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The Synthesis of D-Allosamine Hydrochloride[1]

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Sirupy D-allosamine hydrochloride has been synthesized from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranose and transformed into the crystalline N-acetyl and N-(2'-hydroxynaphthylidene) derivatives.

The increasing importance of glucosamine and galactosamine as components of biological systems and the recent isolations of new amino sugars from microörganisms[2] makes desirable the synthesis of 2-aminohexoses, to be used for reference or as biological substrates.

Fifty years ago, Fischer and Leuchs,[3] applied to D-arabinosimine the cyanohydrin method of lengthening the carbon chain and obtained impure D-glucosamine. In a series of papers, Levene later extended Fischer's work and prepared the eight possible 2-aminohexonic acids, but the yield was sometimes small and inconsistent.[4] Reduction of the aminohexonic acid generally gave poor yields and impure products. Only syntheses of D-glucosamine,[5] D-galactosamine[6] and D-mannosamine[7] were reported. A contaminated amino sugar was obtained from xylose. After treatment with phenylhydrazine it gave gulosazone, whereas benzoylation produced a crystalline pentabenzoate in small yield, not further identified.[5,8]

Recently catalytic hydrogenation of lactosazone led to the isolation of a 2-amino sugar in very minute amounts.[9]

The synthesis of 2-aminohexoses with the best yield takes place by addition of ammonia to 2,3-epoxy derivatives of hexoses.[10] However, only amino sugars with the 2,3-*trans* configuration are obtained and the final yield of the 2-amino isomer is controlled by the conformation of the molecule. Baker, *et al.*,[11] extending Winstein's work[12] on the influence of neighboring groups on the solvolysis of sulfonyl esters, have greatly increased the usefulness of the above described method. They were able to show, in the pentose series, that sulfonyl ester groups in transvicinal position to an acetylamino group can be split with concurrent Walden inversion, thus synthesizing 3-aminoribose from 3-aminoxylose. Application of the same method in the hexoses series to natural or synthetic 2-aminohexoses possessing *trans* configuration in positions 2,3 would make possible the preparation of the 2-aminohexoses possessing the 2,3-*cis* configuration. It is the aim of the present paper to describe this application to the naturally occurring D-glucosamine to obtain the new 2-amino-sugar, D-allosamine.

Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[13] treated with methanesulfonyl chloride in the presence of pyridine gave the 3-*O*-methylsulfonyl derivative II. When II was heated in the presence of sodium acetate in methyl Cellosolve solution, it yielded a small amount of I, and the crystalline methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-allopyranoside (III) characterized as its crystalline 3-monoacetate IV. Hydrolysis of III with 60% acetic acid afforded a crystalline methyl 2-acetamido-2-deoxy-α-D-allopyranoside (V), from which a crystalline 3,4,6-triacetate VI could be prepared. 2-Amino-2-deoxy-D-allose hydrochloride (D-allosamine hydrochloride) (IX) was obtained as a sirup by treatment of IV with hydrochloric acid. IX was characterized as two crystalline compounds, the 2-acetamido VII and the 2-(2'-hydroxynaphthylideneamino) (VIII) derivatives. Degradation with ninhydrin in presence of pyridine[14] gave rise to D-ribose (X). The latter compound, owing to lack of material, was identified by paper chromatography only. However, this additional evidence and the mode of preparation leaves little doubt as to the structure of the amino sugar.

Experimental[15]

Methyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methylsulfonyl-α-D-glucopyranoside (II).—To a solution of 11.5 g. of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[13] (I) in 50 ml. of anhydrous pyridine was added a solution of 6 ml. of methanesulfonyl chloride in 50 ml. of anhydrous pyridine, both solutions being previously cooled to 0°. After standing 8 days at 0°, ice was added, and after a few hr. the mixture was diluted with 2 l. of ice-cold water and left overnight in the refrigerator. The precipitate was filtered, washed well with ice-cold water and dried in a desiccator. After recrystallization from acetone, it yielded 13.0 g. (90%) of needles, m.p. 213–214°, with slight decomposition. The m.p. varied with the speed of heating. Very slow heating gave m.p. as low as 199–200°;

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(2) J. T. Parks, *J. Biol. Chem.*, **194**, 885 (1952); R. E. Strange and F. A. Dark, *Nature*, **177**, 186 (1956); E. E. Van Tamelen, J. R. Dyer, H. E. Carter, J. V. Pierce and E. E. Daniels, THIS JOURNAL, **78**, 4817 (1956).

(3) E. Fischer and H. Leuchs, *Ber.*, **36**, 24 (1903).

(4) P. A. Levene, *J. Biol. Chem.*, **36**, 73 (1918); P. A. Levene and E. P. Clark, *ibid.*, **46**, 19 (1921).

(5) P. A. Levene, *ibid.*, **26**, 155 (1916).

(6) P. A. Levene, *ibid.*, **31**, 609 (1917).

(7) P. A. Levene, *ibid.*, **39**, 69 (1919).

