light yellow oil at 216–218° (5 mm.). This ester was more readily prepared from the phenol and benzoyl chloride by the pyridine method.

Summary

1. *o*-Cresol was benzylated by means of benzyl alcohol in the presence of aluminum chloride. The main product was 2-methyl-4-benzylphenol. Smaller amounts of 2-methyl-6-benzylphenol and 2-methyl-4,6-dibenzylphenol were formed.

2. Benzylation by the Claisen method gave an excellent yield of 2-methyl-6-benzylphenol.

3. The monobromo derivatives and benzoyl derivatives of the two monobenzylcresols were prepared.

EAST LANSING, MICHIGAN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

RESEARCHES ON PYRIMIDINES. CXVII. A METHOD FOR THE SYNTHESIS OF NUCLEOSIDES

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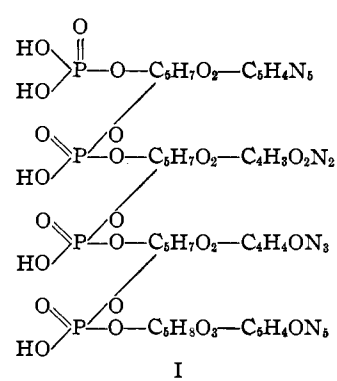
The present generally accepted structure of a nucleic acid molecule is based chiefly on the results of experimental work by Levene and his coworkers. They represent it as being a tetranucleotide construction, in which the individual nucleotides are combined through phosphoric acid ester linkages as expressed in the formula of yeast nucleic acid I. In this acid the functioning sugar is *d*-ribose.



I

The approach to a synthesis of a nucleic acid molecule calls first for methods of preparation of the pyrimidine and purine nucleosides, which may be considered as the glucosidic constructions characterizing the structure of nucleosides. If one accepts Levene's conception of the constitution of pyrimidine-nucleosides, in support of which he has contributed considerable indirect experimental evidence, the problem of nucleoside synthesis resolves itself into one of finding a practical method of coupling a sugar with a pyrimidine (uracil, thymine or cytosine) at the 3-position of the ring. The purpose of this paper is to describe a method of synthesis which has made it possible to obtain such a glucoside.

A comprehensive study of the behavior of 2,6-dioxy- and 2-thio- or 2-alkyl mercapto-6-oxypyrimidines toward different alkyl halides in alkaline

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solution by Johnson and his co-workers[2] led to results which did not enable one to formulate definite rules predicting the favorable position of substitution in such alkylation reactions. The active positions were 1 and 3 of the pyrimidine cycle forming nitrogen derivatives, and also the oxygen of the lactam grouping –CONH– forming cyclic imino ethers. Mixtures of mono- and di-substitution products were formed in varying proportions in practically every case examined. Other alkylating agents have also been used. Davidson and Baudisch[3] found that dimethyl sulfate interacted with uracil to form exclusively 1,3-dimethyluracil. Case and Hill[4] employed diazomethane as an alkylating agent, but here also they obtained results quite in accord with the previous observations of Johnson.

That direct alkylation of the pyrimidine and purine cycles is an unsuccessful method for the synthesis of nucleosides has been shown by Fischer[5] and co-workers and more recently by Levene and Sobotka.[6] The same general method was used by all these workers, namely, the action of acetobromoglucose or related combinations on the alkali or silver salts of purines and pyrimidines. The presence of a CONH linkage in both the pyrimidine and purine cycles favored the formation of glucosides which were easily decomposed by hydrolysis. These products were without doubt glucosides in which the sugar was attached to the ring in ether linkage. Fischer was able to obtain glucoside derivatives of purines in which the sugar was attached to the nitrogen of the imidazole ring only in those cases where the enolic isomerization of the cyclic acid amide grouping CONH of the ring was excluded, as in the case of theophyllin. Fischer confined his attempts at synthesis chiefly to purine combinations and only a few experiments were conducted with pyrimidines.[7] In both cases no products were obtained which resembled the natural nucleosides.

