However, when it was mixed with a sample of myristic acid, a m.p. depression was noted.

Isolation of Myristic Acid.—Approximately 0.7 g. of the ligroin-soluble fatty acid fraction was dissolved in 5 ml. of ethanol, the solution neutralized with *N* aqueous sodium hydroxide, 0.2 g. of *p*-bromophenacyl bromide added, and the mixture heated under refluxing conditions for one hour with the occasional addition of sufficient ethanol to prevent the separation of an oily phase. The oil which separated upon cooling was induced to crystallize by scratching and the solid was recrystallized twice from ethanol to give 0.064 g. of a product, m.p. 74–77°. This material was again recrystallized from ethanol to give the *p*-bromophenacyl ester of myristic acid, m.p. 77–78.5°, and which when mixed with an authentic sample caused no m.p. depression. When mixed with the corresponding derivative of palmitic acid, the m.p. was depressed.

Isolation of Palmitic Acid.—Short path fractional distillation of a 0.7-g. sample of the ligroin-soluble fatty acids, at 30 μ, gave a main fraction, b.p. 130–135°, from which palmitic acid, m.p. 62–63°, was obtained by recrystallization from aqueous acetone. A mixed m.p. with an authentic sample showed no depression.

Nature of the Acetone-soluble Fraction.—The acetone-soluble fraction resulting from the partial hydrolysis of the hemorrhagic agent, *vide ante*, was a dark semi-solid substance which appeared to consist largely of fatty acids. Recrystallization of this material from aqueous acetone gave palmitic acid, m.p. 60–62°.

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

D-Xylosamine[1]

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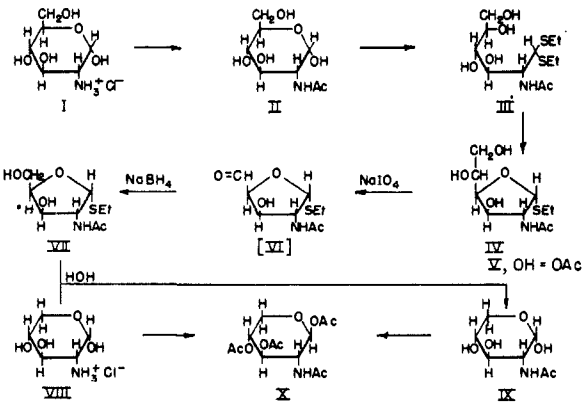
The first known pentosamine, 2-amino-2-deoxy-D-xylose or D-xylosamine, has been synthesized as the hydrochloride through glycol cleavage of ethyl 2-acetamido-2-deoxy-α-D-glucothiofuranoside (IV) with sodium metaperiodate followed by reduction with sodium borohydride to yield ethyl 2-acetamido-2-deoxy-α-D-xylothiofuranoside (VII) which on hydrolysis in the presence of mercuric chloride gave N-acetyl-α-D-xylosamine (IX) and on acid hydrolysis gave α-D-xylosamine hydrochloride (VIII), further characterized as its β-D-tetraacetyl derivative (X). All products were obtained in the crystalline state.

Hitherto, the only known amino sugars have been hexosamines. We report herein the synthesis of a pentosamine, 2-amino-2-deoxy-D-xylose or D-xylosamine, characterized as the hydrochloride VIII and the N-acetyl (IX) and β-D-tetraacetyl (X) derivatives. The starting material was D-glucosamine hydrochloride whose conversion to the dicarbonyl derivative VI (not isolated) has been previously reported.[3,4] This sulfur-containing product (VI) was immediately subjected to carbonyl reduction with sodium borohydride[5-7] and ethyl 2-acetamido-2-deoxy-α-D-xylothiofuranoside (VII) was obtained. It was found that the monobed resins[8] are most suitable for the removal of ionic materials from either the periodate oxidation or the borohydride reduction reaction mixtures without hydrolysis of the thioethoxyl group. The xylothiofuranoside VII was strongly dextrorotatory, $[\alpha]_D$ +222°, in aqueous solution.

