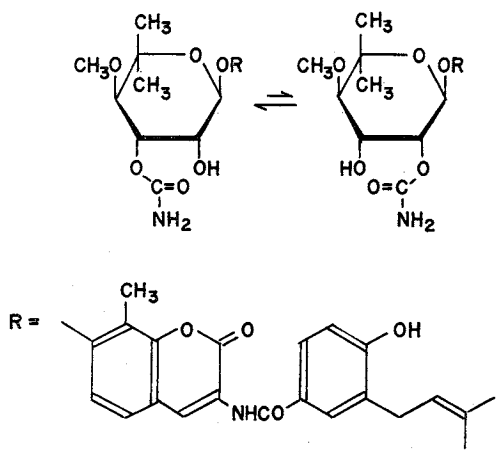
CHEMISTRY OF BLEOMYCIN. XII
ISO-BLEOMYCIN A_2, A PRODUCT OF CARBAMOYL GROUP MIGRATION

Sir:

The bleomycins are a group of related glycopeptide antitumor antibiotics[1], with a sugar moiety containing one mole each of L-gulose and 3-O-carbamoyl-D-mannose[2], as the disaccharide, 2-O-(3-O-carbamoyl-α-D-mannopyranosyl)-L-gulose. This disaccharide is linked to the hydroxyl group of L-*erythro*-β-hydroxyhistidine[3] through an α-glycosidic linkage[4].

The structural similarity of 3-O-carbamoyl-D-mannose to 3-O-carbamoyl-noviose, the sugar moiety of novobiocin[5], suggested that under basic conditions the carbamoyl function of bleomycin could be shifted to the vicinal C-2 hydroxyl group of the mannose moiety. With novobiocin, the carbamoyl group migrates from the C-3 to the C-2 hydroxyl group under alkaline conditions, and finally an equilibrium is established (Fig. 1). The carbamoyl function of novobiocin plays an important role in the antibacterial activity. Decarbamoyl-novobiocin and *iso*-novobiocin, with the O-carbamoyl group at C-2 of noviose, have no antibacterial activity[6]. OMOTO *et al.* synthesized methyl 2-O- and 3-O-carbamoyl-α-D-mannopyranosides as model compounds and studied the carbamoyl migration[7] (Fig. 2). Under alkaline conditions, they were interconverted with about 3 times as much 3-O-carbamoyl as 2-O-carbamoyl at equilibrium.

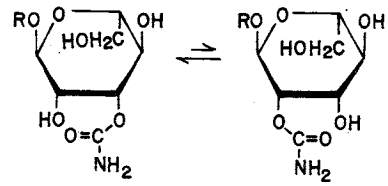
Fig. 1. The equilibrium between novobiocin and *iso*-novobiocin under alkaline conditions.



We studied the behavior of bleomycin A_2 under basic conditions and found the formation of *iso*-bleomycin A_2, which has an O-carbamoyl group at the C-2 of the mannose moiety. In this communication, formation, isolation and properties of *iso*-bleomycin A_2 are presented.

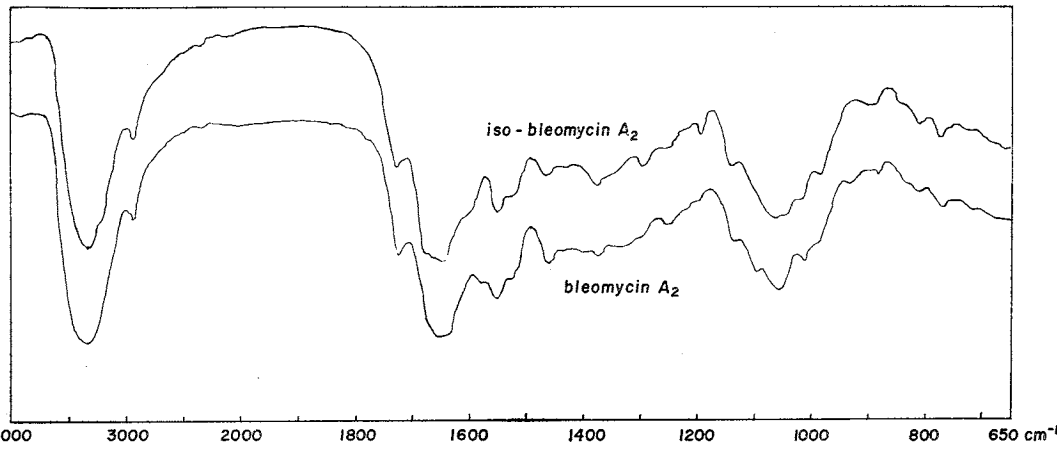
In preliminary experiments, it was found that carbamoyl group migration occurred only in copper-free bleomycin A_2, but not in copper-chelated bleomycin. Copper-free bleomycin A_2 was dissolved in aqueous alcohol (H_2O/EtOH= 4/10 in volume) and the apparent pH of the solution was adjusted to 10.3 with triethylamine as measured by a glass electrode pH-meter. The solution was kept at 20°C for 5 days and then concentrated under reduced pressure to remove ethanol and triethylamine. The concentrate was adjusted to pH 5.0 and treated with cupric carbonate to form the copper complex. The copper complex was subjected to column chromatography on CM-Sephadex C-25 with a linear gradient (0.1~0.2 M) of ammonium chloride. Copper-chelated *iso*-bleomycin A_2 was eluted after unchanged copper-chelated bleomycin A_2. The ratio of *iso*-bleomycin A_2 to unchanged bleomycin A_2 was about 0.5. The UV spectrum of copper-chelated *iso*-bleomycin A_2, $\lambda_{max}^{H_2O}(E_{1cm}^{1\%})$: 242.5 nm (161), 292.5 nm (134), was almost the same as that of copper-chelated bleomycin A_2. The IR spectra were slightly different (Fig. 3). On thin-layer chromatography on silica gel developed with methanol, 10% ammonium acetate solution and 10% ammonia (10 : 9 : 1 in volume), *iso*-bleomycin A_2 (Rf 0.39) was separated from bleomycin A_2 (Rf 0.46). The antibacterial activity measured by the cylinder-

