BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 40 2371—2375 (1976)

Studies of Antibiotics and Related Substances. XXXII. Syntheses of Neamine and Its Analogue[1]

Kuniaki TATSUTA, Eiichi KITAZAWA and Sumio UMEZAWA

Department of Applied Chemistry, Faculty of Engineering, Keio University, Koganei-shi, Tokyo

(Received April 21, 1967)

Neamine, an antibiotic glycoside, was synthesized from paromamine. The preferential tosylation of the primary hydroxyl group of tri-*N*-acetylparomamine was followed by the replacement of the tosyloxy group with an azido group. The azido derivative was catalytically hydrogenated and acetylated to give 4-*O*-(2, 6-diacetamido-2, 6-dideoxy-α-D-glucopyranosyl)-*N*, *N*'-diacetyldeoxystreptamine. De-*N*-acetylation of the product gave neamine. Furthermore, a structural isomer of neamine, namely 6-*O*-(3,6-diamino-3, 6-dideoxy-α-D-glucopyranosyl)-deoxystreptamine, was synthesized from 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-deoxystrept amine which was obtained from kanamycin by partial hydrolysis.

Aminosugar glycosides of 2-deoxystreptamine have been found to have antibacterial activities, for example, neamine,[2] paromamine,[3] 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-deoxystreptamine[4] (6AD), and 5-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-deoxystreptamine[5] (5-*O*-2AD). Among them, paromamine[6] and 5-*O*-2AD[5] have recently been synthesized.

In connection to our investigation[7] of the relationship between the structural and biochemical characteristics of these aminoglycosides, the present

1) Part XVI of "Studies of Aminosugars," by S. Umezawa. A brief communication has already been given of the synthesis of neamine: S. Umezawa, K. Tatsuta, T. Tsuchiya and E. Kitazawa, *J. Antibiotics*, **A20**, 53 (1967). A part of this paper was presented at the 20 th Annual Meeting of the Chemical Society of Japan, Tokyo, March, 1967.

2) a) R. L. Peck, C. E. Hoffhine, Jr., P. Gale and K. Folkers, *J. Am. Chem. Soc.*, **71**, 2590 (1949); b) R. L. Peck, C. E. Hoffhine, Jr., P. H. Gale and K. Folkers, *ibid.*, **75**, 1018 (1953).

3) T. H. Haskell, J. C. French and Q. R. Bartz, *ibid.*, **81**, 3480 (1959).

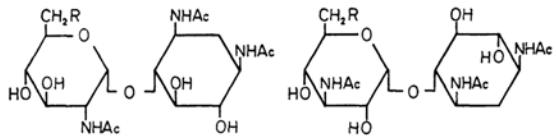
4) S. Umezawa and T. Tsuchiya, *J. Antibiotics*, **A15**, 51 (1962).

5) S. Umezawa, T. Tsuchiya and H. Fujita, *ibid.*, **A19**, 222 (1966).

6) S. Umezawa and S. Kotō, This Bulletin, **39**, 2014 (1966); *J. Antibiotics*, **A19**, 88 (1966).

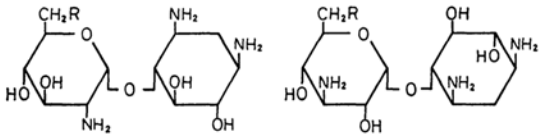
7) S. Umezawa, T. Tsuchiya, S. Nakada and K. Tatsuta, This Bulletin, **40**, 395 (1967).

paper describes the synthesis of neamine (V) from paromamine and another synthesis of 6-*O*-(3, 6-diamino-3, 6-dideoxy-α-D-glucopyranosyl)-deoxystreptamine (X), which is a structural isomer of the neamine, from 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-deoxystreptamine[8] (3AD).



I : R=-OH
II : R=-OTs
III : R=-N_3
IV : R=-NHAc

VI : R=-OH
VII : R=-OTs
VIII : R=-N_3
IX : R=-NHAc
XI : R=-I
XII : R=-H



Paromamine : R=-OH
V : R=-NH_2 (Neamine)

3AD : R=-OH
X : R=-NH_2

Tri-*N*-acetylparomamine[6] (I) contains one primary and four secondary hydroxyl groups. By the preferential tosylation of C-6 of I with *p*-toluenesulfonyl chloride, 4-*O*-(2-acetamido-2-deoxy-6-*O*-tosyl-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (II) was obtained in a 43% yield. In the next step, the replacement of the tosyloxy group of II with an azido group was carried out by heating II in dimethylformamide with sodium azide, thus affording 4-*O*-(2-acetamido-6-azido-2, 6-dideoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (III). The azido compound (III) was hydrogenated catalytically over platinum oxide in methanol and then *N*-acetylated with acetic anhydride to give 4-*O*-(2, 6-diacetamido-2, 6-dideoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (IV) in a 85% yield.

