PREPARATION OF 1,6-ANHYDRO-2,3-DIDEOXY--2,3-EPIMINO-β-D-MANNOPYRANOSE AND ITS CONVERSION TO 2-AMINO-1,6-ANHYDRO-2-DEOXY-β-D-MANNOPYRANOSE*

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From 1,6 : 2,3-dianhydro-4-O-benzyl-β-D-allopyranose (*I*) 1,6-anhydro-3-azido-4-O-benzyl--3-deoxy-β-D-glucopyranose (*III*) was prepared on reaction with sodium azide, and *III* was converted to 3-amino-1,6-anhydro-4-O-benzyl-3-deoxy-2-O-*p*-toluenesulfonyl-β-D-glucopyranose (*XII*). Its *p*-nitrobenzamide *XVI* was reacted with sodium 2-propoxide to afford the corresponding 2,3-epimino derivative *XVII*. On catalytic debenzylation of compound *XVII* 1,6-anhydro-2,3-dideoxy-2,3-epimino-β-D-mannopyranose (*XVIII*) was obtained which was hydrolysed with 5% KOH at 100°C to 2-amino-1,6-anhydro-2-deoxy-β-D-mannopyranose (*XXVII*). Catalytic hydrogenation of *III* on palladium or ammonolysis of 1,6 : 3,4-dianhydro-β-D-allopyranose (*XXII*) gave 3-amino-1,6-anhydro-3-deoxy-β-D-glucopyranose (*IV*).

From epimino derivatives of sugars described in literature only those most accessible were investigated, mainly methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-epimino-α-D-allopyranoside, its *manno* analogue and some similar derivatives[1-5]. With the exception of methyl 2,3-dideoxy-2,3-epimino-α-D-mannopyranoside, which was not satisfactorily characterised[1], no such epimino sugar derivatives have been prepared which would contain a free *trans* oriented hydroxyl group in the neighbourhood of the aziridine ring (a certain analogy of this system in the aliphatic series is represented by N,C-substituted aziridinemethanols[6]). In these compounds the question of a possible existence of equilibrium between the α-hydroxyepimine and α-aminoepoxide (Scheme 1) interested us mainly, because it would represent an analogy of the well known isomerisation of α-hydroxyepoxides, often called epoxide migration[7-12].

The most suitable sterical arrangement of the hydroxyl group and the epimino ring for the considered isomerisation is evidently that in which the hydroxyl group is *trans*-axial: this creates the necessary conditions for intramolecular S_N2 cleavage of the epimine cycle. An oxygen model for a similar situation is 1,6 : 2,3-dianhydro--β-D-mannopyranose (*XXIV*) which isomerises readily in alkaline medium at room

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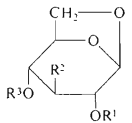
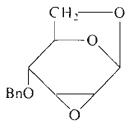
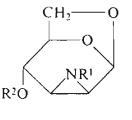
temperature to 1,6 : 3,4-dianhydro-β-D-altropyranose, and the reaction equilibrium is shifted appreciably toward the *altro* derivative[10,12]. Therefore we thought that the undescribed 1,6-anhydro-2,3-dideoxy-2,3-epimino-β-D-mannopyranose (*XVIII*) could be a suitable compound for our study. The starting material for its synthesis, 1,6 : 2,3--dianhydro-4-O-benzyl-β-D-allopyranose[13] (*I*) was prepared in 80% yield from 1,6-anhydro-4-O-benzyl-3-O-methanesulfonyl-2-O-*p*-toluenesulphonyl-β-D-glucopyranose[13] (*II*) under the effect of sodium methoxide in dioxan.

An important step in our synthesis consisted in the introduction of an amino group on $C_{(3)}$ in dianhydro derivative *I*. The current procedure consisting in the opening of the oxiran ring with methanolic ammonia[14], applied successfully in the synthesis of 2-amino-1,6-anhydro-4-O-benzyl-2-deoxy-β-D-glucopyranose[15] from 1,6 : 2,3-dianhydro--4-O-benzyl-β-D-mannopyranose (*XXV*), failed, however, and dianhydro derivative *I* was regenerated. Increase in temperature or ammonia concentration, or the prolongation of the reaction time was not successful either; under drastic conditions, when liquid ammonia and sodium amide were employed a complex mixture was formed which could not be used for preparative purposes. Therefore sodium azide in boiling aqueous 2-ethoxyethanol in the presence of excess ammonium chloride was chosen for the oxiran ring cleavage[16,17]. In this arrangement dianhydro derivative *I* reacts under formation of a single product, 1,6-anhydro-3-azido-4-O-benzyl-3-deoxy-β-





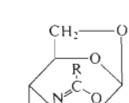


II, R^1 = Ts, R^2 = OMs, R^3 = Bn
III, R^1 = H, R^2 = N_3, R^3 = Bn
IV, R^1 = H, R^2 = NH_2, R^3 = H
V, R^1, R^3 = Ac, R^2 = AcNH
VI, R^1 = H, R^2 = NH_2, R^3 = Bn
VII, R^1 = Ac, R^2 = AcNH, R^3 = Bn
VIII, R^1 = Ac, R^2 = AcNH, R^3 = H
IX, R^1 = Ms, R^2 = N_3, R^3 = Bn
X, R^1 = Ts, R^2 = N_3, R^3 = Bn
XI, R^1 = Ms, R^2 = NH_2, R^3 = Bn
XII, R^1 = Ts, R^2 = NH_2, R^3 = Bn
XIII, R^1 = Ms, R^2 = BzNH, R^3 = Bn
XIV, R^1 = Ts, R^2 = BzNH, R^3 = Bn
XV, R^1 = Ms, R^2 = *p*-NO_2BzNH, R^3 = Bn
XVI, R^1 = Ts, R^2 = *p*-NO_2BzNH, R^3 = Bn

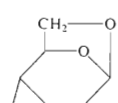
I

XVII, R^1 = H, R^2 = Bn
XVIII, R^1, R^2 = H
XIX, R^1 = Bz, R^2 = Bn
XX, R^1, R^2 = Bz

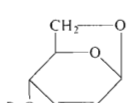
Ac = CH_3CO, Bn = $C_6H_5CH_2$, Bz = C_6H_5CO, Ms = CH_3SO_2, Ts = *p*-$CH_3C_6H_4SO_2$



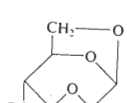
XXI, R = C_6H_5



XXII

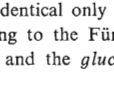


XXIII

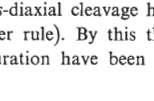


XXIV, R = H
XXV, R = Bn

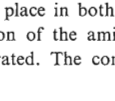
-D-glucopyranose (*III*) in approximately 80% yield.* The reaction course was followed chromatographically on thin layer of silica gel, while Buchanan's reagent[8] and 50% sulfuric acid were used for detection of the azido derivative *III*. The latter specific detection is based on the reduction of the azido group with hydriodic acid under formation of elemental iodine[19]. The proposed structure of azido derivative *III* was in agreement with the IR spectrum ($\nu(N_3)$ 2120 cm^{-1}) and it was demonstrated by its conversion to 3-amino-1,6-anhydro-3-deoxy-β-D-glucopyranose (*IV*) hydrochloride by reduction of the azido group and simultaneous hydrogenolysis of the benzyloxy group with hydrogen under catalysis with palladium on charcoal in ethanolic hydrogen chloride. Amino derivative *IV* was found identical with the sample prepared on reaction of 1,6 : 3,4-dianhydro-β-D-allopyranose[13] (*XXII*) with ethanolic ammonia. (The reaction of dianhydroallose *XXII* with ethanolic ammonia represents a new synthesis of 3-amino-3-deoxy-D-glucose (kanosamine) which is formed from 1,6-anhydro derivative on hydrolysis with hydrochloric acid). If the possibility of the oxiran ring *cis* cleavage is excluded, then the amino derivatives obtained independently from the two isomeric 1,6-anhydro-*allo*-epoxides *I* and *XXII* can be identical only if a *trans*-diaxial cleavage has taken place in both instances (according to the Fürst–Plattner rule). By this the position of the amino group on $C_{(3)}$ and the *gluco* configuration have been demonstrated. The confirmation



