PREPARATION OF 6-AMINO-6-DEOXY--2,3,4,5-TETRA-O-METHYL-D-GLUCONIC ACID AND ITS DERIVATIVES

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6-Amino-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconic acid (*I*) was prepared by catalytic reduction of 6-azido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconic acid (*XVI*) obtained by alkaline hydrolysis of the corresponding dimethylamide *VIII* or a mixture of dimethylamide *VIII*, amide *XIV* and methylamide *XV*. Amides *VIII*, *XIV* and *XV* are the products of alkylation of 6-azido--6-deoxy-D-gluconamide (*VII*) or 6-azido-6-deoxy-3,5-di-O-methyl-D-gluconamide (*XIII*), carried out with methyl iodide and silver oxide in N,N-dimethylformamide. Amides *VII* and *XIII* were prepared by amonolysis of 6-azido-6-deoxy-D-glucono-1,5-lactone (*III*) or 6-azido-6-deoxy--3,5-di-O-methyl-D-glucono-1,4-lactone (*XII*). Methylation of 6-amino-6-deoxy-D-gluconolactam (*II*) with methyl iodide and silver oxide in N,N-dimethylformamide afforded 6-deoxy-2,3,4,5--tetra-O-methyl-6-methylamino-D-gluconolactam (*V*) from which 6-deoxy-2,3,4,5-tetra-O-methyl--6-methylamino-D-gluconic acid (*VI*) was prepared.

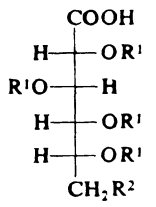
In the continuation of the study of biologically active substances of the general structure of ω-aminoaldonic acids[1] we concentrated in this paper on their permethyl ethers. In view of the material obtained in the preceding study[1] we selected 6-amino--6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconic acid (*I*) as the first representative of these compounds.

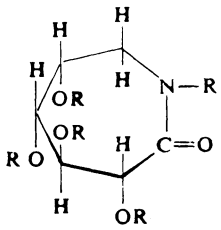
For the preparation of the mentioned compound 6-amino-6-deoxy-D-gluconolactam (*II*) seemed a suitable intermediate which can be obtained from D-glucose by synthesis consisting of several steps, either *via* 6-azido-6-deoxy-D-glucono-1,5-lactone (*III*) (ref.[1-3]) or *via* 6-amino-6-deoxy-D-gluconic acid (*IV*) as described in this paper. In compound *II* the carboxylic and the amino group are mutually protected by the lactam bond and all hydroxyl groups are free for methylation. The assumption of the protection of the NH group against methylation, when the methylation reagent acted on lactam *II*, follows from the analogy of the —$NHCOCH_2$-grouping with the —$NHCOCH_3$ group in amino sugars the methylation of which on free hydroxyl groups only has been described many times[4,5]. On the other hand, the papers[6-8], describing the methylation of the —NH-group in the molecule of ω-caproactam should also be taken into consideration. In our experiments the assumption

