VALILACTONE, AN INHIBITOR OF ESTERASE, PRODUCED BY ACTINOMYCETES

Sir:

We have reported that specific inhibitors of various enzymes located on the cellular membrane, had important effects on cellular functions[1~3]. These inhibitors included esterastin which inhibited esterase and suppressed immune responses[4] (as well as) ebelactones which inhibited esterase but enhanced immune responses[5]. Continued screening for esterase inhibitors has resulted in the discovery of another inhibitor which we have named valilactone. Valilactone has no effect on immune responses. In this paper, we report on the isolation and characterization of valilactone.

In the screening study, culture filtrates of many strains of various species of soil actinomycetes showed the activity to inhibit esterase. We have isolated a new inhibitor from the strain MG147-CF2. This strain, closely related to *Streptomyces albolongus*, was isolated from a soil sample collected in Shirane Mountain, Gunma Prefecture.

The methods for determining the inhibitory activity against esterase were previously reported[5].

Valilactone was produced by shaken culture and jar fermentation of the strain MG147-CF2 in the media containing various carbon and nitrogen sources. A typical medium used for production contained soy bean oil 1.6%, maltose 1.0%, soy bean meal 2.5%, yeast extract 0.2%, K_2HPO_4 0.05% and $MgSO_4 \cdot 7H_2O$ 0.05%.

The maximum production was attained in 2 days in the shaken culture.

Valilactone was present both in mycelium and broth filtrate. The broth filtrate was passed through a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited). Most of the active substance was eluted with 95% MeOH. This eluate was combined with the MeOH extract of the mycelium and concentrated under reduced pressure to a syrup. It was dissolved in distilled water and extracted with EtOAc. The yellowish oily residue remaining when the EtOAc was removed under reduced pressure, was chromatographed on a silica gel column using hexane - $CHCl_3$ - EtOAc (5 : 5 : 1). The active fractions were combined and concentrated under reduced pressure. The concentrate obtained by distillation of the combined active fraction under reduced pressure was subjected to reversed phase silica gel column chromatography with MeOH - water (4 : 1). The concentrate of active fractions from this chromatography was subjected to Sephadex LH-20 column chromatography with MeOH. Further purification of the main

Fig. 1. IR spectrum of valilactone (KBr).

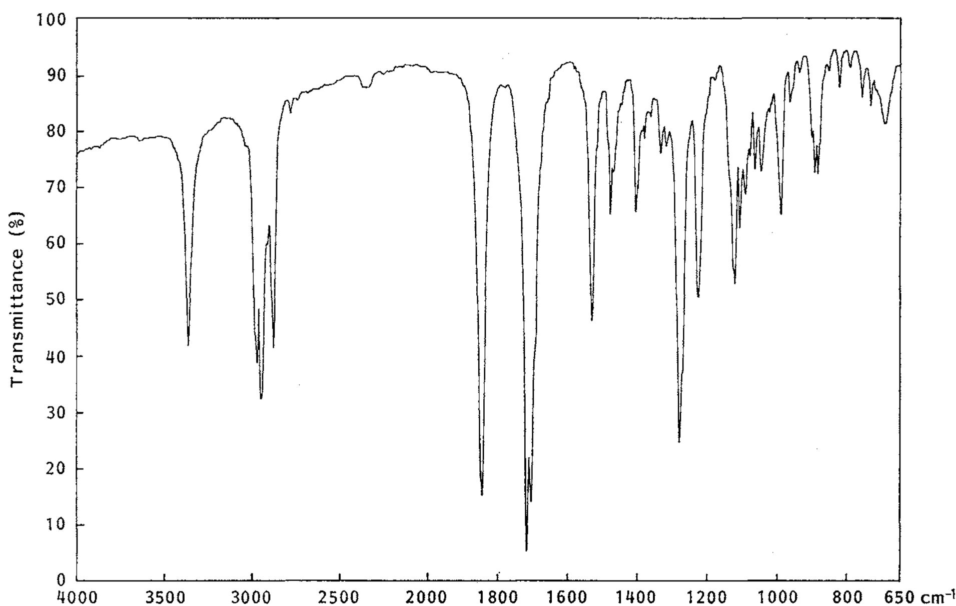
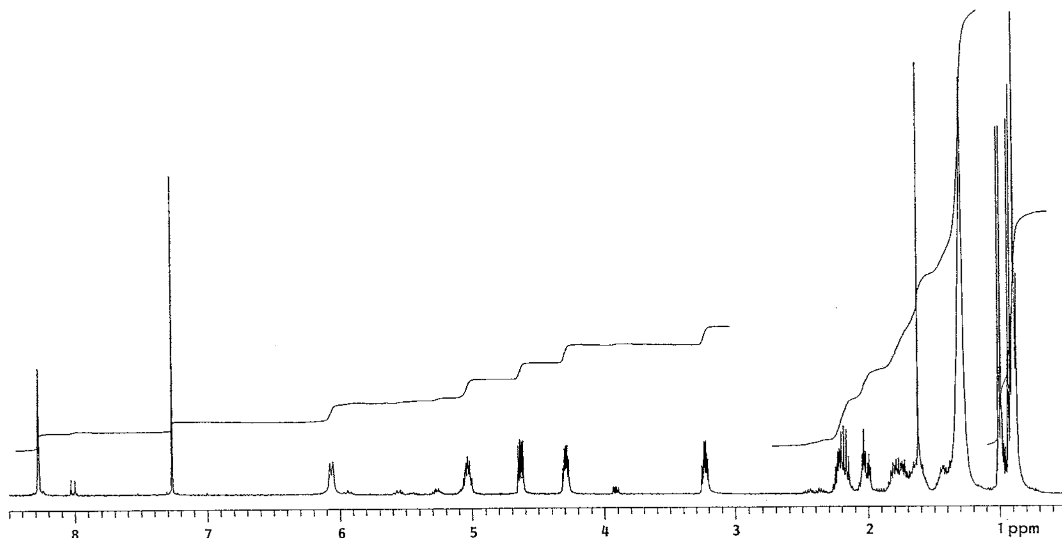


