STRUCTURAL STUDIES ON EBELACTONE A AND B, ESTERASE INHIBITORS PRODUCED BY ACTINOMYCETES

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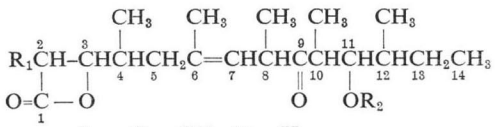
(Received for publication July 26, 1982)

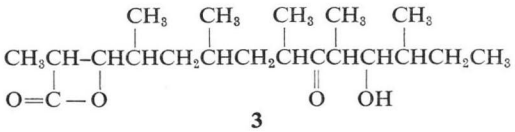
The structures of ebelactone A and B, esterase inhibitors produced by *Streptomyces* sp. MG7-G1 strain, have been determined to be 3,11-dihydroxy-2,4,6,8,10,12-hexamethyl-9-oxo-6-tetradecenoic 1,3-lactone and 2-ethyl-3,11-dihydroxy-4,6,8,10,12-pentamethyl-9-oxo-6-tetradecenoic 1,3-lactone, respectively, by their spectral analyses.

In the course of our studies on enzyme inhibitors produced by microorganisms, two potent esterase inhibitors named ebelactone A and B, were isolated from the cultured broth of the strain MG7-G1 which was closely related to *Streptomyces aburaviensis*[1]. They were determined to be new members of mycolic acid β-lactone. As reported in our previous papers[2,3], esterastin, an esterase inhibitor produced by *Streptomyces lavendulae* MD4-C1, was also structurally a mycolic acid β-lactone. Thus, again the β-lactone structure was suggested as an active group that inhibits esterase. In this paper, we report on structural elucidation of ebelactone A and B.

Ebelactone A (**1a**) was obtained as colorless needles[1], mp 86°C, $[\alpha]_D^{20}$ −221° (*c* 1, methanol), UV maximum at 291 nm in methanol. The molecular formula for **1a** was established by elementary analysis and mass spectrometry as $C_{20}H_{34}O_4$. In the mass spectrum, the molecular ion peak (*m/z* 338) and the peak of a decarboxylation product (*m/z* 294) were observed. The IR spectrum indicated the presence of hydroxyl (3500 cm^{-1}), β-lactone (1820 cm^{-1}) and carbonyl (1695 cm^{-1}) groups. The ^1H NMR spectral analysis (Table 1) showed the presence of a partial structure $CH_3CHCHCHCH_3$. By comparison with the spectral data of the β-lactone moiety of esterastin[3] and antibiotic 1233 A[4] (Table 2), the structure was shown to be A in Fig. 1. The ^1H NMR spectrum (Table 1) also showed the presence of partial structures B and C in Fig. 1. The spectral data of 8-H (δ 3.59, doublet of quartets) and 10-H (δ 2.86, doublet of quartets) indicated that these two fragments should be linked through a carbonyl group. In the ^{13}C NMR spectrum, two carbonyl, seven methine, two methylene, seven methyl and two olefinic carbons were observed as shown in Table 3.



1a: $R_1=CH_3$, $R_2=H$
1b: $R_1=CH_3CH_2$, $R_2=H$
2a: $R_1=CH_3$, $R_2=CH_3CO$
2b: $R_1=CH_3CH_2$, $R_2=CH_3CO$



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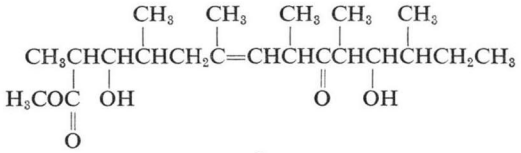

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Table 1. ^{1}H NMR spectral data of ebelactones and their acetyl derivatives.

