Short Communication

The Structure of Leucanicidin,
a Novel Insecticidal
Macrolide Produced by
Streptomyces halstedii

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In the course of our screening search for insect growth regulators, we found that the mycelial extract of *Streptomyces halstedii* markedly showed insecticidal activity in an aseptic feeding assay with the common armyworm, *Leucania separata*.¹⁾ In this paper we wish to make a preliminary report on the structural elucidation of the insecticidal principle named leucanicidin (**1**).

The mycelia of *Streptomyces halstedii* was extracted with acetone. After removal of the solvent *in vacuo*, the active substance was transferred to chloroform. Leucanicidin (**1**) was isolated with Silicar CC-7 column chromatography (benzene-ethyl acetate, 60:40) and crystallized as needles from chloroform-hexane, mp 130~132°C; C₄₂H₇₀O₁₃; FABMS *m/z*: 805 (M+Na)⁺; λ_{max}^{MeOH} nm (ε): 248 (23,500), 287 (10,500); ν_{max}^{KBr} cm⁻¹: 3500~3400, 1680, 1640, 1615, 1240; [α]_D²⁶ -47° (c=1.0, CHCl₃).

The ¹³C-NMR spectrum of **1** (25 MHz in CDCl₃)²⁾ revealed 42 carbons of the following functional groups: CH₃ × 10, CH₂ × 2, CH × 6, OCH₃ × 3, CH-O × 10, O-C-O × 1, O-CH-O × 1, CH= × 5, C= × 3 and COO × 1, accounting for 65 protons. The remaining five protons should be in hydroxyl groups and all the 70 protons including the five hydroxyls

were observed in the 400 MHz ¹H-NMR spectrum of **1** in CDCl₃.

Analysis of the 400 MHz COSY spectra³⁾ of **1** in CDCl₃ and in pyridine-*d*₅, together with conventional double resonance and NOE experiments, revealed three partial structures, A (C₁~C₁₈), B (C₁₉~C₂₅) and C (C₁'~C₆'), as shown in Fig. 1. The presence of secondary hydroxyl groups at C-7 and C-17 was revealed by the coupled hydroxy protons in the COSY spectrum in CDCl₃. The location of methoxyl groups at C-2 and C-14 was determined by NOE enhancement observed at CH₃ (C-4) and H-14 when methoxyls at δ 3.64 and 3.24 were irradiated, respectively. The H-15 proton, which resonated in the lowest field (δ 4.96) among the methine protons bearing an oxygen function, should be esterified with the C-1 carbonyl. Proof of the junctures of C-8~C-9 and C-15~C-16~C-17 was uncertain in the spectrum in CDCl₃ because of overlapping signals between H-8 and one of the H-9 methylene protons, and H-16 and another one of the H-9 methylene protons. However, these signals were clearly separated in pyridine-*d*₅,

FIG. 1. Partial Structures A, B and C for **1**.

Chemical shifts are indicated as ppm from TMS in CDCl₃, and the values in parenthesis are those in pyridine-*d*₅.

Fig. 2. Partial Structure for **2**.

The spectrum was obtained in CDCl_3 .

and the connections were established. The configurations of the double bonds in the partial structure A were established through NOE enhancement and the coupling constant.

In the partial structure B, the connection of C-19 and C-20 was manifested by a W-type coupling ($J=2\text{ Hz}$) between one of the H-20 protons and the hydroxyl proton at C-19, which was a quaternary and hemiketal carbon. The carbon chain from C_1 to C_6 of the partial structure C was determined mainly from a COSY spectrum in pyridine- d_5 and this fragment was thought to contain two hydroxyls and probably the third methoxyl group. The partial structure C has a ketal and one more ether oxygen, suggesting that this part comprised some 6-deoxy hexose and was connected to C-21 or C-23 through a glycosyl linkage. The partial structure B was also deduced to take a cyclic system from the degree of unsaturation of **1**. These were confirmed by the following chemical reactions.

When **1** was treated with 0.5N HCl and followed by acetylation, 2-*O*-methyl-1,3,4-*O*-triacetyl- α -L-rhamnose^{4,5)} was produced, indicating the position of two hydroxyls and one methoxyl in the partial structure C, which was extended to the 2-*O*-methyl-L-rhamnosyl moiety.

On reduction with NaBH_4 , **1** gave two products, the minor one being the reduced product at C-19. In the ^{13}C -NMR spectrum of the major product (**2**) ($\text{C}_{35}\text{H}_{58}\text{O}_8$, FABMS (diethanolamine matrix) m/z : 712 ($\text{M}+\text{H}+\text{NH}(\text{C}_2\text{H}_4\text{OH})_2^+$), the carbons ascribable to the sugar moiety as well as the hemiketal carbon at $\delta 98.8$ disappeared and a pair of olefinic carbons were newly observed at $\delta 132.3$ (d) and 133.6 (d). From the COSY spectrum, the partial structure as shown in

Fig. 2 was clarified for **2**. The position of the newly formed double bond was confirmed to be at C-20 and C-21, and the vicinal relationship of C-18 and C-19 was also established, this being difficult to determine in the ^1H -NMR spectrum of **1**. By treatment with NaBH_4 , the sugar moiety was removed from **1** through β -elimination of the potential ketone at C-19 by the basicity of NaBH_4 , and the resulting α,β -unsaturated ketone was reduced by NaBH_4 . This result established that the sugar moiety was linked to C-21, and that C-19~C-23 formed the tetrahydropyran ring in **1**. The partial structures A, B and C were thus rigorously connected to give the total structure

of leucanicidin as **1**. The orientations of the substituents at C-21, C-22 and C-23 on the six-membered ring are suggested to be all equatorial from the J values of the H-21, H-22 and H-23 protons, and that of the hydroxy proton at C-19 was established to be axial⁶⁾ from the W-type coupling with one of the H-20 protons as already described. These relative stereochemistry is illustrated in Fig. 3. The anomeric configuration of the 2-*O*-methyl-L-rhamnosyl moiety was determined to be α -form from the J_{CH} value (167 Hz) of the anomeric carbon.⁷⁾

Leucanicidin (**1**) caused all the test insects to die within four days at a dose of 20 ppm in the diet when applied to the fourth instar larvae of

FIG. 3. Relative Stereochemistry of the Six-membered Ring (C-19~C-23).

L. separata.

Leucanicidin (**1**) is a 16-membered macrolide, and the aglycone is very similar to hyglolidin,⁸⁾ L-681,110⁹⁾ and bafilomycins,¹⁰⁾ but, as a glycoside type, **1** is the first case. Screening of other biological activities is now in progress.

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