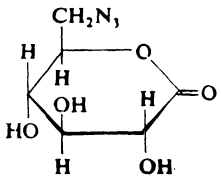
of the protecting effect of the lactam bond was not confirmed and 6-deoxy-2,3,4,5-tetra-O-methyl-6-methylamino-D-gluconolactam (*V*) was the product in the reaction of lactam *II* with methyl iodide and silver oxide in N,N-dimethylformamide. The substitution on nitrogen in the molecule of lactam *V* is also confirmed in addition to elemental analysis by the IR spectrum, containing a characteristic band of the tertiary amide at $\tilde{\nu}$ 1 660 cm^{-1} and the absence of absorption in the region of stretching vibrations of the NH bonds, and by the ^1H NMR spectrum containing a signal of N—CH_3 protons (δ 3·03 ppm) in addition to four distinctly different signals of the protons in OCH_3, in the δ 3·43 – 3·53 ppm interval. On acid hydrolysis lactam *V* afforded 6-deoxy-2,3,4,5-tetra-O-methyl-6-methylamino-D-gluconic acid (*VI*). Hence for the preparation of amino acid *I*, unsubstituted on nitrogen, the amide of 6-azido-6-deoxy-D-gluconic acid (*VII*) was tested which is a product of amonolysis of lactone *III*. The reaction of amide *VII* with methyl iodide and sodium hydride in acetonitrile or with methyl iodide and silver oxide in N,N-dimethylformamide gave a syrupy dimethylamide of 6-azido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconic acid (*VIII*). Its IR spectrum, with a single band of the C=O group at $\tilde{\nu}$ 1 640 cm^{-1} and the absence of peaks in the region of O—H and N—H absorptions and ^1H NMR spectrum with 2 singlets for $N(CH_3)_2$ and four singlets of the OCH_3 groups indicated a complete methylation. Reduction and acetylation of dimethylamide *VIII* gave 6-acetamido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconodimethylamide (*IX*) the structure of which was confirmed by IR and ^1H NMR spectra. For the preparation of compound *VIII* the procedure was found more suitable, where 6-azido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (*X*) serves as starting material. The free hydroxyl groups in compound *X* were etherified with methyl iodide and sodium hydride in acetonitrile. The 6-azido-6-deoxy-3,5-di-O-methyl-α-D-glucofuranose (*XI*) obtained was submitted to acid hydrolysis and oxidation to afford 6-azido-6-deoxy-3,5-di-O-methyl-D-glucono-1,4-lactone (*XII*), which was amonolysed to 6-azido-6-deoxy-3,5-di-O-methyl-D-gluconamide (*XIII*). Methylation of amide *XIII* with an excess of methyl iodide and sodium hydride in acetonitrile or with methyl iodide and silver oxide in N,N-dimethylformamide led to dimethylamide *VIII*, while methylation with a small excess of the reagents, monitored by thin-layer chromatography on silica gel, afforded a product which was found to be a mixture of 6-azido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconamide (*XIV*), 6-azido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconomethylamide (*XV*) and dimethylamide *VIII* on the basis of thin-layer chromatography, elemental analyses and IR data.

Hydrolysis of amides *VIII*, *XIV* and *XV* to 6-azido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconic acid (*XVI*) turned out to be the most difficult step of the whole synthesis. An attempt at nitrosation of amides *XIV* and *XV* and subsequent liberation of acid *XVI* by decomposition of N-nitroso derivatives[9-11] was unsuccessful; under the conditions used nitrosation of the amide nitrogen did not take place. Acid hydrolysis of amides in 2·5M-H_2SO_4 took place very slowly even at about 100°C. Under these

conditions a considerable decomposition took place even under nitrogen, so that the product, isolated in low yield, was not pure. Alkaline hydrolysis of dimethylamide *VIII* in 1M-NaOH required about 7 h of heating at 100–110°C. It was more convenient to work with a mixture of amides *VIII*, *XIV* and *XV* and to terminate the hydrolysis, controlled by chromatography, after 5 h. The rest of the unreacted dimethylamide *VIII* was extracted from the alkaline reaction mixture with chloroform. Acid *XVI* was then extracted from the acidified and concentrated aqueous phase again with chloroform. With this method of hydrolysis the amount of azido acid *XVI* amounted to 50% of the weight of the reacted amides. Azido acid *XVI* was not isolated but reduced immediately with hydrogen gas on palladium catalyst. Amino acid *I* was isolated in the form of a crystalline hydrochloride.





