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THE STRUCTURE OF BEAUVERICIN, A NEW DEPSIPEPTIDE ANTIBIOTIC TOXIC TO *ARTEMIA SALINA*

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Artemia salina (brine shrimp) has recently been shown to be a useful organism for the detection of new biologically active compounds not readily found by the use of conventional assays (1). We have found that a strain of the fungus *Beauveria bassiana*, previously known to be a potent insect pathogen (2,3), produces a substance very toxic to brine shrimp. The active principle was isolated from cultures of this fungus and shown to be a new depsipeptide (4), which we call *beauvericin* (5). Its structure is the subject of this paper.

Beauveria bassiana (NRRL 3352) produced the new substance when grown in submerged culture, utilizing the following nutrient medium: sucrose, 25 g; edible molasses, 25 ml; cornsteep liquor, 5 ml; malt extract, 10 g; NZ Case, 10 g; K_2HPO_4, 2 g; and distilled water to make 1000 ml at pH 6.2. The organism was grown for 76 hours at 26°C to achieve maximum yields of the desired compound. Beauvericin was extracted from the mycelium with methanol, and subsequently was extracted into ethyl acetate after removal of the alcohol under vacuum.

The ethyl acetate was concentrated to an oil which was chromatographed on basic alumina (Woelm) using benzene:ethyl acetate (19:1) as the eluent. After removal of solvent from the active chromatographic fractions, methanol-water was added; beauvericin was obtained from the chilled solution as white needles m.p. 93-94°C, $[\alpha]_D^{25}$+65.8° (C=1, methanol). The compound is freely soluble in most organic solvents and has no titratable groups. The elemental analysis (C=68.14%, H=7.45%, N=5.95%, and O=8.36%) and mass spectral molecular weight (M^+=783) indicated the empirical formula to be $C_{45}H_{57}N_3O_9$. The depsipeptide nature of beauvericin was inferred from the two carbonyl bands present in its infrared spectrum (Fig. 1) at 1740 cm^{-1} (ester) and 1670 cm^{-1}

(amide). The amide is fully substituted, as seen from the absence of N-H or O-H absorption in the 3000 cm^{-1} region of this spectrum. A band at 697 cm^{-1} indicated the presence of monosubstituted benzene which is consistent with the ultraviolet spectrum: λ_{max}^{EtOH} 204, 248, 256 and 263 mμ (ε 13,550, 1320, 1140 and 870).

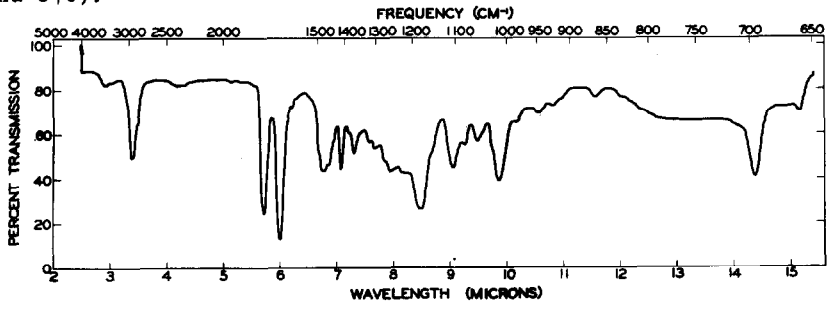


Figure 1. Infrared spectrum of beauvericin in $CHCl_3$.

The simplicity of the nmr spectrum (Fig. 2) of beauvericin in $CDCl_3$ is indicative of the symmetry and cyclic nature of the depsipeptide. The singlet at δ,3.04 showed that the amide nitrogens are substituted by methyl groups. Two types of carbon substituents are present on the alternating lactone-lactam ring. One of these is an isopropyl moiety having two methyl doublets (J=7.5 Hz) at δ,0.4 and 0.8 respectively and a complex methine multiplet centered at δ,1.95. The attachment of the isopropyl moiety to a carbon atom of the

