Mixed with an equimolar quantity of oxalic acid in absolute alcohol, it yielded **hydroecgonidine ethyl ester binoxalate** which was purified from the same solvent: irregular prisms, m.p. 143°.

Anal. Calcd. for $C_{13}H_{21}NO_6$: C, 54.4; H, 7.39. Found: C, 54.2; H, 7.25.

When the ester (165 mg.) was added to a solution of chloroauric acid (330 mg.) in alcohol (1.5 ml.), **hydroecgonidine ethyl ester chloroaurate** precipitated. It was purified from alcohol: platelets some of which appeared to be parallelopipeda. In a bath the temperature of which was rising at a rate of less than a degree per minute this derivative melted at 177.5–181° (reported[1,2] 167°, 173–174°).

The liquid picrate, treated in the same manner as the crystalline isomer, furnished impure **pseudohydroecgonidine ethyl ester** (1.1 g.). Combined with an equimolar quantity of oxalic acid in absolute alcohol, the foregoing oxalate (m.p. 143°) crystallized slowly. The non-crystalline portion was mixed with dilute alkali and extracted with ether. The recovered ester was treated in dry ether solution with dry hydrogen chloride, but the liquid hydrochloride which separated did not crystallize. Regenerated once more the ester (0.50 g.) was added to a mixture of chloroauric acid (0.50 g.) and alcohol (1.5 ml.). The ensuing precipitate (m.p. 119°) was crystallized to constant melting point (three times) from alcohol: short, yellow prisms, m.p. 122.5° (reported[1,3] 122°).

BETHESDA, MD.

[CONTRIBUTION NO. 1746 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

An Agent from *E. coli* Causing Hemorrhage and Regression of an Experimental Mouse Tumor. III. The Component Fatty Acids of the Phospholipide Moiety[1]

BY MIYOSHI IKAWA, J. B. KOEPFLI, S. G. MUDD AND CARL NIEMANN[2]

RECEIVED OCTOBER 22, 1952

Lauric acid, myristic acid, palmitic acid and an optically active β-hydroxymyristic acid have been isolated from hydrolysates of the phospholipide moiety of the hemorrhagic agent obtained from *E. coli*. The optically active β-hydroxymyristic acid appears to possess the D-configuration.

The agent which is synthesized by *E. coli* and which produces a hemorrhagic response in and causes the regression of the experimental mouse sarcoma 180 has been shown to be a complex polysaccharide which contains both a peptide and a phospholipide component.[3] It is the purpose of this communication to comment upon the nature of the phospholipide moiety particularly in respect to its component fatty acids.

When the hemorrhagic agent was subjected to partial hydrolysis, with aqueous sulfuric acid, a fraction was obtained that was insoluble in water but which was soluble in chloroform. This chloroform-soluble fraction was further fractionated into acetone-soluble and acetone-insoluble fractions. The latter fraction was found to contain phosphorus and, from its solubility behavior, may be classified as a phosphatide. Hydrolysis of the phosphatide, *i.e.*, the chloroform-soluble and acetone-insoluble fraction, with aqueous hydrochloric acid and subsequent extraction of the hydrolysate with ether gave an ether-soluble fatty acid fraction.[4]

When an ethereal solution of the fatty acid fraction was triturated with ligroin an optically active acid, m.p. 73–74°, was obtained. It was shown that this compound had the molecular formula $C_{14}H_{28}O_3$, that it contained, in addition to the carboxyl group, a hydroxyl group, and probably only one terminal C-methyl group. The failure of the hydroxy acid to react with lead tetraacetate indicated that the compound was not an α-hydroxy acid.[5] Reaction of the hydroxy acid with thionyl chloride followed by hydrogenolysis gave myristic acid, and when the hydroxy acid was oxidized with alkaline permanganate, lauric acid was obtained. These data suggested that the acid of m.p. 73–74° was one of the optical isomers of β-hydroxymyristic acid. The synthesis of β-hydroxymyristic acid has been described[6,7] but apparently no attempt has been made to resolve the synthetic acid. Therefore β-hydroxymyristic acid was prepared, from lauraldehyde and ethyl bromoacetate, and resolved into its optical isomers with the aid of *d*-α-methyl-β-phenylethylamine. The *l*-acid obtained from the less soluble salt proved to be identical with the naturally occurring acid.