XVIII



XXVI



XXVII

SCHEME 1

* Benzyldianhydromannose *XXV* does not react with sodium azide under the same conditions[15]. The unreactivity of the negatively charged ions during S_N2 substitution on $C_{(2)}$ of pyranose systems is explained by the unfavourable interaction of dipoles in the transition state[18].

of the identity of both amino derivatives and also of their configuration was also carried out by optical rotation measurements (Table I). Hydrochloride of amine *IV*, prepared from dianhydro derivative *XXII* with ammonia, was converted to triacetyl derivative *V* on treatment with acetic anhydride in pyridine. Derivative *V* was identical with the triacetyl derivative obtained from azido compound *III* in the following manner: On catalytic reduction of the azido group with hydrogen in the presence of Raney nickel amine *VI* was prepared which on acetylation afforded benzyloxydiacetate *VII*, and its debenzylation with hydrogen on palladium on charcoal yielded hydroxydiacetate *VIII*. Its acetylation with acetic anhydride in pyridine gave triacetyl derivative *V*.

Further procedure in the synthesis of epimino derivative *XVIII* from azido compound *III* was an analogy of the generally used method[20], *i.e.* cyclisation of β-sulfonyloxyethyl derivatives of acylamides in alcoholate solution, that was also found suitable in the case of saccharides[1,2,21,22]. Azido compound *III* was converted to mesyl ester *IX* or tosyl ester *X* on reaction with sulfonyl chlorides in pyridine. The esters obtained were reduced to corresponding sulfonyl esters of 3-amino-1,6-anhydro-4-O-benzyl-3-deoxy-β-D-glucopyranose *XI* and *XII*. Several methods of reduction of azide compounds were tried[23] of which reduction with zinc and hydrochloric acid in acetone[24] was best for mesyl ester *IX*, and catalytic reduction on palladium on charcoal for tosyl ester *X*. When hydrazine in the presence of Raney nickel was used, especially if its activity was high, mesyl ester *IX* afforded in addition to amine *XI* also 1,6-anhydro-4-O-benzyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranose (*XXIII*) the identity of which with an authentic specimen[25] was proved chromatographically on a thin layer and further by coincidence of physical constants and IR spectra. (The formation of epimine during this reaction, described in literature[16,26], was not observed in our case.) We did not investigate in detail this elimination reaction, described[27] for methyl 2-azido-4,6-O-benzylidene-2-deoxy-3-O-*p*-toluenesulfonyl-α-D-altropyranoside; we only found that the unsaturated substance *XXIII* is not formed by a subsequent reaction from amino derivative *XI*.

Mesyl ester *XI* or tosyl ester *XII* were acylated with benzoyl chloride or *p*-nitrobenzoyl chloride to benzamides *XIII* and *XIV*, or *p*-nitrobenzamides *XV* and *XVI*. Reaction of these amides with alkoxides gave 1,6-anhydro-4-O-benzyl-2,3-dideoxy-2,3-epimino-β-D-mannopyranose (*XVII*), and in some cases 1,6-anhydro-4-O-benzyl--2,3-dideoxy-2,3-(2-phenyl-1-oxa-3-azaprop-2-eno)-β-D-mannopyranose (*XXI*) was formed as a by-product in various amounts depending on the starting compound and the reaction conditions[21,28–30]. For the preparation of epimino derivative *XVII* *p*-nitrobenzamide *XVI* and also *p*-nitrobenzamide *XV* were most suitable; on their reaction with sodium 2-propoxide in dioxan[22] oxazoline derivative *XXI* was practically not formed. The structure of benzylepimine *XVII* follows from the method of its preparation and is in agreement with the IR spectrum that shows a characteristic band[31] at 3330 cm^{-1} due to ν(NH). Benzoylepimine *XIX* (prepared from com-

pound *XVII*) has in its IR spectrum the ν(C=O) band at 1690 cm^{-1}, shifted in comparison with the ν(C=O) bands of common benzamides to higher frequencies[32]. Among chemical properties of benzylepimine *XVII* its relative stability during the heating in alkaline medium should be mentioned which contrasts with its behaviour in chloroform in which it decomposes on standing even at room temperature. The side-product formed during the preparation of benzylepimine *XVII* is considered to be oxazoline derivative *XXI* on the basis of literature data[21] and according to its IR spectrum in which the ν(N=C) band[31] was found at 1660 cm^{-1} and not the ν(NH) band.

After hydrogenolytic debenzylation of benzylepimine *XVII* in the presence of 10% palladium on charcoal in ethanolic hydrogen chloride (the reaction does not go smoothly either in ethanol or acetic acid) 1,6-anhydro-2,3-dideoxy-2,3-epimino-β-D-mannopyranose (*XVIII*) was isolated using an anion exchanger. Its structure was proposed on the basis of its synthesis, IR spectrum and mainly PMR spectrum which was very similar to that of dianhydromannose[33] *XXIV* (Table II). The upfield shift of protons $H_{(2)}$ and $H_{(3)}$ of epimine *XVIII* (about 1 p.p.m.) in comparison with corresponding protons of dianhydromannose *XXIV* is due to the lower electronegativity of the nitrogen in comparison with oxygen, which also manifests itself in the increase of coupling constants $J_{1,2}$ and $J_{2,3}$. From the difference of the coupling constants $J_{5,6}$ between dianhydromannose *XXIV* and epimine *XVIII*

TABLE I

$[M]_D$ Values (in water) of 1,6-Anhydro-β-D-hexopyranoses and Hydrochlorides of Amino--1,6-anhydro-deoxy-β-D-hexopyranoses

Configuration	1,6-Anhydro-hexoses (lit.)	1,6-Anhydro-x-aminohexoses		
		2- (lit.)	3- (lit.)	4- (lit.)
allo	−123° (42)	—	—	—
altro	−345° (43)	−316° (51)	−340° (54)	—
gluco	−108° (44)	− 98° (52,15)	−108° [a]	−204° (40)[b]
manno	−207° (45)	−211° [a]	—	−198° (41)[c]
gulo	+ 81° (46)	+ 89° (38)	+ 91° (39)	—
ido	−150° (47)	−158° (53)	−150° [d] (39,41)	—
galacto	− 36° (48)	− 31° (41)	− 18° (55)	—
talo	−131° (49,50)	—	—	−130° (56)

[a] This paper; [b] for 4-acetamido-2,3-di-O-acetyl-1,6-anhydro-4-deoxy-β-D-glucopyranose lit.[40] gives m.p. 181·5—182·5°C, $[\alpha]_D^{27}$ −62° (*c* 0·83; methanol); [c] for 4-acetamido-2,3-di-O-acetyl--1,6-anhydro-4-deoxy-β-D-mannopyranose lit.[41] gives m.p. 180°C, $[\alpha]_D^{18}$ −76° (*c* 0·54; chloroform); [d] $[M]_D$ value of free amine.