Assignment	δ ppm (J Hz)			
	Ebelactone A (**1a**)	Ebelactone B (**1b**)	Acetylebelactone A (**2a**)	Acetylebelactone B (**2b**)
2′-H_3	—	1.06 t (7)	—	1.06 t (7)
1′-H_2	—	~1.86	—	~1.85
2-CH_3	1.38 d (7.5)	—	1.39 d (7.5)	—
2-H	3.29 dq (7.5, 4)	3.20 dt (7, 4)	3.27 dq (7.5, 4)	3.21 dt (7, 4)
3-H	3.88 dd (4, 8)	3.92 dd (4, 8)	3.89 dd (4, 8.5)	3.84 dd (4, 8)
4-H	~2.0	~2.0	~2.0	~2.0
4-CH_3	0.87 d (6.5)	0.86 d (6.5)	0.86 d (6.5)	0.86 d (6.5)
5-H_2	~1.8 2.35 m (10)	~1.8 2.38 m (10)	~1.8 2.38 m (10)	~1.8 2.4 m (10)
6-CH_3	1.73 d (2)	1.73 d (2)	1.73 d (2)	1.72 d (2)
7-H	5.04 m (2, 10)	5.04 m (2, 10)	5.13 m	5.12 m
8-H	3.59 dq (10, 7)	3.58 dq (10, 7)	3.53 dq (10, 7)	3.54 dq (10, 6.5)
8-CH_3	1.12 d (7)	1.12 d (7)	1.12 d (7)	1.12 d (6.5)
10-H	2.86 dq (7.5, 3)	2.86 dq (7.5, 3)	2.98 dq (6.5, 6.5)	2.98 dq (7, 7)
10-CH_3	1.10 d (7.5)	1.10 d (7.5)	1.05 d (6.5)	1.05 d (7)
11-H	3.50 m	3.51 m	5.12 m	5.12 m
11-OH	3.03 m (2)	3.04 m (2.5)	—	—
12-H	~1.4	~1.4	~1.4	~1.4
12-CH_3	0.79 d (6.5)	0.78 d (6.5)	0.86 d (6.5)	0.86 d (6.5)
13-H_2	~1.7	~1.7	~1.7	~1.7
14-H_3	0.87 t (7)	0.87 t (7)	0.85	0.85
11-Ac	—	—	2.04 s	2.04 s
1-OCH_3	—	—	—	—

Chemical shifts, δ (ppm) were measured in $CDCl_3$ using TMS as the internal reference.

Treatment of **1a** with acetic anhydride in pyridine gave acetylebelactone A (**2a**); an ester absorption at 1735 cm^{-1}; a singlet acetyl methyl signal at δ 2.04. The 11-H signal (δ 3.50) of **1a** shifted to δ 5.12, indicating the acetylation of the 11-hydroxyl group.

Catalytic hydrogenation of **1a** gave dihydroebelactone A (**3**). In the spin decoupling experiment of **3**, irradiation at δ 2.93, which was between the signals of the 10-H (δ 2.86) and 11-OH (δ 3.0), caused a collapse of the multiplet signal of the 11-H at δ 3.53 to doublet (J=8 Hz), suggesting the 12-methine.

Methanolysis of **1a** with 0.01 N NaOH in methanol gave a methyl ester (**4**), showing the presence of hydroxyl (3500 cm^{-1}), ester (1730 cm^{-1}) and carbonyl (1705 cm^{-1}). In the ^{1}H NMR spectrum of **4**, a multiplet signal at δ 2.41, which had the long-range coupling (J=2 Hz) with the olefinic proton signal

Fig. 1. Partial structures of ebelactones.

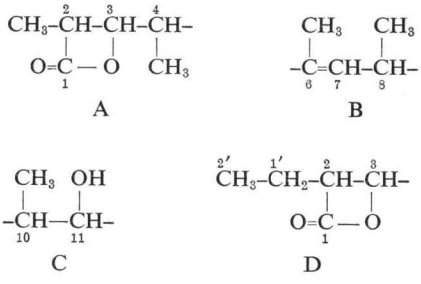


Table 2. Chemical shifts and coupling constants of β-lactones.