The absolute structure of the synthetic product IV and its identity with the tetra-*N*-acetylneamine[2b] were confirmed by the copper complex method.[9] Tetra-*N*-acetylneamine includes two pairs of adjacent hydroxyl groups which make the projected angles of about +60° and −60°; one in the glucose moiety and the other in the deoxystreptamine moiety. Accordingly, the $\Delta[M]_{CuAm}$ of tetra-*N*-acetylneamine may be expected to be nearly zero as a result of internal compensation. The determination of the $\Delta[M]_{CuAm}$ values of the synthetic product and the corresponding tetra-*N*-acetyl derivative of natural neamine gave values of +34 and +59 respectively. Thus, the observed values for $\Delta[M]$'s of IV and tetra-*N*-acetylneamine agreed within the range of experimental error, and the result indicated that the product IV is identical to tetra-*N*-acetylneamine, in which the four acetamido groups are located at C-2- and 6-positions of glucose moiety and at C-1- and 3-positions of 2-deoxystreptamine moiety. Thin-layer chromatography of the synthetic product IV illustrated that its R_f-value corresponds to that of tetra-*N*-acetylneamine.

In the next step, de-*N*-acetylation of IV with hydrazine, followed by chromatography on a column of Dowex 1×2 (OH form), gave a free base, which was then neutralized with hydrochloric acid to afford the tetrahydrochloride of 4-*O*-(2, 6-diamino-2, 6-dideoxy-α-D-glucopyranosyl)-deoxystreptamine (V) in a 72% yield. On paper chromatography, the synthetic product V and the natural neamine showed identical mobilities. The antibiotic spectra and minimal inhibitory concentrations (MIC) of the synthetic product V against test organisms were in agreement with those of the natural neamine as shown in Table 1. Since the synthesis of paromamine[6] has been established, the above synthesis may be regarded as a total synthesis of neamine.

6-*O*-(3, 6-Diamino-3, 6-dideoxy-α-D-glucopyranosyl)-deoxystreptamine (X) was prepared likewise from 3AD, which was obtained from kanamycin by partial hydrolysis. Tri-*N*-acetyl derivative[8] (VI) of 3AD was preferentially tosylated to give 6-*O*-(3-acetamido-3-deoxy-6-*O*-tosyl-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (VII) in a 44% yield. The tosyloxy group of VII was then replaced by an azido group to afford 6-*O*-(3-acetamido-6-azido-3, 6-dideoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (VIII) in a 83% yield. The azido compound (VIII) was converted to 6-*O*-(3, 6-diamino-3, 6-dideoxy-α-D-glucopyranosyl)-deoxystreptamine (X) *via* tetra-*N*-acetylated derivative (IX), in the same way as described in the preparation of V.

Structural proof was obtained from the nuclear magnetic resonance spectrum of 6-*O*-(3-acetamido-3, 6-dideoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (XII), which was prepared from mono-*O*-tosylated derivative (VII) *via* deoxyiodo derivative (XI). The signal at τ 8.79 (doublet, J=7 cps) may be assigned to three methyl protons, the singlets at τ 8.04, 8.01 and 7.97, with the relative intensities of 3 : 3 : 3 may be ascribed to nine *N*-acetyl protons, and the doublet at τ 5.01 (J=3 cps), with a relative intensity of 1, may be assigned to the anomeric hydrogen. These results indicated that the primary alcoholic group in the 3-amino-3-deoxy-D-glucose moiety of 3AD is

8) S. Umezawa, K. Tatsuta and T. Tsuchiya, *ibid.*, **39**, 1244 (1966).
9) S. Umezawa, T. Tsuchiya and K. Tatsuta, This Bulletin, **39**, 1235 (1966).

preferentially tosylated and consequently aminated by replacing the tosyloxy group with an amino group.

As shown in Table 1, the synthetic product V (or natural neamine) and 6AD, in which amino-glucose moieties were linked to C-4-position of 2-deoxystreptamine, have antibacterial activities. On the other hand, compound X and 3AD did not show any antibacterial activity and their amino-glucose moieties were linked to C-6-position of 2-deoxystreptamine. The structural similarities of V and X did not reflect any biochemical similarities. It seems, therefore, that C-4-linked amino-glycosides of 2-deoxystreptamine (neamine or V, paromamine, and 6AD) have antibacterial activities but C-6-linked derivatives (X and 3AD) are hardly endowed with antibacterial activities.

