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Note

Chemical modification of neamine. Part III*

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In connection with the preceding papers of this series[2,3], the introduction of a pentofuranosyl and a hexopyranosyl group β-D-anomerically linked at O-5 of the 2-deoxystreptamine moiety of neamine has been attempted in order to elucidate structure–antimicrobial activity relationships.

Ribostamycin[6] (**3**), an aminocyclitol antibiotic produced by *Streptomyces ribosidificus*, was reported in 1970 and its structure has been established by Niida *et al.*[10] as *O*-β-D-ribofuranosyl-(1→5)-*O*-[2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)]-2-deoxystreptamine. Syntheses of it have been described[7,8], and we now report a facile synthesis of this antibiotic in 47% overall yield by employing 6,3′,4′-tri-*O*-acetyl-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine[2] (**1**) as the starting material.

As 5-*O*-(α-D-glucopyranosyl)neamine, obtained by transglycosylation of neamine with maltose and Clarase® (ref. 9), was more active against microorganisms than the parent neamine, we have attempted to prepare the anomer, namely 5-*O*-(β-D-glucopyranosyl)neamine (**5**), by using the same starting material **1**.

RESULTS AND DISCUSSION

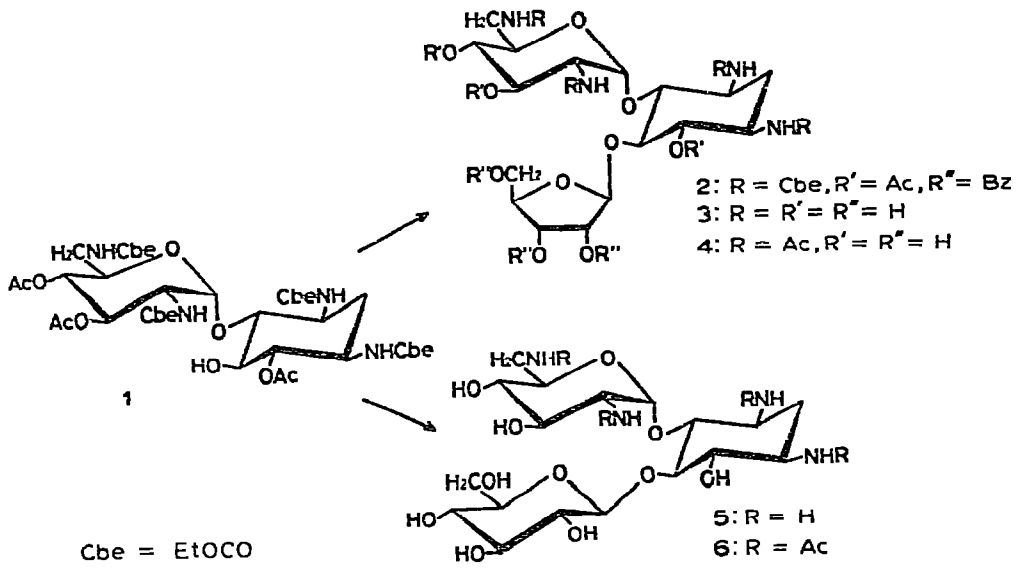
When **1** (ref. 2) was condensed with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[4] in dry benzene in the presence of mercuric cyanide and "Drierite" under reflux for 22 h, and the product purified on a silica gel column, 6,3′,4′-tri-*O*-acetyl-2″,3″,5″-tri-*O*-benzoyl-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)ribostamycin (**2**) was obtained in 75% yield. *O*-Deacylation of **2** in ethanolic sodium ethoxide, followed by hydrolysis in aqueous barium hydroxide, gave **3** in 63% yield as an amorphous solid. The amorphous base was obtained crystalline from methanol, and identified with natural ribostamycin by comparing its physical properties and antimicrobial activities with those of an authentic sample.

Similar condensation of **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[5], was followed by acetylation and subsequent purification by column chromatography. The desired fraction was hydrolyzed in aqueous barium hydroxide,

*For a preliminary report, see ref. 1.

and the hydrolyzate was purified on a column of Amberlite CG-50 (NH_4^+) to give **5** in 14% overall yield.

The structure of **5** was confirmed by its ^{1}H-n.m.r. spectrum. Presence of the β-D-anomeric linkage was demonstrated by the coupling constant (7 Hz) exhibited by the doublet at δ 5.18 in the spectrum.



