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SYNTHESIS OF AMINO DERIVATIVES OF CYCLOHEPTAAMYLOSE HAVING STRONG ANTIMICROBIAL ACTIVITIES

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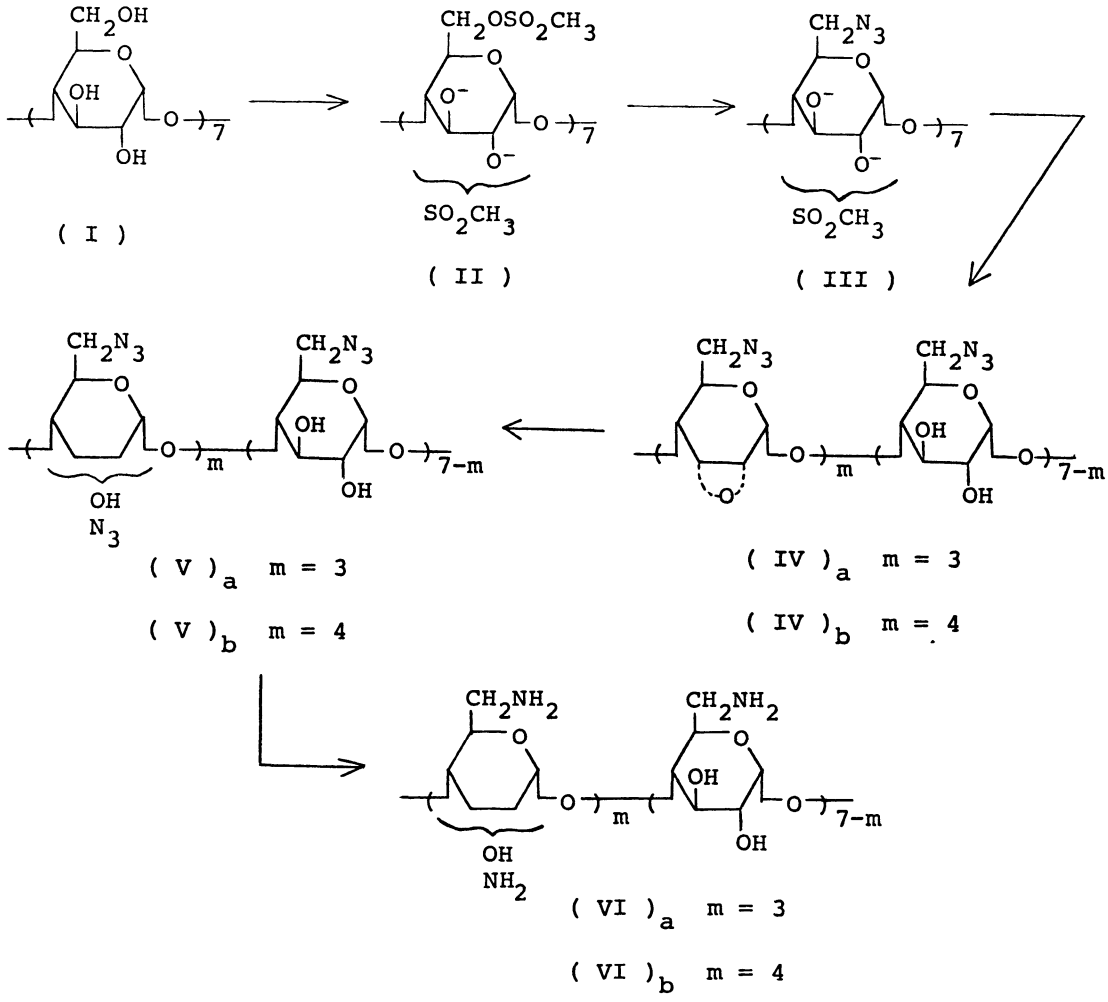
In a study of the relationship between a number of amino groups in cycloheptaamylose and their antimicrobial activity, the polyamino derivatives of cycloheptaamylose, VI_a and VI_b possessing ten and eleven amino groups respectively were synthesized. VI_a and VI_b showed strong antimicrobial activities against such gram negative bacteria as Escherichia, Shigella and Pseudomonas species.