There is no record of the previous isolation of β-hydroxymyristic acid from a natural source. However Bergström, Theorell and Davide[8] have reported that *l*-β-hydroxydecanoic acid may be obtained from a metabolic product of *Pseudomonas pyocyanes*, and Jarvis and Johnson[9] isolated the same compound from the hydrolysate of a glycolipide obtained from *Ps. aeruginosa*. Furthermore *d*-β-hydroxycaproic and *d*-β-hydroxycaprylic acid have been obtained as hydrolysis products of the glycolipids produced by the corn smut *Ustilago zeae*,[10] and the mycolic acids from the human tubercle and the diphtheria bacillus are believed to be β-hydroxy acids.[11,12] From these observations it

(1) Supported from 1938 to 1943 by grants from the Argonaut Foundation and from 1948 onwards by grants from the National Cancer Institute of the U. S. Public Health Service.

(2) To whom inquiries regarding this article should be sent.

(3) M. Ikawa, J. B. Koepfli, S. G. Mudd and C. Niemann, *J. Nat. Cancer Inst.*, **13**, 157 (1952).

(4) The other fractions obtained at this stage, *cf.* Experimental section, were reserved for an investigation of the nitrogenous components of the phosphatide. The results of this study will be described in a separate communication.

(5) R. Criegee, "Newer Methods of Preparative Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1948.

(6) H. Thaler and G. Geist, *Biochem. Z.*, **302**, 369 (1939).

(7) F. L. Brensch, *Fortschr. Chem. Forsch.*, 1, 567 (1950).

(8) S. Bergström, H. Theorell and H. Davide, *Arch. Biochem.*, **10**, 165 (1946); *Arkiv. Kemi, Mineral., Geol.*, **23A**, No. 13 (1946).

(9) F. G. Jarvis and M. J. Johnson, THIS JOURNAL, **71**, 4124 (1949).

(10) R. U. Lemieux, *Can. J. Chem.*, **29**, 415 (1951).

(11) J. Asselineau and E. Lederer, *Biochem. Biophys. Acta*, **7**, 126 (1951).

(12) E. Lederer, V. Portelance and K. Serck-Hanssen, *Bull. soc. chim.*, [5] **19**, 413 (1952).

is clear that quite a number of lipides of microbial origin contain one or more β-hydroxy fatty acids.

Lemieux and Giguere[13] have related the configuration of *l*-β-hydroxycaproic acid to that of D-lactic acid. On the basis of their respective specific rotations in chloroform solutions both the *l*-β-hydroxymyristic acid obtained from *E. coli* and the *l*-β-hydroxydecanoic acid obtained from *Ps. pyocyanea* and *Ps. aeruginosa* may be provisionally assigned the D-configuration.

The mixture of fatty acids remaining after the separation of the D-β-hydroxymyristic acid was subjected to frontal analysis.[14] A plot of the change in refractive index *versus* the volume of the emergent solution, *cf.* Fig. 1, showed three well-defined steps thus indicating the probable presence of only three major components. Since in the frontal analysis technique only the first component emerges in a pure state,[14] the solution corresponding to the emergence of the first step was collected separately and from this solution lauric acid was obtained. The frontal analysis diagram, *i.e.* Fig. 1, suggested that myristic and palmitic acids were the two other major components and their presence was confirmed, by the isolation of palmitic acid, by fractional distillation of a sample of the fatty acid mixture, and by the isolation of myristic acid *via* its *p*-bromophenacyl ester.