α-D-Xylosamine hydrochloride (VIII) was obtained from the thiofuranoside VII by rather vigorous acid hydrolysis or by alkaline deacetylation followed by a milder acid hydrolysis of the thioglycosidic group. The hydrochloride was dextrorotatory and showed downward mutarotation, $[\alpha]_D$ +80° → +40° in water. The substance was strongly reducing and reacted positively in the Elson–Morgan color test for amino sugars,[9,10] hitherto considered characteristic of hexosamines. Its stability toward ordinary storage conditions was about the same as that of an acetohalogen sugar derivative.

Hydrolysis of the thiofuranoside VII in the presence of mercuric chloride produced N-acetyl-α-D-xylosamine (IX), $[\alpha]_D$ +56° → +9° (water), which reacted positively in the Elson–Morgan test. Acetylation of N-acetyl-α-D-xylosamine or of α-D-xylosamine hydrochloride with acetic anhydride and sodium acetate yielded a tetraacetyl-D-xylosamine (X), which was presumably the β-D-form, $[\alpha]_D$ − 48° (chloroform).



Experimental

Ethyl 2-Acetamido-2-deoxy-α-D-xylothiofuranoside (VII).—An amount of 3.91 g. (10 millimoles) of ethyl 2-acetamido-

(1) Reported in *Abstracts Papers Am. Chem. Soc.*, **122**, 8R (1952).

(2) Special Postdoctoral Research Fellow of the National Institutes of Health, United States Public Health Service.

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(5) S. W. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).

(6) M. L. Wolfrom and H. B. Wood, *ibid.*, **73**, 2933 (1951).

(7) M. L. Wolfrom and Kimiko Anno, *ibid.*, **74**, 5583 (1952).

(8) Amberlite MB-3, a mixture of cation and anion exchange resins produced by the Rohm and Haas Co., Resinous Products Division, Philadelphia 5, Pa.

(9) L. A. Elson and W. T. J. Morgan, *Biochem. J.*, **27**, 1824 (1933).

(10) J. W. Palmer, Elizabeth M. Smyth and K. Meyer, *J. Biol. Chem.*, **119**, 491 (1937).

triacetyl-2-deoxy-α-D-glucothiofuranoside (V)[3,4] was dissolved in 100 ml. of absolute methanol and anhydrous ammonia was passed into the solution for 30 min. at −5 to 0°. After standing for 2 hr. at room temperature, the solvent was removed under reduced pressure and the resultant sirup crystallized on standing overnight in a desiccator. The crystals were dissolved in 40 ml. of water and 42 ml. of 0.2509 *M* (10.5 millimoles) of sodium metaperiodate was added and the volume was made up to 100 ml. After 30 min. at 10–12°, an aliquot showed that all of the periodate had been consumed and the reaction mixture was neutral. The solution was passed through a column of Amberlite MB-3,[8] (130 × 25 mm., diam.) and the strongly reducing effluent was concentrated under reduced pressure to 30 ml. To this was added dropwise a solution of sodium borohydride (0.30 g.) in 20 ml. of water over a period of 10 min. at room temperature (28–30°). After stirring for an additional 30 min., the reaction mixture was non-reducing. The remaining hydride was neutralized with 7 ml. of *N* sulfuric acid, the solution was diluted with one volume of water and passed through a column of Amberlite MB-3[8] (130 × 25 mm., diam.). The effluent was concentrated under reduced pressure to a colorless sirup which crystallized on dehydration in a desiccator; yield 2.0 g., m.p. 125–135°. Fine needles were obtained on crystallization from ethanol–ether; yield 0.43 g. (18%), m.p. 154–155°, $[\alpha]^{27}D$ +212° (*c* 1, water). One further crystallization gave pure material; m.p. 157–158°, $[\alpha]^{21}D$ +222° (*c* 1, water).

Anal. Calcd. for $C_9H_{17}O_4NS$: C, 45.94; H, 7.28; N, 5.95; S, 13.62. Found: C, 45.89; H, 7.32; N, 5.88; S, 13.47.

