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DIAMINOSUGARS—IV[1]

THE SYNTHESIS OF 2,6-DIAMINO-2,6-DIDEOXY-L-IDOSE[2]

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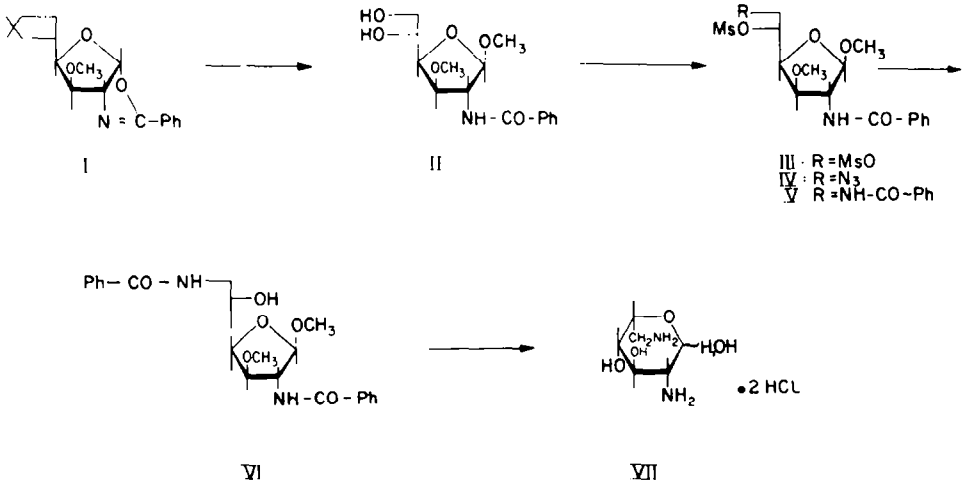
Abstract—The synthesis of the title compound could be achieved by inversion of C-5 in a suitably substituted derivative of 2,6-diamino-2,6-dideoxy-D-glucose (V). Purification of the product by preparative thin layer chromatography and its apparent conformational instability are being emphasized. Its identity with natural paromose has been established.

DURING the last years interest in diamino-dideoxy-hexoses has been growing rapidly and an increasing number of these sugars has been isolated from natural sources. Our attention was drawn particularly to compounds obtained by hydrolysis of metabolites of microorganisms among which are paromomycin[3] and the neomycins.[4] Two diamino-dideoxy-hexoses have been isolated from these antibiotics one of which (neosamine C) has been shown[5] to be 2,6-diamino-2,6-dideoxy-D-glucose and was synthesized in several laboratories.[6] The other diamino-dideoxy-hexose could be obtained from both paromomycin[7] and neomycin B[8] and was called paromose and neosamine B resp. Material from both sources has been compared, found to be identical,[9] and shown to be a 2,6-diamino-2,6-dideoxy-hexose.[7,8] The configuration of this sugar has been suggested to be that of L-idose[10] a proposal recently born out by degradation[11] of N,N'-diacetyl-paromose dithioacetals yielding a mixture of 5-acetamido-5-deoxy-L-xylofuranose and N-acetyl-L-xyloazapyranose. In the following we describe the synthesis of paromose (neosamine B).

L-Idose can be obtained from D-glucose by inversion of the configuration at C-5 in the latter[12] a principle which could successfully be applied to a suitably

[1] Part III: W. Meyer zu Reckendorf, *Angew. Chem.* **75**, 573 (1963); ibid., *Internat Edit.* **2**, 398 (1963).
[2] A preliminary report has been published; s. 1.
[3] Parke, Davis & Company, Belgian Patent 547 976 (Oct. 12, 1956)
[4] E. A. Swart, D. Hutchison and S. A. Waksman, *Arch. Biochem.* **24**, 92 (1949).
[5] H. Weidmann and H. K. Zimmerman, *Liebigs Ann.* **644**, 127 (1961).
[6] *a* H. Weidmann and H. K. Zimmerman, *Angew. Chem.* **72**, 750 (1960; *b* K. L. Rinehart, M. Hichens, K. Striegler, K. R. Rover, T. P. Culbertson, S. Tatsuoka, S. Horii, T. Yamaguchi, H. Hitomi and A. Miyake, *J. Amer. Chem. Soc.* **83**, 2964 (1961); *c* W. Meyer zu Reckendorf, *Chem. Ber.* **96**, 2017 (1963).
[7] Th. H. Haskell, J. C. French and Q. R. Bartz, *J. Amer. Chem. Soc.* **81**, 3481 (1959).
[8] K. L. Rinehart, A. D. Argoudelis, W. A. Goss, A. Sohler and C. P. Schaffner, *J. Amer. Chem. Soc.* **82**, 3938 (1960).
[9] K. L. Rinehart, W. S. Chilton, M. Hichens and W. von Phillipsborn, *J. Amer. Chem. Soc.* **84**, 3216 (1962).
[10] K. L. Rinehart, M. Hichens, A. D. Argoudelis, W. S. Chilton, H. E. Carter, M. P. Georgiadis, C. P. Schaffner and R. T. Schillings, *J. Amer. Chem. Soc.* **84**, 3218 (1962).
[11] Th. H. Haskell and S. Hanessian, *Abstr. 144th* Meeting. *Amer. Chem. Soc.* Los Angeles 1963, 19C.
[12] A. S. Meyer and T. Reichstein, *Helv. Chim. Acta* **29**, 152 (1946).