Levene and Sobotka[8] made a rather comprehensive study of the action of

[2] Johnson and Johns, *Am. Chem. J.*, **34**, 182 (1905); Johnson and Heyl, *ibid.*, **37**, 628 (1907); **38**, 237 (1907); Wheeler and Liddle, THIS JOURNAL, **30**, 1152 (1908); Johnson and Clapp, *J. Biol. Chem.*, **5**, 49 (1908); Johnson and Derby, *Am. Chem. J.*, **40**, 444 (1908); Johnson and Jones, *ibid.*, **40**, 538 (1908); Wheeler and Liddle, *ibid.*, **40**, 233 (1908); Johnson and Jones, THIS JOURNAL, **31**, 590 (1909); Wheeler and Johnson, *Am. Chem. J.*, **42**, 30 (1909); Wheeler and McFarland, *ibid.*, **42**, 431 and 1011 (1909); **43**, 10 (1909); Johnson and Moran, *ibid.*, **48**, 307 (1912); Johnson and Zee, *ibid.*, **49**, 287 (1913); Johnson and Bailey, THIS JOURNAL, **35**, 1007 (1913); Johnson and Haggard, *ibid.*, **37**, 177 and 2591 (1915); Johnson and Matsuo, *ibid.*, **41**, 782 (1919); Johnson and Joyce, *ibid.*, **38**, 1385 (1916).

[3] Davidson and Baudisch, THIS JOURNAL, **48**, 2379 (1926).

[4] Johnson, Hill and Case, *Proc. Nat. Acad. Sci.*, **8**, 44 (1922); Case and Hill, THIS JOURNAL, **52**, 1536 (1930).

[5] Fisher and Helferich, *Ber.*, **47**, 210 (1914); Fischer, *ibid.*, **47**, 1377 (1914); Fischer and Fodor, *ibid.*, **47**, 1058 (1914); Helferich and von Kühlewein, *ibid.*, **53**, 17 (1920).

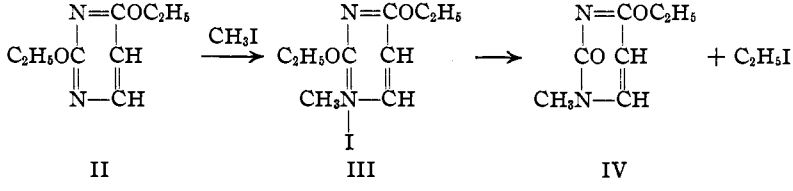
[6] Levene and Sobotka, *J. Biol. Chem.*, **65**, 463, 469 (1925).

[7] Fischer, *Ber.*, **47**, 1377 (1924).

[8] Levene and Sobotka, *J. Biol. Chem.*, **65**, 469 (1925).

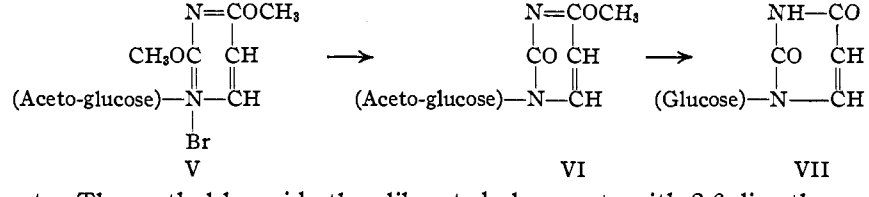
acetobromoxylose on salts of pyrimidines. A great variety of substituents in the pyrimidine cycle were used so as to cover any possible change in reactivity of the molecule. The silver salts of 1-methyluracil, 5-nitrouracil and 2-ethylmercapto-6-oxypyrimidine on treatment with acetobromoxylose led to the formation of oxygen ethers. The silver salt of cytosine did not react. The use of the potassium instead of the silver salts was also unsuccessful. The great difference in behavior toward hydrolytic agents of these synthetic nucleosides and the natural uridine and cytidine was assumed as offering further indirect evidence that the sugar in the pyrimidine nucleosides is linked in the 3-position of the ring.

