133 mg) obtained from the first Si gel column to remove the dye and thiourea products was separated on a Si gel (6 g) column and monitored by tlc with $CHCl_3$-MeOH-H_2O (18:1:1, lower phase). The first of five eluted fractions gave, on crystallization from hexane-Me_2CO (1:1), 31 mg (21%) of **5** identical (mp, ir, ^1H-nmr, specific rotation, and tlc mobility) to the prepared peracetate of **1**. The third fraction (17 mg, 12%) gave alcohol **6**; the other fractions were complex mixtures or polar decomposition products which were not investigated further.

2α,6α,9,16,19-Pentahydroxylabda-8(20),13-dien-15-oic acid, γ-lactone,2,6,19-triacetate [**6**].—A heavy oil: $[\alpha]^{23.5}D$ +121° (*c*=0.12, MeOH); ir (neat) ν max 3479 (OH), 1780, 1734 (C=O), 1637 (C=C), 1250, 1027 (C-O), 758 cm^{-1}; uv (MeOH) λ (end absorption) 205 nm (log ε 4.21); fabms (glycerol) *m/z* 493 (2, MH^+), 433 (1, MH^+−HOAc), 373 (1, MH−2HOAc), 355 (0.6, MH^+−H_2O−2HOAc), 313 (3, MH^+−3HOAc), 295 (3, MH^+−H_2O−3HOAc); eims *m/z* 432.2071 (1, M^+−HOAc, $C_{24}H_{32}O_7$ requires 432.2149), 372 (2, M^+−2HOAc), 330 (2, M^+−CH_2CO−2HOAc), 312 (5, M^+−3HOAc), 294.1561 (1, M^+−H_2O−3HOAc, $C_{20}H_{22}O_2$ requires 294.1621), 43 (100, CH_3CO); ^1H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

PHOTO-OXYGENATION OF **4**.—Triacetate **4** (690 mg) and methylene blue (15 mg) were dissolved in 15 ml of absolute EtOH, photo-oxygenated for 1 h, and the dye removed over a Si gel column as described above. The $CHCl_3$-eluted orange oil (570 mg) gave from hexane-Me_2CO (1:1) 278 mg (40% yield) of crystalline **5** and a mother liquor residue (fraction A). $CHCl_3$-MeOH (19:1) eluted a brown oil (200 mg, fraction B).

Fraction A was separated on a Si gel (10 g, 230–400 mesh) column with $CHCl_3$ and $CHCl_3$-MeOH (499:1, 199:1). Five pooled fractions (A1 to A5) were formed from tlc analysis (see experiment with thiourea). Fractions A1 and A2 eluted with $CHCl_3$-MeOH (499:1) crystallized from $CHCl_3$ to yield epoxides **9** (21 mg, 3%) and **10** (16 mg, 2%), respectively. Fraction A3, eluted with $CHCl_3$-MeOH (199:1), gave grindelane **11** (40 mg, 6%) as a heavy oil.

Fraction B in MeOH (1 ml) was applied to a reversed-phase RP-8 column (E. Merck) equilibrated with H_2O and eluted with MeOH-H_2O (1:5, 2:5, 3:5). The (2:5) eluted fraction showed on tlc two peroxide zones when sprayed with *N,N*-dimethyl-1,4-phenylenediamine (9). The first zone (17 mg) decomposed rapidly and was not characterized, but the second zone (40 mg, 6%) was hydroperoxide **7**.