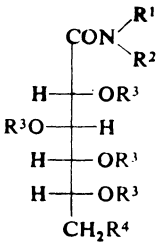


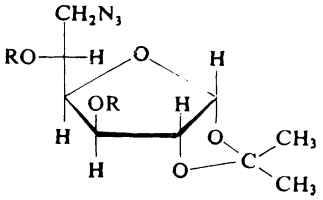
I, $R^1 = CH_3$, $R^2 = NH_2$
IV, $R^1 = H$, $R^2 = NH_2$
VI, $R^1 = CH_3$, $R^2 = NHCH_3$
XVI, $R^1 = CH_3$, $R^2 = N_3$

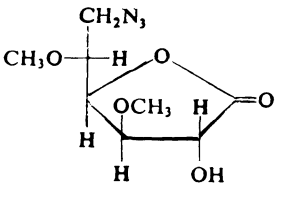
II, R = H
V, $R = CH_3$

III

In view of the difficult hydrolysis of amide *VIII*, *XIV* and *XV* the methylation of azido lactones *III* and *XII* or the salts of corresponding acids with dimethyl sulfate in aqueous sodium hydroxide was also tried. Only after fourfold methylation of lactone *III* traces of the fully substituted compound appeared in the reaction mixture,







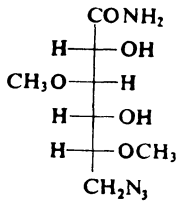
VII, $R^1 = R^2 = R^3 = H$, $R^4 = N_3$
VIII, $R^1 = R^2 = R^3 = CH_3$, $R^4 = N_3$
IX, $R^1 = R^2 = R^3 = CH_3$, $R^4 = NHCOCH_3$
XIV, $R^1 = R^2 = H$, $R^3 = CH_3$, $R^4 = N_3$
XV, $R^1 = H$, $R^2 = R^3 = CH_3$, $R^4 = N_3$

X, R = H
XI, $R = CH_3$

XII

but the major part of the mixture consisted of lower substituted derivatives. Owing to the difficult separation and isolation of the methylation products from the ballast inorganic salts, the attempts with dimethyl sulfate were not carried out up to a complete etherification of the starting substances.

Among the syntheses of amino acid *I* tested the most successful variant was expressed by the sequence $X \rightarrow XI \rightarrow XII \rightarrow XIII \rightarrow (VIII, XIV, XV) \rightarrow XVI \rightarrow I$, which afforded the required product in approximately a 14% yield.



XIII

EXPERIMENTAL

The melting points were measured on a Kofler block and they are not corrected. Optical rotations were measured on an Opton instrument at 20°C and 0·5—1·5 g/100 ml concentration. The IR spectra were recorded on a Perkin–Elmer 325 instrument in KBr pellets (solid substances) or in chloroform. The ^{1}H NMR spectra were measured on a Varian XL 100—15 instrument in deuteriochloroform with tetramethylsilane as internal reference. The chemical shifts are given in δ-scale (ppm), the coupling constants in Hz. Samples for elemental analysis were dried at 30 to 35°C and 10 Pa. Thin-layer chromatography used for the monitoring of the reaction course was carried out on silica gel G plates (Merck, GFR) (25 × 75 mm) with a layer thickness of 0·2 to 0·3 mm, using the following elution solvents: chloroform–methanol 100 : 1 (S_1), chloroform–methanol 100 : 5 (S_2), chloroform–methanol 100 : 10 (S_3), chloroform–methanol 100 : 20 (S_4), benzene–ethyl acetate 1 : 1 (S_5), acetone–methanol–water 16 : 4 : 1 (S_6). Detection was carried out by spraying the plates with a 1% cerium-IV sulfate solution in 10% sulfuric acid and heating. For the chromatography of amino acids commercial foils with a cellulose layer (Lucefol-Quick), (a product of Kavalier, Votice, Czechoslovakia) were used. For development the system n-butanol–acetic acid–water 4 : 1 : 5 (S_7) was used and for detection a bath (0·25% ninhydrin in acetone) and heating were used. Column chromatography was carried out on silica gel CH, particle size 100—160 μm (Lachema, Czechoslovakia).

