

LACTONES. XVI.*

REACTION OF 2,3-ANHYDRO-4,6-DIDEOXY-L-ribo-HEXONIC ACID LACTONE WITH AMMONIA AND DIMETHYLAMINE

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The reaction of the lactone of 2,3-anhydro-4,6-dideoxy-L-ribo-hexonic acid (*I*) with ammonia or dimethylamine at elevated temperature gives rise to the products substituted at C₍₃₎ exclusively. The chemical proof of the position of the amino group was carried out by converting the reaction product to 3-acetamido-3,4,6-trideoxy-L-xylo-hexitol (*VII*) which is oxidable with periodic acid. The configuration of the substances has been confirmed by NMR-spectra. A difference in composition of the equilibrium acid-lactone mixture, attained in aqueous solutions of the hydrochloride of 3-amino-3,4,6-trideoxy-L-xylo-hexonic acid lactone and of its dimethylamino analogue has been determined. The stereoselective opening of the epoxide ring during the reaction of epoxy lactone *I* with bases is explained by the inductive effect of the carboxyl group.

In an earlier paper¹ we described the preparation of the lactone of 2,3-anhydro-4,6-dideoxy-L-ribo-hexonic acid (*I*) from natural parasorbic acid. In connection with further synthetic utilization of this lactone we investigated its reaction with ammonia or dimethylamine, which should lead, after the opening of the anhydro ring, to the substances derived from aminotrideoxyhexoses. Reaction of epoxy lactone *I* with anhydrous ammonia in an autoclave at room temperature gave a mixture of two substances having R_F values 0.05 and 0.28 on thin layer chromatogram (silica gel G; developed in benzene-ethanol 3 : 2). From this mixture we isolated by preparative chromatography on silica gel a substance having the higher R_F value. Its elemental composition corresponded to that of the amide of 2,3-anhydro-4,6-dideoxy-L-ribo-hexonic acid (*II*). At elevated temperature the reaction of epoxy lactone *I* with liquid ammonia led to the amide of 3-amino-3,4,6-trideoxy-L-xylo-hexonic acid (*IIIa*) exclusively. Its mobility on a thin layer of silica gel was identical with that of the substance with the lower R_F value described above. On acetylation of amide *IIIa* with acetic anhydride in methanol or pyridine we prepared crystalline N-acetyl derivative *IIIb* and peracetyl derivative *IIIc*. In aqueous medium amide *IIIa* undergoes hydrolysis extremely easily giving the corresponding 3-amino-3,4,6-trideoxy-L-xylo

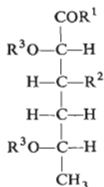
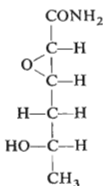
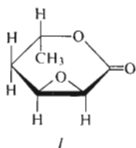
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hexonic acid (*IV*). The same acid is also formed from epoxy amide *II* by its ammonolysis at elevated temperature and the hydrolysis of the reaction product. After the neutralisation of the amino group of the acid *IV* with an equivalent of hydrochloric acid the lactone ring is closed under formation of the hydrochloride of lactone of 3-amino-3,4,6-trideoxy-*L*-xylo-hexonic acid (*V*) which may be obtained quantitatively on evaporation of its aqueous solution in which it exists in equilibrium with the corresponding acid. The closure of the lactone ring also took place when an attempt was made to prepare the peracyl derivative of the amino acid *IV* by acetylation in pyridine. In this case the lactone of 3-acetamido-2-*O*-acetyl-3,4,6-trideoxy-*L*-xylo-hexonic acid (*VI*) was formed.

