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BASSIANOLIDE, A NEW INSECTICIDAL CYCLODEPSIPEPTIDE FROM *BEAUVERIA BASSIANA* AND *VERTICILLIUM LECANII*

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In the course of our screening search for insecticidal metabolites of fungi, we succeeded in the isolation of a new insecticidal cyclodepsipeptide, bassianolide (1), from mycelia of *Beauveria bassiana*, a pathogenic fungus for various insects. In addition to 1, beauvericin, which was reported as a toxic substance to brine shrimps and mosquito larvae[1], was also isolated from the same fungus. This paper discloses that the structure of 1 has been determined as a cyclodepsipeptide composed of four moles each of L-N-methyl-leucine and D-α-hydroxyisovaleric acid (1a).

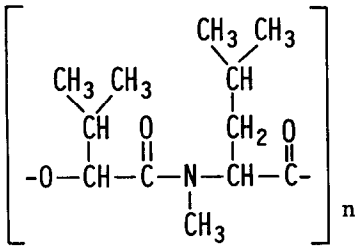
B. bassiana was cultured stationarily on Czapek-Dox medium containing 2% yeast extract at 26.5°C for 10 days. The active principle was extracted from the mycelia with methanol. A neutral fraction separated from the extract was applied succesively to a silicic acid column (benzene-ethyl acetate, 1:1), a neutral alumina column (benzene-ethyl acetate, 96:4), a silicic acid TLC (benzene-ethyl acetate, 2:1) and a Sephadex LH-20 column (methanol) to give pure 1 as an amorphous solid: $[\alpha]_D^{22}$ -73° ($CHCl_3$, c=3.3); IR ν_{max}^{Nujol} 1745 (ester C=O), 1660 cm^{-1} (amide C=O). In the PMR spectrum of 1 in $CDCl_3$ five N-methyl signals were observed at δ 2.86, 2.89, 3.01, 3.05

and 3.25 ppm and no exchangeable proton was detected with D_2O. The CMR spectrum of 1 in the same solvent revealed totally sixty signals. On the other hand, in C_6D_6 solution at 70°C, the PMR spectrum showed only one N-methyl signal at 2.84 ppm and the CMR spectrum was simplified to twelve signals: 171.2(s), 169.3(s), 75.1(d), 54.9(d), 37.3(t), 30.7(q), 30.4(d), 25.5(d), 23.4(q), 21.7(q), 18.7(q) and 18.0(q) ppm. These facts implied that 1 was constituted of several C_{12} moieties.

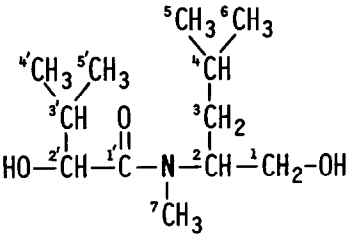
On acid hydrolysis, 1 gave only one amino acid, which was identified as L-N-methylleucine through the comparison with an authentic sample. An ether extract of the hydrolyzate solely yielded α-hydroxyisovaleric acid, which was identified by the PMR spectrum and gas chromatography (PEG 20M) of its methyl ester. Thus 1 was presumed to be a cyclic depsipeptide consisting of L-N-methylleucine and α-hydroxyisovaleric acid.

Treatment of 1 with $LiBH_4$ gave a compound 2 as a sole product: one spot (Rf, 0.27) on a silicic acid TLC (benzene-ethyl acetate, 1:3) and a single peak in the chromatography on Sephadex LH-20 column (methanol); IR ν_{max}^{Film} 3420 (OH), 1630 cm^{-1} (amide C=O). The high resolution mass spectrometry of 2 indicated the peaks at m/e 200.1678 (Calcd. for $C_{11}H_{22}NO_2$, 200.1650; M-CH_2OH) and m/e 188.1222 (Calcd. for $C_9H_{18}NO_3$, 188.1287; M-C_3H_7). Then the molecular formula of 2 was determined as $C_{12}H_{25}NO_3$. The structure of 2 was established as α-hydroxyisovaleryl-L-N-methylleucinol by the PMR spectrum with its double irradiation experiments and the CMR spectrum (Table 1).

The condensation of D-α-acetoxyisovaleric acid and L-N-methylleucine methyl ester with DCCI followed by the reduction with $LiBH_4$ afforded D-α-hydroxyisovaleryl-L-N-methylleucinol, which was completely identical with 2 in all respects. In contrast L-α-hydroxyisovaleryl-L-N-methylleucinol synthesized in a similar manner was distinguishable in TLC (Rf, 0.48) and the CMR spectrum from 2. Accordingly, the structure of 1 was determined as a cyclic repeating sequence of D-α-hydroxyisovaleryl-L-N-methylleucyl ($C_{12}H_{21}NO_3$) units.



1a n=4
1b n=5



2

Table 1 PMR and CMR spectral data of 2

C. No.	PMR		CMR
	Chemical shift, ppm (multiplicity)	Multiplicity change by irradiation(frequency, ppm)	Chemical shift ppm
1 CH_2	3.55 (m)	br. s (4.8)	63.1
2 CH	4.8 (m)	d. d. (3.55) (J=5.0, 9.0) d. d. (1.5) (J=5.0, 8.0)	54.2
3 CH_2	1.5 (m)		36.7
4 CH	1.6 (m)		24.9
5 CH_3	0.91 (d, J=5.9)	s (1.6)	21.7†
6 CH_3	0.93 (d, J=5.9)	s (1.6)	23.5†
7 CH_3	2.84 (s)		28.9
1' C=O	---		176.1
2' CH	4.25 (d, J=2.5)	s (1.9)	72.7
3' CH	1.9 (m)		31.1
4' CH_3	0.80 (d, J=6.7)	s (1.9)	14.8
5' CH_3	1.80 (d, J=6.7)	s (1.9)	20.0

† These assignments may be reversed.

FD- and EI mass spectra of 1 indicated the molecular ion peak at m/e 908, which was in accordance with a molecular formula of $C_{48}H_{84}N_4O_{12}$. The apparent discrepancy between the molecular formula and the NMR spectra suggested the idea that 1 in $CDCl_3$ existed in the mixtures of several conformers[2,3].

Thus, the structure of 1 was elucidated as a cyclodepsipeptide composed of four D-α-hydroxyisovaleryl-L-N-methylleucyl units (1a), although structure 1b was not entirely neglected for 1 by considering the NMR spectra.

Fifth instar larvae of silkworm, *Bombyx mori*, were killed when fed with an artificial diet containing 1 at a dose of 13 ppm; while beauvericin was not lethal to the larvae even at a dose of 1000 ppm.

1 was also isolated from mycelia of *Verticillium lecanii*, a pathogenic fungus for insects.

Acknowledgements

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References

1) R. L. Hamill, C. E. Higgins, H. E. Boaz and M. Gorman, Tetrahedron Letters, 4255(1969).

2) Yu. A. Ovchinnikov, V. T. Ivanov and A. M. Shkrob, "Membrane-active Complexones", BBA Library Vol. 12, Elsevier Scientific Publishing Co., 1974, pp 140-156.

3) The details of NMR studies will be published in near future.