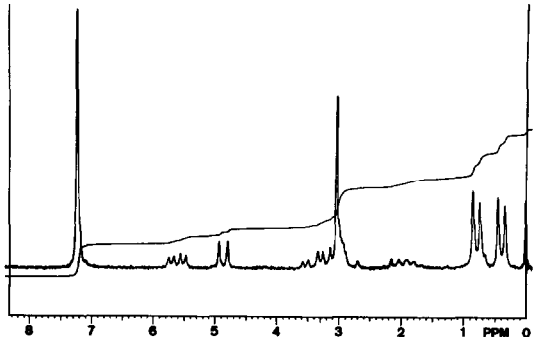
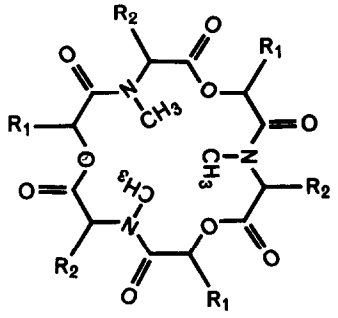


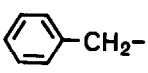
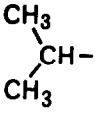
Figure 2. Nmr spectrum of beauvericin in $CDCl_3$.

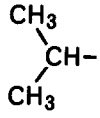
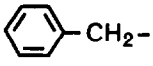
depsipeptide ring was corroborated by the coupling (J=8 Hz) of the isopropyl

methine to a proton (doublet) down field at δ,4.87. On the basis of the molecular weight there must be three units with each substitution; therefore, three of the six units comprising beauvericin must be either 2-hydroxyisovaleric acid or N-methyl valine.

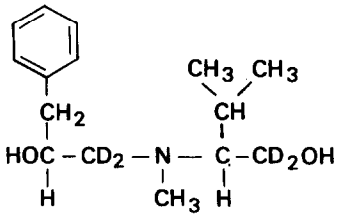
A five proton singlet, resulting from the mono-substituted benzene mentioned above, is seen at δ,7.25. The aromatic ring is attached to a methylene (this assumption is supported by the observation of a large m/e 91 peak in the mass spectrum of beauvericin) that forms part of a typical ABX pattern. The AB protons are observed at δ,3.02 and 3.39, and the X-proton is seen as a quartet centered at δ,5.60 (J_{AX}=12.1 Hz, J_{BX}=5.0 Hz). The carbon bearing this last methine is again part of the ring system. Therefore, the three benzyl groups are present as either 2-hydroxy-3-phenylpropionic acid or N-methyl phenylalanine. Based on this information, beauvericin can have either structure I or II.



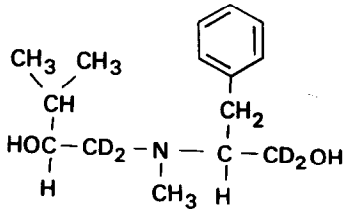
I R_1=  , R_2= 

II R_1=  , R_2= 

A decision between these two possibilities was reached by looking at the product obtained from the lithium aluminum deuteride reduction of beauvericin. This product should possess, if derived from I, structure III and, if derived from II, structure IV. The compound was obtained as a chromatographically homogeneous oil which showed a molecular ion, M^+=255.



III



IV

The nmr spectrum of the reduction product was consistent with, but did not differentiate between, III and IV; however, acetylation (acetic anhydride-pyridine) produced an O-diacetyl derivative (M^+=339) whose nmr spectrum ($CDCl_3$) showed the shift of a single proton doublet at δ,3.35 in the diol to 4.78. This methine was coupled to only one other proton, at δ,1.95. Thus, the reduction product has structure IV. Confirmation of structure IV was obtained from the mass spectral fragmentation pattern of the diol which gave the ions expected from both structures: m/e, 233 (loss of CD_2OH); m/e, 212 (loss of $-CH(CH_3)_2$); m/e, 164 (loss of $-CH_2-C_6H_5$, also a large m/e, 91 peak); and a major peak at m/e, 182 (M^+-73, loss of $-CHOHCH[CH_3]CH_3$) which could only arise from structure IV. The ion composition at m/e, 182 was supported by the occurrence of a shift in the molecular ion from 255 to 257, and a shift in the 182 peak to 183 after deuterium oxide exchange was performed in the mass spectrometer.

Structure IV leads to the conclusion that the structure of beauvericin is represented by II, a cyclic repeating sequence of three alternating molecules of N-methyl phenylalanine and three molecules of 2-hydroxyisovaleric acid. Acid hydrolysis of the depsipeptide yielded the two expected acids. These were shown to be L-N-methyl phenylalanine and D-2-hydroxyisovaleric acid through a comparison of their ord curves with those of known standards.

In addition to the toxic action of beauvericin on brine shrimp, moderate inhibitory activity was seen toward gram-positive bacteria, fungi, and mosquito larvae.

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