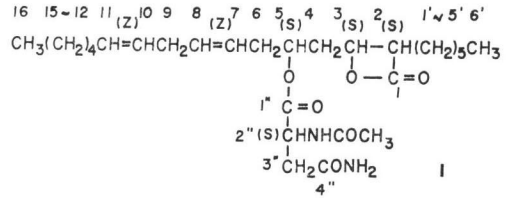
Communications to the editor

THE STRUCTURE OF ESTERASTIN, AN INHIBITOR OF ESTERASE

Sir:

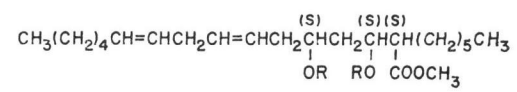
Esterastin found in the culture of *Streptomyces lavendulae* No. MD4-Cl by UMEZAWA *et al.*[1] exhibits a strong inhibition of pancreatic esterase. In this communication, its structure (**1**) is reported.



Esterastin (**1**) is obtained as a colorless powder, mp 99~100°C; $[\alpha]_D^{23}$ +11° (*c* 1, $CHCl_3$); UV 265 nm (sh) in 95% aqueous methanol; IR (KBr) 1840 (β-lactone), 1730, 1185 (ester), 1645, 1610 and 1545 (amide) cm^{-1}; positive RYDON-SMITH and anisaldehyde-H_2SO_4 reactions and negative ninhydrin reaction.[1] The formula $C_{28}H_{46}N_2O_6$ was established for **1** by high-resolution mass spectroscopy and elemental analysis. The ^1H NMR spectrum of **1** in $CDCl_3$ showed two C-methyl groups (δ 0.90, t), an N-acetyl group (δ 2.03, s) and four olefinic protons (δ 5.4~5.7, m) as shown in Table 1. Catalytic hydrogenation of **1** in methanol with PtO_2 at room temperature for 2 hours in a Parr apparatus gave a colorless tetrahydroesterastin (**2**), mp 102.5~104°C; *m/e* 511, $(M+1)^+$; UV 260 nm (sh) in 95% aqueous methanol; IR (KBr) 1830 (β-lactone), 1720, 1185 (ester), 1650, 1610 and 1545 (amide) cm^{-1}.

Alkaline hydrolysis of **1** (498 mg, 0.98 mmole) with 1 N KOH (4 ml) in dioxane (250 ml) under gently refluxing for 4 hours afforded a colorless oil (175 mg) which was determined to be a δ-lactone (**3**) of a member of mycolic acids*, $[\alpha]_D^{27}$ +21.5° (*c* 1, $CHCl_3$); *m/e* 350.2785 (M^+, calcd. for $C_{22}H_{38}O_3$, *m/e* 350.2818); IR (KBr) 1700 and 1175 (δ-lactone) cm^{-1}. Catalytic hydrogenation of the δ-lactone (**3**) gave a colorless tetrahydro derivative (**4**), mp 107.5°C; *m/e* 355, $(M+1)^+$. Acetylation of **3** and **4** with acetic anhydride in pyridine overnight at room temperature gave their crystalline mono-O-acetyl derivatives **5** (*m/e* 392, M^+) and **6** (*m/e* 397, $(M+1)^+$), respectively.

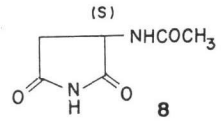
Treatment of **1** (255 mg, 0.50 mmole) with 55 ml of 0.01 N NaOH in methanol at room temperature for 50 minutes afforded the δ-lactone **3** (35 mg), a colorless oil of a δ-hydroxymycolic acid methyl ester (**7**, 65 mg) and a crystalline powder of N^{α}-acetylaspartimide (**8**, 50 mg) which was identified as the L-enantiomer, $[\alpha]_D^{23}$ −41° (*c* 0.44, CH_3OH) (Lit.,[2] $[\alpha]_D^{20}$ −56.6°). The methyl ester **7** showed *m/e* 383, $(M+1)^+$; IR (KBr) 1725 and 1165 (ester) cm^{-1}. The di-O-acetyl derivative **9** (*m/e* 467, $(M+1)^+$) was obtained by acetylation of **7** with acetic anhydride in pyridine.



7 R = H

9 R = CH_3CO

Mild hydrolysis of **1** (275 mg, 0.54 mmole) with 0.01 N NaOH in a mixture (55 ml) of dioxane and water (1 : 1) overnight at room temperature afforded a δ-hydroxymycolic acid β-lactone (**10**, 140 mg) and an N-acetyl amino acid (**11**, 63 mg).



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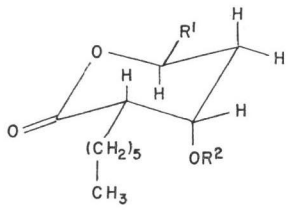

3 R^1 = $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2$. R^2 = H

4 R^1 = $CH_3(CH_2)_{10}$. R^2 = H

5 R^1 = $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2$. R^2 = CH_3CO

6 R^1 = $CH_3(CH_2)_{10}$. R^2 = CH_3CO

The former (**10**) was obtained as a colorless oil by extraction with *n*-hexane followed by column chromatography on silica gel, $[\alpha]_D^{26}$ −10° (*c* 1, $CHCl_3$); *m/e*

* Mycolic acid is the generic name of higher fatty acids possessing α-alkyl and β-hydroxyl groups.

