Communications to the editor

THE STRUCTURES OF MINOR COMPONENTS OF THE DETOXIN D GROUP

Sir:

The detoxin D group is one of the main active component of detoxin complex[1,2], elaborated by *Streptomyces caespitosus* as selective antagonists of blasticidin S.

The antagonists show several interesting biological activities which antagonize blasticidin S both in plant and animal cells.

Detoxin D_1[3] is a unique depsipeptide with the new amino acid detoxinine, L-valine and (+)-S-2-methylbutyryl-L-phenylalanine as structural components as shown in Fig. 1.

The detoxin D group is separated as a mixture of closely related compounds by buffered ion-exchange chromatography on Dowex 50 W X-2[2] from the detoxin complex containing eight groups of active substances.

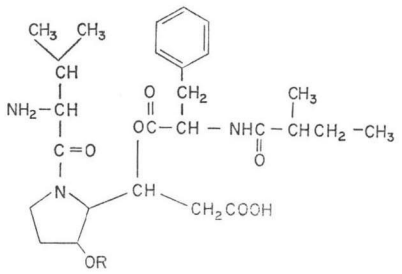
In the detoxin D mixture, detoxin D_1 is the main active component with highest specific activity. The presence of at least three minor components in the mixture was demonstrated by gel filtration and other chromatographic procedures.

In this paper we wish to communicate the separation of these minor components by GLC as well as the structual elucidation by gaspectrometry-mass spectrometry (GC-MS).

At the beginning of chemical studies on the detoxin D group, the use of GC-MS for structural elucidation was attempted but was unsuccessful due to the low volatility of the detoxin D compounds and some instrumental defects. Since it is well known that N-trifluoroacetyl peptide methyl esters[4] have appreciable volatility, detoxin D_1 and the detoxin D group mixture were treated with trifluoroacetic anhydride in methanol followed by an excess of ethereal diazomethane to afford the N-trifluoroacetate methyl esters of detoxin D_1 (TFA-D_1) and the detoxin D group (TFA-D-mixt).

The oily products obtained after removal of the solvents were purified by preparative TLC using benzene-ethylacetate (1 : 1) and the corresponding yellowish band was collected.

Fig. 1. The detoxin D group



Detoxin $D_1R = CH_3CO-$
$D_2R = CH_3CH_2CO-$
$D_3R = \begin{matrix} CH_3 \\ CH_3 \end{matrix} > CHCO-$
$D_4R = CH_3CH_2CH_2CO-$
$D_5R = \begin{matrix} CH_3 \\ CH_3 \end{matrix} > CHCH_2CO-$

Structural confirmation of these derivatives was made by comparison of the NMR spectra with that of N-acetyl detoxin D_1 methyl ester[3].

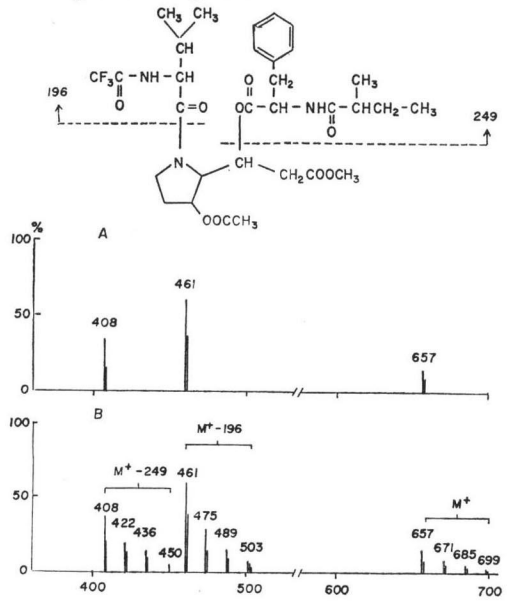
The mass spectra of the two samples were measured (Hitachi RMU-6 L Mass Spectrometer) at a vaporizing temperature of 180~190°C as shown in Fig. 2. In the mass spectrum of TFA-D_1, a distinct molecular ion peak was observed at *m/e* 657 together with two characteristic strong peaks at *m/e* 461 and *m/e* 408. Clearly, the peak at *m/e* 461 is formed by loss of the N-trifluoroacetylvalyl moiety and that at *m/e* 408 is derived by removal of the 2-methylbutyrylphenylalanyl group from the parent molcule as shown in Fig. 2.

In consequence, the two key peaks derived from the cleavages of known moieties are designated P—I (M-196) P—II (M-249), respectively.