substituted derivative of 2-amino-2-deoxy-D-glucose. The blocked oxazoline I[13] on methanolysis with 0·0005 N hydrogen chloride in absol. methanol[14] yielded a mixture of two compounds as shown by thin layer chromatography. Recrystallization effected the purification of the main product (II) which was shown to be the desired furanoside by comparison with the analogous pyranoside synthesized in these Laboratories before.[15] A considerable amount of product collected from the mother liquors of II seemed to the impure methyl 2-benzamido-2-deoxy-5,6-O-isopropylidene-3-O-methyl-β-D-glucofuranoside which could be converted into II by treatment



I II III · R=MsO; IV · R=N_3; V R=NH-CO-Ph

VI VII

with hydrogen chloride in aqueous tetrahydrofuran.[14] Mesylation of II did not yield a crystalline product. Thin layer chromatography showed the presence of small amounts of byproducts one of which might have been identical with the corresponding pyranoside. The crude mesylate (III) was, therefore, transformed[6c] into the azide IV which could easily be purified by chromatography. Reduction and subsequent benzoylation yielded the benzamide V which should be capable of inversion reactions at C-5.

Heating of V with sodium methoxide in methanol produced a sirup consisting of two components as shown by thin layer chromatography none of which was identical with the oxazoline expected to be formed[16] by rearface attack at C-5 by the benzamide group at C-6. Heating with sodium benzoate in dimethylformamide[17] or sodium acetate in ethanol[18]—procedures developed by Baker for the displacement of sulfonyloxy groups by a neighboring amide function—yielded the same syrupy mixture acetylation of which afforded a product with an even more complex composition. We, therefore, subjected the crude material very smoothly produced by refluxing V with sodium acetate in 95% ethanol overnight and supposed to be

[13] R. Gigg and P. M. Carroll, *Nature, Lond.* **191,** 495 (1961).
[14] S. Konstas, I. Photaki and L. Zervas, *Chem. Ber.* **92,** 1288 (1959).
[15] W. Meyer zu Reckendorf, *Chem. Ber.* **96,** 2019 (1963).
[16] W. Meyer zu Reckendorf and W. A. Bonner, *Chem. Ber.* **95,** 1917 (1962).
[17] E. J. Reist, L. Goodman and B. R. Baker, *J. Amer. Chem. Soc.* **80,** 5775 (1958).
[18] B. R. Baker and R. E. Schaub, *J. Org. Chem.* **19,** 646 (1954).

mainly VI to the final deblocking procedure. Reaction with boron trichloride in dichloromethane[19] removed the 3-O-methyl group and yielded a syrup[20] which was directly hydrolyzed with 5 N hydrochloric acid to produce the free amino sugar (VII) contaminated with several ninhydrin-positive components. As VII could not be purified by crystallization we separated it from byproducts by preparative thin layer chromatography on cellulose. This method was found to be very convenient and superior to column chromatography. We obtained VII as an amorphous, hygroscopic, white powder with $[\alpha]_D^{24}$: $+17{\cdot}8°$ in close agreement with reported values.

For further characterization, VII was selectively N-acetylated[21] and the amorphous N,N′-diacetyl derivative reduced to the crystalline 2,6-diacetamido-2,6-dideoxy-L-iditol the rotation and m.p. of which agreed closely with values already published.[7] The amorphous N,N′-diacetyl derivative of VII was further transformed into the crystalline *p*-nitrophenyl-hydrazone[7] which was proved to be identical with an authentic sample[22] by comparison of m.p., IR spectra and the mixed m.p.

