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THE STRUCTURE OF DETOXIN D_1
A SELECTIVE ANTAGONIST OF BLASTICIDIN S

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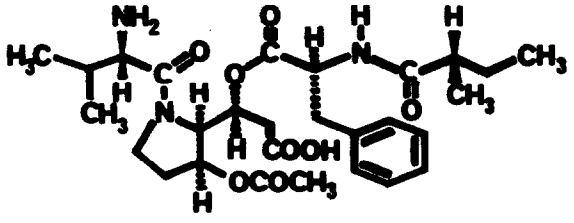
Detoxin D_1, one of the most active principle of detoxin complex produced by Streptomyces caespitosus var. detoxicus, is a selective antagonist of blasticidin S and shows several interesting biological activities to counteract the toxicity of the antibiotic against plant and animal cells.[1),2)] This communication concerns with the structural elucidation of detoxin D_1.

Detoxin D_1 I, $C_{28}H_{41}N_3O_8$, mp. 156 - 158°C, $[\alpha]_D^{25}$ -16° (C 1, MeOH), is an amphoteric compound with pKa 4.0 and 8.0 and shows positive ninhydrin reaction. I is a kind of peptide (ν_{max}^{nujol} 3400, 2750, 1740, 1650, 1600 cm^{-1}) exhibiting the signals of one phenyl nucleus (δ^{CDCl_3} 7.22), one acetyl (δ 2.00) and four methyl groups (near δ 1.0) in the NMR spectrum.

Detoxin D_1 acetate methyl ester II, $C_{31}H_{45}N_3O_9$ (M^+: m/e=603) was obtained by acetylation of I with acetic anhydride in pyridine followed by esterification with diazomethane.

Acid hydrolysis of I with 5.7N HCl at 110°C for 16 hrs provided each one mole of L-valine and L-phenylalanine accompanying an unknown amino acid designated detoxinine which was coloured to yellow with ninhydrin. The N-terminus was determined to the amino group of L-valine by DNP and diazotization methods.

Fig. 1 I



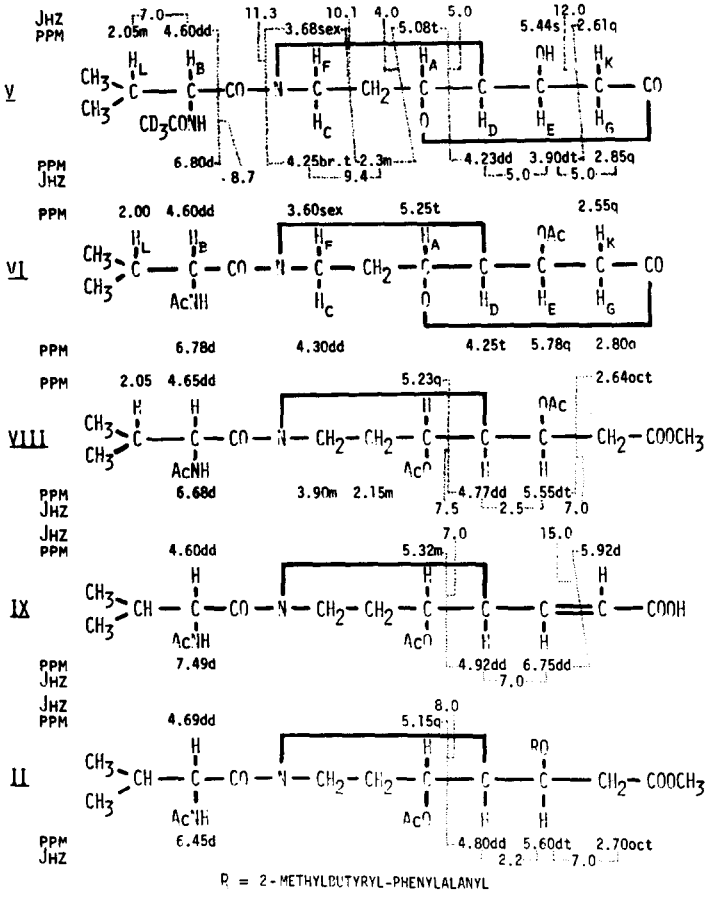


Fig. 2

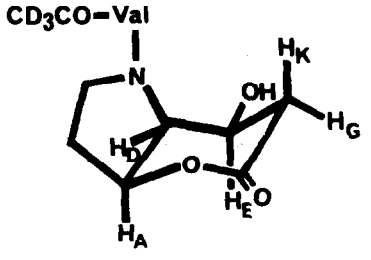


Fig. 3

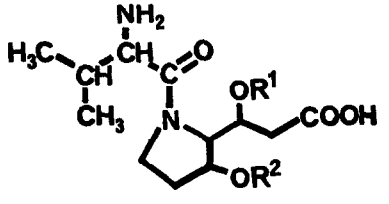


Fig. 4

VII : $R_1 = R_2 = H$

Ia : R_1=Ac, R_2=2-methylbutyrylphenylalanyl

Ib : R_2=Ac, R_1=2-methylbutyrylphenylalanyl

Alkaline hydrolysis of I with 0.1N NaOH at room temperature for a week, followed by ethereal extraction at acidic condition, gave crystalline needles III, $C_{14}H_{19}NO_3$ (M^+: m/e=249) mp. 122-123°C, ν_{max}^{nujol} 3300, 2800, 1740, 1650 cm^{-1}. L-Phenylalanine and (+)-S-2-methylbutyric acid, $[\alpha]_D^{22}$ + 19.2° (C 1.2, MeOH)[3], were obtained by acid hydrolysis of III. These evidences together with the NMR and mass spectral data are consistent with the structure of (+)-2-methylbutyryl-L-phenylalanine for III.