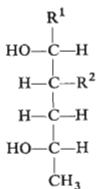
The reaction of epoxy lactone *I* with dimethylamine in an autoclave and at elevated temperature leads in analogy to the preceding reaction to the dimethylamide of 3,4,6-trideoxy-3-dimethylamino-*L*-xylo-hexonic acid (*VIIIa*). However, the isolation of this compound in a pure state was difficult, due to appreciable hygroscopicity of the reaction mixture. Therefore it was more convenient to submit the reaction mixture to hydrolysis and to isolate the product in the form of the crystalline 3,4,6-trideoxy-3-dimethylamino-*L*-xylo-hexonic acid (*IX*). We were able to obtain the crystalline dimethylamide of acid *IX* in a low yield only in the form of its di-*O*-acetyl derivative *VIIIb* by acetylation of the crude reaction mixture (obtained on reaction of lactone *I* with dimethylamine and following evaporation of the excess base) with acetic anhydride in pyridine. Deacetylation and hydrolysis of this 2,5-di-*O*-acetyl-3,4,6-trideoxy-3-dimethylamino-*L*-xylo-hexonic acid dimethylamide (*VIIIb*) also led to dimethylamino acid *IX*. In analogy to amino acid *IV* the dimethylamino acid *IX* also refuses to close the lactone ring; only when the basic group was neutralised the evaporation of the aqueous solution led to crystalline hydrochloride of the lactone of 3,4,6-trideoxy-3-dimethylamino-*L*-xylo-hexonic acid (*X*). Basing our considerations on the known configuration of the starting epoxy lactone *I* and the generally accepted mechanism of cleavage of the epoxide ring with nucleophilic agents we were able to suppose that in the reaction of ammonia with lactone *I* two isomeric products could be formed, which are derived from 2-amino-2,4,6-trideoxy-*L*-arabino-hexonic acid (*XI*) and 3-amino-3,4,6-trideoxy-*L*-xylo-hexonic acid (*IV*).^{*} We carried out the chemical proof of the position of the amino group in the products of ammonolysis by reducing lactone *VI* with bis(2-methoxyethoxy)sodium aluminum hydride. The formed, 3-acetamido-3,4,6-trideoxy-*L*-xylo-hexitol (*VII*) consumed on microanalytical oxidation 1.48 mol/mol of potassium periodate and liberated approximately 1 mol of formaldehyde. As the second possible product of cleavage of the epoxy lactone *I* with ammonia would give by the same reaction sequence the alcoholic sugar *XII*, which cannot be oxidised with periodic acid, it is evident that the amino group

* An analogous consideration is also valid for the products of the reaction of epoxy lactone *I* with dimethylamine.

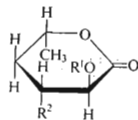
in lactone VI (and hence also in compounds IIIa, IIIb, IIIc, IV, and V) is bound to the third carbon. In view of the above consideration the L-xylo configuration may be assigned to all these compounds. In the NMR spectrum of lactone VI the signal of the NH-group proton is present at lower field (5.94 p.p.m.), split indistinctly by proton at C₍₃₎. The proton on the second carbon produces a doublet at 5.12 p.p.m. ($J_{2,3} = 11$ Hz). Signals of protons on C₍₃₎ and C₍₅₎ form mutually superposed multi-



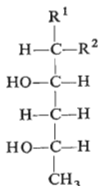
- IIIa, $R^1 = R^2 = \text{NH}_2$, $R^3 = \text{H}$
 IIIb, $R^1 = \text{NH}_2$, $R^2 = \text{NHCOCH}_3$, $R^3 = \text{H}$
 IIIc, $R^1 = \text{NH}_2$, $R^2 = \text{NHCOCH}_3$, $R^3 = \text{COCH}_3$
 VIIIa, $R^1 = R^2 = \text{N}(\text{CH}_3)_2$, $R^3 = \text{H}$
 VIIIb, $R^1 = R^2 = \text{N}(\text{CH}_3)_2$, $R^3 = \text{COCH}_3$



- IV, $R^1 = \text{COOH}$, $R^2 = \text{NH}_2$
 VII, $R^1 = \text{CH}_2\text{OH}$, $R^2 = \text{NHCOCH}_3$
 IX, $R^1 = \text{COOH}$, $R^2 = \text{N}(\text{CH}_3)_2$