β-Lactones	Chemical shifts (δ, ppm) O=C—O / —CH—CH-		J value (Hz)
Ebelactone A	3.29	3.88	4
Ebelactone B	3.20	3.92	4
Esterastin[3]	3.21	4.34	4
Antibiotic 1233A[4]	3.32	4.50	4

Table 3. ^{13}C NMR chemical shifts of ebelactone A and B.

Carbon	δ (ppm)		Carbon	δ (ppm)	
	1a	**1b**		**1a**	**1b**
1	171.7 s	171.7 s	12	36.6 d	36.6 d
2	49.1 d	56.0 d	13	24.9 t	24.9 t
3	82.9 d	81.1 d	14	13.5 q	13.7 q
4	35.5 d	35.5 d	4-CH_3	10.9 q	10.9 q
5	42.9 t	42.9 t	6-CH_3	16.4 q	16.4 q
6	135.5 s	135.5 s	8-CH_3	16.4 q	16.4 q
7	126.5 d	126.5 d	10-CH_3	9.5 q	9.5 q
8	45.3 d	45.4 d	12-CH_3	14.9 q	14.9 q
9	217.5 s	217.5 s	2-CH_3	12.9 q	
10	45.2 d	45.2 d	1′		21.4 t
11	74.6 d	74.6 d	2′		11.4 q

Chemical shifts were measured in $CDCl_3$, using TMS as the internal reference.
Assignments, s, d, t and q show multiplicity of off-resonance experiment.

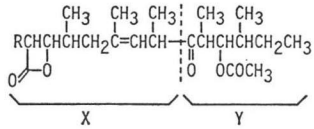
(7-H) at δ 5.00, was assigned to the 5-H. Furthermore, in the ^1H NMR spectrum of **1a** (Table 1) the signal of 5-H (δ 2.35) was collapsed by the irradiation at δ 2.0 (4-H).

From the chemical and spectral data described above, the structure of ebelactone A was proposed to be 3,11-dihydroxy-2,4,6,8,10,12-hexamethyl-9-oxo-6-tetradecenoic 1,3-lactone (**1a**). This structure was confirmed by X-ray crystallographic analysis of its *p*-bromobenzoate, which also disclosed the stereochemistry (2*S*, 3*S*, 4*S*, 6*E*, 8*R*, 10*S*, 11*R*, 12*R*). The X-ray analysis will be reported in the other paper.

Ebelactone B (**1b**) was obtained as colorless needles[1], mp 77°C, $[\alpha]_D^{25}$ −203° (*c* 1, methanol), UV maximum at 291 nm in methanol, IR hydroxyl (3540 cm^{-1}), β-lactone (1810 cm^{-1}) and carbonyl (1700 cm^{-1}). These spectral data were very similar to those of **1a**. Its molecular formula was determined by elementary analysis and mass spectrometry as $C_{21}H_{36}O_4$. In accordance with the molecular formula, the ^{13}C NMR spectrum of **1b** (Table 3) showed one more methylene carbon signal at δ 21.4 other than the signals of **1a**. In the ^1H NMR spectrum of **1b** (Table 1) a partial structure D (Fig. 1) was shown. Thus, the structure of ebelactone B was proposed to be 2-ethyl-3,11-dihydroxy-4,6,8,10,12-pentamethyl-9-oxo-6-tetradecenoic 1,3-lactone (**1b**).

These structures of **1a** and **1b** were in good agreement with the high-resolution mass spectroscopic data of their acetyl derivatives **2a** and **2b** (Table 4).

Table 4. Significant fragment ions of acetylebelactone A and B in high-resolution mass spectra.