As a result of the recent work of Hilbert and Johnson,[9] it is now possible to apply a method of alkylation which enables one to control substitution in the 3-position of the pyrimidine ring. This involves the reaction between a 2,6-dialkoxypyrimidine and an alkyl halide which interact in accordance with the equation below, giving a 3-substituted pyrimidine IV. Constructions of this type, IV, easily undergo hydrolysis, giving a normal uracil derivative of known structure.



II III IV

It has now been found that an analogous reaction takes place by treating 2,6-dimethoxypyrimidine with acetobromoglucose, yielding 2-oxy-6-methoxy-3-tetra-acetylglucosido-pyrimidine VI. The intermediate addition product V is unstable at 50°, at which temperature the reaction is carried



V VI VII

out. The methyl bromide thus liberated also reacts with 2,6-dimethoxypyrimidine, yielding a large quantity of 2-oxy-3-methyl-6-methoxypyrimidine. The acetylated glucoside VI and 2-oxy-3-methyl-6-methoxypyrimidine were separated by taking advantage of their different solubilities in water, the former being insoluble and the latter very soluble. It was then easily purified by recrystallizing from 50% alcohol, from which it separated in long needles melting at 220–221°.

The 2-oxy-6-methoxy-3-tetra-acetylglucosido-pyrimidine VI was simultaneously de-ethylated and deacetylated on treatment with alcoholic

[9] Hilbert and Johnson, THIS JOURNAL, 52, 2001 (1930).

hydrochloric acid, yielding 3-glucosido-uracil VII. In its chemical behavior this is similar in every respect to the natural uridine. It is stable toward dilute hydrochloric acid, however, when the double bond is reduced, forming 4,5-dihydro-3-glucosido-uracil; the latter is hydrolyzed with 3% sulfuric acid, yielding glucose and hydrouracil. This remarkable reaction was originally shown to take place by Levene and LaForge[10] with uridine, and the fact that our 3-glucosido-uracil parallels in its stability their findings would seem to be experimental evidence confirming the structure assigned to uridine.

3-Glucosido-uracil was brominated, forming 5-bromo-3-glucosido-uracil, and when acetylated yielded 3-tetra-acetylglucosido-uracil. It also gave a negative Wheeler–Johnson color reaction,[11] as is to be expected with a 3-substituted uracil.

This method for introducing a sugar on nitrogen should also be applicable to other ring systems such as quinolines, pyridines and pyrazolones and also to straight-chain compounds of the imino ether type.

Experimental Part

2-Oxy-6-methoxy-3-tetra-acetylglucosido-pyrimidine.—A solution of 2 g. of acetobromoglucose in 2 cc. of 2,6-dimethoxypyrimidine was placed in a stoppered tube and heated at 50° for forty-eight hours. After twenty-four hours the contents of the tube solidified to a colorless crystalline mass. The odor of methyl bromide was noticeable. The reaction mass was triturated three times with 5-cc. portions of water. This water extract on standing deposited colorless needles which were identified as 3-methyluracil;[12] yield, 1 g. The crude acetylated glucoside after treatment with water was usually in the form of a thick paste which solidified upon the addition of 5 cc. of ether. The solid was filtered by suction and thoroughly washed with ether to remove any unchanged acetobromoglucose. It was then recrystallized twice from 50% alcohol, from which it separated as a mass of long needles melting at 220–221° without decomposition; yield, 0.5–0.7 g. (23–32% of the theoretical). 2-Oxy-6-methoxy-3-tetra-acetylglucosido-pyrimidine is sparingly soluble in ether, slightly soluble in hot water, soluble in hot alcohol and very soluble in cold chloroform. The statement made in *Science*[13] that it reduces Fehling's solution after treatment with hydrochloric acid is in error.