it may be concluded that the half-chair conformation $^5H_0$ of both compounds is deformed differently, which is reflected in different optical rotation values: epimine *XVIII* $[\alpha]_D$ −67° (water) and dianhydromannose[10] *XXIV* $[\alpha]_D$ −35° (water); similarly benzylepimine *XVII* $[\alpha]_D$ −45° (chloroform) and benzylepoxide[34] *XXV* $[\alpha]_D$ −27° (chloroform). In contrast to this for 4,6-O-benzylidene derivatives of hexopyranosides with 2,3-epimino and 2,3-epoxy ring of the same configuration an excellent agreement of $[\alpha]_D$ values has been described[3]. Epimine *XVIII* was converted to dibenzoyl derivative *XX* in the IR spectrum of which ν(C=O) bands at 1730 cm^{-1} and 1690 cm^{-1} were present due to the ester and amide bond of the benzoyl group, and the ν(NH) band at 3330 cm^{-1}, found in the spectrum of free epimine *XVIII* and its O-benzyl derivative *XVII*, was absent.

Further we endeavoured to isomerise epimine *XVIII* to amino-epoxide *XXVI* (Scheme 1). In 5% potassium hydroxide at 25°C (*i.e.* under the conditions when dianhydromannose *XXIV* isomerises to 1,6 : 3,4-dianhydro-β-D-altropyranose[10]) epimine *XVIII* does not change even after 24 hours. When this reaction mixture was heated for 6 hours at 90–100°C under nitrogen, in addition to the starting material with R_F 0·4 (solvent system S_3) two other substances could be proved in it by thin-layer chromatography. One of them, with R_F 0·15, was the predominant component. It was isolated chromatographically on a silica gel column and characterised as hydrochloride. We assigned it the structure of 2-amino-1,6-anhydro-2-deoxy-β-D-mannopyranose hydrochloride (*XXVII*) on the basis of elemental analysis, comparison of its trimethylsilyl derivative with the same derivatives of authentic samples of other 1,6-anhydroaminohexoses by means of gas chromatography, and agreement of its $[M]_D$ value with that of 1,6-anhydro-β-D-mannopyranose (Table I). The correctness of the proposed structure was supported by PMR spectrum (Table II) which was compared with the spectra of 1,6-anhydro-β-D-hexopyranoses[35] and was in agreement with the supposed $^1C_4$ conformation. The chemical shift of the proton $C_{(2)}$—H and the values of interaction constants $J_{1,2}$ and $J_{2,3}$ correspond to the position of the amino group on $C_{(2)}$ and axial or equatorial orientation of protons $H_{(2)}$ and $H_{(3)}$, respectively. The coupling constants $J_{3,4}$ and $J_{3,5}$ indicate the equatorial orientation of the corresponding protons. The parameters for the methylene group of the 1,6-anhydride bridge are also in agreement with earlier measurements of 1,6-anhydro-β-D-hexopyranoses[35]. Acid hydrolysis of anhydromannosamine *XXVII* with 6M-HCl gave hydrochloride of 2-amino-2-deoxy-D-mannose.

The formation of anhydromannosamine *XXVII* from epimine *XVIII* cannot be explained by the *trans* cleavage of the aziridine ring with hydroxide ions because it would lead to an amino derivative of 3-amino-*gluco*- (*IV*) or 2-amino-*altro* configuration. A *cis*-cleavage is improbable and it was not observed in the reactions of epimino sugar derivatives with sodium azide[2,30,36], ammonium halogenides[1], potassium acetate and thioacetate[36], *etc.*[5]. Therefore we suppose that the reaction takes place in the following manner: In alkaline solution epimine *XVIII* isomerises

TABLE II

Chemical Shifts and Coupling Constants of the PMR Spectra of 1,6-Anhydro-β-D-hexopyranose Derivatives

The PMR spectra were measured in deuterated dimethyl sulfoxide on Varian HA-100 apparatus. Chemical shifts are given in δ values (p.p.m.), tetramethylsilane was used as internal reference. J values are given in Hz and they were determined with a $\pm$ 0·5 Hz accuracy. The assignment of the signals of single protons was carried out by the double resonance method.

H-1	H-2	H-3	H-4	H-5	H-6 (*endo*)	H-6′ (*exo*)
			Compound *XVIII*			
5·55 $J_{1,2} = 4·25$ $J_{1,4} + J_{1,6} = 0·7$ $J_{1,6'} = 0·3$	2·38 $J_{2,1} = 4·25$ $J_{2,3} = 6·05$ $J_{2,4} = 0·5$	1·85 $J_{3,5} = 1·4$ $J_{3,2} = 6·1$ $J_{3,4} \neq 0 < 0·3$	3·55 $J_{4,5} = 1·05$ $J_{4,2} = 0·5$ $J_{4,3} \neq 0 < 0·3$	4·12 $J_{5,4} = 1·05$ $J_{5,6} = 1·95$ $J_{5,6'} = 6·6$ $J_{5,3} = 1·35$	3·52 $J_{6,5} = 2·0$ $J_{6,1} = 0·4$ $J_{6,6'} = 6·9$	3·34 $J_{6,6'} = J_{6',5} = 6·9$
			Compound *XXIV*			
5·60 $J_{1,2} = 3·2$	3·26 $J_{2,1} = 3·2$ $J_{2,3} = 3·8$ $J_{2,4} = 0·9$	2·90 $J_{3,5} = 1·5$ $J_{3,2} = 3·8$ $J_{3,4} = 0·6$	3·70 $J_{4,5} = 1·1$ $J_{4,3} = 0·6$ $J_{4,2} = 0·9$	4·23 $J_{5,6'} = 4·8$ $J_{5,6} = 3·7$ $J_{5,3} = 1·5$	3·37 $J_{6,5} = 3·7$	3·37 $J_{6',5} = 4·8$
			Hydrochloride *XXVII*			
5·42 $J_{1,2} = 1·8$ $J_{1,3} = 1·2$ $J_{1,6} \neq 0 < 0·5$	3·18 $J_{2,1} = 1·8$ $J_{2,3} = 5·8$	3·79 $J_{3,4} = 1·5$ $J_{3,2} = 5·8$ $J_{3,1} = 1·2$ $J_{3,5} = 1·5$	3·61 $J_{4,3} = 1·5$ $J_{4,5} = ?$	4·41 $J_{5,6'} = 6·0$ $J_{5,6} = 0·8$ $J_{5,4} = ?$ $J_{5,3} = 1·5$	4·13 $J_{6,5} = 0·8$ $J_{6,6'} = 7·0$ $J_{6,1} \neq 0 < 0·5$	3·54 $J_{6',5} = 6·0$ $J_{6,6'} = 7·0$

to 2-amino-1,6 : 3,4-dianhydro-2-deoxy-β-D-altropyranose (*XXVI*) by course of intramolecular attack of the aziridine ring at the position $C_{(3)}$ with the hydroxy group anion at $C_{(4)}$. The aminoepoxide *XXVI* thus formed then hydrolyses to anhydromannosamine *XXVII*. A similar mechanism is evidently operative in alkaline hydrolysis of the analogous dianhydromannose *XXIV* to 1,6-anhydro-β-D-mannopyranose[37]. However, the supposed intermediate, the as yet undescribed aminoepoxide *XXVI*, could not be detected with certainty in the reaction mixture. Hence, if the aminoepoxide *XXVI* is formed at all, then the rate of its hydrolysis is approximately the same or greater then the rate of isomeration of epimine *XVIII*.