- V, $R^1 = \text{H}$, $R^2 = \text{NH}_2 \cdot \text{HCl}$
 VI, $R^1 = \text{COCH}_3$, $R^2 = \text{NHCOCH}_3$
 X, $R^1 = \text{H}$, $R^2 = \text{N}(\text{CH}_3)_2 \cdot \text{HCl}$



- XI, $R^1 = \text{COOH}$, $R^2 = \text{NH}_2$
 XII, $R^1 = \text{CH}_2\text{OH}$, $R^2 = \text{NHCOCH}_3$

plets at 4.53 p.p.m. Two protons of the methylene group at $C_{(4)}$ display multiplets in the 2.22–2.52 p.p.m. and 1.52–1.92 p.p.m. regions, where the signals of the acetyl groups distinctly protrude ($=NAc$ at 1.96 p.p.m., OAc at 2.16 p.p.m.). In the region of higher field intensity a doublet is present due to the methyl group at $C_{(6)}$ (1.40 p.p.m., $J_{5,6} = 6.3$ Hz). The assignment of the signals, confirmed by decoupling experiments, as well as the observed values of the vicinal couplings support the structure *VI* and confirm thus the conclusions of chemical analysis. The configuration of substances formed on reaction of epoxy lactone *I* with dimethylamine was demonstrated by their relation to the products of ammonolysis: amino acid *IV* gave on methylation with formaldehyde and formic acid and subsequent evaporation of a solution in dilute hydrochloric acid the hydrochloride of dimethylaminolactone which was identical (according to its melting point and infrared spectrum) with substance *X* obtained in the above described manner from epoxy lactone and dimethylamine. The NMR spectrum of hydrochloride of dimethylaminolactone *X*, which is in broad outline identical with the spectrum of acetamido lactone *VI*, also corroborated the mentioned structure.

The unwillingness of acid *IV* and *IX* to form δ -lactone, which was not observed in the case of hydroxy analogues^{2,3}, is evidently caused by their character of internal salts. In the infrared spectra of amino acid *IV* and dimethylamino acid *IX* the absorption of the carboxyl band is shifted to the region of 1600 cm^{-1} frequency, characteristic of the carboxyl anion⁴. After neutralisation of aqueous solutions of the acids with one equivalent of hydrochloric acid lactonisation takes place which is evident from the change in optical rotation which proceeds up to the attainment of the equilibrium state. The same equilibrium is attained in aqueous solutions of

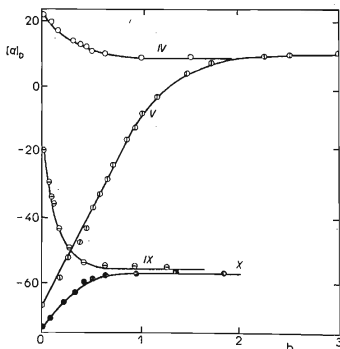


FIG. 1
Change of Specific Rotations of Hydrochlorides of Acids *IV* and *IX* and Lactones *V* and *X* in Time

TABLE I

Initial and Final Values of Specific Rotations of Hydrochlorides of Lactones *V* and *X* and Amino Acids *IV* and *IX* in Water^a

Substance	Conc. g/100 ml	Initial ^b [α] _D <i>t</i> = 0	Equilibrium state		
			[α] _D	time, h	% lactone
<i>IV</i> ^c	1.20	+21.3	+ 8.8	1.0	—
<i>V</i>	1.20	-65.8	+ 9.5	3.1	13.5
<i>IX</i> ^c	1.22	-19.7	-55.2	0.65	—
<i>X</i>	1.22	-73.7	-56.7	1.6	66.6

^a Temperature $23 \pm 1^\circ\text{C}$; ^b Initial values of the rotations were calculated by extrapolation of the values measured during the first 15–20 min according to the described method⁶; ^c Amino acids were neutralised before measurement with one equivalent of hydrochloric acid.