A large number of studies on chemical modifications of aminoglycoside antibiotics have been reported.[1] There have also been the reports on syntheses of amino-deoxy derivatives from natural oligosaccharides with α-anomeric configuration, in order to study a relationship between structures and biochemical characteristics of aminoglycosides.[2]

However, attempts to induce oligosaccharides to have strong antimicrobial activities by means of a replacement of hydroxyl groups by amino groups have been unsuccessful. Recently we reported[3] that amino derivatives of cycloheptaamylose possessing six or seven amino groups at C-6 position of each glucose residue showed significant antimicrobial activities against such gram negative bacteria as Escherichia, Shigella and Pseudomonas species. It became of great interest for us to examine the relationship between a number of amino groups in cycloheptaamylose and their antimicrobial activity. In this communication we report the synthesis of cycloheptaamylose derivatives possessing ten or eleven amino groups and their antimicrobial activity.



Cycloheptaamylose (I) was allowed to react with methanesulfonyl chloride (2.15 eq mole per glucose residue) in pyridine to give tetradeca(O-methanesulfonyl)-cycloheptaamylose (II) in a 98% yield as a white powder which gave only one spot in its TLC, mp 189-190° (decom.). IR(Nujol): 3350, 1350, 1170, 1050 cm^{-1}. Found: C, 29.78; H, 4.41; S, 19.92. Calcd for $C_{56}H_{98}O_{63}S_{14}$: C, 30.19; H, 4.41; S, 20.11. II was heated with sodium azide in DMF at 85° for 7 h to give hepta(2 or 3-O-methanesulfonyl)-hepta(6-azido-6-deoxy)cycloheptaamylose (III) in a 96% yield as a white powder, mp 193-195° (decom.). IR(Nujol): 3350, 2100, 1350, 1170, 1040 cm^{-1}. Found: C, 31.42; H, 4.35; N, 15.35; S, 11.87. Calcd for $C_{49}H_{77}O_{42}N_{21}S_7$: C, 31.70; H, 4.15; N, 15.85; S, 12.07.

Treatment of III with sodium methoxide in methanol or methanol-chloroform, generally employed for epoxidation of carbohydrates,[4] did not make the progress of the reaction sufficiently. However, the formation of the oxirane ring occurred when DMF was used as cosolvent. Thus, III was treated with sodium ethoxide (1.3 eq mole per glucose residue) in DMF-ethanol solution at room temperature for

3 days. The reaction mixture was poured into ice-water and the separated pale yellow precipitate was filtered to give a mixture of many kinds of epoxides. From this mixture cyclo[tri(2,3-anhydro-6-azido-6-deoxy-O-α-pyranosyl(1→4))·tetra-(6-azido-6-deoxy-O-α-D-glucopyranosyl(1→4))] (IV_a) (yield, 18%) and cyclo[tetra-(2,3-anhydro-6-azido-6-deoxy-O-α-pyranosyl(1→4))·tri(6-azido-6-O-α-D-glucopyranosyl(1→4))] (IV_b) (yield, 23%) were isolated as main products by silica gel column chromatography. VI_a was a white powder having mp 152-163° (decom.). IR(Nujol) 3400, 2100, 1290, 1250(weak), 1060, 920, 810 cm^{-1}. The bands at 1350, 1170 cm^{-1} due to the methanesulfonyloxyl group disappeared completely. Found: C, 40.63; H, 4.64; N, 23.57. Calcd for $C_{42}H_{57}O_{25}N_{21}$: C, 40.20; H, 4.54; N, 23.43. R_f value was 0.26 (Silica gel plate; Solvent: ethyl acetate-chloroform = 6 : 1). IV_b was a white powder having mp 145-153° (decom.). IR(Nujol): 3350, 2100, 1290, 1250, 1060, 920, 815 cm^{-1} (no absorption bands at 1350, 1170 cm^{-1}). Found: C, 41.08; H, 4.60; N, 23.60. Calcd for $C_{42}H_{55}O_{24}N_{21}$: C, 40.80; H, 4.45; N, 23.75. R_f value was 0.37 (Silica gel plate; Solvent: ethyl acetate-chloroform = 6 : 1).