(8) P. A. Levene, *ibid.*, **36**, 73 (1918).

(9) R. Kuhn and W. Kirschenlohr, *Ber.*, **87**, 1547 (1954).

(10) W. N. Haworth, W. H. G. Lake and S. Peat, *J. Chem. Soc.*, 271 (1939); S. P. James, F. Smith, M. Stacey and L. F. Wiggins, *ibid.*, 625 (1946).

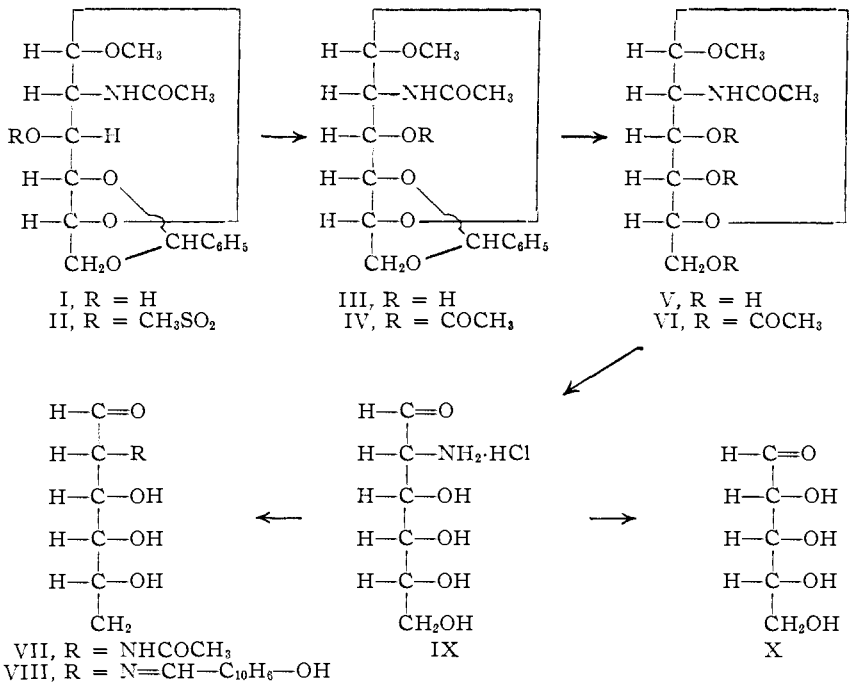
(11) B. R. Baker and R. E. Schaub, THIS JOURNAL, **75**, 3864 (1953); B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, *ibid.*, **76**, 4044 (1954).

(12) See Winstein, *et al.*, *ibid.*, **64**, 2796 (1942), and later publications.

(13) A. Neuberger, *J. Chem. Soc.*, 50 (1941).

(14) P. J. Stoffyn and R. W. Jeanloz, *Arch. Biochem. Biophys.*, **52**, 373 (1954).

(15) R. W. Jeanloz, THIS JOURNAL, **76**, 555 (1954); R. W. Jeanloz and D. A. Jeanloz, *ibid.*, **79**, 2579 (1957).



$[\alpha]^{24}D$ +42 ± 1° (in chloroform, *c* 1.33). *Anal.* Calcd. for $C_{17}H_{23}O_8NS$: C, 50.86; H, 5.77; S, 7.99. Found: C, 50.80; H, 5.92; S, 7.88.

Methyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-allopyranoside (III).—A solution of 450 mg. of II and 400 mg. of sodium acetate trihydrate in 10 ml. of 95% methyl cellosolve was heated under reflux for 40 hr. After cooling, the solution was extracted with 150 ml. of chloroform. The chloroform layer was washed three times with water and dried over sodium sulfate. After evaporation *in vacuo*, the residual crystalline mixture was dissolved in chloroform and chromatographed on 20 g. of silicic acid. Mixtures of chloroform and ether 4:1, 2:1, 1:1 and pure ether eluted fractions, which after crystallization from a mixture of acetone, ether and pentane gave 42 mg. (10%), m.p. 202–206° (with decomposition), which showed no depression in admixture with the starting material (II).

Mixtures of ether and ethyl acetate varying from 19:1 to 1:1 eluted fractions, which were crystallized from a mixture of acetone and pentane to give 236 mg. (66%) of fine needles III, m.p. 214–215°; $[\alpha]^{24}D$ +64 ± 2° (in chloroform, *c* 0.95). *Anal.* Calcd. for $C_{16}H_{21}O_6N$: C, 59.43; H, 6.55. Found: C, 59.31; H, 6.55. The test for S was negative.

Ethyl acetate eluted fractions weighing 50 mg. (14%) with a m.p. of 259–261° after crystallization from a mixture of chloroform, ether and pentane. When mixed with methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside,[13] the m.p. was not depressed.

The same reaction carried out with a tenfold amount gave an identical yield.