6-Amino-6-deoxy-gluconolactam (*II*)

A solution of 11 mmol of hydrogen chloride in 5 ml of methanol was added to a stirred suspension of 388 mg (2 mmol) of amino acid *IV*, ref.[1], in 25 ml of methanol at room temperature. After dissolution of the reaction component the solution was refluxed for 5 h. After evaporation and drying the residue weighed 513 mg (99%). The product, hydrochloride of methyl 6-amino-6-deoxy-D-gluconate, was dissolved in 25 ml of methanol and neutralized with 1·84 ml of 1·09M-sodium methoxide in methanol. The separated lactam *II* (319 mg, 92%), m.p. 214°C (lit.[3] gives 212—214°C) had an IR spectrum identical with a preparation described earlier[1].

6-Deoxy-2,3,4,5-tetra-O-methyl-6-methylamino-D-gluconalactam (*V*)

A suspension of 306 mg (1·7 mmol) of lactam *II*, 15 g (65 mmol) of silver oxide in 42 ml (96 g) of methyl iodide in 50 ml of N,N-dimethylformamide was shaken at room temperature and the reaction course followed by TLC in system S_1. After 6 h the reaction mixture pattern on thin layer was constant, the main spot between two trace spots had R_F 0·47. After a conventional working up of the reaction mixture 403 mg of a syrup were obtained which was chromatographed on a silica gel column (30 g) with S_1 to give a chromatographically pure product. Crystallization from a mixture of diethyl ether–light petroleum afforded 260 mg (65%) of lactam *V*, m.p. 69 to 70°C, $[\alpha]_D^{20}$ ~0°, $[\alpha]_{365}^{20}$ −22·2° (chloroform). IR spectrum: 2 820, 1 665 cm^{-1}. ^1H NMR spectrum: 3·4–4·07 (6 H, m); 3·03 (3 H, s, N—CH_3); 3·42, 3·46, 3·48, 3·52 (4 × 3 H, 4 s, O—CH_3). For $C_{11}H_{21}NO_5$ (247·3) calculated: 53·42% C, 8·56% H, 5·67% N; found: 53·42% C, 8·76% H, 5·38% N.

6-Deoxy-2,3,4,5-tetra-O-methyl-6-methylamino-D-gluconic Acid (*VI*)

A solution of 193 mg (0·78 mmol) of lactam *V* in 10 ml of 1M-HCl was stirred at 110°C for 25 h. Chromatography on thin layer in system S_1 was used to indicate the disappearance of the starting substance. After 60 h standing at room temperature the solution was evaporated, the residue dissolved in water was filtered through a column of Dowex 2 × 8 (CH_3COO^- form). The dry residue of the eluate was crystallized from a mixture of ethanol and ether, m.p. 176°C (a partial melting and recrystallization) and then 192–194°C, $[\alpha]_D^{20}$ +72° ± 1° (water). On a thin layer of cellulose acid *VI* has in system S_7 R_F 0·58. For $C_{11}H_{23}NO_6$ (265·3) calculated: 49·80% C, 8·74% H; found: 49·77% C, 8·66% H.

6-Azido-6-deoxy-D-gluconamide (*VII*)

A solution of 1·92 g (8·7 mmol) of 6-azido-6-deoxy-D-glucono-1,5-lactone (*III*), ref.[1], in 40 ml of a mixture of 2-propanol and ethanol 1 : 1, was cooled to −25°C and then saturated with dry ammonia. After 2 h standing at room temperature thin-layer chromatography in S_4 could no longer detect the presence of the starting lactone. The residue after evaporation of the solvents was recrystallized twice from 15 ml of ethanol. The obtained amide *VII* (1·29 g, 63%) had m.p. 122·5–123·5°C, $[\alpha]_D^{20}$ +30·2 ± 1° (water). For $C_6H_{12}N_4O_5$ (220·2) calculated: 32·73% C, 5·49% H, 25·45% N; found: 32·75% C, 5·77% H, 25·59% N.