IV_a was heated with sodium azide and ammonium chloride in DMF at 90-95°C for 10 hours and the reaction mixture was poured into water. The resultant precipitate was purified by silica gel column chromatography to give cyclo[tri-(2 or 3-azido-2 or 3-deoxy-6-azido-6-deoxy-O-α-pyranosyl(1→4))·tetra(6-azido-6-deoxy-O-α-D-glucopyranosyl(1→4))] (V_a) in a 65% yield as a white powder having mp 136-140° (decom.). IR(Nujol): 3400, 2100, 1290, 1040 cm^{-1}. Found: C, 37.08; H, 4.42; N, 30.78. Calcd for $C_{42}H_{60}O_{25}N_{30}$: C, 36.42; H, 4.34; N, 30.35. Cyclo[tetra(2 or 3-azido-2 or 3-deoxy-6-azido-6-deoxy-O-α-pyranosyl(1→4))·tri(6-azido-6-deoxy-O-α-D-glucopyranosyl(1→4)) (V_b) was obtained from IV_b in a similar manner as described above. Yield 62%, a white powder mp 125-133° (decom.). IR(nujol): 3400, 2100, 1290, 1040 cm^{-1}. Found: C, 36.18; H, 42.0; N, 32.07. Calcd for $C_{42}H_{59}O_{24}N_{33}$: C, 35.77; H, 4.19; N, 32.79.

Catalytic hydrogenation of V_a with platinum dioxide in hydrochloric acid-methanol solution at room temperature for 4 days under 3 atmospheric pressure of hydrogen gas gave the hydrochloride of cyclo[tri(2 or 3-amino-2 or 3-deoxy-6-amino-6-deoxy-O-α-pyranosyl(1→4))·tetra(6-amino-6-deoxy-O-α-D-glucopyranosyl-(1→4))] (VI_a) in a 90% yield as a white amorphous powder having mp 204-208° (decom.). IR(Nujol): 3300(broad), 1950, 1600, 1500, 1380, 1030 cm^{-1}. $[\alpha]_D^{24}$+79.8° (water). Found: C, 34.40; H, 5.96; N, 9.08; Cl, 24.68. Calcd for

$C_{42}H_{80}O_{25}N_{10}\cdot 10HCl$: C, 33.85; H, 6.04; N, 9.40; Cl, 23.84. R_f value was 0.59 (Toyo's filter paper No. 51. Solvent: butanol-acetic acid-pyridine-water = 1 : 2 : 5 : 5). The hydrochloride of cyclo[tetra(2 or 3-amino-2 or 3-deoxy-6-amino-6-deoxy-O-α-pyranosyl(1→4))·tri(6-amino-6-deoxy-O-α-D-glucopyranosyl(1→4))] (VI_b) was obtained similarly from V_b in a 87% yield as a white amorphous powder having mp 203-207° (decom.). IR(Nujol): 3300(broad), 1950, 1600, 1500, 1380, 1030 cm^{-1}. $[\alpha]_D^{22}$+69.3°(water). Found: C, 32.16; H, 5.98; N, 10.65; Cl, 26.29. Calcd for $C_{42}H_{81}O_{24}N_{11}\cdot 11HCl$: C, 33.06; H, 6.03; N, 10.10; Cl, 25.61. Rf value was 0.53 (the same solvent system as described above).

The hydrochlorides of the amino compounds VI_a and VI_b thus synthesized showed strong antimicrobial activity, as tested by the two-fold dilution method in a heart-infusion agar medium. They inhibited the growth of Staphylococcus aureus at concentrations of 6.25-100 μg/ml, of Escherichia coli at 1.56-6.25 μg/ml, of Shigella flexneri and S. sonnei at 0.39-6.25 μg/ml, and of Pseudomonas aeruginosa at 1.56-3.12 μg/ml. It was found that when more amino groups were introduced into cycloheptaamylose, stronger antimicrobial activity resulted.

Further work on precise structural elucidation of VI_a and VI_b and syntheses of many kinds of amino derivatives are now in progress.

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