2-O-(3-O-CARBAMOYL-D-MANNOSYL)-6-DEOXY-L-GULOSE, THE SUGAR MOIETY OF THE ANTIBIOTIC YA-56*

Sir:

In the previous communication[1], we have reported that methyl 3-O-carbamoylmannoside and an unidentified sugar derivative were found in the methanolysis product of two main components (X and Y) of YA-56, a phleomycin-bleomycin-zorbamycin[2] group antibiotic.

Recently we have succeeded in isolating anomeric pairs of the unidentified sugar derivatives (S-II, S-III) and pairs of anomeric disaccharide derivatives (S-V, S-VI) together with methyl 2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranoside (S-IV) from the fully acetylated methanolysis product of both components of YA-56.

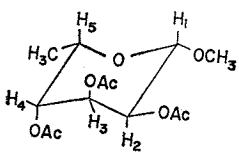
This communication concerns the structure elucidation of the above acetylalated sugar derivatives which leads us to conclude the sugar moiety of YA-56 is 2-O-(3-O-carbamoyl-D-mannosyl)-6-deoxy-L-gulose.

Methanolysis was carried out according to the procedure applied to bleomycin[3]. The methanolysis products were acetylated and then separated on a silica gel column using $CHCl_3$ - MeOH (97 : 3). Two fractions showing Rf values at 0.55 and 0.13 on TLC (silica gel G Merck, $CHCl_3$ - MeOH (97 : 3)) were obtained. Each fraction was further separated on a silica gel column using a ethylacetate - toluene (2 : 1) solvent system. Thus, three compounds, S-I**, S-II and S-III could be isolated from the first fraction (Rf 0.55) and the other three compounds, S-IV, S-V and S-VI could be isolated from the second fraction (Rf 0.13). The Rf values on silica gel G (Merck) plates using ethylacetate-toluene (2 : 1) were as follows: S-I, 0.82; S-II, 0.73; S-III, 0.62; S-IV, 0.40; S-V, 0.29; S-VI, 0.18.

Table 1.

H	(ppm)	J (Hz)	
H-1	4.66	$J_{1,2}$	8.0
H-2	5.02	$J_{2,3}$	3.7
H-3	5.20	$J_{3,4}$	4.0
H-4	4.86	$J_{4,5}$	1.6
H-5	4.09	J_{5,CH_3}	6.4
CH-C$\underline{H}_3$	1.22		

Fig. 1.



The compound S-II was obtained as colorless syrup; $[\alpha]_D^{23}$ +35° (*c* 1, $CHCl_3$). The mass spectrum of S-II showed a fragmentation pattern typical of a methyl tri-O-acetyl-deoxy-hexopyranoside (*m/e* 273, 184, 142). The NMR spectrum of S-II (60 MHz in $CDCl_3$, TMS reference) indicated one secondary methyl (δ 1.22, 3H, doublet), two axial acetoxy (δ 2.10 and 2.13, each 3H, singlets), one equatorial acetoxy (δ 1.99, 3H, singlet) and one equatorial methoxyl (δ 3.51, 3H, singlet) group as well as five ring protons (δ 3.85~5.50). Analysis of the 100 MHz NMR spectrum (in $CDCl_3$, TMS) and spin decoupling experiments made it possible for us to assign the 6-deoxy-gulo configuration to S-II as shown in Table 1 and Fig. 1. The molecular rotation of S-II, +107°, was in agreement with the L-β (1C) conformation of S-II. Thus the structure of S-II was proposed to be methyl 2,3,4-tri-O-acetyl-6-deoxy-β-L-gulopyranoside.

De-O-acetylation of S-II with sodium methoxide in absolute MeOH followed by acid hydrolysis gave a free sugar (S-II-A) as a colorless syrup. Rm values[4] on cellulose plate (Merck) were as follows: 0.91 (H_2O std. MEK) and 0.11 (H_2O std. BuOH). The sugar was characterized as its crystalline *p*-bromophenylhydrazone (S-II-B). M.p. 135~137°C, $[\alpha]_D^{25}$ +13±2° (*c* 1, EtOH, after 1 hour). Anal. calcd. for $C_{12}H_{16}N_2O_5Br$: C 43.26, H 5.14, N 8.41. Found: C 43.17, H 5.39, N 8.03. The IR spectrum of S-II-B was superimposable with that of newly synthetized 6-deoxy-L-gulose *p*-bromophenylhydrazone***. Moreover, the authentic sample of 6-deoxy-D-gulose[5] was not distinguished from S-II-A on TLC using the above solvent systems. Thus, the structure of S-II was established. Although occurrence of 6-deoxy-D-gulose was described in literature[5], the present finding would be

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** Since the yield of S-I was quite low, no further study has been carried out.

*** The synthesis of 6-deoxy-L-gulose by a new route will be reported elsewhere.

the first recognition of 6-deoxy-L-gulose as a component of natural products.