Experimental

Thin-layer Chromatography, Silica-gel Column Chromatography, and Paper Chromatography. Thin-layer chromatography was conducted by the use of silica gel (Daiichi Pure Chemicals Co.); the prepared plate was activated at 110°C and then stored in a desiccator. The spray reagent used was concentrated sulfuric acid. Solvent systems used: ethanol-benzene (3 : 1) (Solvent A) and ethanol-benzene (5 : 1) (Solvent B). Silica-gel column chromatography was carried out by the use of silica gel (Kanto Chemical Co.) activated at 110°C before use. Paper chromatography was conducted by the descending technique on Toyo filter paper No. 50, and the substances were detected by the use of ninhydrin spray (0.25% in pyridine). Solvent system used: *n*-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) (Solvent C).

General Procedure for Nuclear Magnetic Resonance Spectrometry. The NMR spectrum was determined at a frequency of 60 Mcps with a Varian A60 spectrometer in deuterium oxide. Sodium 2, 2-dimethyl-2-silapentane-5-sulfonate was used as an internal reference in the sample. Peak positions are given in τ-values.

4-*O*-(2-Acetamido-2-deoxy-6-*O*-tosyl-α-D-gluco-pyranosyl)-*N*,*N*′-diacetyldeoxystreptamine (II). To a cold (−15°C) solution of tri-*N*-acetylparomamine[6] (I, 1.22 g, 2.71 mmol) in dry pyridine (49 m*l*) was added *p*-toluenesulfonyl chloride (1.08 g, 5.67 mmol), and the mixture was stirred at the same temperature for 1 hr and allowed to stand overnight at 0°C and then 5°C for 2 days. After the addition of a small volume of water, the mixture was left for 30 min at 0°C. The mixture was evaporated and the residue was dried by coevaporation with toluene. The solid obtained was treated with methanol (3 m*l*) - acetone (20 m*l*) to separate the unchanged I (440 mg), which is insoluble in acetone. Concentration of the filtrate afforded a crude mono-tosyl derivative, which virtually showed a single spot (R_f 0.42) on a thin-layer chromatogram with Solvent A. The crude product was dissolved in methanol and chromatographed on a silica-gel column (48×265 mm) with a solvent mixture of ethanol-benzene (3 : 1). Every fraction (15 g) was tested by thin-layer chromatography. The substance having an R_f 0.42 appeared in the fractions of tube Nos. 29—36, which were combined and evaporated under reduced pressure, and the residue was recrystallized from methanol-ether; yield 710 mg (43%); mp 169—172°C (decomp.), $[\alpha]_D^{25}$ +68° (*c* 0.5, methanol). IR spectrum (KBr disk): 3400 (νOH), 3320 (νNH), 1650 (amide I), 1600 (phenyl), 1550 (amide II), 1175 ($\nu_s SO_2$) cm^{-1}.

Found: C, 49.45; H, 5.86; S, 5.66%. Calcd for $C_{25}H_{37}O_{12}N_3S$: C, 49.74; H, 6.18; S, 5.31%.

4-*O*-(2-Acetamido-6-azido-2, 6-dideoxy-α-D-gluco-pyranosyl)-*N*, *N*′-diacetyldeoxystreptamine (III). To a solution of II (431 mg) in dry *N*, *N*-dimethylformamide (4.3 m*l*) was added sodium azide (434 mg), and the suspension was vigorously agitated at 100°C for 6.5 hr. The resulting dark solution was filtered, and the filtrate was evaporated and coevaporated with toluene to obtain a solid substance, which showed essentially a single spot (R_f 0.39) on a thin-layer chromatogram with Solvent A. The dark solid substance was dissolved in methanol and chromatographed on a silica-gel column (32×221 mm) with a solvent mixture of ethanol-benzene (3 : 1). Fractions (15 g each) were tested by thin-layer chromatography. The substance having an R_f 0.39 was eluted in the fractions of tube Nos. 34—41. The fractions were combined and evaporated to give a solid substance which was recrystallized from methanol-ether; yield 119 mg (35%); mp 241—245°C (decomp.), $[\alpha]_D^{25}$ +114° (*c* 0.5, water). IR spectrum (KBr disk): 3440 (νOH), 3300 (νNH), 2110 (azide), 1650 (amide I), 1550 (amide II) cm^{-1}.

