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Aminocyclitols. XXIII. A Synthetic Study of Streptozotocin Analogs*1

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Streptozotocin is an antibiotic which exhibits wide antimicrobial and antitumor activities, and its structure has been established to be *N*-carbamyl-*N'*-methyl-*N'*-nitroso-D-glucosamine. In the present article, streptozotocin analogs in which the pyranose ring was substituted for a cyclohexane ring were prepared. These compounds showed considerable activities against Ehrlich ascites tumor.

Streptozotocin,[1-4] an antibiotic produced by *Streptomyces achromogenes var.* 128 exhibits wide antimicrobial and antitumor activities *in vitro* and *in vivo*. The structure has been established to be *N*-carbamyl-*N'*-methyl-*N'*-nitroso-D-glucosamine, and this was further confirmed by the syntheses.[5,6]

In the present article, we wish to report a synthesis of *N*-carbamyl-*N'*-methyl-*N'*-nitroso derivatives of *scyllo*-inosamine,[7] *epi*-inosamine-2,[8-10] and *myo*-inosadiamine-1,3.[11,12]

When anisaldehyde was added to a mixture of *scyllo*-inosamine hydrochloride (I)[7] and 1N sodium hydroxide solution, *N*-[*p*-methoxybenzylidene]-*scyllo*-inosamine (II) was obtained in 84% yield. Acetylation of II in a mixture of acetic anhydride and pyridine afforded the pentaacetate (III), which was further converted to penta-*O*-acetyl-*scyllo*-inosamine hydrochloride (IV) by warming in 5N hydrochloric acid.

IV was treated with methyl isocyanate in the presence of silver carbonate to give penta-*O*-acetyl-*N*-carbamyl-*N'*-methyl-*scyllo*-inosamine (V) in a yield of 87%. Nitrosation of V with a mixture of nitrosyl chloride and pyridine afforded penta-*O*-acetyl-*N*-carbamyl-*N'*-methyl-*N'*-nitroso-*scyllo*-inosamine (VI) in 91% yield. An attempted deacetylation of VI in methanolic ammonia resulted in the formation of *N*-carbamyl-*scyllo*-isnosamine (VII) with a loss of diazomethane. Therefore, deacetylation was carried out before the nitrosation to give *N*-carbamyl-*N'*-methyl-*scyllo*-inosamine (VIII) in 64% yield from V.

An alternative method to prepare VIII (64% yield) was successfully carried out by treating I with methyl isocyanate in boiling aqueous acetonitrile in the presence of silver carbonate.

Nitrosation of VIII was achieved with sodium nitrite in aqueous acetic acid to give *N*-carbamyl-*N'*-methyl-*N'*-nitroso-*scyllo*-inosamine (IX) in 72% yield. Acetylation of IX by a conventional method afforded a penta-*O*-acetyl derivative which was identified to be VI.

N-Carbamyl-*N'*-methyl-*N'*-nitroso-*epi*-inosamine-2 (XI) and di-*N*,*N'*-(*N*-methyl-*N*-nitroso-carbamyl)-*myo*-inosadiamine-1,3 (XV) were prepared from *epi*-inosamine-2 hydrochloride (X) and *myo*-inosadiamine-1,3 dihydrochloride (XIII) respectively by analogous reactions described above.

These three compounds: IX, XI and XV, were active against Ehrlich ascites tumor and Hela carcinoma.

Experimental

The melting points reported were determined on a Mitamura Riken micro hot stage. The NMR spectra were determined with a Varian A-60D spectrometer.

***N*-[*p*-Methoxybenzylidene]-*scyllo*-inosamine (II).** To a solution of 949 mg of *scyllo*-inosamine hy-

*1 A part of this study has been presented at the 22nd Annual Meeting of the Chemical Society of Japan, Tokyo, April 1969.

