

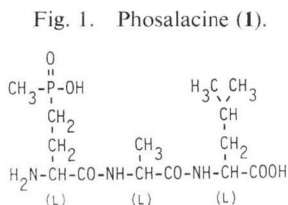
THE STRUCTURE OF
PHOSALACINE, A NEW HERBICIDAL
ANTIBIOTIC CONTAINING
PHOSPHINOTHRICIN

Sir:

In the course of our screening program for novel antimetabolites competitive with L-glutamine from soil actinomycetes, a new herbicidal antibiotic was isolated from the culture filtrate of *Kitasatospora phosalacinea* sp. nov. KA-338. The taxonomy, isolation and biological activities will be reported in separate papers^{1,2}. In the present communication, the structure elucidation of the new phosphorous-containing tripeptide herbicide is described.

Phosalacine (**1**) is a water-soluble amphoteric peptide: mp >225°C (dec), $[\alpha]_D^{25} -38.8^\circ$ (c 0.65, H₂O), FD-MS m/z 366 (M+1)⁺. The molecular formula C₁₄H₂₉N₈O₆P was confirmed by the high resolution EI-MS spectrum (obsd m/z 489.182, Calcd for C₁₈H₃₁N₉O₇PF₃ m/z 489.185) of *N*-trifluoroacetylphosalacine dimethylester. IR spectrum suggested the presence of carboxylic acid (1658 and 3000~3500 cm⁻¹) and amide carbonyl groups (1545 cm⁻¹).

The ¹³C NMR spectrum showed the signals of



four methyl, three methylene, four methine (three methine attributed to be α -carbon of α -amino acid by their chemical shifts), two amide carboxy and a carboxylic acid. Among them, two signals split to doublets in the proton noise decoupling spectrum (Fig. 2), *i.e.* a methyl (δ_c 16.3, $J=93$ Hz) and a methylene (δ_c 27.5, $J=90$ Hz). These observations suggested that **1** was a tripeptide containing a methylphosphorous-methylene skeleton.

By the amino acid analysis of the acid hydrolysate (6 N HCl, 110°C, 18 hours), phosalacine was found to be composed of alanine (1 mol), leucine (1 mol) and an unusual amino acid. Since the unusual amino acid had to be constructed with a methylene, an α -carbon of α -amino acid, an amide carbonyl and the above-mentioned phosphorous-containing partial structure, it was revealed to be phosphinothricin which is a component of a known glutamine antimetabolite, phosphinothricylalanylalanine³⁾ (bialaphos)^{4,5)}.

The direct comparison of the hydrolysate of **1** with that of phosphinothricylalanylalanine in silica gel and cellulose TLC using BuOH - AcOH - H₂O (4:1:1) as developing solvent supported the existence of the unusual amino acid.

The EI-MS spectrum of *N*-trifluoroacetyl dimethylester of **1** showed several characteristic fragment ion peaks as shown in Fig. 3, and indicated that the amino acid sequence of **1** is phosphinothricylalanylleucine. All amino acids isolated from the hydrolysate of **1** were assigned to be L-configuration by the optical

Fig. 2. ¹³C NMR spectrum of phosalacine (22.5 MHz, D₂O).