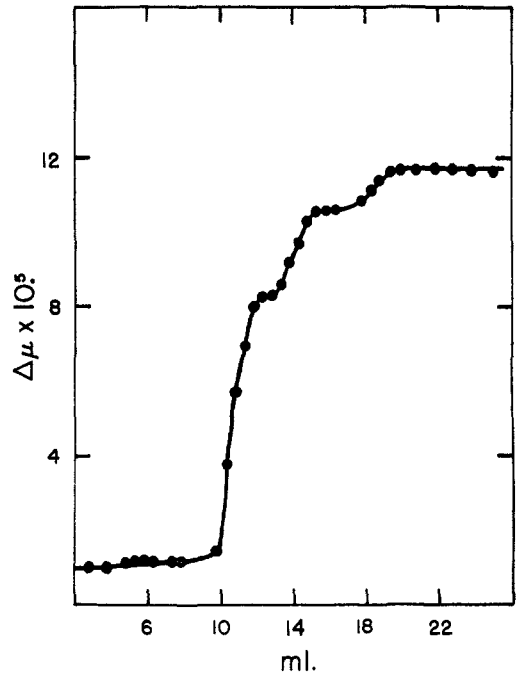


Fig. 1.—Frontal analysis diagram of the ligroin-soluble fatty acid fraction.

The amount of the phosphatide fraction obtained from the hemorrhagic agent by partial hydrolysis with aqueous sulfuric acid was *ca.* 19%; *cf.* Table I. The remainder of the chloroform-soluble material, *i.e.*, *ca.* 6%, was also soluble in acetone. A study of several other hydrolytic procedures, *cf.* Table I, failed to reveal a procedure as satisfactory as the aqueous sulfuric acid technique used initially.

(13) R. U. Lemieux and J. Giguere, *Can. J. Chem.*, **29**, 678 (1951).
(14) S. Claesson, *Arkiv. Kemi, Mineral., Geol.*, **23A**, No. 1 (1946).

TABLE I

LIPIDE FRACTIONS OBTAINED FROM *E. Coli* PREPARATIONS

	Conditions of hydrolysis			Chloroform-soluble[b]	Acetone-insoluble[c]
Preparation	Concn.[a]	Reagent	Time 100°, hr.		
Fraction B_1	0.5	1 *N* H_2SO_4	1	23.9	18.4
	.5	1 *N* H_2SO_4	1	25.2	19.0
	.5	1 *N* H_2SO_4	1.5	25.9	18.9
	.5	0.1 *M* Cl_3CCO_2H	1.5	23.4	18.1
		.1 *M* Cl_3CCO_2H	1.5	21.3	16.7
	.5	.1 *M* $ClCH_2CO_2H$	1.5	9.4	
		.1 *M* $ClCH_2CO_2H$	6	18.8	
		.1 *M* $(CO_2H)_2$	1.5	5.6	
Cell debris	5	1 *N* H_2SO_4	2	4.2	0.8
	7	1 *N* H_2SO_4	2	4.7	0.8
	6.5	1 *N* H_2SO_4	2	3.3	0.6
Fraction A_2	5.5	1 *N* H_2SO_4	2	24.5	15.3
	5	1 *N* H_2SO_4	2	34.9	19.4
Fraction B_2	1	0.1 *M* Cl_3CCO_2H	1.5	11.3	2.0

[a] w./v. %. [b] % of original preparation present in the chloroform-soluble fraction. [c] % of original preparation present in the chloroform-soluble and acetone-insoluble fraction.

The chloroform- and acetone-soluble fraction, obtained by the partial hydrolysis of the hemorrhagic agent with aqueous sulfuric acid, *vide ante*, was found to be a mixture principally composed of fatty acids. When this mixture was subjected to fractional recrystallization palmitic acid was obtained. It is not obvious whether the acetone-soluble fraction is derived from the acetone-insoluble fraction or whether its formation is a reflection of the presence of two kinds of lipide fragments in the hemorrhagic agent.