In this study we made use of the correlation between the configuration and the optical rotation of aminodeoxy derivatives of 1,6-anhydro-β-D-hexopyranoses. The supposition that the substitution of the hydroxy group by an amino group or ammonium group under the preservation of configuration does not practically change the molecular rotation value[38,39] is documented by Table I. The agreement of $[M]_D$ values is good, with the exception of $[M]_D$ for 4-amino-1,6-anhydro-4-deoxy-β-D-glucopyranose differing by $-97°$ from the $[M]_D$ value of 1,6-anhydro-β-D-glucopyranose. Regarding its indirect preparation from 1,6-anhydro-2,4-di-O-*p*-toluenesulfonyl--β-D-glucopyranose under the effect of ammonia, and in view of the circumstance that its structure was not convincingly demonstrated[40], we suppose that this compound has a different configuration. According to the $[M]_D$ value, melting point[40,41], and the properties of its peracetylated derivative[40,41], we consider it to be 4-amino--1,6-anhydro-4-deoxy-β-D-mannopyranose[41].

EXPERIMENTAL

The melting points were determined on a micromelting point apparatus Boetius. Optical rotations were measured on an automatic Bendix-Ericsson ETL 143 A polarimeter at 23–25°C. The IR spectra were measured in chloroform (concentration 5–7%) on a Zeiss-Jena UR-20 spectrophotometer, unless otherwise stated. Gas chromatography was performed on a Chrom 3 apparatus of Laboratorní přístroje, Prague, provided with FID. Trimethylsilyl derivatives were separated on a 192 × 0·4 cm column packed with Chromosorb W-TMCS coated with 6·6% of the OV-101 phase. The PMR spectra were measured in deuterated dimethyl sulfoxide containing traces of CD_3COOD on a Varian HA-100 apparatus, using tetramethylsilane as internal reference. Thin-layer chromatography was carried out on silica gel according to Stahl (layer thickness 0·2–0·3 mm) in the following systems: S_1 ether–chloroform (4 : 1), S_2 chloroform–methanol (9 : 1), S_3 2-propanol–chloroform–ammonia–water (10 : 10 : 1 : 1). The spots were detected by mineralisation with 50% sulfuric acid. In addition to this the azido compounds were detected specifically as brown spots on a white background by spraying the plate first with Buchanan's reagent[8] (a solution of 5 g of NaI and 0·01 g of methyl red in 100 ml of butanol) and then by 50% sulfuric acid. Column chromatography was carried out with the above mentioned solvent systems S_1–S_3 on silica gel (particle diameter 40–60μ), activated at 120–140°C. Paper chromatography was carried out on Whatman No 4 paper, descending arrangement, in S_4 water saturated 1-butanol and S_5 1-butanol–pyridine–water (6 : 4 : 3). Before development the chromatograms were equilibrated overnight with the stationary phase vapours; distance of the

front from the start: 32 to 34 cm. The spots of the epimino and epoxy derivatives were detected with Buchanan's reagent, and the hexosamine spots with ninhydrin. The solvents were evaporated under reduced pressure at 40—60°C. The light petroleum used was a 40—60°C fraction. Samples for analysis were dried over phosphorus pentoxide at 0·1 Torr.

1,6 : 2,3-Dianhydro-4-O-benzyl-β-D-allopyranose (*I*)

To a solution of 20 g of ester[13] *II* in 60 ml of dioxan a solution of 4 g of sodium in 100 ml of methanol was added dropwise under stirring. After 24 hours' standing at room temperature the mixture was neutralised with 10% hydrochloric acid and evaporated. Water (100 ml) was added to the residue and the mixture was extracted with three 100 ml portions of chloroform. The combined extracts were dried over calcium chloride and evaporated to a syrupy residue. Its crystallisation from an acetone–ether–light petroleum mixture gave 7·4 g (80%) of dianhydro derivative *I* the physical constants of which coincided with those from literature[13].

1,6-Anhydro-3-azido-4-O-benzyl-3-deoxy-β-D-glucopyranose (*III*)

A mixture of 5 g of dianhydro derivative *I*, 5 g of sodium azide, and 25 g of ammonium chloride in 50 ml of 2-ethoxyethanol and 15 ml of water was refluxed for about 7 hours. The reaction course was controlled by thin-layer chromatography in S_1 (R_F of compound *I* was 0·6, R_F of azido derivative *III* was 0·7). The reaction mixture was filtered and the residue on the filter washed with 50 ml of chloroform. The combined filtrates were evaporated and the residue triturated with 100 ml of water. The precipitated product was filtered off under suction, dissolved in ethanol, the solution decolorised with charcoal and evaporated to dryness. After crystallisation from ethanol–water 4·8 g (81%) of compound *III* were obtained, m.p. 74—76·5°C, $[\alpha]_D$ −13° (*c* 2·6; chloroform). IR spectrum: 2120 cm^{-1} $\nu(N_3)$, 3570 cm^{-1} ν(OH). For $C_{13}H_{15}N_3O_4$ (277·3) calculated: 56·31% C, 5·45% H, 15·15% N; found: 56·50% C, 5·50% H, 15·22% N.

Hydrochloride of 3-Amino-1,6-anhydro-3-deoxy-β-D-glucopyranose (*IV*)

A) A mixture of 0·1 g of compound *III* and 50 mg of 10% palladium on charcoal was hydrogenated in 2 ml of 5% ethanolic hydrogen chloride at normal pressure and room temperature. The course of the reduction and subsequent debenzylation was followed chromatographically on thin layer in S_3 (R_F of 4-O-benzylamine *VI* 0·7, R_F of amine *IV* 0·25). After 24 hours of reaction when debenzylation was over (reduction of the azido group was much faster) the catalyst was filtered off with suction, washed with ethanol, and the filtrate evaporated to dryness. The residue was dissolved in ethanol and the solution concentrated to a syrup. Crystallisation of the residue from a mixture of water saturated ether and ethanol gave hydrochloride of 3-aminoglucosan *IV* in the form of a monohydrate. Yield 56 mg (72%), m.p. 66—84°C, $[\alpha]_D$ −50° (*c* 0·76; water). The substance does not give a positive reaction with Fehling's reagent. For $C_6H_{12}NO_4Cl.H_2O$ (215·6) calculated: 33·42% C, 6·54% H, 6·50% N, 16·44% Cl; found: 33·72% C, 6·64% H, 6·84% N, 16·34% Cl.

B) A solution of 0·1 g of dianhydroallose[13] *XXII* in 5 ml of ethanolic ammonia (saturated at −15°C) was heated in a sealed tube at 100—110°C for 120 hours. The mixture was then evaporated, the residue dissolved in ethanol and 5% ethanolic hydrochloric acid was added to it dropwise until acid. After evaporation of ethanol the residue was crystallised from a mixture of water saturated ether and ethanol. Yield 105 mg (70%) of amine *IV* hydrochloride identical according to physical constants and IR spectra in nujol with the product prepared under *A*).

