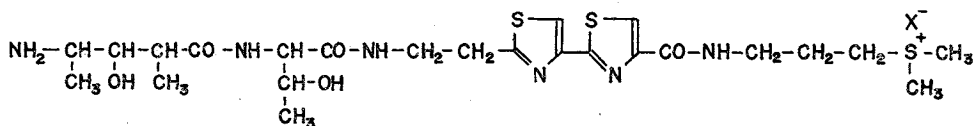


Fig. 2. The structure of tetrapeptide S.



ble DNP-III and free I, VI, and VII, which were identified by thin-layer chromatographic comparison with authentic samples.

The DNP-tetrapeptide S dissolved in concentrated hydrochloric acid was kept at 37°C for 3 days. The reaction mixture was diluted with water, and then extracted with ether. The ether extract contained DNP-III, and the aqueous layer contained tripeptide S, a trace of unreacted DNP-tetrapeptide S and a new yellow substance. The yellow substance (Rm 0.92) was more basic than DNP-tetrapeptide S (Rm 0.47) and retransformed to DNP-tetrapeptide S by alkaline treatment. Thus, the yellow substance is suggested to be the N→O acyl shifted product of DNP-tetrapeptide S. Thus, tetrapeptide S can be assigned to the structure shown in Fig. 2.

The recovery yield of tetrapeptide S after CM Sephadex column chromatography was calculated from the UV absorption at 291.5 nm. The molecular extinction coefficient (ϵ 12,850) of crystalline tripeptide S dihydrobromide at 291.5 nm was used as the basis of calculation. The maximum yield reached 44.8% when 6 moles of NBS was used for one mole of bleomycin A₂.

The fact that the N-terminus of tetrapeptide S is compound III indicates that tetrapeptide S was formed by NBS cleavage at the carboxyl peptide bond of β -hydroxy histidine (IV), because the peptide bond between IV and III is already shown in

tetrapeptide A. Then, it was established that the C-terminus of III is connected with the N-terminus, I, of tripeptide S.

YASUHIKO MURAOKA*

TOMOHIISA TAKITA**

KENJI MAEDA

HAMA O UMEZAWA

Institute of Microbial Chemistry,
Shinagawa-ku, Tokyo, Japan

* Research Laboratory,
Pharmaceutical Division,
Nippon Kayaku Co., Ltd.,
Kita-ku, Tokyo, Japan

** Author to whom correspondence
should be addressed.

(Received January 14, 1972)

References

- 1) TAKITA, T.; Y. MURAOKA, K. MAEDA & H. UMEZAWA: Selective cleavage of bleomycin. Proceedings of the 8th Symposium on Peptide Chemistry. Osaka. pp. 179~183, 1970
- 2) KUHN, R.; I. LÖW & H. TRISCHMANN: Die Konstitution der Lycotetraose. Chem. Ber. 90: 203~218, 1957
- 3) SHALTIEL, S. & A. PATCHORNIK: Cleavage of histidyl peptide bonds by N-bromosuccinimide. J. Am. Chem. Soc. 85: 2799~2806, 1963
- 4) TAKITA, T.; T. YOSHIOKA, Y. MURAOKA, K. MAEDA & H. UMEZAWA: Chemistry of bleomycin. V. Revised structure of an amine component of bleomycin A₂. J. Antibiotics 24: 795~796, 1971