Anal. Calcd. for $C_{19}H_{24}O_{11}N_2$: C, 50.00; H, 5.3; N, 6.14. Found: C, 50.50; H, 5.8; N, 6.16.

The influence of the variation of the temperature and of the proportions of the reactants upon the yield of the nucleoside was thoroughly investigated. If the reaction was carried out at room temperature, two weeks were required for completion of the reaction; the yield was 5% of that required by theory. At a temperature of 80° crystallization started within a few hours. However, the yield was only 9%. If equivalent amounts of the reactants were used the yield was not only very poor but the product was also more difficult to obtain pure because of the large preponderance of 3-methyl-

[10] Levene and LaForge, *J. Biol. Chem.*, 13, 507 (1912–1913).

[11] Wheeler and Johnson, *ibid.*, 3, 183 (1907).

[12] 2-Oxy-6-methoxy-3-methyl-pyrimidine on standing in the aqueous extract is hydrolyzed to 3-methyluracil.

[13] Johnson and Hilbert, *Science*, 69, 579 (1929).

uracil. In large quantity production the reaction was generally smooth. In one case, however, the end-product was partially deacetylated. This on treatment in the appropriate manner with acetic anhydride yielded the desired 2-oxy-6-methoxy-3-tetra-acetylglucosido-uracil.

3-Glucosido-uracil.—A solution of 9 g. of 2-oxy-6-methoxy-3-tetra-acetylglucosido-pyrimidine in 125 cc. of hot absolute methyl alcohol was treated with 30 cc. of a solution of dry hydrochloric acid in absolute ethyl alcohol (22% hydrochloric acid by weight). The reaction mixture was allowed to stand at room temperature for twenty-four hours. During this time a solid cake of colorless chunky crystals separated. The product was filtered and after drying weighed 5.6 g. (97% of the theoretical). It was recrystallized from 90% alcohol, from which it separated in the form of hexagons which contained water of crystallization and melted between 195–203°, depending upon the rate of heating. The water of crystallization was very firmly bound and required heating at 115° at 2-mm. pressure for its complete removal.[14] The anhydrous substance melted at 207–209° with decomposition to a milky liquid. Its rotation is $[\alpha]_D^{23} = +21.4$ in aqueous solution.

Anal. Calcd. for $C_{10}H_{14}O_7N_2(H_2O)_{1/2}$: H_2O, 3.18. Found: H_2O, 3.27, 3.41, 3.48.

Anal. Calcd. for $C_{10}H_{14}O_7N_2$: C, 43.80; H, 5.11; N, 10.22. Found: C, 43.56; H, 5.25; N, 10.12.

3-Glucosido-uracil does not reduce Fehling's solution after boiling with various concentrations of hydrochloric acid for several hours. It also gives a negative Wheeler–Johnson color reaction for pyrimidines of the uracil type.

3-Tetra-acetylglucosido-uracil.—A gram of 3-glucosido-uracil was treated with a solution of 0.5 g. of anhydrous sodium acetate in 10 g. of acetic anhydride and heated on a steam-bath for two hours. The pyrimidine derivative rapidly dissolved, forming a solution with a pale yellow color. On pouring the reaction mixture into 40 cc. of ice water, an oil separated which on standing at 0° slowly crystallized. The solid was filtered, thoroughly washed with water and dried. After treatment with boneblack it was recrystallized twice from 50% alcohol, from which it separated in clusters of prisms; melting point, 154–155°; yield, 1 g.

Anal. Calcd. for $C_{18}H_{22}O_{11}N_2$: N, 6.34. Found: N, 6.33.