Found: C, 45.26; H, 6.49; N, 17.53%. Calcd for $C_{18}H_{30}O_9N_6$: C, 45.56; H, 6.37; N, 17.71%.

4-*O*-(2, 6-Diacetamido-2, 6-dideoxy-α-D-gluco-pyranosyl)-*N*, *N*′-diacetyldeoxystreptamine (IV), (Tetra-*N*-acetylneamine). A sample (200 mg) of III was dissolved in methanol (20 m*l*) and hydrogenated over platinum oxide (100 mg) under 3.5 atm of hydrogen-pressure at 40°C for 4 hr in a Paar shaker-type apparatus. After removal of the catalyst, the ninhydrin-positive solution was *N*-acetylated with acetic anhydride (3.5 m*l*) for 30 min. The resulting mixture was evaporated *in vacuo* to give a solid substance which was recrystallized from methanol; yield 176 mg (85%); mp 326°C (decomp.), $[\alpha]_{589}^{15}$ +95° (*c* 0.50, water), $[\alpha]_{436}^{15}$ +178° (*c* 0.50, water), $[\alpha]_{436}^{15}$ +185° (*c* 0.50, CuAm),[10] $\Delta[M]_{CuAm}$ +34. IR spectrum (KBr disk): 3450 (νOH), 3330 (νNH), 1665 (amide I), 1555 (amide II) cm^{-1}.

Found: C, 48.50; H, 7.25; N, 11.02%. Calcd for $C_{20}H_{34}O_{10}N_4$: C, 48.97; H, 6.99; N, 11.42%.

The tetra-*N*-acetylneamine[2b] obtained from natural neamine by *N*-acetylation showed $[\alpha]_{589}^{15}$ +94° (*c* 0.49, water), $[\alpha]_{436}^{15}$ +172° (*c* 0.49, water), $[\alpha]_{436}^{15}$ +184° (*c* 0.49, CuAm), $\Delta[M]_{CuAm}$ +59.

On a thin-layer chromatogram with Solvent B, the product IV showed a single spot (R_f 0.30) and the R_f-value agreed with that of tetra-*N*-acetylneamine.

4-*O*-(2, 6-Diamino-2, 6-dideoxy-α-D-glucopyrano-syl)-deoxystreptamine (V), (Neamine). A mixture of IV (50 mg) and 80% hydrazine hydrate (1 m*l*) in

10) CuAm: Cuprous chloride in aqueous ammonia; cuprous chloride (1.60 g) was dissolved in 15 N ammonia (100 m*l*) at 15°C.

$\Delta[M]_{CuAm}$ =

$$([\alpha]_{436}\text{CuAm} - [\alpha]_{436}\text{water}) \times \frac{\text{Mol wt}}{100}$$

; see Ref. 9.

a sealed tube was heated in a boiling water bath for 20 hr. After the hydrazine had been removed *in vacuo*, the residue was dissolved in water and chromatographed on a column (11×42 mm) of Dowex 1X2 (OH form) with water. A ninhydrin-positive eluate between 3—6 m*l* was neutralized with hydrochloric acid to pH 2 and evaporated to dryness. The residue was recrystallized from aqueous methanol-acetone affording the tetrahydrochloride of V; yield 34 mg (72%); mp 233°C (decomp.), $[\alpha]_D^{25}$ +86.8° (*c* 0.50, water). IR spectrum (KBr disk): 3400—3300 (νOH, NH), 2900 (νCH), 1600 ($\delta_{as}NH_3^+$), 1500 ($\delta_sNH_3^+$) cm^{-1}.

Found: C, 30.64; H, 6.94; N, 11.64%. Calcd for $C_{12}H_{26}O_6N_4 \cdot 4HCl$: C, 30.78; H, 6.46; N, 11.97%.

On a paper chromatogram with Solvent C, the product V showed a single spot ($R_{f\ paromamine}$ 0.47) and the R_f-value agreed with that of natural neamine. Infrared spectra of the product V and the natural neamine were completely superimposed.