hydrochlorides of lactones *V* and *X*. In Table I the starting (extrapolated) and final values of specific rotations of the above mentioned substances are given, as well as the percentual composition of the equilibrium mixtures, calculated from the values of their rotation. From Fig. 1 in which the curves of the time dependence of specific rotations of hydrochlorides of amino acids *IV* and *IX* and lactones *V* and *X* are represented, the faster equilibration of dimethylamino derivative in comparison with the amino derivative is evident. The same is true of the acids as compared with lactones. This fact can be explained by the difference in basicity or acidity of these substances. The difference in the composition of the equilibrium mixtures is remarkable (Table I). In the case of 3-amino derivatives it is close to the composition of their 3-hydroxy analogues⁵, while in the case of 3-dimethylamino derivatives it is strongly shifted in favour of the lactone. In the above described reactions of epoxy lactone *I* with ammonia and dimethylamine we were unable to detect the products of the cleavage of the anhydro ring at the second carbon atom in the reaction mixtures. This fact is not surprising, considering the theoretical possibilities of the effect of sterical and polar factors in the opening of the epoxide ring. For the evaluation of the effect of sterical factors we can use the results of the experiments carried out at room temperature and at elevated temperature. On their basis we can judge that the opening of the epoxy ring takes place only in the stage when the acyclic epoxy amide was formed, *i.e.* the controlling sterical effects of the six-membered ring, known from the reactions of anhydro pyranosides, do not come into effect. Sterical factors on the acyclic chain perhaps cannot play a decisive role. A much more important role is probably played by the inductive effect of the amidocarboxylic group, causing a relative electronic deficiency on the third carbon atom. In the reaction with nucleophilic substituents this leads unambiguously to the products substituted on the third carbon atom.

EXPERIMENTAL

The melting points of solid substances were determined on a Kofler block and they are not corrected. The samples for analysis were dried *in vacuo* at 20–40°C for 10 hours. Optical rotation was measured on Opton polarimeter, using the subjective method of the determination of the angle of rotation. The infrared spectra were measured on a Perkin-Elmer-325 spectrophotometer. Chromatographic analysis of the reaction mixtures and of the products was carried out on microplates coated with a MN-silica gel G layer (Macherey and Nagel, Germany), developed in benzene-ethanol 3 : 2 (S₁), or chloroform-methanol 5 : 1 (S₂); detection by spraying with conc. sulfuric acid and heating. The NMR spectra were measured on a Varian HA-100 apparatus in deuteriochloroform, using tetramethylsilane as the internal standard, if not stated otherwise.

2,3-Anhydro-4,6-dideoxy-L-ribo-hexonic Acid Amide (II)

Epoxy lactone *I* (1.57 g; 12.3 mmol) and approx. 20 ml of liquid ammonia reacted at room temperature in a stainless-steel autoclave for 48 hours. After the evaporation of excess ammonia the syrupy residue (1.94 g) gave on thin layer chromatography in system S₁ two spots of *R_F* 0.28 and 0.05 (*R_F* of the starting substance was 0.7). The reaction mixture was chromatographed on a column of 30 g of silica gel with benzene-ethanol 1—10%. The fractions containing the substance of *R_F* 0.28 weighed 867 mg (48.5%), which was crystallised from chloroform-tetrachloromethane. M.p. 67–68°C, $[\alpha]_D^{22} -25.0 \pm 1.1^\circ$ (*c* 0.92, ethanol). IR spectrum (KBr pellet): 1660 (CONH₂), 1250, 845 cm⁻¹ (epoxide ring). For C₆H₁₁NO₃ (145.2) calculated: 49.65% C, 7.64% H, 9.65% N; found: 49.89% C, 7.57% H, 9.60% N.