9(Z)-8-Hydroperoxy-2α,6α,16,19-tetrahydroxy-8βH-labda-9(11),13-dien-15-oic acid, γ-lactone, 2,6,19-triacetate [**7**].—A heavy oil: $[\alpha]^{23.5}D$ +11° (*c*=0.6, MeOH); ir ($CHCl_3$) ν max 1790, 1740 (C=O), 1645 (C=C), 1260 (acetate C-O), 1030 (C-O) cm^{-1}; uv (MeOH) λ (end absorption) 206 nm (log ε 4.13); fabms (glycerol) *m/z* 509.2435 (0.6, MH^+, $C_{26}H_{37}O_{10}$ requires 509.2387), 492 (0.6, MH^+−OH), 448 (1.5, M^+−HOAc), 389 (0.9, MH^+−2HOAc), 355 (2, MH^+−2HOAc−H_2O_2), 329 (1, MH^+−3HOAc), 93 (100); ^1H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

9(Z)-2α,6α,8,16,19-Pentahydroxy-8βH-labda-9(11),13-dien-15-oic acid, γ-lactone, 2,6,19-triacetate [**8**].—Hydroperoxide **7** (38 mg) in 3 ml of Me_2CO was treated with Ph_3P (85 mg) (19) at room temperature for 20 min while stirring. The residue after solvent removal at reduced pressure was chromatographed on Si gel (5 g) with $CHCl_3$. The $CHCl_3$eluted the Ph_3PO and the product was obtained with $CHCl_3$-MeOH (199:1). Alcohol **8** was obtained as a heavy oil: $[\alpha]^{23.5}D$ +4° (*c*=0.6, MeOH); ir ($CHCl_3$) ν max 1790, 1740 (C=O), 1370, 1250, 1040 cm^{-1}; fabms (glycerol) *m/z* 493.2454 (0.4, MH^+, $C_{26}H_{37}O_9$ requires 493.2438), 433 (0.3, MH^+−HOAc), 373 (0.4, MH^+−2HOAc), 355 (0.5, MH^+−H_2O−2HOAc), 313 (0.6,

$MH^+-3HOAc$), 295 (0.7, $MH^+-H_2O-3HOAc$), 93 (100); eims *m/z* 279 (0.4), 213 (1), 199 (1), 175 (4), 97 (8), 83 (16), 57 (21), 43 (100, Ac); ^1H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

(11E)-8,9-Epoxy-2α,6α,16,19-tetrahydroxy-8αH,9αH-labda-11,13-dien-15-oic acid, γ-lactone, 2,6,19-triacetate [**9**].—A cystalline product: mp 133–135°, $[\alpha]^{23.5}D$ +94° (*c*=0.5, MeOH); ir ($CHCl_3$) ν max 1790, 1750 (C=O), 1650, 1600 (C=C), 1250, 1215, 1040 (C-O) cm^{-1}; uv (MeOH) λ max 259 nm (log ε 4.39); fabms (glycerol) *m/z* 491.2324 (0.4, M^+, $C_{26}H_{35}O_9$ requires 491.2281), 93 (100); eims *m/z* 448 (2, $M-CH_2CO$), 430 (13, M−HOAc), 388 (4, $M-CH_2CO-HOAc$), 370 (10, $M^+-2HOAc$), 355 (6, M−Me−HOAc), 310 (8, $M^+-3HOAc$), 190 (100); ^1H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

(11E)-8,9-Epoxy-2α,6α,16,19-tetrahydroxy-8βH,9βH-labda-11,13-dien-15-oic acid, γ-lactone, 2,6,19-triacetate [**10**].—A crystalline product: mp 145–147°; $[\alpha]^{23.5}D$ +1° (*c*= 0.4, MeOH); ir ($CHCl_3$) ν max 1790, 1750 (C=O), 1650, 1600 (C=C), 1260, 1215, 1040 (C-O) cm^{-1}; uv (MeOH) λ max 260 nm (log ε 4.34); fabms (glycerol) *m/z* 491.2319 (0.3, MH^+, $C_{26}H_{35}O_9$ requires 491.2281), 431 (0.3, MH^+-HOAc), 371 (0.4, $MH^+-2HOAc$), 311 (0.6, $MH^+-3HOAc$), 93 (100); ^1H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

