N^{10}-methylpteroylglutamic acid and N^{10}-methylpteroic acid, have been synthesized in pure crystalline form, and found to be antagonists for pteroylglutamic acid.

2. The purified N^{10}-ethyl- and butyl-, and crude N^{10}-carboxymethyl-, benzyl-, and phenacylpteroic acids and N^{10}-phenacylpteroylglutamic acid have also been prepared. These have a lower order of antagonist activity.

3. Convenient methods of obtaining pure N-monosubstituted aminobenzoic and aminobenzoylglutamic acids have been devised.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF COLORADO]

The Synthesis of Thymine Nucleosides[1,2]

BY DONALD W. VISSER,[3] IRVING GOODMAN AND KARL DITTMER

A method for the synthesis of pyrimidine nucleosides was described by Hilbert and Johnson[4] who prepared 1-glucosyluracil by the reaction of 2,4-diethoxypyrimidine with acetobromoglucose followed by hydrolysis. By this method these and other investigators prepared the uracil nucleosides of D-ribose,[5] L-arabinose,[6] D-xylose,[6] D-glucose[4] and D-galactose.[6] However, the literature contains no conclusive report of the synthesis of thymine nucleosides. The syntheses of the D-ribose, D- and L-arabinose, D-glucose and D-galactose nucleosides of thymine by a modification of this procedure are reported in this paper.

When Schmidt–Nickles and Johnson[7] treated 2,4-diethoxy-5-methylpyrimidine with D-acetobromoglucose at 50° for seven days, they obtained a small amount of a crystalline substance which melted at 316°. We repeated this reaction under similar conditions and isolated a small amount of material which, when crystallized from water, melted at 326° and proved to be thymine. Since no other product was isolated from this reaction mixture, various modifications of the original procedure were studied.

It seemed desirable to provide conditions which would enhance the removal of ethyl bromide, a by-product, which might enter into undesirable side reactions.[7] For this reason the reaction between the acetobromoglucose and 2,4-diethoxy-5-methylpyrimidine was carried out at a pressure of 2 mm. for four days at 50°. Since ethyl bromide was collected in a Dry Ice trap, it was assumed that the desired product was formed, even though it could not be isolated. The complete reaction mixture was then hydrolyzed with dry hydrogen chloride in absolute methanol. After the solvents were removed *in vacuo*, glucosylthymine was crystallized from absolute ethyl alcohol.

In a similar manner the other thymine nucleosides were formed, although each behaved differently. The acetoarabinosylethoxythymine crystallized directly in the reaction mixture. Ribosylthymine did not crystallize upon hydrolysis of the reaction mixture. Therefore, an impure intermediate was isolated by fractional precipitation at low temperatures before hydrolysis. Hydrolysis of this intermediate yielded ribosylthymine.

Experimental

2,4-Diethoxy-5-methylpyrimidine.—The 2,4-diethoxy-5-methylpyrimidine was prepared from thymine (commercial source) according to the directions given by Schmidt–Nickles and Johnson.[7]

D-Acetobromoribose.—The directions of Levene and Tipson[8] were followed for the preparation of D-acetobromoribose except that petroleum ether was not used to facilitate crystallization of the product. The ether solution of the acetobromoribose, after treatment with Norite, was slowly concentrated *in vacuo* to about one-third of its original volume. The large colorless crystals were washed with a small amount of cold, dry ether, and dried over phosphorus pentoxide in a vacuum desiccator. Contact with moisture was avoided throughout the procedure. If the proper precautions were not taken, the acetobromoribose began to decompose within ten to fifteen minutes. Unless it was redissolved in dry ether, treated with Norite and recrystallized, complete decomposition took place within a few hours.

D-Acetobromoarabinose.—This compound was prepared according to the directions of Anderson and Snell.[9]

D-Acetobromoglucose.—D-Acetobromoglucose was prepared by a method similar to that described by Karjala and Link.[10]

D-Acetobromogalactose.—A modification of the method of Levene and Raymond[11] was used for the synthesis of D-acetobromogalactose. One hundred grams of glacial acetic acid was saturated with dry hydrogen bromide and cooled to 0°. To this solution 25 g. of dry, finely-powdered D-galactosepentaacetate was added and anhydrous hydrogen bromide was passed through the suspension at 0–10° with stirring until all the galactosepentaacetate had dissolved. The flask was loosely stoppered and allowed to stand for one hour at room temperature. The hydrogen bromide was removed under vacuum, and the product isolated as described for the preparation of acetobromoribose.