Alkaline hydrolysis of I with 1N NaOH followed by resin chromatography on Dowex 50W x 2 [H^+] gave valyl-detoxininolactone IV, which was acylated with CD_3COCl in pyridine to crystalline N-deuteroacetate V, $C_{14}H_{19}N_2O_5D_3$ (M^+: found; m/e=301.1744, calcd.; 301.1715), mp. 174 - 175°C, ν_{max}^{KBr} 1730 cm^{-1} and with acetic anhydride in pyridine to diacetate VI, $C_{16}H_{24}N_2O_6$ (M^+: m/e=340)

The results of NMR and spin decoupling experiments of V and VI together with the IR and mass spectral data provided the evidences of their structures as formula V and VI (Fig. 2 and 3).

On the other hand, the resin chromatography on Dowex 50W x 2 of the alkaline hydrolysate of I after neutralization with dilute hydrochloric acid provided amorphous powder of valyl-detoxinine VII, $C_{12}H_{22}N_2O_5$. Unlike the lactone IV, VII has an amphoteric nature with a free carboxyl group (ν_{max}^{nujol} 2700, 1730 cm^{-1}), which was converted to the corresponding triacetate methyl ester VIII, $C_{19}H_{30}N_2O_8$ (M^+: m/e=414), on acetylation followed by esterification. The NMR spectrum of VIII is also shown in Fig. 2 and is very similar to that of II except the signals of 2-methylbutyryl-phenylalanyl moiety. Accordingly, the structure of VII was established (Fig. 4).

These accumulated informations lead to the structure Ia or Ib for detoxin D_1, and the binding position of III remains to be settled unequivocally. Several attempts in effecting partial hydrolysis of I or transesterification of bulky group III were unsuccessful. Since the β-acyloxycarboxylic group is present in the structure of Ia or Ib, β-elimination provoked by the nucleophiles to form an α,β-unsaturated carboxylic acid would be expected. Fortunately, treatment of I with acetic anhydride at 70°C for 1 hr yielded the α,β-unsaturated acid N-acetate IX $C_{16}H_{24}N_2O_6$ (M^+: m/e=340), λ_{max}^{EtOH} 210 nm (ε 14,000), δ 5.92 (1H,d), 6.75 (1H,dd).

This result demonstrated that the bulky group III was removed by the β-elimination and consequently, the structure of detoxin D_1 is established as depicted in Fig. 1.

The absolute configuration of I was determined as follows. There are six asymmetric carbons in I and three of their configurations have been determined already. Remaining three are present in detoxinine moiety. Their absolute configurations were determined by the application of Klyne's lactone sector rule[4] to V.

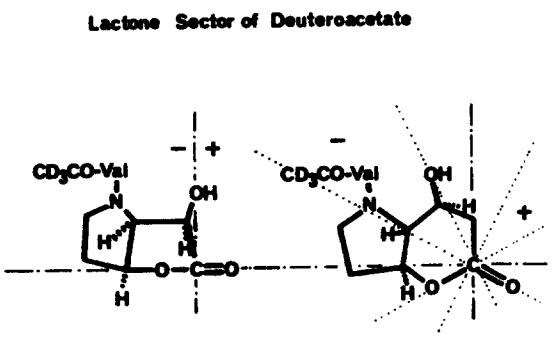


Fig. 5

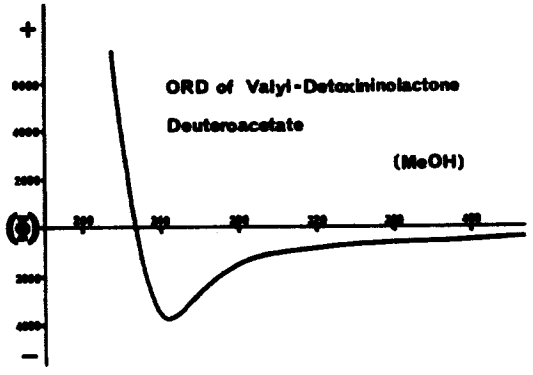


Fig. 6

The relative configuration of V was elucidated by its NMR coupling constant. The ring juncture of this lactone is cis ($J_{H_A,H_D}=5_{HZ}$) and it has a chair conformation. The lactone sectors are shown in Fig. 5. The ORD of V in MeOH showed clear negative Cotton effect at 243 nm as was expected from the sectors (Fig. 6). The coupling constants between H_A and H_D in II and VIII are 7.5 $_{HZ}$ and 8.0_{HZ} in $CDCl_3$, respectively. The theoretical values calculated from the models are 7.7_{HZ} for cis and 4.9 $_{HZ}$ for trans configuration. Therefore, it is apparent that H_A and H_D in II are located in cis configuration since the inversion of configuration on the carbon adjacent to lactone oxygen did not take place.

On the basis of the evidences described above, the absolute structure of detoxin D_1 was established conclusively as I which is a kind of depsipeptide containing a new amino acid with pyrrolidine nucleus.

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