(13S,14S)-9,13-Epoxy-2α,6α,14,16,19-pentahydroxylabda-8(17)-en-15-oic acid, γ-lactone,2,6,19-triacetate [**11**].—A heavy oil: $[\alpha]^{23.5}D$ +33° (*c*=0.1, MeOH); ir ($CHCl_3$) ν max 3585, 3420 (OH), 1795, 1740 (C=O), 1655 (C=C), 1385, 1375, 1260, 1085, 1035, 1020 cm^{-1}; uv (MeOH) λ (end absorption) 200 nm (log ε 3.44); fabms (glycerol) *m/z* 509.2418 (2, MH^+, $C_{26}H_{37}O_{10}$ requires 509.2387), 449 (2, MH^+-HOAc), 389 (6, $MH^+-2HOAc$), 329 (9, $MH^+-3HOAc$), 93 (100); eims *m/z* 448 (67, M^+-HOAc), 388 (93, $M^+-2HOAc$), 346 (11, $M^+-CH_2CO-2HOAc$), 328 (69, $M^+-3HOAc$), 43 (100); ^1H- and ^{13}C-nmr data, see Tables 1 and 2, respectively.

Acetylation of compound **11**.—Compound **11** (5 mg) was acetylated as described by O'Mathúna and Doskotch (1) to yield 5 mg of tetraacetate **12** as a heavy oil; ^1H nmr (250 MHz, $CDCl_3$) δ 5.79 (1H, br s, H-14), 5.40 (1H, br s, H-17A), 4.92 (1H, br s, H-17B), 4.20 (1H, d, *J*=9 Hz, H-19A), 4.08 (1H, d, *J*=9 Hz, H-19B), 2.214 (3H, s, Ac), 2.211 (1H, d, *J*=12 Hz, H-5), 2.07 (3H, s, Ac), 2.03 (3H, s, Ac), 2.02 (3H, s, Ac), 1.20 (3H, s, Me), 0.93 ppm (3H, s, Me).

HOREAU PROCEDURE ON COMPOUND **11**.—Grindelane **11** (4 mg, 8 μmol) in 0.4 ml of 1.25% solution of 2-phenylbutanoic anhydride (5 mg, 16 μmol) in anhydrous pyridine was reacted at room temperature for 48 h; then 3 drops of H_2O were added and after 30 min the solvents were removed at reduced pressure. The residue was partitioned between saturated aqueous $NaHCO_3$ (5 ml) and $CHCl_3$ (3×5 ml). The combined $CHCl_3$ phase was reduced to dryness to give the esterified grindelane (3.8 mg): fabms ["magic bullet" (20)] *m/z* 677 (0.5, MNa^-), 595 (0.3, MH^+-HOAc), 535 (1, $MH^+-2HOAc$), 475 (2, $MH^+-3HOAc$), 311 (2, $MH^+-3HOAc-PhCH(Et)CO_2H$), 55 (100); ^1H nmr (250 MHz, $CDCl_3$) δ 7.32 (5H, s, ArH), 5.64 (1H, s, H-14), 5.03 (1H, br s, H-17A), 4.86 (1H, br s, H-17B), 4.18 (1H, d, *J*=9 Hz, H-19A), 4.05 (1H, br s, H-16), 4.04 (1H, d, *J*=9 Hz, H-19B), 3.58 (1H, t, *J*=8 Hz, H-2′), 2.60 (1H, dd, *J*=5 and 12 Hz, H-7β), 1.19 (3H, s, Me), 0.95 (1H, t, *J*=7 Hz, H-4′), 0.87 (3H, s, Me).

The $NaHCO_3$ solution was acidified with 10% HCl and extracted with $CHCl_3$ (3×5 ml). After washing the combined $CHCl_3$ extract with H_2O (2×20 ml), the organic phase was evaporated to dryness to give 3.8 mg of α-phenylbutanoic acid, $[\alpha]^{23.5}D$ +2.5° (*c*=0.4, MeOH), identical (mp and ^1H-nmr data) to an authentic sample.

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