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(2) This work was supported in part by a contract with the Office of Naval Research.

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(4) Hilbert and Johnson, THIS JOURNAL, **52**, 4489 (1930).

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(7) Schmidt–Nickles and Johnson, *ibid.*, **52**, 4511 (1930).

(8) Levene and Tipson, *J. Biol. Chem.*, **92**, 109 (1931).

(9) Anderson and Snell, "Organic Syntheses," Vol. VIII, John Wiley and Sons, New York, N. Y., 1926, p. 18.

(10) Karjala and Link, THIS JOURNAL, **62**, 917 (1940).

(11) Levene and Raymond, *J. Biol. Chem.*, **90**, 247 (1931).

1-D-**Ribosylthymine.**—Eight grams (0.044 mole) of 2,4-diethoxy-5-methylpyrimidine was heated with 8.1 g. (0.024 mole) of D-acetobromoribose in an oven at 50° for four days at 3–4 mm. pressure. To the cloudy, viscous sirup was added 50 ml. of anhydrous ether, and the solution was kept at −10° for three days. A white crystalline material (1.7 g.) was filtered and recrystallized from 50% aqueous ethanol, m. p. 126°. Hydrolysis of this compound in anhydrous methanol and hydrogen chloride yielded thymine. This compound was believed to be 2-D-acetoribosido-4-ethoxy-5-methylpyrimidine. The isolation, purification and properties of this compound will be reported elsewhere. The filtrate was cooled in a Dry Ice–acetone-bath, and the material (3.0 g.) which separated was filtered in a funnel cooled with Dry Ice and acetone. The white amorphous material was hydrolyzed for three days with hydrogen chloride in methanol, and the solvent completely removed *in vacuo*. The residue was dissolved in 10 ml. of absolute ethanol and cooled overnight. The 1-D-ribosylthymine, 0.5 g. (yield 8.1%), was filtered and recrystallized from absolute alcohol, m. p. 252°. The specific rotation was $[\alpha]^{25}D$ −110° (*C*, 2 in water).

Anal. Calcd. for $C_{10}H_{14}O_6N_2$: C, 46.52; H, 5.46; N, 10.82. Found: C, 47.10; H, 5.78; N, 11.02.

1-D-**Arabinosylthymine.**—Ten grams (0.055 mole) of 2,4-diethoxy-5-methylpyrimidine was heated with 10.5 g. (0.031 mole) of D-acetobromoarabinose at 50° for four days at 3–4 mm. pressure. Crystals separated at the end of the first day and a solid cake was formed in the bottom of the reaction flask after the fourth day. Twenty milliliters of anhydrous ether was added and the mixture stirred thoroughly and cooled to 0°. The white needles were filtered and recrystallized from 50% aqueous ethanol. The yield of 1-D-acetoarabinosyl-4-ethoxy-5-methylpyrimidine was 4.5 g. (43%), m. p. 181°. The specific rotation was $[\alpha]^{29}D$ −93.6° (*C*, 3 in 95% ethanol).

The 1-D-acetoarabinosyl-4-ethoxythymine was hydrolyzed with anhydrous hydrogen chloride in methanol. From 3.50 g. of the intermediate, 1.42 g. of 1-D-arabinosylthymine was obtained, m. p. 250–251°. The specific rotation was $[\alpha]^{25}D$ −69° (*C*, 3 in water).

Anal. Calcd. for $C_{10}H_{14}O_6N_2$: C, 46.52; H, 5.46; N, 10.82. Found: C, 45.72; H, 5.93; N, 10.92.

1-L-**Arabinosylthymine.**—According to the procedure just described the 1-L-arabinosylthymine was prepared in yields identical with those obtained in the preparation of the 1-D-arabinosylthymine. The 1-L-acetoarabinosylthymine, m. p. 181°, had a specific rotation, $[\alpha]^{29}D$ + 93.5° (*C*, 3 in 95% ethanol). When this intermediate was hydrolyzed, the 1-L-arabinosylthymine was obtained, m. p. 250–251°; $[\alpha]^{25}D$ + 69° (*C*, 3 in water).

Anal. Calcd. for $C_{10}H_{14}O_6N_2$: C, 46.52; H, 5.46; N, 10.82. Found: C, 46.75; H, 5.52; N, 10.80.

