[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Synthesis of 2-Amino-2-deoxy-L-Arabinose (L-Arabinosamine)

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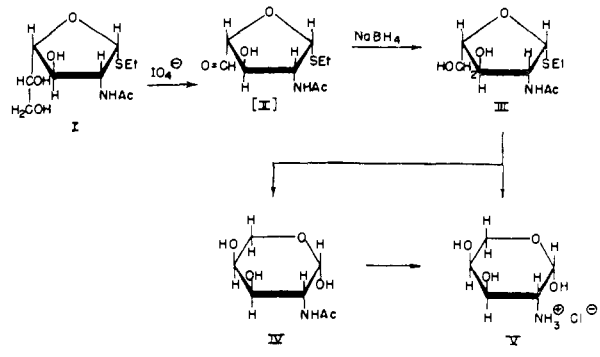
Oxidation of ethyl 2-acetamido-2-deoxy-1-thio-α-D-galactofuranoside (ethyl 2-acetamido-2-deoxy-1-thio-D-*glycero*-β-L-*arabino*-hexafuranoside) (I) with sodium metaperiodate, followed by reduction with sodium borohydride, produced ethy 2-acetamido-2-deoxy-1-thio-β-L-arabinofuranoside (III), which was hydrolyzed with aqueous mercuric chloride solution t 2-acetamido-2-deoxy-β-L-arabinose (IV) and with hydrochloric acid to 2-amino-2-deoxy-β-L-arabinose hydrochloride (V).

The amino pentoses have come into prominence as components of antibiotics[2,3] and of possible carcinolytic agents. The first pentosamine, 2-amino-2-deoxy-α-D-xylose hydrochloride, dec. 165–167°, $[\alpha]_D$ +80 → +40° (water), was synthesized by Wolfrom and Anno.[4] 3-Amino-3-deoxy derivatives[5–7] of D-ribose, D-xylose and D-arabinose have been synthesized. Three more 2-amino pentoses,[8] 2-amino-2-deoxy-α-L-ribose hydrochloride, m.p. 142–148° dec., $[\alpha]_D$ −15.6 → +6.7° (water), its enantiomorph, m.p. 144–149°, $[\alpha]_D$ +14.1 → −2.75° (water), and 2-amino-2-deoxy-α-D-lyxose hydrochloride, dec. 148–155°, $[\alpha]_D$ +54 → −36° (water), have been reported. Also, a 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose *p*-toluenesulfonate has been described.[8]

We wish to report herein the synthesis of 2-amino-2-deoxy-β-L-arabinose ("L-arabinosamine"), characterized as the hydrochloride V and the N-acetyl derivative IV. The synthesis was carried out by an extension of the method employed by Wolfrom and Anno[4] for the synthesis of 2-amino-2-deoxy-D-xylose.

Ethyl 2-acetamido-2-deoxy-1-thio-α-D-galactofuranoside (ethyl 2-acetamido-2-deoxy-1-thio-D-*glycero*-β-L-*arabino*-hexafuranoside) (I),[9] was oxidized in the dark at 0–3° with one mole of sodium metaperiodate. Under these conditions the molecule is cleaved very rapidly at the C5–C6 bond.[9] The oxidation was followed immediately by reduction with sodium borohydride to yield ethyl 2-acetamido-2-deoxy-1-thio-β-L-arabinofuranoside (III), m.p. 127–129°, $[\alpha]_D$ +172° (water). Hydrolysis of III with aqueous mercuric chloride produced 2-acetamido-2-deoxy-β-L-arabinose (IV), m.p. 154–156°, $[\alpha]_D$ +147.5 → +94.1° (water). It gave a positive Morgan–Elson[10] color test for N-acetylamino sugars. Hydrolysis of III and IV with hydrochloric acid provided 2-amino-2-deoxy-β-L-arabinose hydrochloride (V), m.p. 153–154° dec., $[\alpha]_D$ +174 → +115° (water). The hydrochloride was strongly reducing to Benedict solution and reacted positively to the Elson–Morgan[11] and ninhydrin[12] color tests. The Dische–Borenfreund[13] color reaction for amino sugars (amino group unsubstituted) showed a maximum absorption at 490 mμ.