In their original publication[8] Rinehart *et al.* have characterized neosamine B and certain other products by the preparation of solid N,N′-bis-(2,4-dinitrophenyl) derivatives. Their procedure when applied to our product (VII) was found to be quite misleading. The purification step described did not seem to be effective at all for thin layer chromatography revealed the presence of at least eight components in the purified product besides traces of 2,4-dinitrophenol and 2,4-dinitrofluorobenzene. As no sample of the original preparation could be obtained for a direct comparison we subjected the mixture to preparative thin layer chromatography on silica gel. By repeated separations it was possible to obtain the four main components in pure form, designated as b, c, d, and e. Components b, c, and d have been available in amounts sufficient for characterization. They were proved by combustion analysis to be isomeric. In its m.p. behavior d seemed to be similar to the product described by Rinehart, however, the optical rotation found ($[\alpha]_D^{24}$: $-154{\cdot}8°$) differs considerably from the value given in the literature[8] ($[\alpha]_D^{26}$: $-44°$). The relationship in the yields of the single components varied and seemed to be dependent on the reaction conditions. However, this observation could not be followed further because of the limited supply of VII. Preliminary experiments showed that the main fractions c and d could be transformed into b and more polar products by boiling in organic solvents such as acetone and methanol. Short heating in 0·01 N methanolic sodium methoxide caused partial decomposition together with the formation of more polar components.

An explanation of these observations may be possible by examining the stereochemistry of 2,6-diamino-2,6-dideoxy-L-idose. Considerable steric strain caused by the axial $-CH_2NH_3^+$ group is to be expected if this sugar would occupy the Cl conformation of the pyranose form. If this conformation is being formed at all a rearrangement into the furanose form or the interaction of the amino group at C-6 with the anomeric center to form an amino acetal can easily be visualized. The latter

[19] T. G. Bonner, E. J. Bourne and S. McNally, *J. Chem. Soc.* 2929 (1960).

[20] Our results obtained in the pyranose series[15] indicated that the 1-O-methyl group is partly retained and probably anomerized during this step. No further purification of the product has been attempted at this stage.

[21] S. Roseman and J. Ludowieg, *J. Amer. Chem. Soc.* **76**, 301 (1954).

[22] Obtained from natural paromose and kindly furnished by Dr. S. Hanessian, Parke, Davis & Company.

formation of a 1,6-anhydro compound has made the isolation of pure L-idose impossible.[12] Furthermore a transformation of L-idose into L-sorbose has been observed.[23] At the present time no conclusive evidence as to the nature of the dinitrophenyl derivatives can be presented and the stereochemistry of L-idose[24] seems to be a promising field for further investigations.

EXPERIMENTAL

M.p.s have been determined in capillaries on a Thomas Hoover apparatus and are uncorrected. Thin layer chromatography has been carried out by the standard technique employing either Silica Gel GF (E. Merck, Germany) or cellulose powder MN 300 (Macherey & Nagel, Germany) as adsorbing materials, using the solvent systems indicated in each case. Spots were detected on silica gel with a spray containing 5% each of ammonium molybdate, sulfuric acid, and phosphoric acid in water, and on cellulose with ninhydrin or anilin phthalate. Column chromatography was carried out on neutral alumina (E. Merck, Germany). "Petroleum ether" means a hydrocarbon fraction with a boiling range from 30–60°.

Methyl 2-benzamido-2-deoxy-3-O-methyl-β-D-glucofuranoside (II). The oxazoline I[12] (20 g) was dissolved in 1 l. of 0.0005 N HCl in methanol and stored overnight. The solution was neutralized with silver carbonate, filtered through celite and evaporated *in vacuo* to give a syrup which on crystallization from ethyl acetate-pet ether yielded 11 g (56%) product. The collected mother liquors were evaporated to give 8 g of a syrup mainly consisting of II and its 5,6-O-isopropylidene derivative as found by thin layer chromatography on silica gel using ethyl acetate–10% methanol as the solvent. It was dissolved in 200 ml tetrahydrofuran, 200 ml water added and the mixture made acidic (0·0005 N) by addition of dil. HCl. After storage at room temp for 5 days the mixture was processed as described above yielding 5·6 g diol II, increasing the total yield to 85%. M.p. 124-125°, $[\alpha]_D^{26}$: −66·0° [*c*, 0·94; in methanol) (Found: C, 58·0; H, 6·8; N, 4·8. $C_{16}H_{21}NO_6$ requires: C, 57·9; H, 6·8; N, 4·5%).