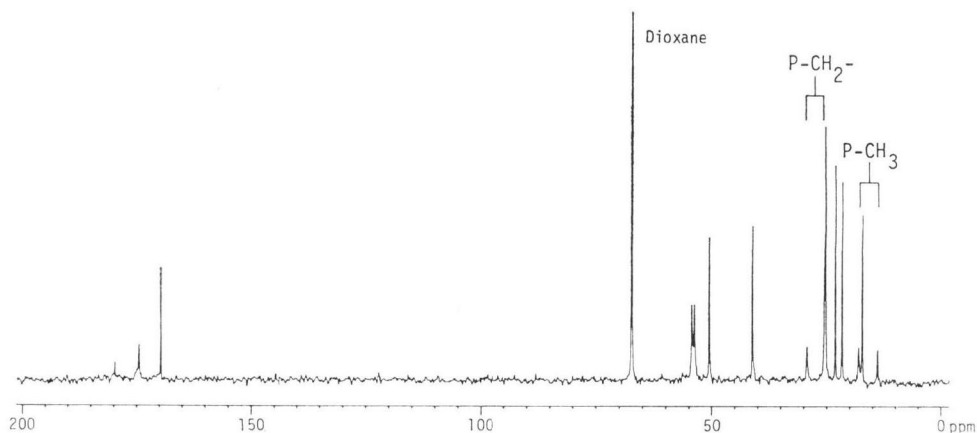
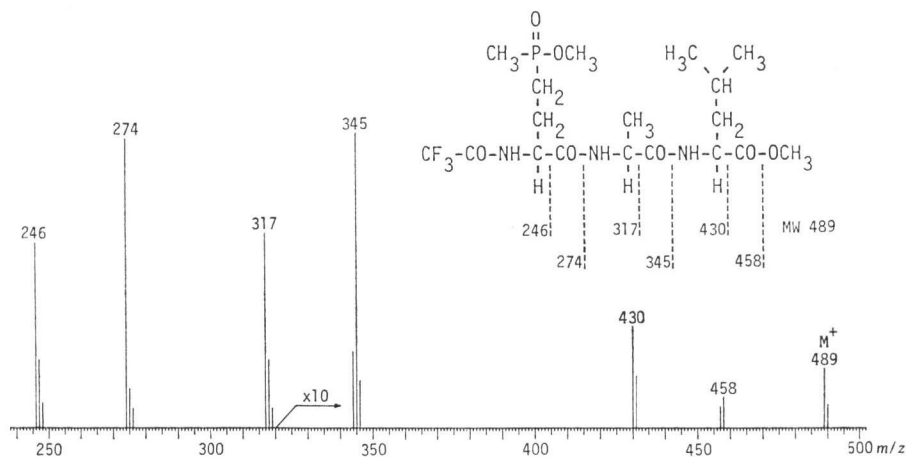


Fig. 3. EI mass spectrum of *N*-trifluoroacetylphosalacine dimethylester.

rotation of each component. Thus, the structure of **1** was determined as shown in Fig. 1.

SATOSHI ŌMURA
KIYOIZUMI HINOTOZAWA
NOBUTAKA IMAMURA
MASATSUNE MURATA

School of Pharmaceutical Sciences,
Kitasato University and
The Kitasato Institute,
Minato-ku, Tokyo 108, Japan

(Received March 29, 1984)

References

- 1) ŌMURA, S.; M. MURATA, H. HANAKI, K. HINOTOZAWA, R. ŌIWA & H. TANAKA: Phosalacine, a new herbicidal antibiotic containing phosphinothricin. Fermentation, isolation, biological activity and mechanism of action. *J. Antibiotics* 37: 829~835, 1984
- 2) TAKAHASHI, Y.; Y. IWAI & S. ŌMURA: Two new species of the genus *Kitasatosporia*, *K. phosalacinea* and *K. griseola*. *J. Gen. Appl. Microbiol.*, submitted.
- 3) BAYER, E.; K. H. GUGEL, K. HÄGELE, H. HAGENMAIER, S. JESSIPOW, W. A. KÖNIG & H. ZÄHNER: Stoffwechselprodukte von Mikroorganismen. 98. Phosphinothricin und Phosphinothricinyl-Alanyl-Alanin. *Helv. Chim. Acta* 55: 224~239, 1972
- 4) KONDO, Y.; T. SHOMURA, Y. OGAWA, T. TSURUOKA, H. WATANABE, K. TOTSUKAWA, T. SUZUKI, C. MORIYAMA, J. YOSHIDA, S. INOUE & T. NIIDA: Studies on a new antibiotic SF-1293. I. Isolation and physico-chemical and biological characterization of SF-1293 substance. *Sci. Reports of Meiji Seika Kaisha* 13: 34~41, 1973
- 5) OGAWA, Y.; T. TSURUOKA, S. INOUE & T. NIIDA: Studies on a new antibiotic SF-1293. II. Chemical structure of antibiotic SF-1293. *Sci. Reports of Meiji Seika Kaisha* 13: 42~48, 1973