3-Amino-3,4,6-trideoxy-L-xylo-hexonic Acid Amide (IIIa)

A mixture of epoxy lactone *I* (2.3 g, 16.7 mmol) and liquid ammonia (25 ml) was heated in an autoclave at 90°C for 6 hours. After cooling and evaporation of excess ammonia the residue was dried *in vacuo* to give 3.06 g of a substance m.p. 130–135°C. Thin-layer chromatography of the crude product in system S₂ demonstrated that the reaction mixture did not contain the initial material. By treble crystallisation from ethanol 1.88 g of product *IIIa* were obtained (67%), m.p. 136–138°C, $[\alpha]_D^{24} +2.0 \pm 0.4^\circ$ (*c* 1.6, water). IR spectrum (KBr pellet): 1670 cm⁻¹ (CONH₂). For C₆H₁₄N₂O₃ (162.2) calculated: 44.44% C, 8.70% H, 17.28% N; found: 44.32% C, 8.66% H, 17.18% N. 3-Acetamido derivative *IIIb*: To a solution of 1.1 g (6.8 mmol) of amide *IIIa* in 15 ml of methanol acetic anhydride (1 ml) was added under cooling and the reaction mixture was allowed to stand at room temperature for 48 hours. After evaporation in vacuum the residue was purified by double crystallisation from ethanol. Yield, 1.01 g (78.5%) of N-acetyl derivative *IIIb*, m.p. 161–163°C, $[\alpha]_D^{23} +27.6 \pm 0.7^\circ$ (*c* 0.79, methanol). For C₈H₁₆N₂O₄ (204.2) calculated: 47.05% C, 7.90% H, 13.72% N; found: 46.81% C, 8.01% H, 13.76% N. Peracetyl derivative *IIIc*: To a solution of 386 mg (2.4 mmol) of amide *IIIa* in 10 ml of pyridine 1 ml of acetic anhydride was added under cooling. The reaction mixture was allowed to stand at room temperature for 48 hours. After evaporation *in vacuo* and drying the residue (687 mg) was crystallised twice from ethanol-light petroleum. Yield, 418 mg (61%) of peracetyl derivative *IIIc*, m.p. 209–211°C, $[\alpha]_D^{25} +28.9 \pm 0.5^\circ$ (*c* 1.2, ethanol). For C₁₂H₂₀N₂O₆ (288.3) calculated: 49.99% C, 6.99% H, 9.72% N; found: 49.87% C, 7.05% H, 9.69% N.

3-Amino-3,4,6-trideoxy-L-xylo-hexonic Acid (IV)

A. A solution of 1.0 g (6.1 mmol) of amide *IIIa* in 25 ml of water was heated at 60°C for 20 hours. After evaporation under reduced pressure the residue was crystallised from ethanol-water 4 : 1. Yield, 788 mg (78%) of amino acid *IV*, m.p. 185–187°C, $[\alpha]_D^{23} +17.1 \pm 1.2^\circ$ (*c* 1.1, water). IR spectrum (KBr pellet): 1640 (COO⁻), 1590, 1520 cm⁻¹ (NH₃⁺). For C₆H₁₃NO₄ (163.2) calculated: 44.16% C, 8.03% H, 8.59% N; found: 44.46% C, 8.22% H, 8.74% N.

B. A mixture of 172 mg (1.19 mmol) of epoxyamide *II* and 8 ml of anhydrous ammonia was heated in a stainless-steel autoclave for 8 hours at 90°C. The syrupy product of ammonolysis (182 mg) was dissolved in 2 ml of water and then heated at 60°C for 30 hours. After evaporation the residue was crystallised from ethanol-water mixture 4 : 1. The product was identical, according to mixture melting point, and IR spectrum, with the amino acid prepared as under *A*.

Hydrochloride of 3-Amino-3,4,6-trideoxy-L-xylo-hexonic Acid Lactone (*V*)

Amino acid *IV* (600 mg, 3.7 mmol) was dissolved in 10 ml of water and titrated with 0.21M-HCl, using Tashiro as indicator. Consumption was 18.2 ml, *i.e.* 3.82 milliequivalents. The solution was evaporated and the residue dried *in vacuo*. The crystalline residue was washed four times with 3 ml of acetone. After drying 616 mg (92%) of hydrochloride of lactone *V* were obtained, m.p. 196–199°C (decomp.), $[\alpha]_D^{23} -65.8^\circ$ (*c* 1.2, water, extrapolated to time *t* = 0), $[\alpha]_D^{23} +9.5^\circ$ (*c* 1.2, water, equilibrium *t* 3.5 h), IR spectrum (KBr pellet): 1735 cm⁻¹ (δ -lactone). For C₆H₁₂ClNO₃ (181.6) calculated: 39.68% C, 6.66% H, 19.50% Cl, 7.71% N; found: 39.49% C, 6.71% H, 19.90% Cl, 7.53% N.