6-Azido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconodimethylamide (*VIII*)

A) Methyl iodide (10 ml) and sodium hydride (1·5 g, in portions) were added to a solution of 2·5 g (11·4 mmol) of amide *VII* dissolved in 40 ml of acetonitrile at about 20°C and the reaction mixture was analyzed by TLC in the systems S_1 and S_5 at this temperature. Over 40 h further amounts of the methylation reagent and the solvent were added in small portions (a total of 10 ml of methyl iodide and 4 g of sodium hydride in about 30 ml of acetonitrile). After decomposition of the mixture with 20 ml of ethanol the volatile substances were evaporated, the residue partitioned between chloroform and water and the chloroform extract washed with an aqueous solution of sodium sulfate and dried over magnesium sulfate. The residue of the filtered chloroform solution was purified by column chromatography on 40 g of silica gel (elution with 500 ml of solvent mixture S_3). The oily residue obtained (2·81 g) was distilled to give 2·52 g (73%) of chromatographically pure compound *VIII*, b.p. 124°C/50 Pa (bath temperature 160–170°C), $[\alpha]_D^{20}$ +21·5° ± 1° (chloroform). IR spectrum: 3 005, 2 950, 2 820, 2 110, 1 645 cm^{-1}. ^1H NMR spectrum: 4·22 (1 H, d, $J_{2,3}$ 6,3,H-2); 3·25–3·70 (17 H, m), from this multiplet the following

peaks protrude: 3·50, 3·42, 3·40, 3·37 (4 s, 4 × OCH_3); 3·15, 2·96 (2 × 3 H, 2 s, $N(CH_3)_2$). For $C_{12}H_{24}N_4O_5$ (304·3) calculated: 47·36% C, 7·95% H, 18·41% N; found: 47·26% C, 7·84% H, 18·19% N.

B) A mixture of 1·56 g (7·1 mmol) of amide *VII*, 15 g of silver oxide, 30 ml of methyl iodide and 30 ml of N,N-dimethylformamide was shaken at room temperature for 12 h. The methylation course was checked simultaneously by thin-layer chromatography (in systems S_2 and S_5). Then the substance with R_F 0·8 was the main component in the reaction mixture, accompanied by an admixture with a higher R_F value. After working up the syrupy product was purified by chromatography on 80 g of silica gel (elution with benzene–ethyl acetate with an increasing proportion of the more polar component, from 10 to 100%). Yield, 1·012 g (47%) of dimethylamide *VIII* the physical constants of which were identical with the preparation prepared under *A*.

C) Methyl iodide (10 ml) and four 0·5 g portions of sodium hydride were added to a solution of 2·9 g (11·7 mmol) of amide *XIII* in 40 ml of acetonitrile over 10 h under stirring. After working up and purification of the product according to the procedure described under *A* 3·1 g (87%) of dimethylamide *VIII* were obtained, identical according to its physical data with the product from procedure *A*.

6-Acetamido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconodimethylamide (*IX*)

A solution of 1·96 g (6·45 mmol) of dimethylamide *VIII* in 50 ml of ethanol was stirred with 0·5 g of 5% Pd/C under hydrogen atmosphere and the process was monitored by thin-layer chromatography in system S_3 (R_F of the starting compound 0·68, R_F of product 0·06). After 75 min the catalyst was filtered off and 2 ml of acetic anhydride were added to the filtrate. According to thin-layer chromatography (in S_2) a single substance was present in the product, with R_F 0·42. Evaporation gave 2·4 g of crude dimethylamide which was purified by rapid chromatography on a column of silica gel (90 g) with chloroform–methanol 100 : 2 as eluent. The chromatographically pure product had the following IR spectrum: 3 450, 3 010, 2 940, 2 820, 1 670, 1 645, 1 510 cm^{-1}. ^1H NMR spectrum: 6·06 (1 H, s, NH—); 4·30 (1 H, d, $J_{2,3}$ 6·5 Hz, H-2); 3·25—3·80 (17 H, m), from this multiplet the following peaks protrude: 3·51, 3·48, 3·30, 3·28 (4 s, 4 × OCH_3); 3·0, 3·18 (2 × 3 H, 2 s, $N(CH_3)_2$; 2·02 (3 H, s, $COCH_3$). $[\alpha]_D^{20}$ +16·3 (chloroform). For $C_{14}H_{28}N_2O_6$ (320·4) calculated: 52·48% C, 8·81% H, 8·74% N; found: 52·31% C, 8·87% H, 8·56% N.