5-Bromo-3-glucosido-uracil.—Nine-tenths of a gram of 3-glucosido-uracil was treated with bromine water until the solution had a permanent pale yellow color. The water was removed by distillation under diminished pressure. A pale yellow, sirupy residue remained, which was dissolved in 15 cc. of absolute ethyl alcohol and the resulting solution evaporated to dryness twice in an open vessel with constant stirring. The solid product was recrystallized twice from 90% alcohol, from which it separated as small colorless glistening prisms; yield 1 g. It contained 1% of water of crystallization and melted at 224° with effervescence, turning dark brown. The water of crystallization was very firmly bound and required drying at 115° at 2-mm. pressure for its complete removal. The decomposition point of the anhydrous 5-bromo-3-glucosido-uracil was 238°. It was extremely soluble in water and insoluble in organic solvents. The rotation was $[\alpha]_D^{25°} + 10.3°$ in aqueous solution.

Anal. Calcd. for $C_{10}H_{13}O_7N_2Br$: C, 33.99; H, 3.68; N, 7.93. Found: C, 34.18; H, 3.59; N, 8.04.

4-5-Dihydro-3-glucosido-uracil.—A solution of 2 g. of 3-glucosido-uracil in 50 cc. of 80% alcohol was shaken with 0.2 g. of Adams and Shriner[15] platinum oxide catalyst

[14] Fischer[5,7] found that the water of crystallization in the purine glucosides was also difficult to remove.

[15] Adams and Shriner, THIS JOURNAL, **45**, 2171 (1923).

and hydrogen under a pressure of 2–3 atmospheres. Reduction was completed in an hour. The catalyst was removed from the solution and the alcohol distilled under diminished pressure. A colorless sirup remained, which on trituration with absolute alcohol and scratching eventually solidified. The product was recrystallized from 100 cc. of 90% alcohol, yielding 1.2 g. of anhydrous colorless diamond-shaped crystals; 4,5-dihydro-3-glucosido-uracil turns slightly brown at 220° and decomposes at 238°. It is very soluble in cold water and insoluble in organic solvents. It showed $[\alpha]_D^{24°}$ +9.3° in aqueous solution.

Anal. Calcd. for $C_{10}H_{16}O_7N_2$: C, 43.48; H, 5.80; N, 10.15. Found: C, 43.65; H, 5.66; N, 10.19.

Hydrolysis of 4,5-Dihydro-3-glucosido-uracil with 3% Sulfuric Acid to 4,5-Dihydrouracil and Glucose.—One and six-tenths grams of 4,5-dihydro-3-glucosido-uracil was dissolved in 50 cc. of 3% sulfuric acid and refluxed for two hours. The brown colored solution was treated with 8 g. of powdered barium carbonate and heated on a steam-bath until effervescence of carbon dioxide ceased and then finally filtered. The water was removed by distillation under diminished pressure and the crystalline residue dissolved in 5 cc. of boiling water, treated with boneblack and filtered. On cooling, colorless plates of 4,5-dihydrouracil separated; yield, 0.2 g. After recrystallizing from hot water the product melted at 276–277°. A mixed melting point with an authentic specimen was unchanged.

The filtrate from 4,5-dihydrouracil was treated with 0.8 g. of phenylhydrazine hydrochloride and 0.6 g. of sodium acetate and heated on a steam-bath for a few hours. After heating a short time yellow needles of phenylglucosazone started to separate. After recrystallization from dilute alcohol it melted at 207–208°. A mixed melting point with a genuine sample was unchanged.

Conclusion

1. A method for the synthesis of pyrimidine nucleosides has been described. 3-Glucosido-uracil has been prepared by this procedure.

2. 3-Glucosido-uracil is not hydrolyzed by dilute acids, whereas its reduction product, 4,5-dihydro-3-glucosido-uracil, is hydrolyzed by 3% sulfuric acid to hydrouracil and glucose. They thus behave similarly to uridine and its reduction product 4,5-dihydrouridine. This is believed to be the strongest direct experimental evidence yet obtained in favor of the structure of uridine, being 3-ribosido-uracil.

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