When equal amounts of 1-L-arabinosylthymine and 1-D-arabinosylthymine were mixed and crystallized from water, 1-DL-**arabinosylthymine** was obtained, m. p. 238–239°; it did not rotate polarized light and was considerably less soluble in water than the optical isomers.

1-D-**Glucosylthymine.**—Fifteen grams (0.082 mole) of 2,4-diethoxy-5-methylpyrimidine was heated with 15 g. (0.037 mole) of recrystallized acetobromoglucose at 50° for seven days at 2–3 mm. pressure. The sirup was dissolved in 375 ml. of absolute methanol containing 13 g. of dry hydrogen chloride. The flask was stoppered and allowed to stand at room temperature. After three days the solvents were completely removed *in vacuo*, and the residue was dissolved in 50 ml. of hot absolute alcohol and placed in the cold overnight. The product was filtered and a second crop obtained from the filtrate. The combined yield was recrystallized from an alcohol and water mixture giving 4.6 g. (43%) of 1-D-glucosylthymine, m. p. 271°. The specific rotation was $[\alpha]^{25}D$ + 14.6° (*C*, 2 in water).

Anal. Calcd. for $C_{11}H_{16}O_7N_2$: C, 45.83; H, 5.60; N, 9.72. Found: C, 45.90; H, 5.69; N, 9.53.

1-D-**Galactosylthymine.**—Seventeen grams (0.041 mole) of D-acetobromogalactose was heated with 17 g. (0.093 mole) of 2,4-diethoxy-5-methylpyrimidine as described for the synthesis of glucosylthymine. The light yellow reaction mixture was hydrolyzed and the solvents removed as previously described. The sirupy residue was taken up with an equal weight of hot absolute alcohol and 3 volumes of hot chloroform were added. Upon cooling the solution, a white material was deposited which was collected by filtration and washed several times with chloroform before it was allowed to dry. The material collected on the funnel was dissolved in absolute alcohol and treated with Norite. To the hot filtrate was added 3 volumes of chloroform. The material separating from the solution was filtered and washed first with chloroform and then with ether. The product, 5.7 g. (yield 48%) was a white powder having an indefinite melting point.

Anal. Calcd. for $C_{11}H_{16}O_7N_2$: N, 9.72. Found: N, 8.74.

Ultraviolet Absorption Spectra of Thymine Nucleosides.—The ultraviolet absorption spectra of the D-ribose, D-arabinose and D-glucose thymine nucleosides were determined with a Beckman spectrophotometer with a hydrogen discharge tube as the source of light. The concentration used for all the measurements was 25 mg. in a liter of distilled water. The maximum and minimum absorption values of the thymine nucleosides are recorded in Table I. The absorption spectrum of glucosylthymine is shown in Fig. 1.

TABLE I

THE MAXIMUM AND MINIMUM ULTRAVIOLET ABSORPTION OF THYMINE NUCLEOSIDES

	Maximum, Å.	Minimum, Å.
1-D-Ribosylthymine	2660	2340
1-D-Arabinosylthymine	2640	2320
1-D-Glucosylthymine	2640	2340

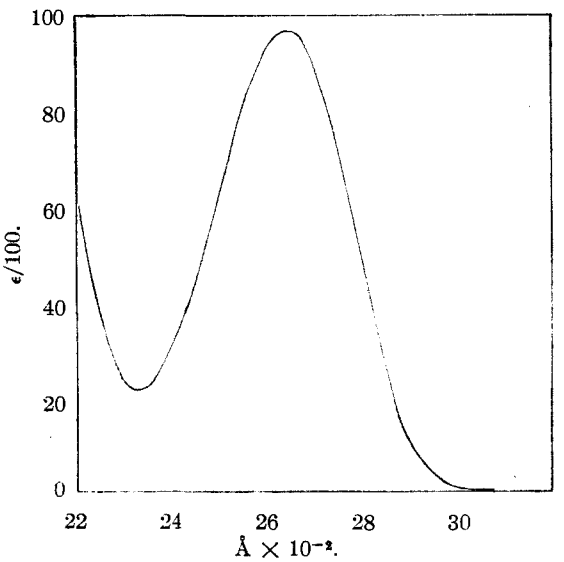

Fig. 1.—Ultraviolet absorption spectrum of 1-D-glucosylthymine. The wave length is plotted against the molecular extinction, ϵ ($\epsilon = E \times$ mol. wt./cd) where E = extinction, mol. wt. = molecular weight of compound, c = concentration in g. per liter, and d = cell thickness in cm.