6-*O*-(3-Acetamido-3-deoxy-6-*O*-tosyl-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (VII). To a cold (−15°C) solution of 6-*O*-(3-acetamido-3-deoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine[8] (VI, 2.00 g, 4.46 mmol) in dry pyridine (80 m*l*) was added *p*-toluenesulfonyl chloride (1.28 g, 6.72 mmol), and the mixture was stirred at the same temperature for 1 hr and kept at 5°C for 5 days. After a small volume of water had been added and the mixture had been set aside for 30 min, the mixture was evaporated and coevaporated with toluene to give a solid substance, which showed three spots having R_f values of 0.18 (unchanged VI), 0.32 (main) and 0.78 (minor) on a thin-layer chromatogram with Solvent A. The solid substance was treated with ethanol (42 m*l*)-acetone (170 m*l*) to separate the unchanged VI (0.69 g), which was insoluble in acetone. After concentration of the filtrate, the residue was dissolved in a solvent mixture of ethanol-benzene (3 : 1) and chromatographed on a silica-gel column (25×380 mm) with the same solvent. After about 230 m*l* of eluate, fractions (15 g each) were tested by thin-layer chromatography. The substance having an R_f value of 0.32 appeared in the fractions of tube Nos. 22—35, which were combined and evaporated under reduced pressure. The residue was recrystallized from ethanol-acetone affording VII; yield 1.18 g (44%); mp 184—189°C (decomp.), $[\alpha]_D^{20}$ +59° (*c* 0.68, methanol). IR spectrum (KBr disk): 3400 (νOH), 3320 (νNH), 1650 (amide I), 1600 (phenyl), 1560 (amide II), 1180 (ν_sSO_2) cm^{-1}.

Found: C, 49.48; H, 6.09; S, 5.36%. Calcd for $C_{25}H_{37}O_{12}N_3S$: C, 49.74; H, 6.18; S, 5.31%.

6-*O*-(3-Acetamido-6-azido-3, 6-dideoxy-α-D-glucopyranosyl)-*N*,*N'*-diacetyldeoxystreptamine (VIII). To a solution of VII (80 mg) in dry *N*, *N*-dimethylformamide (0.8 m*l*) was added sodium azide (80 mg), and the suspension was treated in a way described in the synthesis of III. The product showed essentially a single spot (R_f 0.25) on a thin-layer chromatogram with Solvent A, and was dissolved in methanol and chromatographed on a silica-gel column (9×150 mm) with a solvent mixture of ethanol-benzene (3 : 1). After elution of about 6 m*l* of the solution, fractions (3g each) were tested by thin-layer chromatography. The substance having an R_f value of 0.25 was eluted in the fractions of tube Nos. 10—19. These fractions were combined and evaporated to give a solid, which was recrystallized from methanol-ether; yield 52 mg (83%); mp 239—241°C (decomp.), $[\alpha]_D^{20}$ +88°C (*c* 0.54, water). IR spectrum (KBr disk): 3400—3340 (νOH, NH), 2110 (azide), 1650 (amide I), 1555 (amide II) cm^{-1}.

Found: C, 45.29; H, 6.59; N, 17.62%. Calcd for $C_{18}H_{30}O_9N_6$: C, 45.56; H, 6.37; N, 17.71%.

6-*O*-(3, 6-Diacetamido-3, 6-dideoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (IX). A sample (100 mg) of VIII was dissolved in methanol (10 m*l*) and hydrogenated over platinum oxide (40 mg), followed by *N*-acetylation with acetic anhydride (1 m*l*) as has been described in the synthesis of IV. The product obtained showed essentially a single spot (R_f 0.23) on a thin-layer chromatogram with Solvent B, and was dissolved in a solvent mixture of ethanol-benzene (5 : 1) and chromatographed on a silica-gel column (10×240 mm) with the same solvent. An eluate between 102—146 m*l* was evaporated to dryness and the residue was recrystallized from methanol affording needles of IX; yield 62 mg (60%); mp 293—296°C (decomp.), $[\alpha]_D^{20}$ +63° (*c* 0.67, water). IR spectrum (KBr disk): 3400—3320 (νOH, NH), 1650 (amide I), 1555 (amide II) cm.$^{-1}$

Found: C, 48.76; H, 7.21; N, 11.12%. Calcd for $C_{20}H_{34}O_{10}N_4$: C, 48.97; H, 6.99; N, 11.42%.