The inspection of the mass spectrum of TFA-D-mixt. reveals, in addition to the molecular ion peak of TFA-D_1 at *m/e* 657, the presence of two peaks of lower intensity at *m/e* 671 and 685 and a weak peak at *m/e* 699 corresponding to the stepwise increment of 14 mass units from the molecular ion peak of TFA-D_1.

Furthermore, these molecular ion peaks are in agreement with those expected for P—1 at *m/e* 475, *m/e* 489, *m/e* 503 and P—II at *m/e* 422, *m/e* 436 and *m/e* 450, respectively.

Fig. 2. The mass spectra of TFA-D_1 (A) and TFA-D-mixtures (B) and the origin of two diagonistic fragments



Accordingly the shifts of 14 mass units with the three diagonistic peaks, *i.e.* the molecular ion, P—I and P—II, are reasonably ascribed to changes in the acyl substituent at C_3 of the detoxinine moiety.*

In order to identify the acyl substituents, the detoxin D mixture (10 mg) was hydrolyzed in 0.5 N sodium hydroxide solution under reflux for one hour and after acidification, the hydrolysate was extracted continuously with ether. The ethereal extract was carefully condensed at 50°C to 1/10 volume and the residual liquid was directly analyzed by GLC (Hitachi-Perkin Elmer F-6 D Gaschromatograph equipped with a Tween 80 (9 %)+H_3PO_4 (1 %) on C-22 SK column).

Five fatty acids in the hydrolysate of the detoxin D mixture were indentified as shown in Fig. 3, namely, in addition to acetic acid and 2-methylbutyric acid known as the common structural components of the detoxin D group, *n*-propionic, isobutyric and *n*-butyric acids were detected. Although a trace of

* The possibility of the binding of an acyl substituent to the detoxinine moiety at other than C_3 is ruled out, because valyldetoxinine was a sole product detectable as a ninhydrin-positive spot on selective alkaline hydrolysis of the detoxin D mixture[3].

Fig. 3. The GL chromatogram of fatty acids contained in the hydrolysate of detoxin D mixture

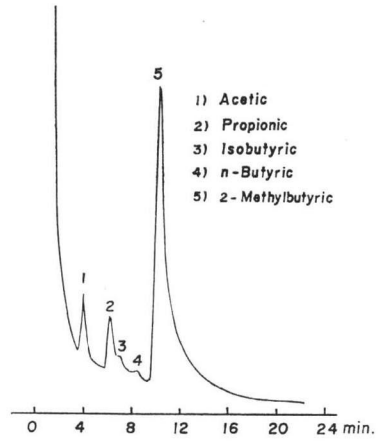
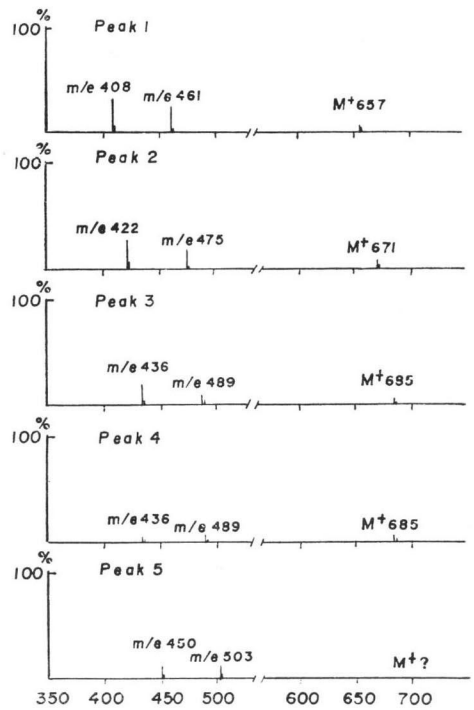


Fig. 4. The GC-MS spectra of TFA-D-mixture



isovaleric acid could be detected on a paper chromatogram (using the solvent system of ethanol - 28 % ammoniacal water; 95:5), it was not identified clearly by GLC, presumably due to overlap of the large peak of 2-methylbutyric acid.

Identification of the fatty acids contained in the hydrolysate of the detoxin D mixture

supports the structural relationship of the congeners varying only in the acyl substituent which causes the 14 mass unit shifts observed.

Finally, the structural relationship of five individual members of the detoxin D group was verified with the GC-MS procedure.

The separation of TFA-D-mixt into five components was achieved by GLC (Hitachi K 53 Gas chromatograph) using an OV-17 column (0.3×100 cm, 2% OV-17 on Chromosorb W) and each component was directly introduced into a mass spectrometer (Hitachi RMU-6 L).

The mass spectra are shown in Fig. 4.

On the basis of the results described above, we wish to propose structures for the members of the detoxin D group as depicted in Fig. 1.

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