ISOLATION AND STRUCTURE ELUCIDATION OF GANCIDIN W

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The antibiotic gancidin W has been shown to be a cyclodipeptide containing leucine and proline moieties.

The antitumor antibiotic gancidin W, mp 163-164°, with the molecular formula C_{11}H_{17}O_{9}N_{2} was first reported by Wakaki and his coworkers²,³ as a fermentation product of Streptomyces No. AAK-84. These authors reported the ultraviolet and infrared spectra and various color reactions for the antibiotic. Subsequently, gancidin W has been referred to as an antibiotic of unclassified nature⁴. During the course of our studies on the fermentation products from a strain of Streptomyces species isolated in our laboratories and designated BC-494, we have isolated closely related crystalline compounds from the bioactive fractions. Guided by the published physico-chemical and biological data,²³ it was apparent that one of our crystalline isolates, C_{11}H_{18}O_{9}N_{2}, mp 160-163°, was identical with the gancidin W reported by the Japanese workers. In this communication we report this comparison and the structure of gancidin W. The gancidin-producing culture of Streptomyces gancidicus (NRRL B-1872) was utilized for the fermentation to prepare an authentic sample of gancidin W; the broth, after clarification, (5 l) was
exhaustively extracted with chloroform (3.5 l) which on evaporation yielded a yellowish-brown gum (139 mg). Paper chromatography (Whatman Paper No. 1; n-butanol-acetic acid-water, 4:1:5) of the chloroform extracts of BC-494 and Streptomyces gancidicus followed by bioautography against Staphylococcus aureus 209P gave zones of inhibition that were comparable in pattern. Additionally, the in vitro assay values (e.g. MIC's 8-16 µg/ml vs. Staphylococcus aureus HH127 and SA910; 63-125 µg/ml vs. E. coli 12140; 16-31 µg/ml vs. Klebsiella pneumoniae) and spectral characteristics (e.g. $\nu_{\text{max}}$ CHCl$_3$ 1667 cm$^{-1}$) of the two extracts were comparable.

Column chromatography of the extract from Streptomyces gancidicus on neutral alumina followed by crystallization of selected fractions yielded gancidin W which was identical with the isolate prepared in an analogous manner from BC-494 (mixed mp 159-162°) in its spectral features (see Table 1).

**TABLE 1**

<table>
<thead>
<tr>
<th>UV spectrum (0.1N HCl)</th>
<th>Gancidin W from Streptomyces gancidicus</th>
<th>Isolate from BC-494</th>
</tr>
</thead>
<tbody>
<tr>
<td>end-absorption</td>
<td></td>
<td>end-absorption</td>
</tr>
<tr>
<td>IR spectrum : $\nu_{\text{KBr}}$</td>
<td>3247, 1678, 1642, 1302 and 709 cm$^{-1}$</td>
<td>3268, 1678, 1639, 1302 and 709 cm$^{-1}$</td>
</tr>
<tr>
<td>mass spectrum: 70 eV/180°</td>
<td>43(98.3), 70(96.0), 86(98.6), 124(100),</td>
<td>43(98.3), 70(100.0), 86(100.0), 124(100),</td>
</tr>
<tr>
<td>m/e (% relative intensity)</td>
<td>154(95.9), 167(73.7), 195(41.5), 210(6.5)</td>
<td>154(100.0), 167(100.0), 195(76.9), 210(10.5)</td>
</tr>
</tbody>
</table>

* Lit. values$^2$ for gancidin W: $\nu$ Nujol 3205, 1664, 1629, 1342 and 699 cm$^{-1}$. $\nu_{\text{max}}$
The ultraviolet spectrum of gancidin W indicated the absence of any characteristic chromophore in the molecule. The presence of a cyclic secondary amide structure was evident from its infrared spectrum. This was further supported by the mass spectrum which showed the molecular ion peak at m/e 210 along with intense fragments at m/e 70, 86, 154 and 167. The course of fragmentation depicted in Scheme I can be rationalized in terms of the structure (I) for gancidin W. The chemical proof in favor of the proposed structure (I) was adduced in the following manner. Acid hydrolysis (HCl/HOAc, 100°, 72 hr) of gancidin W (from BC-694 and Streptomyces gancidicus) followed by paper chromatography (Whatman Paper No. 1; n-butanol-acetic acid-water, 4:1:5) resulted in two ninhydrin positive spots (yellow: Rf 0.23; purple: Rf 0.58) identified as proline and leucine, respectively, by mixed spot analysis. The structure (I) for gancidin W was also supported by the FT-nmr spectrum. Besides showing two sets of doublets partially superimposed in the region 1.0-1.15 δ due to gem-dimethyls, there were two well-defined multiplets in the ratio of 1:1, one set in the region 3.7-3.5 δ assigned to the hydrogen on the carbon flanked by the amide and alkyl groups, and the second set in the region 4.25-4.0 δ assigned to the hydrogen on the bridgehead carbon.

![Structure I](image-url)
SCHEME I

MASS SPECTRAL FRAGMENTATION MECHANISM OF CYCLO (Leu-Pro) I

I

\[ \text{CH}_3\text{CH} \quad \text{CH}_2 - \text{CH} - \text{CH}_2 \quad \text{CH}_3 \]

m/e 43

\[ \text{CH}_3\text{CH} \quad \text{CH}_2 - \text{CH} - \text{CH}_2 \quad \text{CH}_3 \]

\[ \text{NH}_2 \]

m/e 86

\[ \text{R} - \text{CH} \quad \text{N} \quad \text{CH}_2 \quad \text{CH}_2 \]

m/e 70

\[ \text{R} = \text{CH}_3\text{CH}-\text{CH}_2^- \]

m/e 154

\[ \text{C} \quad \text{C} \quad \text{NH} \quad \text{C} \quad \text{C} \]

\[ \text{H} \quad \text{O} \quad \text{C} \quad \text{N} \quad \text{C} \]

\[ \text{CH}_2 - \text{CH} - \text{N} \quad \text{C} \quad \text{CH}_2 \]

\[ \text{HN} \quad \text{N} \quad \text{CH}_2 \quad \text{CH}_2 \]

m/e 154
In summary, the antibiotic gancidin W is a member of a common class of naturally occurring cyclopeptides.\textsuperscript{5,6} Since the physical constants for the isolate [mp 160-163\degree, [\alpha]_D^{25} = 149.8\degree (c, 0.25 in ethanol)] match very well with those of the L, L-isomer\textsuperscript{7} [mp 158-161\degree, [\alpha]_D^{21} = 142.4 \pm 0.5\degree (c, 3.33 in ethanol); [\alpha]_D^{20} = 143.4\degree]\textsuperscript{8}, it is safe to conclude gancidin W is cyclo (L-leu-L-pro).

ACKNOWLEDGEMENT We are grateful to the members of our Microbiology and Analytical & Physical Chemistry Departments for their help in this project. We are especially thankful to Marcia Shearer, R. Mehta and L. Fare for procuring the gancidin-producing culture and working out the fermentation conditions.

REFERENCES AND NOTES
1 This paper is dedicated to Professor R. B. Woodward on the anniversary of his sixtieth birthday.

8. J. L. Johnson, W. G. Jackson and T. E. Eble, J. Amer. Chem. Soc., 1951, 73, 2947. The authors report previous softening of the crystalline material at about 145° — a phenomenon observed in our work which is largely dependent upon the temperature of the Kofler Hot Stage when the crystals are placed for the melting point determination.

Received, 16th July, 1977