Lactone of 3-Acetamido-2-O-acetyl-3,4,6-trideoxy-L-xylo-hexonic Acid (*VI*)

To a suspension of 422 mg (2.6 mmol) of amino acid *IV* in 12 ml of pyridine acetic anhydride (2.5 ml) was added and the reaction mixture was shaken at room temperature for 24 h. After evaporation to dryness and drying in vacuum the residue was crystallised from ether and ethanol. Yield 255 mg (43%) of lactone *VI*, m.p. 118–120°C, $[\alpha]_D^{22} -61.7 \pm 0.5^\circ$ (*c* 1.05, ethanol). IR spectrum (KBr): 1760, 1750 (δ -lactone, OCOCH₃), 1660, 1550 cm⁻¹ (NHCOCH₃). For C₁₀H₁₅NO₅ (229.2) calculated: 52.40% C, 6.60% H, 6.11% N; found: 52.44% C, 6.88% H, 6.09% N.

3-Acetamido-3,4,6-trideoxy-L-xylo-hexitol (*VII*)

To a solution of 4.7 g (23 mmol) of sodium bis(2-methoxyethoxy) aluminum hydride in benzene a solution of lactone *VI* (834 mg; 3.66 mmol) in 12.5 ml tetrahydrofuran was added dropwise over 60 min under cooling and stirring, and the mixture was stirred at 10–15°C for another 60 minutes. After this the reaction did not contain any initial substance, as shown by thin-layer chromatography with system S₂. The reaction mixture was decomposed, under cooling, with 50% acetic acid (10 ml) and then diluted with methanol and filtered through a column of 100 ml of Dowex 50W (H⁺). The residue of the first 500 ml of eluate, which was decolorized with charcoal, weighed 530 mg and was crystallised from acetone-n-hexane 2 : 1. Yield 395 mg (57%) of hexitol *VII*, m.p. 135–138°C, $[\alpha]_D^{20} +56.7 \pm 1.1^\circ$ (*c* 0.94, ethanol). IR spectrum (KBr pellet): 1620, 1575 cm⁻¹ (NHCOCH₃). For C₈H₁₇NO₄ (191.2) calculated: 50.25% C, 8.96% H, 7.33% N; found: 50.57% C, 8.97% H, 7.42% N. *Oxidation*: The consumption of potassium periodate in the microanalytical determination according to⁷ was 8.36 μ mol per 1.08 mg (5.64 μ mol) of hexitol *VII*. This corresponds to 1.48 mol of periodate per 1 mol of hexitol *VII*. This value did not change on prolonged standing with the reagent. In a preparative experiment 8.0 mg (42 μ mol) of hexitol were dissolved in 10 ml of 0.01M-KIO₄. After the addition of 1 ml of 20% ethanolic dimedone solution the reaction mixture was allowed to stand overnight. The precipitated crystals were filtered and dried. Yield 10.5 mg (86%) of formaldehyde derivative, m.p. 190–192°C, identical according to mixture melting point with an authentic sample.