In order to determine whether a phosphatide component was present in fractions other than that designated as the hemorrhagic agent, *i.e.*, fraction B_1[3] or its precursors, several of the fractions obtained during the course of the isolation of the hemorrhagic agent were subjected to partial hydrolysis, with aqueous sulfuric acid, or aqueous trichloroacetic acid, and an attempt made to isolate a phosphatide fraction from these hydrolysates; *cf.* Table I. Both the lyophilized cell debris and fraction B_2[3] proved to be poor sources in that the yields of the phosphatide fractions were *ca.* 0.8 and 2%, respectively. However, fraction A_2[3], the water-insoluble residue obtained after electrodialysis and high speed centrifugation of the solution from which fraction B_1 was ultimately obtained by fractional precipitation with ethanol, appeared to be as rich in lipid components as was the hemorrhagic agent although the relative amount of the acetone-soluble fraction was considerably greater in the former instance. If the amount of the phosphatide fraction obtained upon partial hydrolysis may be taken as an index of the amount of the hemorrhagic agent present in the various fractions it may be concluded that the procedure developed for the preparation and isolation of the hemorrhagic agent[3] is very effective in obtaining all of the agent elaborated in a water-soluble form and leaving practically none associated with the cellular debris.

Experimental[15,16]

Partial Hydrolysis of the Hemorrhagic Agent and Extraction of Lipides.—Fraction B_1^3, 2.2 g., was dissolved in 440 ml. of water, 12.5 ml. of concd. sulfuric acid added and the solution heated under refluxing conditions for 1–1.5 hours. The hydrolysate was cooled, shaken with chloroform, the phases separated by centrifugation, the chloroform extract filtered and the solvent evaporated to give a waxy residue. This residue was triturated with acetone to give an acetone-insoluble and an acetone-soluble fraction. The yields of the various fractions are given in Table I.

Properties of the Acetone-insoluble Fraction.—The acetone-insoluble fraction obtained above was dissolved in chloroform and reprecipitated with acetone to give a soft waxy pale yellow to light brown solid which melted in the region of 175–180°. This solid was readily soluble in chloroform, benzene and ligroin, somewhat less soluble in ether and was relatively insoluble in acetone, ethanol and water.

Anal. Found: C, 57.3; H, 10.1; N, 3.3; P, 1.6.

The glucosamine equivalent was estimated as approximately 12%.

Hydrolysis of the Acetone-insoluble Fraction.—Approximately 300 mg. of the acetone-insoluble fraction and 10 ml. of 5 *N* aqueous hydrochloric acid was placed in a sealed tube and the mixture heated in an oven at 100° for 10–16 hours. The hydrolysate was chilled in an ice-bath, the aqueous phase withdrawn, extracted with ether and evaporated to dryness over sodium hydroxide pellets in a vacuum desiccator. The ethereal extract was added to the oil, the mixture triturated, the flocculent precipitate removed by centrifugation, and the clear ethereal extract evaporated to dryness to give a semi-solid fatty acid fraction in amount equivalent to 55–58% of the starting material, *i.e.*, the acetone-insoluble fraction. The flocculent precipitate and the dried aqueous phase were reserved for an investigation of the component nitrogenous bases.

Isolation of *l*-β-Hydroxymyristic Acid.—The fatty acid fractions obtained from a number of hydrolytic experiments were combined and 1.013 g. of the fatty acid mixture was triturated at 25° with a small amount of 60–70° ligroin to give a ligroin-soluble and a ligroin-insoluble fraction. The latter fraction was recrystallized from hot 60–70° ligroin to give 265 mg. (26%) of an acid, m.p. 72–73°. Further recrystallization from the same solvent gave a product, m.p. 73–74°, $[\alpha]^{25}D$ −9.6° (*c* 1.6% in pyridine), $[\alpha]^{25}D$ −16° (*c* 2% in chloroform). The neut. equiv. was found to be 245, the molecular weight, by the Rast method with exaltone, 253; and the number of C-methyl groups 1.2. Calcd. for one alcoholic hydroxyl group, 7.0; found,[17] 6.7.