3-Acetamido-2,4-di-O-acetyl-1,6-anhydro-3-deoxy-β-D-glucopyranose (*V*)

A) To a solution of 0·1 g of diacetyl derivative *VIII* in 1 ml of pyridine 0·1 ml of acetic anhydride was added and the mixture was allowed to stand overnight at room temperature. After addition of 0·5 ml of water the mixture was evaporated and crystallised from ethanol–ether mixture to afford 0·1 g (86%) of a product, m.p. 177—178°C (under sublimation), $[\alpha]_D$ −65·5° (*c* 0·85; chloroform). IR spectrum: 1690 and 3450 cm^{-1} ν(NHC=O), 1750 cm^{-1} ν(C=O). For $C_{12}H_{17}NO_7$ (287·3) calculated: 50·16% C, 5·96% H, 4·87% N; found: 50·35% C, 6·08% H, 5·06% N.

B) Hydrochloride of amine *IV*, prepared by ammonolysis of dianhydroallose *XXII*, was acetylated with acetic anhydride in pyridine. The product obtained was identical according to its melting point, IR spectrum and $[\alpha]_D$ value with the product obtained from azide *III* by catalytic reduction, debenzylation and subsequent acetylation according to procedure *A*).

3-Amino-1,6-anhydro-4-O-benzyl-3-deoxy-β-D-glucopyranose (*VI*)

To a solution of 1·0 g of azide *III* in 20 ml of ethanol 16 ml of a Raney nickel suspension[57] in ethanol was added and the mixture hydrogenated for 2 hours at normal pressure and room temperature. When the reaction was over (controlled by thin-layer chromatography in S_1) the catalyst was filtered of with suction through a layer of silica gel and charcoal, and washed with saturated methanolic ammonia solution. The filtrate was evaporated and the residue crystallised from an ethanol–ether–light petroleum mixture to give 0·65 g (72%) of product melting at 128 to 134°C, suitable for further work. Crystallisation from ethanol (with charcoal) and further recrystallisation from water gave amino derivative *VI*, m.p. 133—134°C, $[\alpha]_D$ −68° (*c* 0·88; methanol). IR spectrum: broad band at 3400 cm^{-1} ν(NH_2), 3560 cm^{-1} ν(OH). For $C_{13}H_{17}NO_4$ (251·3) calculated: 62·14% C, 6·81% H, 5·57% N; found: 62·02% C, 6·86% H, 5·69% N.

3-Acetamido-2-O-acetyl-1,6-anhydro-4-O-benzyl-3-deoxy-β-D-glucopyranose (*VII*)

To a solution of 0·4 g of amino derivative *VI* in 3 ml of pyridine 1·2 ml of acetic anhydride was added and the mixture was allowed to stand at room temperature for 6 hours. Water (1 ml) was then added and the mixture evaporated. The remaining syrup, when crystallised from an acetone–ether–light petroleum mixture, gave 0·43 g (81%) of acetate *VII*, m.p. 117·5—119°C, $[\alpha]_D$ +19° (*c* 0·87; chloroform). IR spectrum: 3450, 1685 cm^{-1} ν(NHC=O), 1750 cm^{-1} ν(C=O). For $C_{17}H_{21}NO_6$ (335·3) calculated: 60·89% C, 6·31% H, 4·17% N; found: 60·83% C, 6·39% H, 4·40% N.

3-Acetamido-2-O-acetyl-1,6-anhydro-3-deoxy-β-D-glucopyranose (*VIII*)

To a solution of 0·3 g of diacetate *VII* in 10 ml of ethanol 0·15 g of 10% palladium on charcoal were added and the mixture was hydrogenated at normal pressure and room temperature for 11 hours (the reaction course was controlled by thin-layer chromatography in S_2). When the reaction was over the catalyst was filtered off and the ethanolic filtrate evaporated to dryness. Crystallisation of the residue from ethanol gave 0·2 g (87%) of product *VIII*, m.p. 168—169·5°C (sublimates about 156°C), $[\alpha]_D$ −45° (*c* 0·88; chloroform). IR spectrum: 1685, 3450 cm^{-1} ν(NHC=O), 1750 cm^{-1} ν(C=O), 3590 cm^{-1} ν(OH). For $C_{10}H_{15}NO_6$ (245·2) calculated: 48·98% C, 6·16% H, 5·71% N; found: 49·09% C, 6·20% H, 5·70% N.

1,6-Anhydro-3-azido-4-O-benzyl-3-deoxy-2-O-methanesulfonyl-β-D-glucopyranose (*IX*)

To a solution of 3·0 g of azide *III* in 40 ml of pyridine 7·2 ml of methanesulfonyl chloride were added at the temperature below 0°C under cooling with acetone-dry ice mixture. After 14 hours' standing at 5°C the mixture was diluted with 200 ml of water under cooling with ice. The precipitated ester *IX* was filtered off and crystallised from ethanol: yield 2·6 g (68%), m.p. 98–99°C, $[\alpha]_D$ +5·5° (*c* 1·7; chloroform). IR spectrum: 2130 cm^{-1} $\nu(N_3)$, 1180, doublet at 1360 cm^{-1} $\nu(SO_2)$. For $C_{14}H_{17}N_3O_6S$ (355·4) calculated: 47·31% C, 4·82% H, 11·82% N, 9·02% S; found: 47·46% C, 4·84% H, 11·93% N, 9·27% S.

1,6-Anhydro-3-azido-4-O-benzyl-3-deoxy-2-O-*p*-toluenesulfonyl-β-D-glucopyranose (*X*)

To a solution of 4·0 g of azide *III* in 15 ml of pyridine 4 g of *p*-toluenesulfonyl chloride were added in several portions under cooling with ice. After 17 hours standing at room temperature the reaction mixture was diluted with 50 ml of water under cooling with ice. The separated ester *X* was filtered off under suction and crystallised from ethanol; yield 5·4 g (87%), m.p. 74–76°C, $[\alpha]_D$ −25·5° (*c* 0·83; chloroform). IR spectrum: 2120 cm^{-1} $\nu(N_3)$, 1180, 1200, 1380 cm^{-1} $\nu(SO_2)$. For $C_{20}H_{21}N_3O_6S$ (431·5) calculated: 55·67% C, 4·91% H, 9·47% N, 7·43% S; found: 55·74% C 4·92% H, 9·70% N, 7·52% S.

3-Amino-1,6-anhydro-4-O-benzyl-3-deoxy-2-O-methanesulfonyl-β-D-glucopyranose (*XI*)

A) To a solution of 0·35 g of ester *IX* in ethanol (4 ml) 0·36 g of 80% hydrazine hydrate and about 0·3 ml of a Raney nickel suspension[57] in ethanol were added and the mixture was refluxed. After 15 minutes when the lively evolution of gases subsided the catalyst was filtered while hot. After cooling the product crystallised out and was crystallised from ethanol to give 0·18 g (55%) of amino derivative *XI*, m.p. 119·5–120·5°C, $[\alpha]_D$ −38° (*c* 0·73; chloroform). IR spectrum: broad band at 3400 cm^{-1} $\nu(NH_2)$, 1180, doublet at 1360 cm^{-1} $\nu(SO_2)$. For $C_{14}H_{19}NO_6S$ (329·4) calculated: 51·05% C, 5·81% H, 4·25% N, 9·73% S; found: 51·17% C, 5·87% H, 3·91% N, 9·66% S.

