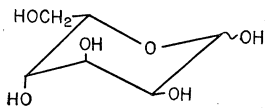
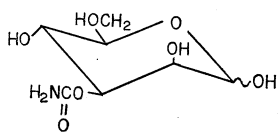
CHEMISTRY OF BLEOMYCIN. III THE SUGAR MOIETIES OF BLEOMYCIN A_2

Sir:

In previous reports[1,2] we described the structures of the amine components of bleomycin A_2. In this communication, we report the occurrence of L-gulose and 3-O-carbamoyl-D-mannose as part of bleomycin A_2 molecule. It is the first recognition of these sugars as components of natural products.



L-Gulose



3-O-Carbamoyl-D-mannose

Sugar moieties of bleomycin A_2 were obtained by refluxing a methanolic solution for 20 hours with a strongly acidic resin (Amberlyst 15). In this process basic methanolysis products were adsorbed on the resin and only neutral products were liberated into solution. A cellulose thin-layer chromatogram using water-saturated *n*-butanol indicated the presence of at least two products in the solution. After evaporation of the solvent, a sticky colorless syrup was obtained. The syrup was acetylated with acetic anhydride and pyridine, and then chromatographed on silica-gel using 100 : 1 chloroform - methanol.

The more rapidly eluted material was further separated into two components by silica-gel chromatography using 4 : 1 toluene - ethyl methyl ketone. The major component (I-a) was eluted faster than the minor (I-b) and the yield of the former was about three times that of the latter. The mass spectrum of I-a showed a fragmentation pattern typical of a methyl tetra-O-acetyl-aldohexopyranoside[3] (m/e 331, 242, 200, 169, 157, 145, 140, 115, 103, 98 and 43). The NMR spectra of I-a and I-b suggested that they were anomers and had the gulose-configuration[4] (Compound I-a: δ_{H-1} 4.72 ppm, J_{1-2} 8.3 Hz; δ_{H-2} 4.98, J_{2-3} 3.1; δ_{H-3} 5.38, J_{3-4} 4.2; δ_{H-4} 4.98, J_{4-5} 1.0. Compound I-b: δ_{H-1} 4.88, J_{1-2} 3.9, J_{1-3} 1.0, J_{1-4} 0.6; δ_{H-2} 5.19, J_{2-3} 3.9; δ_{H-3} 5.28, J_{3-4} 3.4; δ_{H-4} 5.03, J_{4-5} 1.5. 100 MHz in $CDCl_3$-TMS).

Compounds I-a and I-b were crystallized from isopropyl alcohol as fine needles. Compound I-a: m. p. 64.0~66.5°C, $[\alpha]_D^{22}$ +33° (*c* 1, $CHCl_3$), Anal. Found: C 50.02, H 6.22. Calcd. for $C_{15}H_{22}O_{10}$: C 49.72, H 6.12. Compound I-b: m. p. 96~97°C, $[\alpha]_D^{14}$ −96.5 (*c* 0.85, $CHCl_3$), Anal. Found: C 49.91, H 6.11. These data indicated that I-a was methyl tetra-O-acetyl-β-L-gulopyranoside and I-b was the α-anomer, [Lit.[5] β-D-anomer: m. p. 66~67°C, $[\alpha]_D$ −32.1° ($CHCl_3$). α-D-anomer: m. p. 98°C $[\alpha]_D$ +97.3° ($CHCl_3$)]. The IR spectrum of I-b (KBr) was identical with that of methyl tetra-O-acetyl-α-D-gulopyranoside, enantiomer of I-b, which was kindly supplied by Dr. H. S. ISBELL.

The material (II) eluted secondly from the silica-gel chromatography ($CHCl_3$-MeOH) was crystallized from isopropyl alcohol, m. p.

Fig. 1. Mass spectrum of II (Methyl 2, 4, 6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranoside)