The assignment of the L-arabinosamine structure to the pentosamine follows from the nature of the reaction and the established configuration of chondrosamine as 2-amino-2-deoxy-D-galactose.[14] Removal of the terminal asymmetric center in the α-D-galactoside I results in an L-arabinoside III with consequent change in anomeric designation from α-D to β-L without any change in the order of groups about C1.



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Experimental

Ethyl 2-Acetamido-2-deoxy-β-L-arabinofuranoside (III).—Ethyl 2-acetamido-2-deoxy-1-thio-α-D-galactofuranoside (ethyl 2-acetamido-2-deoxy-1-thio-D-*glycero*-β-L-*arabino*-hexofuranoside) (I)[9] (1.06 g., 4 millimoles) was dissolved in 21 ml. of water and treated with 4.1 millimoles of sodium metaperiodate in 19 ml. of water, at 0–3°, in the dark, for 30 min. The reaction mixture was passed through a column (180 × 13 mm., diam.) of Amberlite MB-3[15] and the column was washed with 100 ml. of water. The combined effluent and washings were concentrated under reduced pressure to 15 ml. To this solution was added dropwise, with stirring, a solution of 0.15 g. of sodium borohydride in 10 ml. of water, over a period of 5 min., at room temperature. After stirring for an additional 30 min., the solution was neutralized with *N* sulfuric acid, passed through a column (180 × 13 mm.) of Amberlite MB-3,[15] and the column was washed with 100 ml. of water. The effluent and washings were combined, concentrated to a sirup and further dried by repeated evaporation with ethanol under reduced pressure. The material was crystallized from alcohol–ether–petroleum ether;

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yield 0.75 g. (80%), m.p. 116–118° (preliminary softening). Pure material was obtained after several recrystallizations from ethanol–ether–petroleum ether; m.p. 127–129° (preliminary softening), $[\alpha]^{21}D$ +172° (*c* 0.5, water); X-ray powder diffraction data[16]: 12.8m, 8.29s(3), 6.97w, 6.42w, 4.88s(1), 4.33m. 4.13s(2), 3.93m, 3.31vw, 3.19w, 2.85vw, 2.26vw, 2.21vw, 2.14vw.

Anal. Calcd. for $C_9H_{17}NO_4S$: C, 45.91; H, 7.28; N, 5.95; S, 13.62. Found: C, 45.94; H, 7.20; N, 5.82; S, 13.41.

2-Acetamido-2-deoxy-β-L-arabinose (IV).—An aqueous solution of 693 mg. of mercuric chloride in 60 ml. of water, at 50°, was added to a solution of 300 mg. of ethyl 2-acetamido-2-deoxy-1-thio-β-L-arabinofuranose (III) in 15 ml. of water and allowed to stand at room temperature for 5 hr. The white precipitate which formed was removed by filtration. The filtrate was passed through a column (200 × 18 mm., diam.) of Amberlite MB-3[15] and the column was washed with 200 ml. of water. The effluent and washings were concentrated to a sirup which was further dried by evaporation repeatedly with ethanol under reduced pressure. The product crystallized from methanol–acetone–ether; yield 158 mg. (65%), m.p. 151–155° (preliminary softening). Pure material was obtained by recrystallization from the same solvents. The compound was strongly reducing and exhibited a positive Morgan–Elson[10] test for *N*-acetylamino sugars; m.p. 154–156° (preliminary softening), $[\alpha]^{24}D$ +147.5° (initial, extrapolated) → +94° (*c* 1, water, final); X-ray powder diffraction data[16]: 9.03m, 7.99s(2), 7.01w, 4.76s(1), 4.60m, 4.39m, 4.10s(3), 3.53s(2), 3.33vw, 3.15w, 3.03m, 2.82w, 2.66vw, 2.48vw, 2.40vw, 2.23vw, 2.18vw, 2.07vw.