2,5-Di-O-acetyl-3,4,6-trideoxy-3-dimethylamino-L-xylo-hexonic Acid Dimethylamide (*VIIIb*)

A mixture of epoxy lactone *I* (3.21 g, 25 mmol) and 25 ml of anhydrous dimethylamine was heated in an autoclave at 80–100°C for 27 hours. After cooling the content of the autoclave was evaporated *in vacuo* and the residual syrup (4.4 g) was acetylated in 30 ml of pyridine and 15 ml of acetic anhydride at room temperature for 48 hours. The reaction mixture was decomposed by pouring it onto crushed ice and then extracted with chloroform. The extract was washed gradually with 2% sulfuric acid, water, saturated sodium hydrogen carbonate solution, and water. After drying and filtering the solution was evaporated to dryness to yield 2.2 g of a syrup which after treble crystallisation from ethanol and light petroleum afforded 530 mg (7%) of dimethylamide *VIIIb*, m.p. 94–95°C, $[\alpha]_D^{25} +23.3 \pm 0.5^\circ$ (*c* 1.42, chloroform). For $C_{14}H_{26}N_2O_5$ (302.4) calculated: 55.61% C, 8.67% H, 9.27% N; found: 55.62% C, 8.71% H, 9.49% N.

3,4,6-Trideoxy-3-dimethylamino-L-xylo-hexonic Acid (*IX*)

A mixture of epoxy lactone *I* (1.7 g; 13.3 mmol) and 10 ml of anhydrous dimethylamine was allowed to react in an autoclave under the same conditions as in the preceding experiment. After the evaporation of excess base in vacuum the syrupy residue was dissolved in 30 ml of water and the solution was heated at 40°C for 40 hours. After the evaporation of the solvent and drying *in vacuo* the substance crystallised from ethanol. After recrystallisation the yield was 1.61 g (61%) (dimethylamino acid *IX*), m.p. 155–157°C, $[\alpha]_D^{22} -16.9^\circ$ (*c* 1.4, water). IR spectrum (KBr pellet): 1600 cm^{-1} (COO⁻). For $C_8H_{17}NO_4$ (191.2) calculated: 50.25% C, 8.96% H, 7.33% N; found: 50.19% C, 9.20% H, 7.33% N.

Hydrochloride of 3,4,6-trideoxy-3-dimethylamino-L-xylo-hexonic Acid Lactone (*X*)

A) To a solution of 195 mg (1.02 mmol) of dimethylamino acid *IX* 4.95 ml of a 0.21M hydrochloric acid (1.03 equivalents) were added and the solution was evaporated to dryness. The residue was crystallised from methanol. Yield 210 mg (quantitative) of lactone *X*, m.p. 232–234°C, $[\alpha]_D^{22} -73.7^\circ$ (*c* 1.22, water, extrapolated to time *t* = 0), $[\alpha]_D^{22} -56.7^\circ$ (*c* 1.22, water, at equilibrium). NMR spectrum (measured on a JNM-3 H-60 JEOL (60 MHz) apparatus in deuterium oxide): H₂ 4.62 p.p.m., $J_{2,3} = 12$ Hz, H₃ 3.94 p.p.m., $J_{3,2} = 12$ Hz, H_{4eq,3} 2.48 p.p.m., $J_{4eq,3} = 4.5$ Hz, H_{4ax} 2.12 p.p.m., $J_{4ax,3} = 13.5$ Hz, H₅ 4.60 p.p.m., $J_{5,6} = 6.8$ Hz, H₆ 1.47 p.p.m., $J_{6,5} = 6.8$ Hz, N(CH₃)₂ 2.95 p.p.m. IR spectrum (Nujol): 1740 cm^{-1} (δ-lactone). For $C_8H_{18}ClNO_3$ (209.7) calculated: 45.83% C, 7.69% H, 6.68% N, 16.91% Cl; found: 46.05% C, 7.87% H, 6.54% N, 16.80% Cl.

B) Amino acid *IV* (102 mg; 0.59 mmol) was heated in a mixture of 2 ml of 30% formaldehyde and 2 ml of 90% formic acid at 120–130°C (bath temperature) for 2.5 hours. The solution was evaporated to dryness and the residue evaporated twice with 5 ml of 10% hydrochloric acid. Yield 123 mg (quantitative) of lactone *X*, identical according to its melting point, mixture melting point, and IR spectrum with the substance prepared under A).

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