350 $(M)^+$; IR (KBr) 1820 and 1120 (β-lactone) cm^{-1}. Acetylation of **10** with acetic anhydride in pyridine gave a mono-O-acetyl derivative (**12**), *m/e* 392 (M^+). The latter **11** which was isolated from the aqueous layer of the hydrolysate was identical with authentic N^{α}-acetyl-L-asparagine (Sigma Chemical Co.) in all respects.

Mild hydrolysis of **2** (76 mg, 0.15 mmole) with 0.01 N NaOH in a mixture (18 ml) of dioxane and water (1 : 1) overnight at room temperature afforded a tetrahydro derivative (**13**, 39 mg) of **10**, mp 64.5~65.5°C; $[\alpha]_D^{26}$ −15° (*c* 1, $CHCl_3$); *m/e* 355, $(M+1)^+$; IR (KBr) 1810 and 1130 (β-lactone) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 t (J= 7 Hz, 16-H_3, 6′-H_3), ~1.3 ($CH_2 \times 8$), 1.7~2.6 m (4-H_2, 6-H_2, 1′-H_2), 3.31 m (J=7, 4 Hz, 2-H), 3.76 m (5-H) and 4.46 m (J=4, 6.5 Hz, 3-H). The β-lactone in **13** was decarboxylated at 200°C for 20 minutes under a nitrogen stream and con-

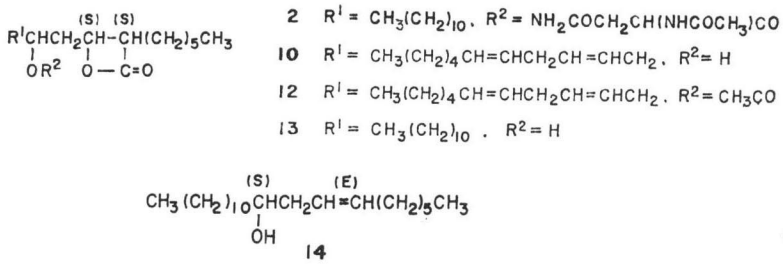

Fig. 1. Spectral analysis of ^1H NMR of the δ-lactone **3**

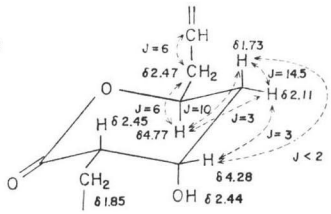

Table 1. Chemical shifts of ^1H NMR spectra

Proton	δ ppm (J Hz)			
	Esterastin (**1**)	δ-Lactone (**3**)	Methyl ester (**7**)	β-Lactone (**10**)
2-H	3.21 m (7, 4)	~2.45	2.46 m (6, 7)	3.32 m (4, 7)
3-H	4.34 m (6, 4)	4.28 m	3.92 m	4.47 m (4, 6)
4-H_2	~2.1	1.73 dd (14.5, 10, <2) 2.11 dd (14.5, 3, 3)	~1.6 m	~2.0 m
5-H	5.02 m	4.77 m (3, 10, 6)	3.92 m (7)	3.79 m (5)
6-H_2	~2.4 m	2.47 t (6)	2.28 m (7, 5)	2.32 m (6, 5)
7, 8-H	5.4~5.7 m	5.15~5.65 m	5.2~5.6 m	5.15~5.72 m
9-H_2	2.78 t (6)	2.80 t (6)	2.80 t (6)	2.80 t (6)
10, 11-H	5.4~5.7 m	5.15~5.65 m	5.2~5.6 m	5.15~5.72 m
12-H_2	~2.1	~2.0	2.05 m	~2.0 m
13~15-H_2	~1.3	~1.33	~1.28	~1.3
2′~5′-H_2	~1.3	~1.33	~1.28	~1.3
16, 6′-H_3	0.90 t (6)	0.90 t (7)	0.88 t (6) 0.89 t (6)	0.89 t (6.5)
1′-H_2	1.74 m (7)	~1.85	~1.55	1.81 m
1-COOMe	—	—	3.72 s	—
3 or 5-OH	—	~2.44	~3.15 ~3.54	~2.0
2″-H	4.72 m (8, 4.5)	—	—	—
3″-H_2	2.75 dd (16, 4.5) 2.97 dd (16, 4.5)	—	—	—
2″-NAc	2.03 s	—	—	—
2″-NH	6.80 d (8)	—	—	—
3″-$CONH_2$	5.73 6.12	—	—	—