Fig. 2. ^1H NMR spectrum of valilactone (100 MHz in $CDCl_3$).



fraction was achieved on a silica gel column chromatography with hexane - $CHCl_3$ - EtOAc (5 : 5 : 1).

The colorless powder obtained by this means was recrystallized from MeOH - water.

Valilactone is obtained as a colorless needle with the following properties: MP 57~58°C; $[\alpha]_D^{28}$ −37° (*c* 1, $CHCl_3$). The UV spectrum shows end absorption in 95% MeOH solution. The IR spectrum and the ^1H NMR spectrum are shown in Figs. 1 and 2, respectively. Calcd for $C_{22}H_{39}NO_5$: C 66.47, H 9.89, N 3.52, O 20.12; found: C 66.86, H 10.28, N 3.46, O 19.84. Valilactone is soluble in MeOH, EtOAc and $CHCl_3$, insoluble in water. On TLC with Silica gel G (E. Merck), valilactone gives a single spot at Rf 0.20 with hexane - $CHCl_3$ - EtOAc (5 : 5 : 1).

The structure of valilactone was shown by X-ray analysis to be 5-(*N*-formyl-L-valinyloxy)-2-hexyl-3-hydroxydecanoic lactone (Fig. 3). The X-ray specimen of approximate dimensions 0.1 × 0.2 × 0.6 mm was cut from a plasmatic crystal. The lattice constants and the intensity data were collected on a Phillips PW1100 four circle diffractometer using graphite monochromated CuKα radiation. The crystal data are: Valilactone, $C_{22}H_{39}NO_5$, MW=397.6. Monoclinic, space group $P2_1$, $Z=2$. Lattice constants, a= 16.797(9), b=5.360(3), c=13.502(7) Å, β=92.50 (5)°, V=1214 Å^3, D_{calc}=1.088 gcm^{-3}, μ for CuKα=5.8 cm^{-1}.

A total of 2271 reflections were measured as above the 2σ (I) level out of 2826 in a 2θ range 6° through 150°, which correspond to about 80% of the theoretically observable reflections in the same angular range. The structure was solved by the direct method using the MULTAN[6] procedure; it was refined by the method of block-diagonal-matrix least-squares to an R value of 0.102. A difference electron-density map calculated at this stage revealed some hydrogen peaks but it was not possible to locate most of hydrogen atoms. This is due to large thermal vibrations of atoms of aliphatic side-chains (Fig. 3).

The final refinement was carried out without introducing hydrogen atoms and the R value was reduced to 0.089†.

The weight system adopted was: $\sqrt{w}=0.1$, when Fo<2; $\sqrt{w}=2/Fo$ when Fo≥2.