Compound **3** showed identical antimicrobial activity against several microorganisms in comparison with natural ribostamycin. Compound **5** was less active than neamine, except against *Mycobacterium smegmatis* ATCC 607. As 5-*O*-(α-D-glucopyranosyl)neamine[9] was 2 to 4 times more active than neamine, **5** was much less active than the α-D anomer (Table I).

TABLE I

ANTIMICROBIAL ACTIVITIES OF **3** AND **5**

Compound (concentration, / mg/ml)	*Diameter of inhibition zone (mm), as determined by the paper-disk method*					
	Staphylococcus aureus *6538P*	Bacillus subtilis *ATCC6633*	Escherichia coli *K-12*	Mycobacterium smegmatis *ATCC 607*	Klebsiella[a] pneumoniae *7*	Escherichia[a] coli *ML-1629*
3	23.0	35.8	36 0	41.0	13.6	14.4
Ribostamycin	22.8	35.4	35.3	40.5	15.0	12.8
5	17.4	28.4	30.6	26 0	0	0
Neamine	22.2	33.9	31.6	24.9	0	0

[a]Kanamycin-resistant strains.

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes and are uncorrected. Solutions were evaporated under diminished pressure. Optical rotations were measured on a Japan Spectroscopic DIP-SL polarimeter. ^{1}H-N.m.r. spectra were recorded on a Varian A-60D spectrometer at 60 MHz in chloroform-*d*, unless otherwise noted, with tetramethylsilane as the internal standard, and the peak positions are given as δ-values. T.l.c. was performed on Wakogel B-10 (Wako Pure Chemical Co. Ltd.) plates. Silica gel (Wakogel C-300) was employed for column chromatography. Elemental analyses were performed by Mr. Saburo Nakada.

6,3′,4′-Tri-O-*acetyl-2″,3″,5″-tri*-O-*benzoyl-1,3,2′,6′-tetra*-N-(*ethoxycarbonyl*)-*ribostamycin* (**2**). — A mixture of 6,3′,4′-tri-*O*-acetyl-1,3,2′,6′-tetra-*N*-(ethoxycarbonyl)neamine[2] (**1**, 1.15 g, 1.6 mmol) and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[4] (10.3 g, 21 mmol) in benzene (40 ml) was heated to boiling under reflux for 22 h with mercuric cyanide (4.5 g) and "Drierite" (4.3 g). The mixture was filtered and the filtrate evaporated. The residue was acetylated with acetic anhydride in pyridine overnight and the solution was poured into ice-cold water. The aqueous solution was repeatedly extracted with chloroform and the combined chloroform layers were washed successively with aqueous sodium hydrogensulfate, sodium hydrogencarbonate, and water. After drying over anhydrous sodium sulfate, the solution was evaporated. The residue was purified on a column of silica gel with 30:1 (v/v) chloroform–ethanol as eluant. Fractions showing a single spot having R_F 0.48 by t.l.c. in the same solvent were combined and evaporated to give 1.38 g (75%) of **2** as an amorphous solid; m.p. 118–124°, $[\alpha]_D^{20}$ +63.2° (*c* 1.43, chloroform); ^{1}H n.m.r. data δ 1.0–1.4 (m, 12, $4CO_2CH_2CH_3$), 1.96 (s, 3, OAc), 1.97 (s, 3, OAc), 2.15 (s, 3, OAc).

Anal. Calc. for $C_{56}H_{68}N_4O_{24}$: C, 56.94; H, 5.80; N, 4.74. Found: C, 56.65; H, 5.67; N, 4.63.

Ribostamycin (**3**). — Compound **2** (1.14 g) was *O*-deacetylated in 0.1M ethanolic sodium ethoxide (20 ml) at room temperature. The solution was neutralized with Amberlite IR-120 (H^+) resin and evaporated. The residue was heated in a mixture of barium hydroxide octahydrate (5 g) and water (16 ml) for 6.5 h under reflux. Carbon dioxide was bubbled into the mixture, and the precipitate was removed by filtration. The filtrate was evaporated, and the residue purified on a column of Amberlite CG-50 (NH_4^+) resin. After washing with water and 0.1M aqueous ammonia, the column was eluted with 0.3M aqueous ammonia to give 274 mg (63%) of **3** as amorphous solid; $[\alpha]_D^{20}$ +45.0° (*c* 1.16, water). A part of the product was crystallized from methanol to give **3** as crystals; m.p. 189–191° (dec.), $[\alpha]_D^{20}$ +45.0° (*c* 0.52, water). The compound was identified by comparing its i.r. spectrum and antimicrobial activity with those of an authentic sample. (*Anal.* Found: C, 44.78; H, 7.47; N, 12.08.) Lit.[6] m.p. 192–195° (dec.), $[\alpha]_D^{23}$ +42° (*c* 1, water).