When this experiment was repeated with 3·0 g of ester *IX* and freshly prepared Raney nickel the reaction could be terminated only after several additions of further hydrazine hydrate. Chromatography on thin layer in S_1 demonstrated a spot with an R_F value higher than that of the starting compound *IX*. Using column chromatography on silica gel with S_1 as eluent 1·11 g (40%) of amino derivative *XI* and 0·3 g (16%) of the by-product *XXIII* were isolated. The latter had m.p. 55–56°C and $[\alpha]_D$ +154° (*c* 0·5; chloroform). According to IR spectra the product *XXIII* was identical with an authentic sample of m.p. 56–57°C, $[\alpha]_D$ +155° (*c* 0·5, chloroform), prepared in a different manner[25].

B) To a solution of 1·0 g of ester *IX* in 20 ml of acetone 0·7 g of zinc dust (200 mesh) were added, followed by dropwise addition of 8 ml of 17% hydrochloric acid. After dissolution of zinc the mixture was evaporated and 12% aqueous ammonia was added to the syrupy residue until zinc hydroxide was dissolved completely. The separated product was filtered off, the filtrate extracted with 50 ml of chloroform and the extract dried over calcium chloride and evaporated to dryness. Both fractions were combined and crystallised from ethanol to afford 0·75 g (80%) of amino derivative *XI*, m.p. 115–120°C. After recrystallisation from ethanol the product was identical in all its physical constants with amino derivative *XI* prepared by procedure *A*).

3-Amino-1,6-anhydro-4-O-benzyl-3-deoxy-2-O-*p*-toluenesulfonyl-β-D-glucopyranose (*XII*)

A solution of 4·0 g of ester *X* in 80 ml of ethanol was hydrogenated in the presence of 2 g of 5% palladium on charcoal at normal pressure and room temperature. The course of the reduction was followed chromatographically on a thin layer, using S_1 as eluent (R_F of the ester 0·80, R_F of the product 0·14). After 23 hours, when the reaction was over, the catalyst was filtered off and the ethanolic solution evaporated to a syrupy consistence. Its crystallisation from a mixture of ether and light petroleum gave amino derivative *XII* in a 79% yield (2·95 g), m.p. 70–72°C; after recrystallisation the m.p. rose to 72–74°C, $[\alpha]_D$ −32° (*c* 1·67; chloroform). IR spectrum: broad band at 3400 cm^{-1} $\nu(NH_2)$, 1180, 1200 and 1380 cm^{-1} $\nu(SO_2)$. For $C_{20}H_{23}NO_6S$ (405·5) calculated: 59·24% C, 5·72% H, 3·45% N, 7·91% S; found: 59·23% C, 5·73% H, 3·52% N, 7·78% S.

1,6-Anhydro-3-benzamido-4-O-benzyl-3-deoxy-2-O-methanesulfonyl-β-D-glucopyranose (*XIII*)

To a solution of 0·3 g of amino derivative *XI* in 3·5 ml of pyridine 0·5 ml of benzoyl chloride were added dropwise at 0°C under cooling and stirring. After two hours' standing the mixture was diluted with 5 ml of a saturated sodium hydrogen carbonate solution and 10 ml of water under cooling with ice. The separated product was crystallised from ethanol; yield 0·29 g (74%), m.p. 181–183°C, $[\alpha]_D$ +8·5° (*c* 0·43; chloroform). IR spectrum: 3460 cm^{-1} $\nu(NH)$, 1670 cm^{-1} $\nu(NHC{=}O)$. For $C_{21}H_{23}NO_7S$ (433·5) calculated: 58·18% C, 5·35% H, 3·25% N, 7·40% S; found: 58·40% C, 5·37% H, 3·24% N, 7·80% S.

1,6-Anhydro-3-benzamido-4-O-benzyl-3-deoxy-2-O-*p*-toluenesulfonyl-β-D-glucopyranose (*XIV*)

Amino derivative *XII* (0·2 g) was converted to amide *XIV* on reaction with 0·1 ml of benzoyl chloride in 2 ml of pyridine using the procedure described for compound *XIII*. Crystallisation of the crude product from acetone–ether gave 0·21 g (83%) of a substance of m.p. 177–179°C (under decomposition), $[\alpha]_D$ +12° (*c* 0·91; chloroform). On heating the substance twice changes its crystal modification, in the intervals 70–90°C and 126–160°C. IR spectrum: 3460 cm^{-1} $\nu(NH)$, 1670, 1720 cm^{-1} ($\nu NHC{=}O$), 1180, 1200 and 1370 cm^{-1} $\nu(SO_2)$. For $C_{27}H_{27}NO_7S$ (509·6) calculated: 63·64% C, 5·34% H, 2·75% N, 6·29% S; found: 63·64% C, 5·55% H, 2·72% N, 6·47% S.

1,6-Anhydro-4-O-benzyl-3-deoxy-2-O-methanesulfonyl-3-*p*-nitrobenzamido-β-D-glucopyranose (*XV*)

To a solution of 1·35 g of amino derivative *XI* in 16 ml of pyridine *p*-nitrobenzoyl chloride (1·6 g) was added under stirring. After three hours' standing at room temperature 2 ml of water were added and the mixture evaporated almost to dryness. The residue was additioned with a saturated sodium hydrogen carbonate solution until alkaline and approximately 50 ml of water. The precipitated product was filtered off under suction and crystallised from acetone–light petroleum. Yield 1·25 g (64%), m.p. 205–207°C (under decomposition), $[\alpha]_D$ −45° (*c* 0·53; acetone). For $C_{21}H_{22}N_2O_9S$ (478·5) calculated: 52·71% C, 4·63% H, 5·69% N, 6·70% S; found: 52·39% C, 4·67% H, 5·87% N, 6·97% S.

1,6-Anhydro-4-O-benzyl-3-deoxy-3-*p*-nitrobenzamido-2-O-*p*-toluenesulfonyl-β-D-glucopyranose (*XVI*)

Amino derivative *XII* (2·2 g) was acylated in 15 ml of pyridine with 1·3 g of *p*-nitrobenzoyl chloride in a manner similar to that described for the preparation of amide *XV*. Crystallisation from a mix-

ture of acetone and light petroleum gave 2·65 g (88%) of amide *XVI*, m.p. 200—202°C (under decomposition), $[\alpha]_D$ −48° (*c* 1·0; acetone). For $C_{27}H_{26}N_2O_9S$ (554·6) calculated: 58·47% C, 4·73% H, 5·05% N, 5·78% S; found: 58·65% C, 4·75% H, 5·14% N, 5·67% S.

1,6-Anhydro-4-O-benzyl-2,3-dideoxy-2,3-epimino-β-D-mannopyranose (*XVII*)

A) To a solution of 0·5 g of sodium in 30 ml of 2-propanol 0·2 g of amide *XIII* were added and the mixture was refluxed for 30 minutes. Thin-layer chromatography in S_1 demonstrated the presence of compounds of R_F 0·75 and 0·14 in the reaction mixture. It was evaporated and the residue triturated with 20 ml of water and the suspension extracted with three 20 ml portions of chloroform. The combined extracts were dried over calcium chloride and evaporated. Chromatography on a silica gel column in S_1 gave oxazoline derivative *XXI* in a 30 mg yield (19%) which was crystallised from a mixture of cyclohexane and ether; m.p. 161·5—163°C, $[\alpha]_D$ −24° (*c* 0·42; chloroform). IR spectrum: 1 660 cm^{-1} ν(N=C). For $C_{20}H_{19}NO_4$ (337·4) calculated: 71·20% C, 5·68% H, 4·15% N; found: 71·47% C, 5·71% H, 4·00% N. After the oxazoline derivative *XXI* epimine *XVII* was eluted from the column with chloroform–ethanol (1 : 1) in a 70 mg yield (65%). Crystallization from ether gave 53 mg (49%) of a product, m.p. 98—99°C, $[\alpha]_D$ −45° (*c* 0·82; chloroform). IR spectrum: 3 330 cm^{-1} ν(NH), the bands for ν(C=O) and $\nu(SO_2)$ are absent. For $C_{13}H_{15}NO_3$ (233·4) calculated: 66·93% C, 6·48% H, 6·01% N; found: 66·79% C, 6·70% H, 5·94% N.