Methyl 6-azido-2-benzamido-2,6-dideoxy-5-O-methanesulfonyl-3-O-methyl-β-D-glucofuranoside (IV). The diol (II; 1 g) was dissolved in pyridine (10 ml), the solution cooled to −80° and methanesulfonylchloride (1 ml) added. After storage at −5° over night the mixture was poured into ice and water and the product obtained as a syrup (0·8 g; 53%) by the usual extraction procedure with chloroform. The syrup was heated in dimethylsulfoxide (20 ml) containing sodium azide (0·8 g) at 100° for 1·5 hr and the crude product obtained in quantitative yield by precipitation with water. It was purified by chromatography on alumina of activity II in benzene and obtained by elution with the same solvent. Its purity was checked by thin layer chromatography on silica gel in ethyl acetate–40% pet ether. M.p. 103–105°, $[\alpha]_D^{26}$: −45·0° (*c*, 1·1; in chloroform) (Found: C, 46·5; H, 5·4; N, 13·7; S, 7·9. $C_{16}H_{22}N_4O_7S$ requires: C, 46·4; H, 5·4; N, 13·5; S. 7·7%).

Methyl 2,6-dibenzamido-2,6-dideoxy-5-O-methanesulfonyl-3-O-methyl-β-D-glucofuranoside (V). The azide (IV; 6 g) in methanol (300 ml) and 1% methanolic HCl (64 ml) was hydrogenated with 1 g 10% palladium on charcoal in a hydrogen atmosphere for 1 hr. After filtration through celite the solution was evaporated *in vacuo* to give a syrup which was dissolved in pyridine (60 ml). After the addition of benzoyl chloride (4·0 g) the mixture was stored at room temp over night, poured into ice and water and worked up by the usual extraction procedure with chloroform to give 5·0 g (71%) of the product after one recrystallization from 2-propanol. It was purified by chromatography on alumina of activity II in benzene and obtained by elution with a 1:1 mixture of benzene and ethyl acetate. The purity was checked by thin layer chromatography on silica gel in ethyl acetate–20% pet ether. M. p. 133–134°, $[\alpha]_D^{27}$: −45·5° (*c*, 1·1; in chloroform) (Found: C, 56·2; H, 5·9; N, 5·8; S, 6·6. $C_{23}H_{28}N_2O_8S$ requires: C, 56·1; H, 5·7; N, 5·7; S, 6·5%).

2,6-*Diamino-2,6-dideoxy-L-idose dihydrochloride* (VII). The benzamide (V; 2 g) was refluxed in 95% ethanol (100 ml) containing anhydrous sodium acetate (2 g) over night. After evaporation *in vacuo* water was added and the product obtained as a syrup by extraction with chloroform. Thin layer chromatography on silica gel in ethyl acetate-20% pet ether showed the presence of two components. The dry syrup was dissolved in dichloromethane (200 ml), cooled to −80° and boron trichloride (80 ml) added. After stirring at −80° for 30 min the mixture was stored at room temp over night, evaporated *in vacuo* and freed of boric acid by distillation with methanol. The residue was

[23] L. Vargha, *Chem. Ber.* **87**, 1351 (1954).

[24] R. Bentley, *J. Amer. Chem. Soc.* **82**, 2811 (1960).

refluxed with 5N HCl (150 ml) for 1·5 hr and the dark solution extracted with ether and treated with charcoal. After evaporation and drying by codistillation with absol. ethanol the product (VII) was obtained in quantitative yield as a brownish, hygroscopic powder. Thin layer chromatography on cellulose in a mixture of t.-butanol–acetic acid–water (2:2:1) revealed the presence of several ninhydrin positive components moving faster than the main spot. The product was dissolved in a 1:1 mixture of methanol and water and chromatographed on cellulose plates (20 × 20 cm) in the above solvent system. The plates were prepared by using a Desaga[25] applicator set for a thickness of 2 mm[26]. After the development (4–5 hours) a small guide strip was sprayed with ninhydrin, the layer containing the main component scratched off the plate and extracted with a 1:1 mixture of methanol and water. There was obtained 30–40 mg per plate of an almost pure product which was rechromatographed to give pure VII as a white, hygroscopic powder. No impurity could be detected when using the solvent mentioned above or the Fischer and Nebel mixture,[27] but due to the hygroscopic nature of VII it could not successfully be analyzed.[28] $[\alpha]_D^{24}$: +14·4° (after 5 min) → +17·8° (after 24 hr) (*c*, 1·18; in water). Lit.[7,8]: $[\alpha]_D$: +19°; +17·5° (in water).