Fig. 2. The equilibria between methyl 3-O- and 2-O-carbamoyl-α-D-mannopyranosides (R: Me) and between bleomycin A_2 and *iso*-bleomycin A_2 (R: the rest of bleomycin A_2 molecule) under alkaline conditions.



* Part XI of this series: in J. Antibiotics 26: 398~399, 1973

Fig. 3. Infrared specta of copper-chelated bleomycin A_2 and copper-chelated *iso*-bleomycin A_2 (KBr).



agar plate method using *Mycobacterium* 607 as the test organism was about one third that of bleomycin A_2: *iso*-bleomycin A_2 was 305 u/mg, bleomycin A_2 was 960 u/mg, taking copper-free bleomycin A_2 free base as standard, 1,000 u/mg.

Copper-free *iso*-bleomycin A_2, obtained by treatment of the copper-chelated material with hydrogen sulfide, was retransformed to bleomycin A_2 under the basic conditions described above.

To confirm the presence of the 2-O-carbamoyl-D-mannose moiety in *iso*-bleomycin A_2 molecule, *iso*-bleomycin A_2 was methanolyzed by reflux overnight with Amberlyst 15 as acid catalyst in methanol solution[2]. The catalyst was removed by filtration and the filtrate was dried, yielding a syrup containing the methyl glycosides of the sugar components. It was already shown[7] that methyl 2-O- and 3-O-carbamoyl-α-D-mannopyranosides are effectively separated by silica gel chromatography of their O-benzoyl derivatives. After treatment of the syrup with benzoyl chloride and pyridine, the benzoyl derivatives were separated by silica gel column chromatography with ethyl acetate and benzene (1 : 7 in volume). Methyl 2-O-carbamoyl-3, 4, 6-tri-O-benzoyl-α-D-mannopyranoside was isolated from the eluate and crystallized from isopropanol, m. p. 189°C (Lit.[7] 189.5°C). Anal. calcd. for $C_{29}H_{27}NO_{10}$: C, 63.38; H, 4.95; N, 2.55. Found: C, 63.51; H, 4.82; N, 2.50. The IR spectrum was identical to that of the synthetic material[7]. The 3-O-carbamoyl-mannose derivative was not detected in the eluate.

Thus, it was confirmed that *iso*-bleomycin A_2 contains a 2-O-carbamoyl-mannose moiety instead of the 3-O-carbamoyl-mannose moiety of bleomycin A_2. The other amine and sugar components are the same as those of bleomycin A_2 The interconversion between bleomycin A_2 and *iso*-bleomycin A_2 proceeds under mild conditions with an equilibrium favoring bleomycin A_2. It can be concluded that only the carbamoyl migration is involved in this interconversion. It should be noted that the interconverison is observed only with the copper-free form of bleomycin and *iso*-bleomycin retains considerable biological activity.

YUYA NAKAYAMA
MAMORU KUNISHIMA
SHOJI OMOTO*
TOMOHISA TAKITA*
HAMAO UMEZAWA*

Research Laboratory,
Nippon Kayaku Co. Ltd.,
3-31-12 Shimo, Kita-ku, Tokyo, 115 Japan
*Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo,
141 Japan

(Received April 14, 1973)

References

1) TAKITA, T.; Y. MURAOKA, T. YOSHIOKA, A. FUJII, K. MAEDA & H. UMEZAWA: The chemistry of bleomycin. IX. The structures of bleomycin and phleomycin. J. Antibiotics 25: 755~758, 1972
2) TAKITA, T.; K. MAEDA, H. UMEZAWA, S.

OMOTO & S. UMEZAWA: Chemistry of bleomycin. III. The sugar moieties of bleomycin A_2. J. Antibiotics 22: 237~239, 1969

3) KOYAMA, G.; H. NAKAMURA, Y. MURAOKA, T. TAKITA, K. MAEDA, H. UMEZAWA & Y. IITAKA: The chemistry of bleomycin. X. The stereo-chemistry and crystal structure of β-hydroxy-histidine, an amine component of bleomycin. J. Antibiotics 26: 109~111, 1973

4) OMOTO, S.; T. TAKITA, K. MAEDA, H. UMEZAWA & S. UMEZAWA: The chemistry of bleomycin. VIII. The structure of the sugar moiety of bleomycin A_2. J. Antibiotics 25: 752~754, 1972

5) HINMAN, J. W.; E. L. CARON & H. HOEKSEMA: The structure of novobiocin. J. Amer. Chem. Soc. 79: 3789~3800, 1957

6) HINMAN, J. W.; E. L. CARON & H. HOEKSEMA: Novobiocin. V. Carbamyl migration and isonovobiocin. J. Amer. Chem. Soc. 79: 5321~5322, 1957

7) OMOTO, S.; T. TAKITA, K. MAEDA & S. UMEZAWA: Synthesis of methyl 3-O- and 2-O-carbamoyl-α-D-mannopyranosides and carbamoyl migration between them. Carbohydrate Research (to be publisted).