1,3,2′,6′-Tetra-N-*acetylribostamycin* (**4**). — Compound **3** (51 mg) was acetylated with acetic anhydride in methanol overnight at ~0° to give 60 mg (85%) of **4**;

m.p. 182° (sintering) 220° (dec.), $[\alpha]_D^{23}$ +41.2° (*c* 1.07, water). (*Anal.* Found: C, 47.12; H, 6.92; N, 8.62). Lit.[6] m.p. 180° (sintering) 205° (dec.), $[\alpha]_D^{23}$ +40° (*c* 1, water).

5-O-(β-D-Glucopyranosyl)neamine (**5**). — Compound **1** (ref. 2, 1.75 g, 2.4 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[5] (20.0 g, 49 mmol) were heated for several days under reflux in benzene (85 ml) containing mercuric cyanide (12.2 g) and "Drierite" (5.5 g). The mixture was filtered and the filtrate evaporated. The residue was acetylated in the conventional manner and the product was purified twice on a column of silica gel with 1.4 (v/v) butanone–toluene as eluant. Fractions showing a single spot [having R_F 0.20 by t.l.c. in 20:1 (v/v) benzene–ethanol] were combined and evaporated. The residue was hydrolyzed in boiling, aqueous barium hydroxide for 6.5 h, and the hydrolyzate was purified on a column of Amberlite CG-50 (NH_4^+) resin as described in the preparation of **3**, to give 158 mg (14%) of **5**; m.p. 189–196° (dec.), $[\alpha]_D^{21}$ +60.2° (*c* 1.28, water). The product showed a single spot at R_F 0.27 by t.l.c. in 5:8:10:7 (v/v) 28% ammonia–1-butanol–ethanol–water; ^{1}H n.m.r. data (D_2O at pD 1): δ 5.18 (d, 1, *J* 7 Hz, H-1″), 6.15 (d, 1, *J* 3.5 Hz, H-1′).

1,3,2′,6′-Tetra-N-acetyl-5-O-(β-D-glucopyranosyl)neamine (**6**). — Compound **5** (32 mg) was acetylated with acetic anhydride in methanol to give 40 mg (90%) of **6**; m.p. 200° (dec.), $[\alpha]_D^{20}$ +74.1° (*c* 1.27, water): ^{1}H n.m.r. data (D_2O): δ 1.98 (s, 6, 2NAc), 2.03 (s, 3, NAc), 2.05 (s, 3, NAc), 5.24 (d, 1, *J* 3 Hz, H-1′).

Anal. Calc. for $C_{26}H_{44}N_4O_{15}\cdot H_2O$: C, 46.56; H, 6.91; N, 8.35. Found: C, 46.81; H, 6.77; N, 8.05.

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REFERENCES

1 Presented at the 8th International Symposium on Carbohydrate Chemistry, Kyoto, Japan, August 17, 1976.
2 T. Suami, S. Nishiyama, Y. Ishikawa, and S. Katsura, *Carbohydr. Res.*, 52 (1976) 187–196.
3 T. Suami, S. Nishiyama, Y. Ishikawa, and S. Katsura, *Carbohydr. Res.*, 53 (1977) 239–246.
4 N. Yung and J. J. Fox, *Methods Carbohydr. Chem.*, 2 (1963) 108–112.
5 R. U. Lemieux, *Methods Carbohydr. Chem.*, 2 (1963) 221–222.
6 T. Shomura, N. Ezaki, T. Tsuruoka, T. Niwa, E. Akita, and T. Niida, *J. Antibiotics*, 23 (1970) 155–161.
7 T. Ito, E. Akita, T. Tsuruoka, and T. Niida, *Agr. Biol. Chem.*, 34 (1970) 980–981; *idem*, *Antimicrobial Agents Chemother.*, (1970) 33–37.
8 H. Fukami, K. Kitahara, and M. Nakajima, *Tetrahedron Lett.*, (1976) 545–548.
9 T. Endo and D. Perlman, *J. Antibiotics*, 25 (1972) 681–682.
10 E. Akita, T. Tsuruoka, N. Ezaki, and T. Niida, *J. Antibiotics*, 23 (1971) 173–183.