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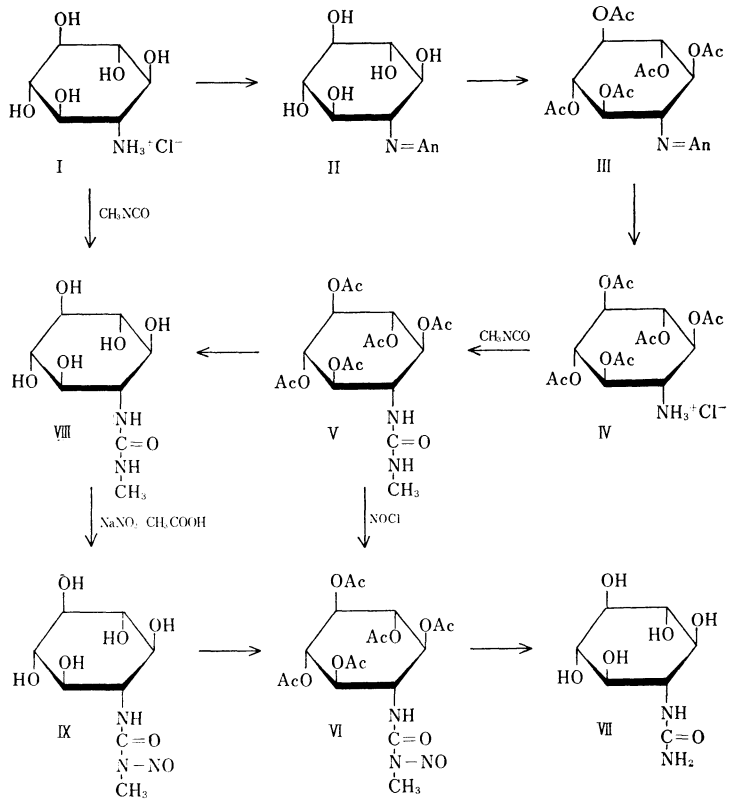
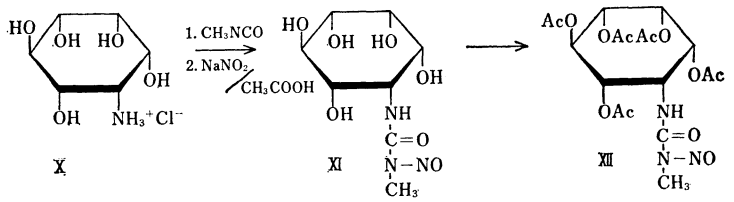
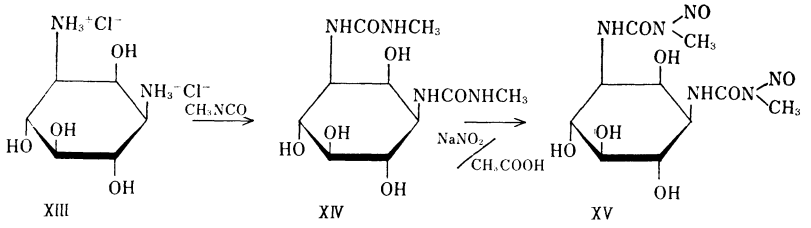


Ac= $-COCH_3$
An= $>CHC_6H_4OCH_3(p)$

Scheme 1



Scheme 2



Scheme 3

drochloride (I)[7] in 4.5 m*l* of 1 N sodium hydroxide, 610 mg of anisaldehyde was added with an occasional shaking. After the reaction mixture was settled overnight in a refrigerator, crystals were collected by filtration and washed with a mixture of ethanol and ether (1 : 1). The product (1.087 g) was obtained in a form of fine powder: mp 243—249°C (dec.). A second crop of the product (92 mg) was obtained from the mother liquor. The total yield was 90.6%.

Penta-*O*-acetyl-*N*-[*p*-methoxybenzylidene]-*scyllo*-inosamine (II). A 1.087 g portion of II was acetylated with 3.3 m*l* of acetic anhydride in 5.9 m*l* of pyridine. After the reaction mixture was settled overnight at room temperature, crystals were collected by

filtration and washed with ethanol to give 1.412 g of the product, mp 228°C (dec.). The filtrate was diluted with 20 ml of ice-cold water to give 0.205 g of the product. The total yield was 87.2%.

The crude product was recrystallized from ethanol to give crystals, mp 228—229°C.

Penta-*O*-acetyl-*scyllo*-inosamine hydrochloride (IV). To a suspension of 1.412 g of III in 14 ml of boiling acetone, 0.57 ml of 5 N hydrochloric acid was added. After cooling, 15 ml of ethyl ether was added to the mixture and the mixture was stored overnight in a refrigerator. Crystals were collected by filtration and washed with ether to give 1.119 g (94.6%) of the product. The crude product was recrystallized from methanol to give needles melting at 220—222°C (dec.).

Found: C, 45.31; H, 5.93; N, 3.31; Cl, 8.45%. Calcd for $C_{16}H_{24}NO_{10}Cl$: C, 45.13; H, 5.68; N, 3.30; Cl, 8.33%.