6-*O*-(3, 6-Diamino-3, 6-dideoxy-α-D-glucopyranosyl)-deoxystreptamine (X). A mixture of IX (200 mg) and 80% hydrazine hydrate (4 m*l*) in a sealed tube was heated at 130°C for 40 hr. The resulting mixture was treated in a way described in the synthesis of V. A ninhydrin-positive eluate was neutralized with hydrochloric acid to pH 2 and evaporated to dryness. The residue was recrystallized from aqueous methanol-ethanol affording the tetrahydrochloride of X; yield 141 mg (74%); mp 170—173°C (decomp.), $[\alpha]_D^{20}$ +69° (*c* 0.66, water). IR spectrum (KBr disk): 3400—3300 (νOH, NH), 1600 ($\delta_{as}NH_3^+$), 1500 ($\delta_sNH_3^+$) cm^{-1}.

Found: C, 30.67; H, 6.77; N, 11.75%. Calcd for $C_{12}H_{26}O_6N_4 \cdot 4HCl$: C, 30.78; H, 6.46; N, 11.97%.

On a paper chromatogram with Solvent C, the product X showed a single spot ($R_{f\ 3AD}$ 0.62).

6-*O*-(3-Acetamido-3, 6-dideoxy-α-D-glucopyranosyl)-*N*, *N'*-diacetyldeoxystreptamine (XII). A solution of the tosylate (VII, 500 mg) in dry *N*, *N*-dimethylformamide (5 m*l*) containing sodium iodide (380 mg) was stirred at 130°C for 6 hr. The resulting mixture was filtered, and the filtrate was evaporated to give a syrup, which was dried by coevaporation with toluene. The dark solid obtained showed essentially a single spot (R_f 0.34) on a thin-layer chromatogram with Solvent A. The solid was dissolved in a solvent mixture of ethanol-benzene (3 : 1) and chromatographed on a silica-gel column (25×230 mm) with the same solvent. An eluate between 416—500 m*l* was evaporated to give a deoxyiodo derivative (XI, 350 mg), which gave a strong positive Beilstein test for halogen. The infrared spectrum of the product showed the absence of the absorption at 1180 cm^{-1} due to a tosyl group.

The product XI (350 mg) was dissolved in 99% ethanol (25 m*l*) and hydrogenated over Raney nickel (W-4) (0.5 m*l*) under 3.5 atm of hydrogen-pressure at 40°C for 2 hr. The mixture was filtered, and the filtrate was again treated with Raney nickel and hydrogen for 4 hr. After removal of the catalyst, the filtrate was

TABLE 1. THE MIC'S OF COMPOUNDS V, X, NEAMINE, PAROMAMINE, 6AD AND 3AD AS DETERMINED BY THE DILUTION METHOD IN SOUILLON, mcg/ml

Test organisms	V	X	Neamine	Paromamine	6AD	3AD
Bacillus subtilis PCI 219	4	>1000	4	125	50	>1000
Mycobacterium tuberculosis 607	30	>1000	30	>1000	200	>1000
M. pyogenes var. aureus 209p	30	>1000	30	>1000	50	>1000
E. coli	125	>1000	62.5	500	1000	>1000

evaporated to give a solid substance, which showed essentially a single spot (R_f 0.18) on a thin-layer chromatogram with Solvent A. The solid was dissolved in a solvent mixture of ethanol-benzene (3 : 1), and chromatographed on a silica-gel column (16×200 mm) with the same solvent. An eluate between 275—480 ml was evaporated to dryness and the residue was recrystallized from methanol affording XII; total yield 180 mg (50%); mp 285°C (decomp.), $[\alpha]_D^{20}$ +67° (*c* 0.65, water). Beilstein test negative. NMR spectrum data: τ 8.79 (doublet, J=7 cps, 3 protons, 6-CH_3) τ 8.9 and 8.4 (multiplets, 1 proton each, 2-CH_2 of deoxystreptamine), τ 8.04, 8.01 and 7.97 (singlets, 3 protons each, NAc), τ 5.01 (doublet, J=3 cps, 1 proton, anomeric hydrogen); IR spectrum (KBr disk): 3430 (νOH), 3300 (νNH), 2910 (νCH), 1650 (amide I), 1555 (amide II) cm^{-1}.

Found: C, 49.64; H, 7.42; N, 9.43%. Calcd for $C_{18}H_{31}O_9N_3$: C, 49.87; H, 7.21; N, 9.69%.

Preliminary Bioassay. The antibiotic spectra and minimal inhibitory concentrations (MIC) of compounds V and X were shown in Table 1 and compared with natural neamine, paromamine, 6AD and 3AD.

The authors are indebted to Dr. Tsutomu Tsuchiya for his helpful advice, to Mr. Saburo Nakada for his microanalysis, to Mr. Mitsuteru Kokubun for his technical assistance, and to Mrs. Setsuko Iriyama for her antibacterial assay.