verted into an *E*-olefin (**14**) in 62% yield, mp 40.5 ~41°C; $[\alpha]_D^{24}$ +1° (*c* 1, $CHCl_3$); *m/e* 310 (M^+); ^1H NMR ($CDCl_3$) δ 5.36 and 5.58 m (J=16 Hz). Consequently, the relative configuration of the β-lactone protons was assigned to be *trans* by application of the thermolysis rule for β-lactones.[3] Oxidation of **14** (60 mg) with $NaIO_4$ (624 mg) and $KMnO_4$ (12 mg) in a mixture of *tert*-butyl alcohol (45 ml) and water (130 ml) adjusted to pH 8~9 with Na_2CO_3, overnight at room temperature afforded two acids; a hydroxy acid (**15**, 18 mg) which was identical with L-3-hydroxymyristic acid, mp 72.5~73°C; $[\alpha]_D^{27}$ +13° (*c* 1, $CHCl_3$) (Lit.,[4] the D-isomer, mp 73~74°C; $[\alpha]_D^{25}$ −16°), and enanthic acid (11 mg) which was identified by gas chromatography (instrument: a Hewlett Packard 402 with a glass column (0.3×100 cm), liquid phase: 10% polyethylene glycol 20 M, support: Uniport B (60~80 mesh, Gasukuro Kogyo Co.), column temperature: 85°C, carrier gas: nitrogen, 15 ml/minute) of its methyl ester. Therefore, the structure of **14** was determined to be (7*E*, 10*S*)-7-heneicosen-10-ol.

As shown in Table 1, a partial structure $-CH_2-CH=CHCH_2CH=CHCH_2CHCH_2CHCHCH_2-$ was shown by the ^1H NMR spectra of **1**, **3**, **7** and **10**. Caproic acid was obtained by periodate-permanganate oxidation of **3**, **7** or **10** and identified by gas chromatography of its methyl ester. The absolute configurations of three derivatives of the mycolic acid are 2*S*, 3*S* and 5*S* as shown by **3**, **7** and **10**, respectively. The *Z*-configurations of the two double bonds were confirmed by the ^1H NMR spectrum of **10**, using $Eu(fod)_3$ as the shift reagent, δ~6.8 and 6.12 ($J_{7,8}$=10 Hz), δ 5.56 and 5.65 ($J_{10,11}$=10 Hz). The *S*-configuration at C-3 was determined by analysis of ^1H NMR spectrum of **3** as shown in Fig. 1. The configuration at C-2 was confirmed by the *E*-double bond formation[3] in **14**.

The ester band in the IR of **1** indicates that the 5-hydroxy of **10** is bound to the carboxyl of **11** by an ester linkage. From the foregoing results, the configuration of esterastin (**1**) can be proposed to be (2*S*, 3*S*, 5*S*, 7*Z*, 10*Z*)-5-[(*S*)-2-acetamido-3-carbamoylpropionyloxy]-2-hexyl-3-hydroxy-7,10-hexadecadienoic lactone. As shown in Table 2, this structure is in agreement with ^{13}C NMR of **1**. Only three natural products have been known to contain a β-lactone group; anisatin and neoanisatin extracted from seeds of *Illicium anisatum* L.[5] and antibiotic 1233A produced by *Cephalosporium* sp.[6] Esterastin is the first β-lactone-containing compound produced by *Streptomyces* sp.

Table 2. Chemical shifts of ^{13}C NMR spectrum

	Carbon	ppm		
		Esterastin (**1**)	δ-Lactone (**3**)	β-Lactone (**10**)
1	1″	172.6 s		
2	Ac-CO	171.3 s		
3	1	170.7 s	173.7 s	171.6 s
4	4″	170.3 s		
5	7, 8, 10, 11	132.2 d	131.8 d	132.3 d
6		131.0 d	130.8 d	130.9 d
7		126.9 d	127.1 d	127.2 d
8		123.1 d	123.3 d	124.6 d
9	3	75.1 d*	64.5 d*	76.1 d
10	5	72.4 d*	75.5 d*	68.5 d
11	2	57.1 d*	46.5 d	56.7 d
12	2″	49.3 d*		
13	6	38.1 t*	35.8 t	40.6 t
14	3″	36.7 t*		
15	4	31.9 t	33.4 t	35.6 t
16	9, 12, 13, 14, 1′, 2′, 3′, 4′	31.5 t	31.8 t	31.6 t
17		31.5 t	31.6 t	31.6 t
18		29.3 t	29.3 t	29.4 t
19		29.0 t	29.3 t	29.1 t
20		27.6 t	27.3 t	28.0 t
21		27.3 t	27.0 t	27.4 t
22		26.7 t	26.5 t	26.9 t
23		25.8 t	25.9 t	25.9 t
24	Ac-CH_3	23.0 q		
25	15, 5′	22.6 t	22.7 t	22.7 t
26		22.6 t	22.6 t	22.7 t
27	16, 6′	14.1 q	14.1 q	14.1 q
28		14.1 q	14.1 q	14.1 q

The ^{13}C FT NMR spectra were taken with a Varian XL-100 spectrometer. Sample were dissolved in $CDCl_3$ containing TMS as the internal reference. Assignments, s, d, t and q, show multiplicity on off-resonance experiment.
* Assignments are given by selective proton decoupling techniques.

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