Anal. Calcd. for $C_{14}H_{28}O_3$ (244.4): C, 68.8; H, 11.5. Found: C, 69.2; H, 11.5.

The addition of benzylamine to an ethyl acetate solution of the acid gave the benzylammonium salt, m.p. 84–86°, after recrystallization from ethyl acetate.

Anal. Calcd. for $C_{21}H_{37}O_3N$ (351.5): C, 71.8; H, 10.6; N, 4.0. Found: C, 71.7; H, 10.6; N, 4.0.

The reaction of the sodium salt of the acid with a solution of *p*-bromophenacyl bromide in ethanol gave the *p*-bromophenacyl ester, m.p. 112.5–113° after recrystallization from aqueous ethanol.

Anal. Calcd. for $C_{22}H_{33}O_4Br$ (441.4): C, 59.9; H, 7.5. Found: C, 59.9; H, 7.6.

Reduction of *l*-β-Hydroxymyristic Acid.—The hydroxy acid, 0.05 g., was dissolved in 2 ml. of thionyl chloride, the solution heated under refluxing conditions for 2 hours, the excess thionyl chloride removed, and the resulting oil washed with water. The oil was then dissolved in 10 ml. of methanol, the solution made alkaline by the addition of a small piece of sodium metal, 25 mg. of platinic oxide catalyst added, the mixture hydrogenated at 25° under one atmosphere of hydrogen, the resulting product filtered, the filtrate acidified with aqueous hydrochloric acid, and evaporated to dryness *in vacuo*. The residue was taken up in ether, the ethereal extract extracted with dilute aqueous sodium hydroxide, the alkaline extract acidified, extracted with ether and the ethereal extract evaporated to dryness. The residue was triturated with ligroin and the residue obtained by evaporation of the ligroin extract was recrystallized from aqueous acetone to give myristic acid; glistening plates, m.p. 51–53°. A mixed m.p. with an authentic sample showed no depression.

Oxidation of *l*-β-Hydroxymyristic Acid.—Treatment of the hydroxy acid with lead tetraacetate in glacial acetic acid at 70° for 45 minutes according to Knoop, *et al.*,[18] resulted in the consumption of less than 0.1 mole of the reagent per mole of hydroxy acid.

The hydroxy acid, 0.066 g., was dissolved in 10 ml. of acetone, 0.250 g. of potassium permanganate added, and the mixture heated under refluxing conditions for one hour. The solvent was removed, dilute aqueous hydrochloric acid added to the residue, the excess permanganate destroyed with sodium bisulfite, the mixture extracted with ether, the ethereal extract evaporated to dryness and the residue recrystallized from aqueous ethanol to give 0.04 g. of a solid, m.p. 29–36°. This product was recrystallized several times from aqueous acetone to give lauric acid; glistening plates, m.p. 40–42°. No m.p. depression was observed with an authentic sample of lauric acid but the m.p. was depressed when the oxidation product was mixed with an authentic sample of *n*-tridecanoic acid. The *p*-bromophenacyl ester of the oxidation product, m.p. 71–73°, when mixed with the *p*-bromophenacyl ester of lauric acid showed no m.p. depression, but a m.p. depression was observed with the corresponding derivative of *n*-tridecanoic acid.

Synthesis of DL-β-Hydroxymyristic Acid.—A Reformatsky reaction between lauraldehyde and ethyl bromoacetate, carried out according to the directions given for the preparation of ethyl β-phenyl-β-hydroxypropionate,[19] gave 38% of ethyl DL-β-hydroxymyristate, b.p. 164–167° (2–3 mm.). The ester was saponified as directed by Thaler and Geist[6] to give DL-β-hydroxymyristic acid, m.p. 78–79°; neut. equiv., 245, calcd. 244.4. Although Thaler and Geist[6] report a melting point of 72–73° for the DL-acid our value agrees with the value of 78° quoted by Brensch.[7]