6-Azido-6-deoxy-3,4-di-O-methyl-1,2-O-isopropylidene-α-D-glucofuranose (*XI*)

A) A solution of 1 g of isopropylidene derivative *X* (ref.[1]) in 10 ml acetonitrile was stirred with 5 ml of methyl iodide and 0·5 g of sodium hydride. According to thin-layer chromatography in S_1 the reaction was over after 2 h. The mixture was decomposed with 10 ml of ethanol, evaporated and the residue partitioned between water and chloroform. The residue of the chloroform layer (1·11 g) was distilled at 145°C bath temperature, b.p. 108°C/40 Pa. The oily dimethyl ether *XI* had $[\alpha]_D^{20}$ −58·4 ± 1° (chloroform). In a patent (ref.[12]) the value $[\alpha]_D^{20}$ −57° ± 1° (chloroform) is given for compound *XI*. For $C_{11}H_{19}N_3O_5$ (273·3) calculated: 48·34% C, 7·00% H, 15·38% N; found: 48·11% C, 6·97% H, 15·29% N.

B) A solution of 1 g (4·7 mmol) of isopropylidene derivative *X* in 10 ml of N,N-dimethylformamide was shaken with 5 ml of methyl iodide and 5 g of silver oxide at room temperature and the reaction course was followed by thin-layer chromatography in S_1. After 3 h the reaction mixture was worked up in the conventional manner. On distillation the crude product gave 1·15 g of oily dimethyl ether *XI*, identical in its physical constants with the preparation obtained by procedure *A*.

6-Azido-6-deoxy-3,5-di-O-methyl-D-gluconamide (*XIII*)

A solution of 9·3 g (34 mmol) of dimethyl ether *XI* in 50 ml of 1M-HCl was stirred at 80°C and the course of hydrolysis was checked by thin-layer chromatography in S_2. After 1 h the reaction mixture was cooled and neutralized on a column of Dowex-3 in OH^- form (180 ml). The residue of the aqueous eluate (7·5 g) was dissolved in 150 ml of water and stirred at room temperature with 20 g of solid barium carbonate and 2 ml of bromine. The oxidation course was checked by thin-layer chromatography in S_2. After 8 h the oxidation was over and the mixture was demineralized and evaporated. The syrupy lactone *XII* (7·5 g) was converted to amide *XIII* in ammonia saturated ethanol. Amide *XIII* was purified by chromatography on a column of silica gel (200 g) using S_3 as eluent. Yield 73%. After crystallization from a mixture of chloroform and diethyl ether m.p. 91–92°C and $[\alpha]_D^{20}$ +27·5 ± 1° (water). For $C_8H_{16}N_4O_5$ (248·2) calculated: 38·70% C, 6·50% H, 22·57% N; found: 38·71% C, 6·55% H, 22·68% N.

Methylation of amide XIII: A mixture of 2·8 g (12 mmol) of amide *XIII*, 10 ml of methyl iodide and 0·6 g of sodium hydride in 40 ml of acetonitrile was stirred at room temperature and analysed at selected time intervals by thin-layer chromatography in S_3. Another two 0·6 g portions of sodium hydride and one of 5 ml of methyl iodide were added to the mixture over 3 h. After the conventional work-up the mixture gave 3·53 g of an oily product which was analysed on thin layer in S_3, showing that it was a mixture of 3 substances of R_F 0·68, 0·61 and 0·57 (R_F of the starting amide was 0·32). IR spectrum (chloroform): 3 440, 3 020, 3 005, 2 940, 2 910, 2 825, 2 100, 1 675, 1 640, 1 535, 1 285 cm^{-1}. Elemental analysis: found: 46·11% C, 7·75% H, 19·01% N. $[\alpha]_D^{20}$ +29·6 ± 1·5° (*c* 1·5, chloroform). The mixture of substances *XIV*, *XV* and *VIII* was used for hydrolysis to free acid without previous separation.