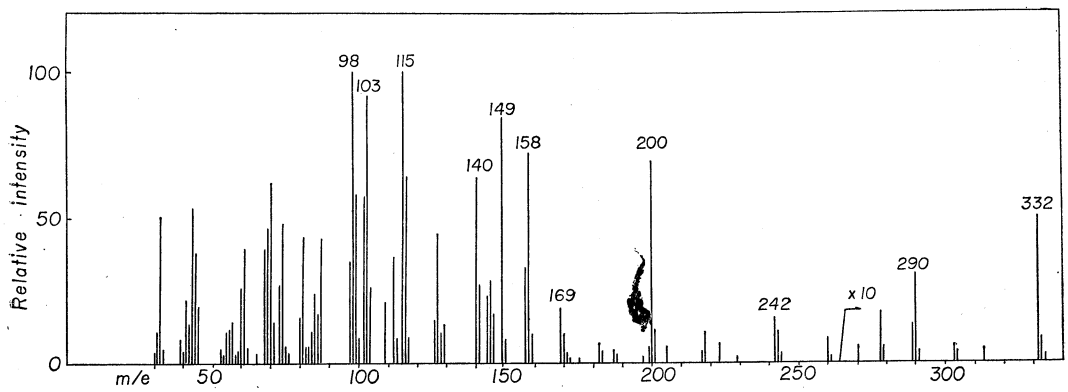
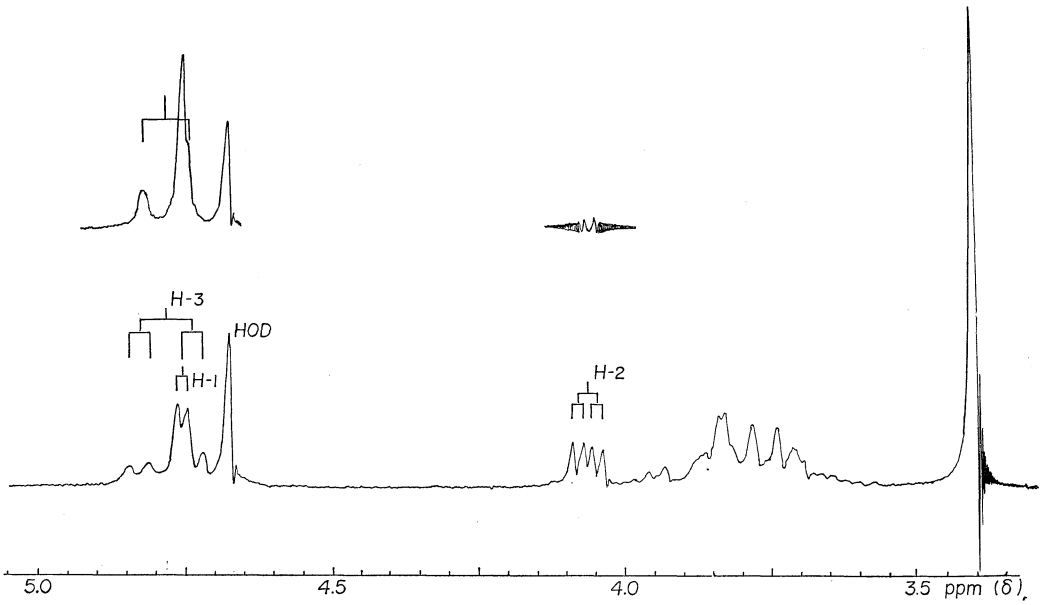
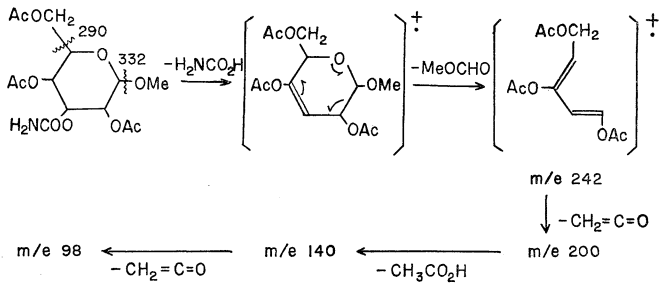


Fig. 2. NMR spectrum of III (Methyl 3-O-carbamoyl-α-D-mannopyranoside) (100 MHz, in D_2O. Chemical shifts were measured relative to DSS as internal reference)



141.0~142.5°C, $[\alpha]_D^{22}$ +35.7° (*c* 1, $CHCl_3$). Anal. Found: C 46.50, H 5.83, N 3.87. Calcd. for $C_{14}H_{21}NO_{10}$: C 46.28, H 5.83, N 3.86. The NMR spectrum of II indicated the presence of three acetyl groups and one methoxy group, and the IR spectrum suggested the presence of a carbamoyl group (1720, 1620 cm^{-1}). The mass spectrum of II is shown in Fig. 1. Interpretation of the mass spectrum suggested that II would be a methyl 2,4,6-tri-O-acetyl-3-O-carbamoyl-aldohexopyranoside. The following fragmentation processes were supported by the high resolution mass spectrum: m/e 332.097 (calcd. 332.098), 290.089 (290.088), 242.077 (242.079), 200.071 (200.068).

Compound II yielded the desacetyl product (III) by treatment with a catalytic amount of sodium methoxide in anhydrous methanol solution. The IR spectrum of III showed absorption bands at 1710 cm^{-1} and 1610 cm^{-1}, characteristic for an O-carbamoyl function[6]. The presence of an O-carbamoyl group was proved further by formation of ammonia and barium carbonate in saturated baryta solution. The decarbamoyl product was acetylated and then crystallized from isopropyl alcohol, m. p. 62.0~63.8°C, $[\alpha]_D^{20}$ +48.7° (*c* 0.77, $CHCl_3$). Anal. Found: C 49.97, H 6.23. Calcd. for $C_{15}H_{22}O_{10}$: C 49.72, H 6.12. It was identified as methyl tetra-O-acetyl-α-D-mannopyranoside, [Lit.[7] m. p. 65°C, $[\alpha]_D^{20}$ +49.1° (*c* 4.7, $CHCl_3$)], by comparison of IR spectra with that of an authentic sample. The position of the O-carbamoyl substituent in III was confirmed to be at C-3 of methyl α-D-mannopyranoside by an NMR study of III, that is, irradiation at the H-2 peak (δ 4.06, J_{1-2} 1.8, J_{2-3} 3.3) brought coalescence of signals of H-1 (δ 4.76) and H-3 (δ 4.78) (Fig. 2). Structure of III, methyl 3-O-carbamoyl-α-D-mannopyranoside, was finally proved by chemical synthesis via ammonolysis of methyl 2,3-O-carbonyl-α-D-mannopyranoside[8].



Bleomycin A_2 has no reducing property and absence of O-acetyl and methoxy groups in this antibiotic is shown by its NMR spectrum. The sugars, L-gulose and 3-O-carbamoyl-D-mannose, were not liberated by a mild alkaline hydrolysis. Therefore, it is suggested that these sugars are bound by glycosidic linkages to the aglycone part of this antibiotic.

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