The compound S-III was obtained as a colorless syrup: $[\alpha]_D^{22}$ −55° (*c* 1, $CHCl_3$). Spectral evidences (IR, NMR & MS) and the $[\alpha]_D$ value of S-III indicated that S-III is an anomer of S-II.

The compound S-IV was crystallized from isopropanol as colorless needles. M.p. 139.5~141°C, $[\alpha]_D^{22}$ +32° (*c* 1, $CHCl_3$). Anal. calcd. for $C_{14}H_{21}NO_{10}$: C 46.28, H 5.83, N 3.86. Found: C 46.03, H 5.89, N 3.81. This data was consistent with the data published for methyl 2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranoside[3]. S-IVwas finally identified with an authentic sample of the natural product derived from bleomycin by comparison of their IR, NMR and mass spectra.

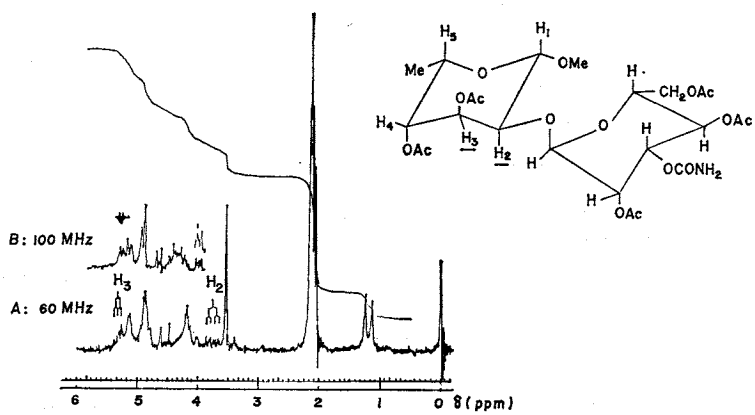
The compound S-V was crystallized from isopropanol-isopropylether as colorless needles. M.p. 179~183°C, $[\alpha]_D^{21}$ +40° (*c* 1, $CHCl_3$). Anal. calcd. for $C_{24}H_{35}NO_{16}$: C 48.57, H 5.97, N 2.36. Found: C 48.84, H 6.27, N 2.45. The presence of the following absorptions in the IR and NMR spectra of S-V suggested that S-V contained the following sugars: 6-deoxy-L-gulose and 3-O-carbamoylmannose. (IR in KBr: 1720 & 1610 cm^{-1} (C=O and NH in $CONH_2$); NMR in $CDCl_3$: δ 1.20 (3H, doublet, $-CH-C\underline{H}_3$), δ 3.52 (3H, singlet, $-OC\underline{H}_3$)).

To prove the structure, methanolysis of de-O-acetylated S-V followed by acetylation was carried out. Examination of the product on TLC revealed the existence of S-II, S-III and S-IV. Thus the constituents of S-V were chemically supported. Since S-V had one methoxyl group and showed no reducing properties, the linkage between both sugars was assumed to be a glycosidic one and the position of the linkage was deduced from the following NMR data. As shown in Fig. 2A (60 MHz) a signal due to the H-3 of 6-deoxy-L-gulose moiety was observed as double doublet at δ 5.31 ($J_{2,3}$ 3.7 Hz, $J_{3,4}$ 4.0 Hz). Irradiation of H-3 at δ 5.34 (Fig. 2B, 100 MHz) caused collapse of the quartet at δ 3.76 to a doublet having J 7.9 Hz. This indicated that the quartet was a signal due to the H-2 of the 6-deoxy-gulose moiety. Compared this to the chemical shift of H-2 (δ 5.02) in S-II, we could interpret that this upfield shift of H-2 in S-V was caused by formation of a glycosidic linkage at H-2 of 6-deoxy-gulose. Since the observed molecular rotation of S-V (+238°) was in closer agreement to the calculated molecular rotation for the α-mannosyl configuration (+341°) rather than β-configuration (−11°), the α-configuration of the glycosidic linkage was tentatively assigned. Consequently, S-V was concluded to be methyl 2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl)-3,4-di-O-acetyl-6-deoxy-β-L-gulopyranoside.

S-VI was crystallized from isopropanol-isopropylether as a crystalline powder. M.p. 149~151.5°C. From the spectral data (NMR, IR and mass) and $[\alpha]_D$ value, S-VI was considered to be an anomer of S-V.

YA-56 X and Y had no reducing properties and the presence of O-acetyl and O-methyl groups in these antibiotics were not shown by their NMR spectra. Therefore, it is

Fig. 2. NMR spectra of the compound S-V. (60 MHz and 100 MHz in $CDCl_3$ (TMS))



suggested that 2-O-(3-O-carbamoyl-α-D-mannosyl)-6-deoxy-L-gulose is present as a sugar moiety in these antibiotic components, while bleomycin is constituted of 2-O-(3-O-carbamoyl-α-D-mannosyl)-L-gulose*.

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