B) To a solution of 0·25 g of sodium in 20 ml of 2-propanol and 60 ml of dioxan 2·0 g of amide *XVI* were added and the mixture was refluxed for one hour. After filtration the filtrate was evaporated and the residue treated with 20 ml of water. The mixture was extracted thrice with 20 ml dichloromethane and the combined extracts dried over calcium chloride and evaporated to a syrupy consistence. Its crystallisation from an ethanol–ether–light petroleum mixture afforded 0·65 g (78%) of 4-O-benzylepimine *XVII* which had identical physical constants with the product prepared under *A*).

C) Amide *XV* (0·5 g) was converted to 4-O-benzylepimine *XVII* on reaction with 0·1 g of sodium in 5 ml of 2-propanol and 15 ml of dioxan carried out under reflux 30 minutes (analogy to procedure *B*). The mixture was filtered and the filtrate evaporated and the residue was extracted three times with 10 ml of warm benzene. The benzene extracts were evaporated and crystallised from ether; yield 0·22 g (90%).

1,6-Anhydro-2,3-dideoxy-2,3-epimino-β-D-mannopyranose (*XVIII*)

To a solution of 0·3 g of benzylepimine *XVII* in 2·5 ml of ethanol and 4 ml of 5% ethanolic hydrogen chloride solution 0·2 g of 10% palladium on charcoal were added and the mixture was hydrogenolysed at normal pressure and room temperature. The reaction course was controlled by thin-layer chromatography in S_3 (benzylepimine *XVII* had R_F 0·9, epimine *XVIII* R_F 0·4). After 48 hours the reaction was over and the catalyst was filtered off and the ethanolic solution freed from hydrogen chloride by filtering through a column of 6·4 g of Amberlite IRA-400 (100—200 mesh, in OH^- cycle). Epimine *XVIII* was washed out with 35 ml of ethanol and the solution evaporated to a syrup which on crystallisation from a mixture of ethanol and ether afforded 106 mg (58%) of prisms, m.p. 102—103°C, $[\alpha]_D$ −67° (*c* 0·78; water). IR spectrum: 3 330 cm^{-1} ν(NH), 3 590 cm^{-1} ν(OH). Paper chromatography in S_4: epimine *XVIII* R_F 0·40, dianhydro derivative *XXIV* R_F 0·58. Concentration of the mother liquors and crystallisation from ethanol–ether–light petroleum gave an additional 28 mg of epimine *XVIII*; total yield 134 mg (73%). For $C_6H_9NO_3$ (143·1) calculated: 50·34% C, 6·34% H, 9·79% N; found: 50·62% C,

6·47% H, 9·79% N. Free epimine *XVIII* is unstable on heating above 50°C, either in solution or neat. By thin-layer chromatography in chloroform–methanol 9 : 1 the formation of a substance of R_F 0·42 was demonstrated (compound *XVIII* has R_F 0·17), as well as of at least another one on the start.

1,6-Anhydro-2,3-benzoylepimino-4-O-benzyl-2,3-dideoxy-β-D-mannopyranose (*XIX*)

To a solution of 0·18 g of benzylepimine *XVII* in 1 ml of pyridine 0·09 ml of benzoyl chloride were added at 0°C and the mixture was allowed to stand at room temperature for one hour. After dilution of the mixture with 3 ml of an aqueous 6% sodium hydrogen carbonate solution and one day's standing in a refrigerator the product crystallised out; it was collected on a filter, washed with water and recrystallised from ethanol. Yield 0·2 g (76%) of benzoyl derivative *XIX*, m.p. 126 to 127°C, $[\alpha]_D$ +33° (*c* 0·72; chloroform). IR spectrum: 1 690 cm^{-1} ν(NC=O), absence of the ν(NH) band. For $C_{20}H_{19}NO_4$ (337·4) calculated: 71·18% C, 5·68% H, 4·15% N; found: 71·11% C, 5·65% H, 4·24% N.

1,6-Anhydro-4-O-benzoyl-2,3-benzoylepimino-2,3-dideoxy-β-D-mannopyranose (*XX*)

An ethanolic solution of epimine *XVIII*, prepared by catalytic debenzylation of 0·1 g of benzylepimine *XVII* (see above), was evaporated without previous elimination of hydrogen chloride. The residue was dissolved in 1 ml of pyridine and additioned with 0·12 ml of benzoyl chloride under cooling with ice. The mixture was allowed to stand at room temperature overnight, diluted with 6% $NaHCO_3$ to pH 7—8 and evaporated. The residue was mixed with 10 ml of water and the mixture extracted with three 5 ml portions of chloroform. The combined extracts were dried over calcium chloride, evaporated and the residue crystallised from a mixture of ethanol, ether and light petroleum. Yield 105 mg (70%) of benzoyl derivative *XX*, m.p. 126—127°C (change of crystal modification in the 107—110°C interval), $[\alpha]_D$ −70° (*c* 0·91; chloroform). IR spectrum: 1 695 cm^{-1} ν(NC=O), 1 730 cm^{-1} ν(OC=O). For $C_{20}H_{17}NO_5$ (351·3) calculated: 68·37% C, 4·88% H, 3·99% N; found: 68·44% C, 4·90% H, 4·13% N.

Hydrochloride of 2-Amino-1,6-anhydro-2-deoxy-β-D-mannopyranose (*XXVII*)

A solution of 50 mg of epimine *XVIII* in 1 ml of a 5% KOH solution was bubbled through with nitrogen and heated in a sealed tube at 90—110°C for 6 hours. After this period the presence of a small amount of starting epimine *XVIII* (R_F 0·4) was detected in the reaction mixture by thin-layer chromatography in S_3, as well as the presence of an unidentified substance of R_F 0·5 and the main component of the mixture of R_F 0·15. The mixture was evaporated with 0·25 g of silica gel and chromatographed on silica gel with S_2 that eluted the starting epimine *XVIII* and the substance of R_F 0·5. The main product with R_F 0·15 was eluted with a mixture of ethanol and conc. ammonia (4 : 1), the eluate was evaporated and the residue dissolved in 5% ethanolic hydrogen chloride. The solution was filtered with charcoal and evaporated. Crystallisation from a mixture of ethanol and moist acetone afforded 24 mg (32%) of hydrate of 2-aminomannosan hydrochloride *XXVII*, m.p. 200—203°C (under decomposition; at 105—120°C loss of crystal water takes place), $[\alpha]_D$ −98° (*c* 0·47; water). PMR spectrum, see Table II. For $C_6H_{12}NO_4Cl.H_2O$ (215·6) calculated: 33·42% C, 6·54% H, 6·50% N; found: 33·68% C, 6·60% H, 6·89% N. Gas chromatography: 1,6-anhydro derivatives were silylated in pyridine according to ref.[58]. After evaporation of the solutions the silyl derivatives were dissolved in chloroform and chromatographed at 160°C (nitrogen flow 40 ml/min; rel. retention time for 2-aminomannosan *XXVII* was 0·70, for 3-aminoglucosan *IV* 0·73, and for 1,6-anhydro-β-D-glucopyranose 1·00 (retention time 810 s).