6-Azido-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconic Acid (*XVI*)

A) A solution of 1·5 g (4·95 mmol) of dimethylamide *VIII* in 100 ml of 1M-NaOH was heated under stirring at 100°C, bubbling nitrogen through it. The liberated dimethylamine was absorbed in a small vessel with water and indicator and it was neutralized continually by titration with 1M-HCl. In addition to this the hydrolysis course was also checked by thin-layer chromatography in S_2. After 7 h heating the pH of the solution in the absorber no longer changed and the starting compound (R_F 0·65) had already disappeared from the reaction mixture. The alkaline solution was neutralized with 2·5M-H_2SO_4, to pH 3. After evaporation the residue with the inorganic salts was extracted with acetone (5 × 50 ml). The residue of the acetone extract (1 g) was dissolved in water and decolorized with charcoal, retained on a Dowex 2 × 8 (OH^-) anion exchanger column (15 ml), and the organic acid was eluted with 15% formic acid. After evaporation of the eluate the syrupy azido acid *XVI* (660 mg, 48%) showed on thin-layer chromatogram in system S_6 an elongated spot of R_F 0·14. The substance was converted to amino acid *I* without further purification.

B. A solution of 1·42 g of a mixture of amides *XIV*, *XV* and *VIII* in 50 ml 1M-NaOH was stirred at 100–110°C. After 5 h it was cooled and then extracted with five 25 ml portions of chloroform. The residue of the chloroform phase (according to thin-layer chromatography it was dimethylamide *VIII*) weighed 289 mg. The aqueous phase was acidified with 1M-H_2SO_4 to pH 3, evaporated in a vacuum to a third of its volume, saturated with ammonium sulfate and extracted with ten 25 ml portions of chloroform. From the chloroform extract 511 mg of azido acid *XVI* were obtained. Several fractions obtained in this manner were purified by ion exchanger chromatography, as described in procedure *A*.

C) A solution of 143 mg of a mixture of amides *XIV*, *XV* and *VIII* in 4 ml of 2,5M-H_2SO_4 was heated under nitrogen at 60°C. According to thin-layer chromatography (S_3) no change

of the reaction mixture could be observed after 3 h. The temperature was increased to 100°C and the experiment continued for another 9 h. After this time the starting compound had disappeared from the reaction mixture. After decolorization with charcoal the acid solution was filtered through a column of Dowex 2X8 (CH_3COO^-) (50 ml). After evaporation of the filtrate 71 mg of a syrup were obtained. Chromatography of this material on a thin layer of silica gel in S_4 indicated that the product of hydrolysis was unhomogeneous.

D) A solution (0·5 ml) of sodium nitrite (280 mg) was added under stirring and cooling at 0°C to 1 g of the mixture of amides *XIV*, *XV* and *XIII* in 1 ml of water and 0·5 ml of hydrochloric acid, and the reaction was monitored by thin-layer chromatography in S_2. After 75 min no change in the composition of the reaction mixture could be observed. After addition of another dose of the same amount of sodium nitrite the mixture was stirred for another 90 min, then alkalized to pH 11·5 and extracted with three 25 ml portions of chloroform. The substance isolated from the chloroform extract (977 mg) gave a similar pattern on thin-layer chromatography as the starting mixture.

6-Amino-6-deoxy-2,3,4,5-tetra-O-methyl-D-gluconic Acid Hydrochloride (*I*)