2a R=CH_3 **2b** R=CH_3CH_2

Fragment ion	Acetylebelactone A (**2a**)			Acetylebelactone B (**2b**)		
	Formula	Observed	Error (mu)	Formula	Observed	Error (mu)
M^+	$C_{22}H_{36}O_5$	380.2522	−3.8	$C_{23}H_{38}O_5$	394.2760	4.3
M^+-CO_2	$C_{21}H_{36}O_3$	336.2653	−0.9			
M^+−AcOH				$C_{21}H_{34}O_3$	334.2525	1.9
$M^+-(CO_2+AcOH)$	$C_{19}H_{32}O$	276.2439	2.8	$C_{20}H_{30}O$	290.2586	−2.1
X	$C_{12}H_{19}O_2$	195.1371	−1.2	$C_{13}H_{21}O_2$	209.1581	4.1
Y	$C_{10}H_{17}O_3$	185.1166	−1.0	$C_{10}H_{17}O_3$	185.1189	1.2
$X-CO_2$	$C_{11}H_{19}$	151.1485	−0.2	$C_{12}H_{21}$	165.1639	−0.3
Y−AcOH	$C_8H_{13}O$	125.0967	0.1	$C_8H_{13}O$	125.0962	−0.3

Experimental

Melting points were uncorrected. UV spectra were recorded on a Hitachi 124 spectrometer, IR spectra on a Hitachi EPI-S2 infrared spectrometer, ^1H NMR and ^{13}C NMR spectra on a Varian XL-100A spectrometer and mass spectra on a Hitachi RMU-6M or M-80 spectrometer. Optical rotations were measured by a Carl Zeiss LEP A2 polarimeter. Column chromatography was carried out by using silica gel (Wakogel C-300) or reversed phase silica gel (Merck, Silica gel 60 silanised).

Ebelactone A (**1a**)

The inhibitor **1a** was purified by the method described in a previous paper[1], and crystallized from methanol-water as colorless needles, mp 86°C, $[\alpha]_D^{20}$ −221° (*c* 1, methanol), UV max. in methanol 291 nm (ε 258), IR (KBr) 3500, 2950, 1820, 1695, 1460, 1125, 980, 870 cm^{-1}. The ^1H NMR spectral data is shown in Table 1. *Anal.* Found: C 70.97, H 10.12, O 18.91, Calcd. for $C_{20}H_{34}O_4$: C 70.97, H 10.20, O 19.07. MS *m/z* 338 (M^+).

Ebelactone B (**1b**)

The inhibitor **1b** was purified by the method described in a previous paper[1], and crystallized from methanol-water as colorless needles, mp 77°C, $[\alpha]_D^{26}$ −203° (*c* 1, methanol), UV max. in methanol 291 nm (ε 278), IR (KBr) 3540, 2950, 1810, 1700, 1460, 1130, 990, 975, 865 cm^{-1}. The ^1H NMR spectral data is shown in Table 1. *Anal.* Found: C 71.72, H 10.31, O 18.37, Calcd. for $C_{21}H_{36}O_4$: C 71.59, H 10.23, O 18.18. MS *m/z* 352 (M^+).

Acetylebelactone A and B (**2a** and **2b**)

Treatment of **1a** (10 mg) with acetic anhydride (0.05 ml) in pyridine (0.1 ml) overnight at room temperature followed by column chromatography on silica gel developed with *n*-hexane - chloroform (3: 1) gave a colorless oil (9.6 mg) of **2a**, $[\alpha]_D^{26}$ −196° (*c* 1, methanol), UV max. in methanol 292 nm (ε 297), IR (KBr) 3450, 2950, 1823, 1735, 1708, 1455, 1380, 1373, 1240, 1125, 980, 866, 755 cm^{-1}. The ^1H NMR and high-resolution mass spectral data are shown in Tables 1 and 4, respectively.

Acetylation of **1b** (25.7 mg) by the same manner yielded a colorless oil (24.0 mg) of **2b**. $[\alpha]_D^{26}$ −184° (*c* 1, methanol), UV max. in methanol 292 nm (ε 287), IR (KBr) 3450, 2950, 1820, 1735, 1705, 1455, 1380, 1370, 1240, 1120, 1000, 962, 870 cm^{-1}. The ^1H NMR and high-resolution mass spectral data are shown in Tables 1 and 4, respectively.