Anal. Calcd. for $C_7H_{13}NO_5$: C, 43.98; H, 6.85; N, 7.33. Found: C, 44.04; H, 6.85; N, 7.39.

2-Amino-2-deoxy-β-L-arabinose Hydrochloride (V).—An amount of 88 mg. of 2-acetamido-2-deoxy-β-L-arabinose (IV) was heated with 2 ml. of 4 *N* hydrochloric acid in a boiling water-bath for 30 min. The hydrolyzate was concentrated to a sirup and further dried by evaporation with methanol–acetone under reduced pressure, after which treatment the sirup crystallized; yield 78 mg. (89%), m.p. 150–154° dec. Pure material was obtained upon recrystallization from methanol–acetone; m.p. 153–155° dec., $[\alpha]^{20}D$ +174 (initial, extrapolated) → 115° (*c* 0.5, water, final); X-ray powder diffraction data[16]: 7.67w, 6.20m, 5.20m, 4.72s(3), 4.11s(1), 3.83s(2), 3.36vw, 3.15w, 2.88w, 2.67vw, 2.50vw.

Anal. Calcd. for $C_5H_{12}ClNO_4$: C, 32.36; H, 6.52; N, 7.55; Cl, 19.10. Found: C, 32.59; H, 6.52; N, 7.46; Cl, 18.84.

The substance was strongly reducing and gave a strong positive reaction to the Elson–Morgan[11] and ninhydrin[12] color tests. In the Dische–Borenfreund test a maximum was developed at λ 490 mμ.[8,13] 2-Amino-2-deoxy-β-L-arabinose hydrochloride was obtained in the same way by the hydrolysis of ethyl 2-acetamido-2-deoxy-1-thio-β-L-arabinofuranoside with 4 *N* hydrochloric acid. The constants were identical with those reported above.

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(16) Interplanar spacing, Å., CuKα radiation. Relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. First three strongest lines are numbered, strongest (1), repeated numbers indicate approximate equality.

COMMUNICATIONS TO THE EDITOR

A STEREOSPECIFIC DIHYDROLIPOIC DEHYDROGENASE FROM *SPINACIA OLERACEA*

Sir:

The oxidation of α-keto acids takes place in a number of integrated steps of which the last two are mediated by dihydrolipoic transacetylase and dihydrolipoic dehydrogenase.[1] The purification and properties of the dihydrolipoic dehydrogenase from animals and microörganisms have been described.[2,3,4,5,6] Diaphorase was also found to have powerful dihydrolipoic dehydrogenase activity.[7] Recently the involvement of flavoprotein in the pyruvate oxidase complex of *E. coli*[8] and of diaphorase in α-ketoglutarate oxidase system of pig heart[9] have been demonstrated. In contrast to dihydrolipoic transacetylase, dihydrolipoic dehydrogenase does not exhibit optical specificity.[1,5]

A stereospecific dihydrolipoic dehydrogenase which has been purified 30–40-fold from the acetone powder of *Spinacia oleracea* is DPN-linked and displays maximum activity at *p*H 8.0. The rate of reaction with dihydrolipoamide is faster than that with dihydrolipoic acid (Table I), as observed by Sanadi and Searls.[4] The enzyme is active with (−)-dihydrolipoic acid (Table I). The slight activity observed with (+)-dihydrolipoic acid may be due to the contamination with the other isomer.

TABLE I

Substrate	DPNH formed in 3 min. (μM.)
(±)-Dihydrolipoic acid	0.044
(−)-Dihydrolipoic acid[a]	0.033
(+)-Dihydrolipoic acid[a]	0.005
(±)-Dihydrolipoamide[a]	0.084
Cysteine	0
Reduced glutathione	0

[a] Prepared from the oxidized compounds by the sodium borohydride reduction method of Wagner, *et al.*[10]

The incubation was carried out at 25° in 1.5 ml. containing 70 μM.TRIS (*p*H 8.0), 0.5 μM. DPN, 2 μM. of substrate and 30 μg. of dihydrolipoic dehydrogenase.

Though the reaction is freely reversible with lipoamide it has not been possible to demonstrate

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