Acid hydrolysis: A solution of 6 mg of 2-aminomannosan *XXVII* in 0·5 ml of 6M-HCl was heated in a sealed tube at 100°C for 18 hours, then filtered with charcoal and evaporated. A part of the residue was dissolved in water and applied onto a sheet of chromatographic paper Whatman No 4 parallelly with authentic samples of hydrochlorides of 2-amino-2-deoxy derivatives of D-altrose, D-galactose, D-glucose, and D-mannose; for development system S_5 was used and for detection ninhydrin. 2-Amino-2-deoxy-D-mannose obtained on hydrolysis of 2-aminomannosan *XXVII* had identical R_F value as the authentic sample. Another part of the residue after the hydrolysis of 2-aminomannosan *XXVII* was converted in the described manner[58] to its silyl derivative by reaction with 0·1 ml of hexamethyldisilazane and 0·05 ml of trimethylchlorosilane in 0·5 ml of pyridine. After evaporation of pyridine the residue was dissolved in dichloromethane and chromatographed parallelly with standards of silyl derivatives of hexosamines on a gas chromatography column at 210°C (nitrogen flow 32 ml/min); relative retention time for D-glucosamine was 1·00 (ret. time 312 s), for D-altrosamine 0·50, and for D-mannosamine 0·85 and 1·00 (a weak band).

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REFERENCES

1. Ali Y., Richardson A. C., Gibbs C. F., Hough L.: Carbohydrate Res. *7*, 255 (1968).
2. Guthrie R. D., Murphy D.: J. Chem. Soc. *1965*, 3828.
3. Buss D. H., Hough L., Richardson A. C.: J. Chem. Soc. *1965*, 2736.
4. Guthrie R. D., King D.: Carbohydrate Res. *3*, 128 (1966).
5. El Shafei Z. M., Guthrie R. D.: J. Chem. Soc. C *1970*, 843.
6. Deyrup J. A., Moyer C. L.: J. Org. Chem. *35*, 3424 (1970).
7. Lake W. H. G., Peat S.: J. Chem. Soc. *1939*, 1069.
8. Buchanan J. G., Schwarz J. C. P.: J. Chem. Soc. *1962*, 4770.
9. Payne G. B.: J. Org. Chem. *27*, 3819 (1962).
10. Černý M., Pacák J., Staněk J.: This Journal *30*, 1151 (1965).
11. Williams N. R.: Advan. Carbohydrate Chem. *25*, 109 (1970).
12. Newth F. H.: J. Chem. Soc. *1956*, 441.
13. Černý M., Trnka T., Beran P., Pacák J.: This Journal *34*, 3377 (1969).
14. Jarý J., Čapek K.: Methods Carbohyd. Chem. *VI*, 238 (1972).
15. Černý M., Juláková O., Pacák J.: This Journal *39*, 1391 (1974).
16. Guthrie R. D., Murphy D.: J. Chem. Soc. *1963*, 5288.
17. Guthrie R. D., Prior A. M., Creasey S. E.: J. Chem. Soc. C *1970*, 1961.
18. Ali Y., Richardson A. C.: J. Chem. Soc. C *1968*, 1764.
19. Hofmann K. A., Hock H., Kirmreuther H.: Ann. *380*, 131 (1911).
20. Wenker H.: J. Am. Chem. Soc. *57*, 2328 (1935).
21. Goodman L.: Advan. Carbohydrate Chem. *22*, 109 (1967).
22. Rhoads W. D., Gross P. H.: Carbohydrate Res. *11*, 561 (1969).
23. Sheradsky T. in the book: *The Chemistry of the Azido Group*, (S. Patai, Ed.) p. 331, Interscience — Wiley, New York 1971.
24. Kirk D. N., Wilson M. A.: Chem. Commun. *1970*, 64.

25. Pecka J., Černý M.: This Journal *38*, 132 (1973).
26. Ponsold K.: Chem. Ber. *97*, 3524 (1964).
27. Guthrie R. D., Wells R. D.: Carbohydrate Res. *24*, 11 (1972).
28. Taguchi T., Kojima M.: J. Am. Chem. Soc. *81*, 4316 (1959).
29. Meyer zu Reckendorf W., Bonner W. A.: Chem. Ber. *95*, 1917 (1962).
30. Cleophax J., Gero S. D., Hildesheim J., Sepulchre A. M., Guthrie R. D., Smith C. W.: J. Chem. Soc. C *1970*, 1385.
31. Buss D. H., Hough L., Richardson A. C.: J. Chem. Soc. *1963*, 5295.
32. Spell H. L.: Anal. Chem. *39*, 185 (1967).
33. Buděšínský M., Černý M.: Unpublished results.
34. Trnka T., Černý M.: This Journal *36*, 2216 (1971).
35. Heyns K., Weyer J.: Ann. *718*, 224 (1968).
36. Meyer zu Reckendorf W.: Chem. Ber. *97*, 325 (1964).
37. Trnka T., Černý M.: This Journal *37*, 3632 (1972).
38. van Tamelen E. E., Dyer J. R., Carter H. E., Pierce J. V., Daniels E. E.: J. Am. Chem. Soc. *78*, 4817 (1956).
39. Richardson A. C., Fischer H. O. L.: J. Am. Chem. Soc. *83*, 1132 (1961).
40. Jeanloz R. W., Rapin A. M. C.: J. Org. Chem. *28*, 2978 (1963).
41. James S. P., Smith F., Stacey M., Wiggins L. F.: J. Chem. Soc. *1946*, 625.
42. Pratt J. W., Richtmyer N. K.: J. Am. Chem. Soc. *77*, 1906 (1955).
43. Richtmyer N. K., Hudson C. S.: J. Am. Soc. *62*, 961 (1940).
44. Karrer P., Smirnoff A. P.: Helv. Chim. Acta *4*, 817 (1921).
45. Knauf A. E., Hann R. M., Hudson C. S.: J. Am. Chem. Soc. *63*, 1447 (1941).
46. Stewart L. C., Richtmyer N. K.: J. Am. Chem. Soc. *77*, 1021 (1955).
47. Sorkin E., Reichstein T.: Helv. Chim. Acta *28*, 1 (1945).
48. Micheel F.: Ber. *62*, 687 (1929).
49. Heyns K., Weyer J., Paulsen H.: Chem. Ber. *100*, 2317 (1967).
50. Horton D., Jewell J. S.: Carbohydrate Res. *5*, 149 (1967).
51. Foster A. B., Stacey M., Vardheim S. V.: Acta Chem. Scand. *12*, 1605 (1958).
52. Micheel F., Michaelis E.: Chem. Ber. *96*, 1959 (1963).
53. Kuhn R., Bister W.: Ann. *617*, 92 (1958).
54. Wiggins L. F.: J. Chem. Soc. *1947*, 18.
55. Heyns K., Weyer J., Paulsen H.: Chem. Ber. *98*, 327 (1965).
56. Chatterjee A. K., Horton D., Jewell J. S., Philips K. D.: Carbohydrate Res. *7*, 173 (1968).
57. Dominguez X. A., Lopez I. C., Franco R.: J. Org. Chem. *26*, 1625 (1961).
58. Sweeley C. C., Bentley R., Makita M., Wells W. W.: J. Am. Chem. Soc. *85*, 2497 (1963).

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