Acetylation of 35 mg. of III with acetic anhydride and pyridine in the usual manner gave, after crystallization from a mixture of acetone, ether and pentane, 28 mg. (70%) of the 3-*O*-acetyl derivative IV, as elongated prisms, m.p. 213–214°; $[\alpha]^{25}D$ +17 ± 3° (in chloroform, *c* 0.71). *Anal.* Calcd. for $C_{18}H_{23}O_7N$: C, 59.17; H, 6.34. Found: C, 59.13; H, 6.29. In admixture with methyl 2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (m.p. 203–205°),[16] the m.p. was depressed to 175–205°. In admixture with III, the m.p. was depressed to 180–195°.

Methyl 2-Acetamido-2-deoxy-α-D-allopyranoside (V).—A solution of 1.0 g. of III in 50 ml. of 60% acetic acid was heated for 1 hr. on a water-bath. The benzaldehyde and the water were removed by distillation *in vacuo*, followed by codistillation with water, then with absolute toluene. After standing overnight in a desiccator, the residual sirup was crystallized from a mixture of ethanol and ether to give 0.71 g. (96%) of stout prisms, m.p. 155–156°; $[\alpha]^{23}D$ +92 ± 1° (in methanol, *c* 1.25). *Anal.* Calcd. for $C_9H_{17}O_6N$: C, 45.95; H, 7.29. Found: C, 45.98; H, 7.14.

Acetylation of 0.36 g. of V with acetic anhydride and pyridine in the usual manner gave, after recrystallization from a mixture of acetone, ether and pentane 0.51 g. (92%) of the 3,4,6-tri-*O*-acetyl derivative VI as stout, hexagonal prisms, m.p. 107–109°; $[\alpha]^{24}D$ +68 ± 1° (in chloroform, *c* 2.21). *Anal.* Calcd. for $C_{15}H_{23}O_9N$: C, 49.86; H, 6.42. Found: C, 49.76; H, 6.35.

D-Allosamine Hydrochloride (2-Amino-2-deoxy-D-allose Hydrochloride (IX)).—A solution of 1.0 g. of V in 5 ml. of 2 *N* hydrochloric acid was heated on the water-bath for 2 hr. After evaporation *in vacuo*, the last traces of hydrochloric acid, water and acetic acid were removed by codistillation *in vacuo* with absolute ethanol and benzene and storage of the residual sirup in a desiccator over soda lime and calcium chloride. After dissolving in methanol, the solution was filtered through a double layer of Darco-G-60 and Celite and evaporated *in vacuo*. A colorless sirup (yield 100%) was obtained which did not crystallize after three years; $[\alpha]^{25}D$ +29 ± 2° (in water, *c* 1.35). *Anal.* Calcd. for $C_6H_{14}O_5NCl$: C, 33.26; H, 6.48; N, 6.50; Cl, 16.44. Found: C, 33.42; H, 6.67; N, 6.43; Cl, 16.48. A small amount, deposited on paper and treated with ninhydrin and pyridine as previously described,[14] gave a spot with an R_f identical to that of D-ribose (X).

2-Acetamido-2-deoxy-β-D-allose (VII).—To a solution of 110 mg. of IX in 1 ml. of methanol was added 83 mg. of silver acetate and 0.1 ml. of acetic anhydride. After standing one day at 0° and 5 hr. at room temperature, the mixture was heated to boiling, filtered through Celite, and the precipitate was washed with 1 ml. of hot water. One drop of 0.1 *N* hydrochloric acid was added to the filtrate. After 2 hr., the solution was filtered through a double layer of Darco G-60 and Celite and concentrated. Crystallization from a mixture of ethanol, acetone and ether gave 87 mg. (77%) of VII, m.p. 205–207° (starting at 190°) with slight decomposition. Starting from room temperature the m.p. was 201–203°. The compound showed mutarotation, from $[\alpha]^{24}D$ −57 (after 6 minutes) to −48 ± 2° (after 16 hr., in water, *c* 0.51). *Anal.* Calcd. for $C_8H_{15}O_6N$: C, 43.44; H, 6.83. Found: C, 43.57; H, 6.85.

2-Deoxy-2-(2′-hydroxynaphthylidenamino)-D-allose (VIII).—A solution of 55 mg. of IX and 30 mg. of sodium acetate trihydrate in 1 ml. of water was treated as previously described[17] with 110 mg. of 2-hydroxynaphthaldehyde in 10 ml. of methanol. Purification was effected by chromatography; the substance was eluted by a mixture of acetone and methanol 49:1. Crystallization from a mixture of pyridine, methanol and acetone gave 32 mg. (38%) of small yellow prisms, m.p. 199–200° (with decomposition above 185°); $[\alpha]^{25}_{5461}$ −80 ± 5° (at equilibrium, in methanol, *c* 0.17). *Anal.* Calcd. for $C_{17}H_{19}O_6N$: C, 61.26; H, 5.75. Found: C, 61.09; H, 5.88.

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(16) L. F. Wiggins, *J. Chem. Soc.*, 18 (1947).

(17) R. W. Jeanloz, THIS JOURNAL, **74**, 4597 (1952).