Penta-*O*-acetyl-*N*-carbamyl-*N'*-methyl-*scyllo*-inosamine (V). To a suspension of 1.00 g of IV in 20 ml of acetonitrile, 1 ml of methyl isocyanate and 780 mg of silver carbonate were added. The reaction mixture was refluxed on a steam bath for 35 min. After cooling, precipitates were removed by filtration and the filtrate was evaporated under reduced pressure to give a crystalline residue. The residue was triturated with ethanol to yield 905 mg (86.8%) of the product, mp 255—257°C.

Recrystallizations from ethanol and from chloroform gave an analytically pure sample, mp 255—257°C.

Found: C, 48.71; H, 6.24; N, 6.19%. Calcd for $C_{18}H_{26}N_2O_{11}$: C, 48.43; H, 5.87; N, 6.27%.

Penta-*O*-acetyl-*N*-carbamyl-*N'*-methyl-*N'*-nitroso-*scyllo*-inosamine (VI). To a suspension of 1.00 g of V in 10 ml of pyridine, 4.0 ml of 1.2 M nitrosyl chloride in acetic anhydride was added drop by drop at 13°C under occasional shaking for 20 min. Then the reaction mixture was poured into 20 ml of ice and water. The yellowish crystals which precipitated in the aqueous solution were collected by filtration and washed with water to yield 975 mg (91.5%) of a crude product.

Recrystallization from acetone gave yellow cubic crystals melting at 192—196°C. IR: 1750 (OAc), 1530 (NH) and 1480 cm^{-1} (N–NO).

Found: C, 45.75; H, 5.53; N, 8.81%. Calcd for $C_{18}H_{25}N_3O_{12}$: C, 45.47; H, 5.30; N, 8.84%.

A presence of nitroso group was supported by a positive Liebermann nitroso test.

***N*-Carbamyl-*scyllo*-inosamine (VII).** A 300 mg portion of VI was added to 30 ml of methanol previously saturated with dry ammonia under ice cooling, and the mixture was settled in a refrigerator overnight. Colorless crystals which appeared in the mixture were collected by filtration and washed with methanol to give 99 mg of the product, mp 243—245°C, 70.7% yield. Recrystallization from aqueous ethanol did not raise its melting point.

Found: C, 37.59; H, 6.63; N, 12.67%. Calcd for $C_7H_{14}N_2O_6$: C, 37.84; H, 6.35; N, 12.61%.

***N*-Carbamyl-*N'*-methyl-*scyllo*-inosamine (VIII).** (a) A 294 mg portion of V was added to 40 ml of ethanol previously saturated with dry ammonia and the mixture was settled in a refrigerator overnight. Crystals which appeared in the mixture were collected by filtration and washed with ethanol to give 99 mg (64.0%) of the product, mp 225—226°C. Recrystallization from aqueous ethanol did not raise its melting point.

Found: C, 40.56; H, 6.98; N, 11.54%. Calcd for $C_8H_{16}N_2O_6$: C, 40.67; H, 6.83; N, 11.86%.

(b) To a solution of 390 mg of I in 10 ml of water, a mixture of 100 mg of methyl isocyanate, 20 ml of acetonitrile and 295 mg of silver carbonate was added. The reaction mixture was heated under reflux on a steam bath for 50 min. After the reaction mixture was cooled, a precipitate was removed by filtration. The filtrate was evaporated under reduced pressure to give a crystalline residue. The residue was recrystallized from aqueous ethanol to give 271 mg (63.8%) of colorless crystals, mp 225—226°C. The product was identified with the product obtained by the method (a), by a mixed melting point determination and a comparison of IR spectra.

***N*-Carbamyl-*N'*-methyl-*N'*-nitroso-*scyllo*-inosamine (IX).** To a solution of 300 mg of VIII in a mixture of 3.8 ml of glacial acetic acid and 5.0 ml of water, 17.5 ml of 0.1 M sodium nitrite solution was added under ice cooling. The reaction mixture was stored in a refrigerator overnight. Crystals (57 mg) appeared in the solution were collected by filtration and washed with acetone to give the product, mp 122—125°C. The filtrate was treated with Amberlite IR-120 to remove a sodium ion and then freeze-dried. The amorphous powder obtained by freeze-drying was recrystallized from aqueous acetone to give 185 mg of the product, mp 123—126°C. The total yield was 72.2%. IR: 1700 (C=O), 1550 (NH) and 1470 cm^{-1} (N–NO). Liebermann nitroso test was positive. NMR (D_2O): τ 6.77 ($N-CH_3$).