α-D-Xylosamine Hydrochloride (VIII).—An amount of 80 mg. of ethyl 2-acetamido-2-deoxy-α-D-xylothiofuranoside (VII) was heated in 3 ml. of 4 *N* HCl in a boiling water-bath for 1 hr. After decolorizing with carbon, the solution was concentrated under reduced pressure to dryness. Recrystallization was effected from methanol–acetone; yield 40 mg. (64%), dec. 160–165°. Further recrystallization from the same solvent produced pure material as clusters of truncated prisms; dec. 165–167°, $[\alpha]^{31}D$ +80° (initial, extrapolated) → +40° (*c* 0.8, water, final). The substance was strongly reducing and exhibited a positive ninhydrin test. It gave a distinct Elson–Morgan color test,[9,10] hitherto considered as characteristic of a hexosamine. It was stable when stored under dry conditions but in moist air it slowly developed a brown coloration.

Anal. Calcd. for $C_5H_{12}O_4NCl$: C, 32.36; H, 6.52; N, 7.55; Cl, 19.10. Found: C, 32.44; H, 6.63; N, 7.57; Cl, 19.05.

α-D-Xylosamine hydrochloride was also obtained from ethyl 2-acetamido-2-deoxy-α-D-xylothiofuranoside (VII) by successive treatment with aqueous alkali and acid. An amount of 0.20 g. of this substance in 5 ml. of 0.8 *N* $Ba(OH)_2$ was heated for 1 hr. in a boiling water-bath. The reaction mixture was neutralized with carbon dioxide, the precipitate was filtered and the filtrate was concentrated under reduced pressure. The residue was extracted with absolute methanol and the methanol solution was concentrated under reduced pressure to a sirup. The sirup was dissolved in 20 ml. of *N* HCl and was heated for 1 hr. in a boiling water-bath. After decolorizing with carbon, the solution was concentrated under reduced pressure and the residue was extracted with a small amount of absolute methanol. Acetone was added to the methanol solution to incipient turbidity and the resultant inorganic precipitate was removed by filtration. This process was repeated several times to remove the inorganic impurities whereupon the methanol–acetone solution was allowed to stand at 15° and crystals formed gradually; yield 0.06 g. (38%), dec. 150–160°. Further recrystallization from the same solvent gave pure material; dec. 165–167°.

N-Acetyl-α-D-xylosamine (IX).—A warm (50°) solution of mercuric chloride (231 mg.) in 20 ml. of water was added to a solution of ethyl 2-acetamido-2-deoxy-α-D-xylothiofuranoside (VII, 100 mg.) in 5 ml. of water. After standing for 5 hr. at room temperature, the separated white precipitate was filtered. The filtrate, diluted with one volume of water, was passed through a column of Amberlite MB-3[8] (50 × 20 mm., diam.). The effluent was concentrated under reduced pressure to a sirup which immediately crystallized. Recrystallization was effected from methanol–acetone–ether; yield 58 mg. (71%), m.p. 184–187° (dec.), $[\alpha]^{29}D$ +8° (*c* 1, water, final). One recrystallization from the same solvents gave pure fine needle-like crystals that were strongly reducing and exhibited a positive Elson–Morgan[9,10] test; m.p. 186–189° (dec.), $[\alpha]^{29}D$ +56° (initial, extrapolated) → +9° (*c* 0.8, water, final).

Anal. Calcd. for $C_7H_{13}O_5N$: C, 43.98; H, 6.85; N, 7.33. Found: C, 43.86; H, 6.93; N, 7.61.

Tetraacetyl-β-D-xylosamine (X).—N-Acetyl-α-D-xylosamine (0.20 g.) was stirred with acetic anhydride (5 ml.) in the presence of sodium acetate (0.20 g.) at 100° until it had been dissolved and then the temperature was maintained at 80° for 1 hr. under stirring. The cooled reaction mixture was poured into 100 ml. of ice and water and extracted with chloroform. The sirup obtained on solvent removal from the washed (with aqueous $NaHCO_3$ and water) and dried extract was crystallized from absolute ethanol–ether–petroleum ether; yield 0.03 g. (9%), m.p. 195–200°. Further recrystallization from absolute ethanol gave pure needles; m.p. 214–215°, $[\alpha]^{30}D$ −48° (*c* 1.3, $CHCl_3$).

Anal. Calcd. for $C_{13}H_{19}O_8N$: C, 49.20; H, 6.03; N, 4.42. Found: C, 49.22; H, 6.22; N, 4.36.

The same substance was obtained from α-D-xylosamine hydrochloride on acetylation with sodium acetate and acetic anhydride as described above.

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