A solution of 1·655 g (6 mmol) of azido acid *XVI* in 50 ml of water was stirred under renewed hydrogen atmosphere in the presence of 300 mg of 5% Pd/C, at room temperature and atmospheric pressure. The reduction course was followed by thin-layer chromatography in S_3. After 10 h hydrogenation the reaction was terminated, the catalyst filtered off and the filtrate acidified with 6·1 ml of 1M-HCl and evaporated. Yield, 1·61 g (94%) of hydrochloride of amino acid *I*. The products of two such reductions were combined (3·13 g) and crystallized from ethanol–diethyl ether 3 : 5. Yield, 1·88 g of hydrochloride of amino acid *I*, m.p. 204–205°C, which on chromatography on a thin layer of cellulose in S_7 gave a spot of R_I 0·54–0·57. On concentration of the mother liquors another fraction of pure product was obtained so that the total yield was 85% (per the starting azido acid). IR spectrum: 3 440, 3 140, 2 940, 2 840, 2 620, 2 560, 2 500, 2 450, 1 720, 1 600, 1 510, 1 465, 1 405 cm^{-1}; $[\alpha]_D^{20} + 29{\cdot}8 \pm 1°$ (*c* 1·3, water). For $C_{10}H_{22}ClNO_6$ (287·8) calculated: 41·74% C, 7·71% H, 12·32% Cl, 4·87% N; found: 41·54% C, 7·97% H, 12·67% Cl, 5·20% N.

Methylation of Lactones *III* and *XII* with Dimethyl Sulfate

Dimethyl sulfate (9 ml; 95 mmol) was added dropwise under nitrogen over 90 min to a stirred solution of lactone *III* (3·4 g, 16·8 mmol) in 100 ml of a 20% aqueous solution of sodium hydroxide and the mixture was stirred for another 4 h. It was neutralized, under cooling, with 2·5M-H_2SO_4 and diluted with 1·5 l of alcohol. After filtration off of the inorganic salts the solution was evaporated and the residue extracted with ethanol. The residue of the ethanolic extract was dissolved in 50 ml of water and a 5 ml portion was withdrawn from it. The remaining solution was combined with the same volume of a 40% NaOH solution and methylated with dimethyl sulfate in the same manner. After fourfold methylation the weight of the product, previously demineralized by ion exchange on a Dowex 2X8 (OH^-) column, elution with 15% formic acid, was 1·4 g. The 5 ml aliquots from each methylation were catalytically hydrogenated as described in the preceding section and then analysed by thin-layer chromatography on cellulose in system S_7, using 6-amino-6-deoxy-D-gluconic acid[1] and its tetramethyl ether *I* as standards.

Lactone *XII* was methylated in the same manner. In no case did a complete methylation take place and in both cases only a small change in the distribution of the partially methylated substances in the direction of the more strongly substituted derivatives was observed.

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REFERENCES

1. Kefurt K., Čapek K., Kefurtová Z., Jarý J.: This Journal *44*, 2526 (1979).
2. Hanessian S.: Chem. Ind. (London) *1966*, 2126.
3. Hanessian S.: J. Org. Chem. *34*, 675 (1969).
4. Stearns D K., Naves R. G., Jeanloz R. W.: J. Org. Chem. *26*, 901 (1961).
5. Horton D. in the book: *The Amino Sugars* (R. W. Jeanloz, Ed.), Vol. 1A, p. 76. Academic Press, New York 1969.
6. Ruzicka L.: Helv. Chim. Acta *4*, 472 (1921).
7. Procházka J.: Chem. Listy *37*, 208 (1943).
8. Benson R. E., Cairns T. L.: J. Amer. Chem. Soc. *70*, 2115 (1948).
9. Kovář J., Novák J.: in the book: *Preparativní reakce v organické chemii* (M. Hudlický, Ed)., Vol. 3, p. 270. Published by Nakladatelství ČSAV, Prague 1956.
10. Henecka H. in the book: *Methoden der Organischen Chemie* (Houben-Weyl) (E. Müller, Ed.) Vol. 8, p. 432. Thieme Verlag, Stuttgart 1952.
11. Schnell H., Nentwick J. in the book: *Methoden der Organischen Chemie* (Houben-Weyl), (E. Müller, Ed.) Vol. 11/2, p. 573. Thieme Verlag, Stuttgart 1958.
12. Japan. Kokai *79* 05909; Chem. Abstr. *91*, P 20987 (1980).

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