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Foundation for generous gifts of thymine and D-ribose. The authors also wish to express their appreciation to Mr. Jack Fox for technical assistance.

Summary

The synthesis of 1-D-ribosyl-, 1-D-arabinosyl-, 1-L-arabinosyl-, 1-D-glucosyl- and 1-D-galactosyl-thymine nucleosides are described. These nucleosides were prepared by reactions between 2,4-diethoxy-5-methylpyrimidine and the proper acetobromo sugar.

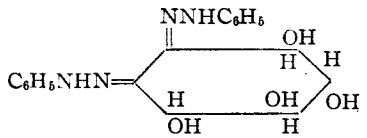
BOULDER, COLORADO RECEIVED DECEMBER 12, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

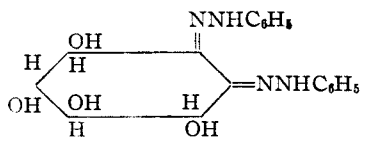
The Action of Periodic Acid on a Cyclohexose Osazone[1]

BY BORIS MAGASANIK[2] AND ERWIN CHARGAFF

It has been shown in a preceding communication[3] that the cyclohexose osazones isolated following the oxidation of *l*- and *d*-inositol by *Acetobacter suboxydans* each consumed three moles of periodic acid per mole of substance. Structure Ia represents the osazone derived from *l*-inositol, Ib that from *d*-inositol.[4]



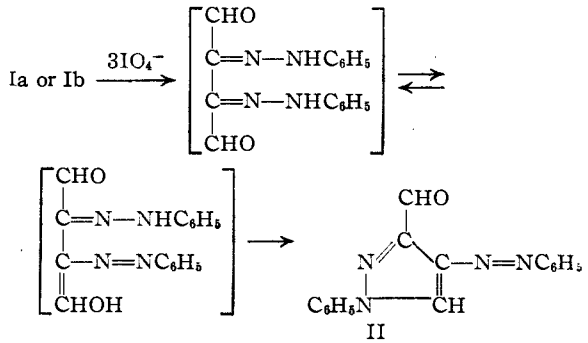
Ia



Ib

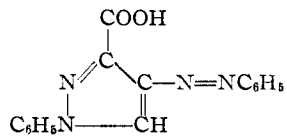
When the oxidation with periodic acid was carried out in a slightly alkaline alcoholic solution, a compound (II) was isolated in a yield of almost 80% whose analytical composition deviated from that of the 2,3-bis-phenylhydrazone of diketosuccinaldehyde, expected by analogy to the behavior of glucose phenylosazone,[5] by the lack of the elements of one molecule of water. The same substance was obtained, though in a lower yield, when the oxidation took place in acidic alcohol. The compound gave the Schiff test and could be oxidized by silver oxide under alkaline conditions to a monocarboxylic acid (III). With semicarbazide it yielded the corresponding semicarbazone.

Compound II appears, therefore, to be derived from the 2,3-bis-phenylhydrazone of diketosuccinaldehyde by the removal of one molecule of water and the concomitant suppression of one aldehyde function. The cyclodehydration of an enolic intermediate would account for the formation of 1-phenyl-4-phenylazo-3-pyrazolecarboxaldehyde (II) from the cyclohexose osazones. This reaction is analogous to the well known formation of 1-phenylpyrazoles from β-diketones and phenylhydrazine.[6]



II

The oxidation of II results in the formation of 1-phenyl-4-phenylazo-3-pyrazolecarboxylic acid (III).



III

The investigation of the absorption spectra of compounds II and III and of IIIa, the sodium salt of compound III, gave results favoring the structures discussed here (see Figure 1). The spectra of II and of the sodium salt IIIa were very similar, showing the low intensity "R band" (molecular extinction ε1060 and 940, at wave lengths of 425 and 435 mμ, respectively, obtained by graphical interpolation), and the high intensity "K band" in the ultraviolet, as found characteristic of phenylazo compounds.[7] The free acid III exhibited a slightly different spectrum, probably

(1) This work was supported in part by a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

(2) This report is from a dissertation to be submitted by Boris Magasanik in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(3) E. Chargaff and B. Magasanik, *J. Biol. Chem.*, **165**, 379 (1946); B. Magasanik and E. Chargaff, *ibid.*, **174**, 173 (1948).

(4) The enantiomorphic osazones originating from *l*- and from *d*-inositol, *i. e.* compounds Ia and Ib, yielded, of course, the same oxidation product with periodic acid.

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