Resolution of DL-β-Hydroxymyristic Acid.—To 0.5 g. of D-α-methyl-β-phenylethylamine sulfate (Dexedrine Sulfate)[20] was added 5 ml. of *N* aqueous sodium hydroxide, the mixture extracted with 5 ml. of ether and the ethereal solution of the amine added to a solution of 0.345 g. of the DL-acid in 30 ml. of dry ether. The solid that formed was suspended in boiling ether and sufficient absolute ethanol added to effect solution. The more insoluble salt, m.p. 123–125°, crystallized in fine silky threads. The more soluble salt, m.p. 85–86°, was obtained from the mother liquors in the form of glistening needles. The salts were suspended in dilute aqueous hydrochloric acid, extracted with ether, the ethereal extracts freed of solvent and the residues recrystallized from 60–70° ligroin to give the respective acids. A mixture of the *l*-acid, m.p. 73–74°, $[\alpha]^{25}D$ −9.4° (*c* 1.9% in pyridine) obtained from the less soluble salt and the *l*-acid isolated from the phosphatide had a m.p. of 73–74°. The benzylamine salt, m.p. 84–86°, and the *p*-bromophenacyl ester, m.p. 111–112°, of the synthetic *l*-acid caused no depression in the m.p. of the corresponding derivatives prepared from the naturally occurring *l*-acid. The *d*-acid, m.p., 71–73°, $[\alpha]^{25}D$ +6.5° (*c* 1.5% in pyridine) isolated from the more soluble salt appeared to contain *ca.* 15% of the *l*-acid.

Isolation of Lauric Acid.—A portion of the fatty acids, *i.e.*, 0.101 g., remaining after the separation of the *l*-β-hydroxymyristic acid, was dissolved in 50 ml. of 95% ethanol and the solution subjected to frontal analysis in a manually operated apparatus similar to that described by Claesson.[14] With 0.153 g. of acid washed and ethanol washed Norite A as the adsorbent the diagram given in Fig. 1 was obtained. The fraction collected between 9–13 ml. was evaporated to dryness and the residue recrystallized from aqueous acetone to give lauric acid, m.p. 40–41.5°. When this sample was mixed with an authentic specimen of lauric acid no m.p. depression was observed.

(15) All melting points are corrected.

(16) Microanalyses by Dr. A. Elek.

(17) J. W. Peterson, K. W. Hedberg and B. E. Christensen, *Ind. Eng. Chem., Anal. Ed.*, **15**, 225 (1943).

(18) F. Knoop, F. Ditt, W. Hecksteden, J. Maier, W. Merz and R. Härle, *Z. physiol. Chem.*, **239**, 30 (1936).

(19) R. L. Shriner, in "Organic Reactions," Vol. I, John Wiley and Sons, New York, N. Y., 1942, pp. 16-17.

(20) Trade Mark of Smith, Kline and French Laboratories.

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°.

PASADENA 4, CALIFORNIA

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

D-Xylosamine[1]

BY M. L. WOLFROM AND KIMIKO ANNO[2]

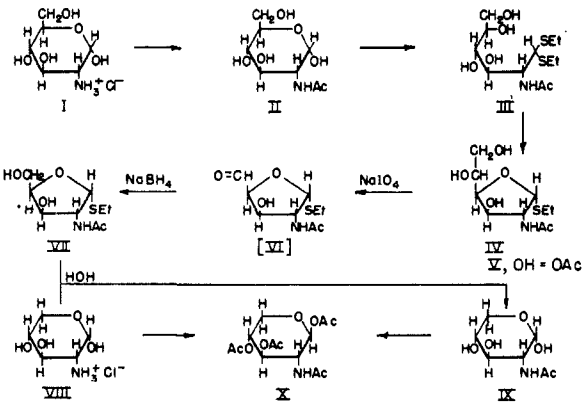
RECEIVED OCTOBER 20, 1952

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.

(3) M. L. Wolfrom, S. M. Olin and W. J. Polglase, THIS JOURNAL, **72**, 1724 (1950).

(4) M. L. Wolfrom and Kimiko Anno, *ibid.*, **74**, 6150 (1952).

(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).