2,6-*Diacetamido*-2,6-*dideoxy*-L-*iditol*. The pure hydrochloride VII (340 mg) was dissolved in water (20 ml) containing Amberlite IRA 400 (HCO_3^-; 20 ml) and acetic anhydride (310 mg) was added with stirring and cooling to 0°. Stirring was continued for 2 hr at 0°, the mixture filtered through Amberlite IR 120 (H^+; 10 ml) and the solution evaporated *in vacuo* to give a syrup (276 mg). This was dissolved in water (10 ml), the solution cooled to 0° and sodium borohydride (50 mg) added. After stirring at 0° for 2 hr acetic acid was added, the solution deionized by filtering through Amberlite MB 1 and evaporated *in vacuo* to give a syrup which crystallized from a mixture of ethanol and ethyl acetate. Yield 160 mg, m.p. 148–149° (after one recrystallization) (Lit.[7] 150·5–151·5°), $[\alpha]_D^{23}$: −19·2° (*c*, 0·52; in water) (Lit.[7]: $[\alpha]_D^{25}$: −17·8° (*c*, 4·0; in acetate buffer) (Found: C, 45·3; H, 7·7; N, 10·3. $C_{10}H_{20}N_2O_6$ requires: C, 45·5; H, 7·6; N, 10·6%.

2,6-*Diacetamido*-2,6-*dideoxy*-L-*idose* p-*nitrophenylhydrazone*. 2,6-Diacetamido-2,6-dideoxy-L-idose (160 mg; prepared from 180 mg of pure VII by the procedure given above) was refluxed with *p*-nitrophenylhydrazine (86 mg) in ethanol (8 ml) for 30 min after adding one drop of acetic acid. After cooling the product crystallized on seeding with an authentic sample[29] and was recrystallized from ethanol–ethyl acetate to give 128 mg (53%). M.p. and mixed m.p. with the authentic sample 221–222° (dec.), $[\alpha]_D^{25}$: −72·2° (*c*, 0·5; in 50% methanol). The authentic sample showed m.p. 226–227° and $[\alpha]_D$: +72·2° (*c*, 0·5; in 50% methanol)[29] (Found: C, 48·2; H, 5·9; N, 17·9. $C_{16}H_{23}N_5O_7$ requires: C, 48·4; H, 5·8; N, 17·6%.

N,N'-Bis-(2,4-*dinitrophenyl*) *derivatives of* VII. The hydrochloride VII (113 mg) was dissolved in 10 ml 80% ethanol, sodium bicarbonate (400 mg) and 2,4-dinitrofluorobenzene (200 mg) added and

	$[\alpha]_D^{20}$ in dimethylsulfoxide	*c*	m.p.	Found: C	H	N
b	−68·0°	0·25	208° (dec.)	42·7	3·1	16·0
c	−29·6°	0·27	205° (dec.)	42·2	3·7	16·0
d	−151·2° −154·8° (in methanol)	0·2 0·42	155–158°	42·0	3·2	16·2
Lit.[8]	−44° (in methanol)	0·31	155–158°			
$C_{18}H_{18}N_6O_{12}$ requires:				42·4	3·6	16·5

[25] C. Desaga, Heidelberg, Germany.

[26] The actual thickness was smaller due to the shrinking of the layer on drying.

[27] F. G. Fischer and H. J. Nebel, Hoppe-Seyler's *Z. physiol. Chem.* **302**, 10 (1955).

[28] R_f values will not be given as they are rather meaningless in thin layer chromatography.

[29] Communicated by Dr. S. Hanessian.

the mixture stirred vigorously over night. It was filtered from inorganic salts, evaporated *in vacuo* and dried by evaporation with absol. ethanol. The residue was dissolved in acetone, filtered, applied to 20 × 20 cm plates of silica gel (thickness 1 mm) and chromatographed in chloroform-10% methanol or chloroform –40% acetone. The separated components were rechromatographed and their purity checked on silica gel plates of 250μ thickness. The components shown in the table were obtained, given in the order of increasing R_f values.

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