Found: C, 34.60; H, 6.03; N, 14.65%. Calcd for $C_8H_{15}N_3O_7 \cdot H_2O$: C, 33.90; H, 6.05; N, 14.84%.

Acetylation of IX (42 mg) with acetic anhydride (1.0 ml) in pyridine (2.0 ml) afforded 53 mg (70.7%) of a product, which was identified to be VI by a comparison of IR spectra.

***N*-Carbamyl-*N'*-methyl-*N'*-nitroso-*epi*-inosamine-2 (XI).** To a solution of 600 mg of *epi*-inosamine-2 hydrochloride (X) in 12 ml of water, a mixture of 200 mg of methyl isocyanate, 783 mg of silver carbonate and 24 ml of acetonitrile was added and the mixture was heated under reflux for 1 hr. The warm mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in a small amount of water and the solution was filtered. The filtrate was again evaporated *in vacuo* to give 720 mg of an oily residue.

The residue was dissolved in a mixture of 1 ml of water and 3 ml of glacial acetic acid. To the solution, 6.0 ml of 0.5 M sodium nitrite solution was added under ice cooling, and the mixture was settled at room temperature overnight. Then the reaction mixture was passed through a column of Amberlite IR-120 to remove a sodium ion, and the aqueous solution was freeze-dried. The crude product was recrystallized from methanol to give 592 mg (81.2%) of nice crystals melting at 104°C (dec.). IR: 1720 (C=O), 1540 (NH) and 1475 cm^{-1} (N–NO).

Found: C, 36.52; H, 6.71; N, 14.50%. Calcd for $C_8H_{15}N_3O_7 \cdot CH_3OH$: C, 36.36; H, 6.40; N, 14.14%.

Penta-*O*-acetyl-*N*-carbamyl-*N'*-methyl-*N'*-nitroso-*epi*-inosamine-2 (XII). Acetylation of XI

(180 mg) with 2 m*l* of acetic anhydride and 2 m*l* of pyridine afforded an oily product. The crude product was crystallized from ethanol to give 265 mg (82.1%) of pale yellow crystals, mp 114—116°C. IR: 1755 (OAc), 1530 (NH) and 1490 cm^{-1} (N–NO).

Found: C, 45.59; H, 5.58; N, 8.71%. Calcd for $C_{18}H_{25}N_3O_{12}$: C, 45.47; H, 5.30; N, 8.84%.

Di-*N*,*N*′-(*N*-methyl-carbamyl)-*myo*-inosadiamine-1,3 (XIV). A mixture of 1.32 g of *myo*-inosadiamine-1,3 dihydrochloride (XIII) and 600 mg of methyl isocyanate in 60 m*l* of 65% aqueous acetonitrile was heated under reflux in the presence of 2.0 g of silver carbonate for 70 min. The warm reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give an oily residue. An insoluble material which was obtained by the filtration was extracted with hot water and from the aqueous extract, 676 mg of the product crystallized out as cubic crystals, mp 230—231°C.

The oily residue was dissolved in a small amount of water and ethanol was added to the solution to give a second crop of the product (222 mg), mp 229—231°C. The total yield was 58.9%.

Recrystallization from aqueous ethanol gave fine crystals, mp 230—231°C.

Found: C, 37.34; H, 7.60; N, 16.90%. Calcd for $C_{10}H_{20}N_4O_6 \cdot 2H_2O$: C, 36.58; H, 7.37; N, 17.07%.

Di-*N*,*N*′-(*N*-methyl-*N*-nitroso-carbamyl)-*myo*-inosadiamine-1,3 (XV). A 202 mg portion of XIV was dissolved in a mixture of 2 m*l* of acetic acid and 15 m*l* of water, and 16.4 m*l* of 0.1 M sodium nitrite solution was added to the solution under ice cooling with an occasional shaking. The mixture was mechanically agitated overnight under ice cooling. Then the clear solution was freeze-dried to give 184 mg (76.4%) of the crude product. Recrystallization from ethanol afforded crystals, mp 158—162°C. IR: 1700 (C=O), 1530 (NH) and 1475 cm^{-1} (N–NO). NMR (D_2O): τ 6.78 (N–CH_3).

Found: C, 34.14; H, 5.71; N, 24.32%. Calcd for $C_{10}H_{18}N_6O_8$: C, 34.28; H, 5.18; N, 23.99%.

Antitumor Activity. A detail of the antitumor activities against Ehrlich ascites tumor and HeLa carcinoma will be published elsewhere.

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