The absolute configuration of the molecule was deduced by assuming the conserved configuration of L-valine at C2″. The structure of the molecule is illustrated in Fig. 3 denoting the bond lengths in the same figure. The molecule is drawn by the ORTEP[7] program to show the thermal vibrations of atoms. The ellipsoid covers the resion where the center of the atom will be found with probability more than 30%. It may be noticed that errors of some bond lengths at the side chain terminals were increas-

† The atomic parameters, bond lengths, and angles have been sent to the Cambridge Crystallographic Data Centre.

Fig. 3. Molecular profile of valilactone.

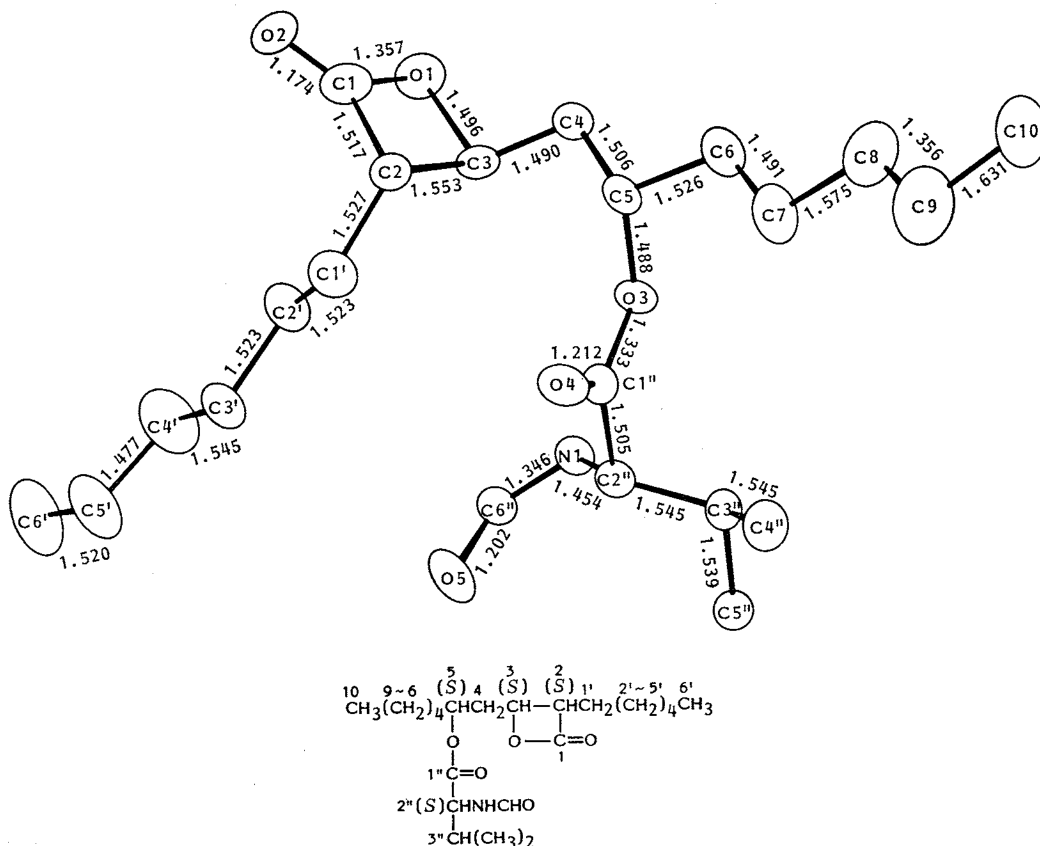


Table 1. Enzyme-inhibitory activity of valilactone, ebelactones and esterastin.

Inhibitor	IC_{50} (μg/ml)[a]		
	Esterase hog liver	Lipase[b] hog pancreas	fMet-AP[c] rat liver
Valilactone	0.029	0.00014	100
Ebelactone A	0.067	0.003	0.07
Ebelactone B	0.00052	0.001	0.02
Esterastin	50	0.0009	100

[a] IC_{50} is 50% inhibition concentration.

[b] The assay of lipase activity was carried out similarly to that of esterase.

[c] fMet-AP was measured by the hydrolysis of fMet β-naphthylamide.

ed by the thermal motions. Thus the lengths, C8-C9 of 1.356 Å, C9-C10 of 1.631Å may be due to the inaccuracy of the position of the atom C9 which has the largest anisotropic temperature factors.

Activities of valilactone in inhibiting esterase, lipase and *N*-formylmethionine aminopeptidase (fMet-AP) are shown in Table 1 in comparison with ebelactones and esterastin. The IC_{50} value of valilactone against esterase is similar to those of ebelactones, and against fMet-AP is similar to that of esterastin.

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