Dihydroebelactone A (**3**)

A solution of **1a** (27.0 mg) in methanol (1 ml) was shaken with PtO_2 catalyst (5 mg) in a Parr apparatus under the pressure of 3.5 kg/cm^2 of hydrogen overnight at room temperature. The product was purified by column chromatography on silica gel eluted with *n*-hexane - chloroform - ethyl acetate (10: 10: 1) to give colorless needles of **3** (10.4 mg), mp 77~78°C, $[\alpha]_D^{23}$ −156° (*c* 0.5, methanol), UV max. in methanol 292 nm (ε 203), IR (KBr) 3450, 2920, 1820, 1690, 1455, 1380, 1123, 978, 863 cm^{-1}, MS *m/z* 340 (M^+), ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, *J*=7 Hz, 14-H), 0.87 (3H, d, *J*=6 Hz), 0.90 (3H, d, *J*=6 Hz), 0.93 (3H, d, *J*=7.5 Hz, 4-CH_3), 1.08 (3H, d, *J*=7 Hz, 8-CH_3), 1.10 (3H, d, *J*=7 Hz, 10-CH_3), 1.39 (3H, d, *J*=8 Hz, 2-CH_3), 2.86 (1H, dq, *J*=2 and 7 Hz, 10-H), 3.0 (1H, m, 11-OH), 3.24 (1H, dq, *J*=4 and 8 Hz, 2-H), 3.53 (1H, m, 11-H), 3.84 (1H, dd, *J*=4 and 8 Hz, 3-H).

Methyl Ester (**4**)

A solution of **1a** (10.1 mg) in 5 ml of 0.01 N NaOH in anhydrous methanol was kept at room temperature for 30 minutes. After addition of 5 ml of water, the solution was extracted with *n*-hexane (5 ml×3) and the extract was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with *n*-hexane - chloroform (3: 7) to yield a colorless oil of **4** (7.2 mg), $[\alpha]_D^{23}$ −172° (*c* 1, methanol), UV max. in methanol 292 nm (ε 325), IR (KBr) 3500, 2950, 1730, 1700, 1458, 1380, 1200, 1173, 990, 970 cm^{-1}, MS *m/z* 370 (M^+). ^{1}H NMR ($CDCl_3$) δ 0.77 (3H, d, *J*=6.5 Hz, 12-CH_3), 0.84 (3H, d, *J*=6.5 Hz, 4-CH_3), 0.87 (3H, t, *J*=7 Hz, 14-H_3), 1.09 (3H, d, *J*=7.5 Hz, 10-CH_3), 1.11 (3H, d, *J*=7 Hz, 8-CH_3), 1.25 (3H, d, *J*=7.5 Hz, 2-CH_3), ~1.4 (1H, m, 12-H), 1.68 (1H, m, 4-H), ~1.7 (2H, m, 13-H_2), 1.71 (3H, d, *J*=2 Hz, 6-CH_3), ~1.8 and 2.41 (2H, m, 5-H_2), 2.72 (1H, dq, *J*=7.5 and 5.5 Hz, 2-H), 2.86 (1H, dq, *J*=7.5 and 3 Hz, 10-H), 3.12 (1H, m, *J*=2 Hz, 11-OH), ~3.5 (1H, m, 11-H), ~3.5 (1H, m, 3-H), 3.56 (1H, dq, *J*=10 and 7 Hz, 8-H), 3.72 (3H, s, 1-OCH_3), 5.00 (1H, m, *J*= 10, 2 and 2 Hz, 7-H).

Acknowledgement

This work was partly supported by a contract NO1-CM-57009 with the Division of Cancer Treatment, the National Cancer Institute, U.S.A.

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