corresponding in composition to a hydrolysate of bovine serum albumin. This suggests that the phosphate itself has no appreciable effect on the hydrolytic destruction of serine. It is apparent from the last column of Table IV that a correction for losses of serine by extrapolation of the results to zero time, assuming first-order kinetics for the decomposition, would lead to considerable error.

Phosphoserylglutamic acid was first isolated by Levene and Hill[40] from an enzymatic digest of casein. de Verdier[39] has reported the presence of phosphoserylglutamic and phosphoserylalanine and several other peptides of phosphoserine from acid digests of casein but was not able to establish that serine was N-terminal.

(39) C. H. de Verdier, *Acta Chem. Scand.*, **8**, 1302 (1954).

PHILADELPHIA, PA.

[CONTRIBUTION FROM THE SECTION ON ENZYMES OF THE LABORATORY OF CELLULAR PHYSIOLOGY, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Synthesis of Some Model Pyrimidine Nucleosides

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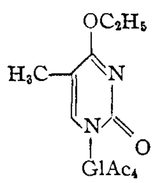
Methods of preparing some N-alkyl and O-alkyl pyrimidine nucleosides are described.

In order to study the tautomeric forms of certain nucleosides and nucleotides in solution[1] by their infrared spectra it was desirable to have derivatives of the possible tautomeric forms in which the labile hydrogen is replaced by methyl or ethyl groups. The present article describes the synthetic methods used for the compounds not previously reported.

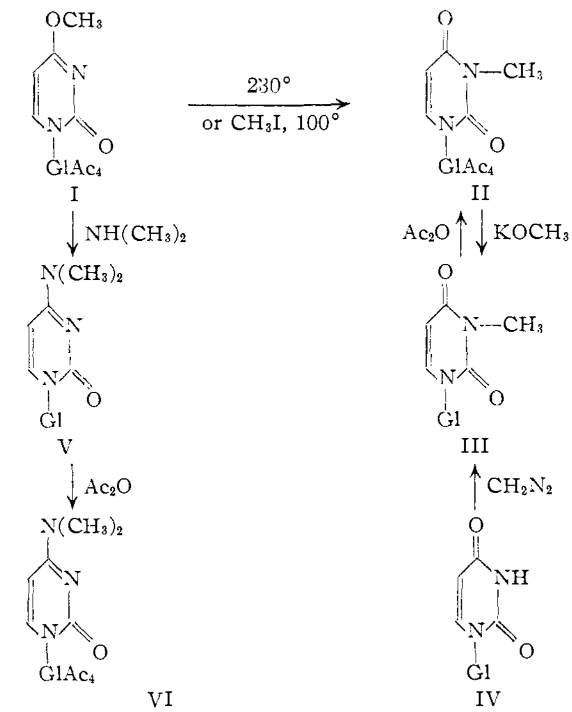
Direct N-Methylation of Nucleosides.—Levene and Tipson[2] reported the preparation of 3-methyluridine by use of diazomethane on 2′,3′-di-O-acetyl-5′-O-triphenylmethyluridine in ether solution. It was found recently[1] that 3-methyluridine could be prepared much more conveniently by direct treatment of the nucleoside in methanol with diazomethane, thus obviating the introduction and subsequent removal of acetyl or trityl groups. By this method 3-methylthymidine, 1-β-D-glucopyranosyl-3-methyluracil and 1-β-D-glucopyranosyl-3-methylthymine have been prepared for the first time. In each case only the product of N-methylation was isolated.

Preparation of the O-Alkyl Nucleosides.—The 1-(2′,3′,4′,6′-tetra-O-acetyl)-β-D-glucopyranosyl-4-alkoxypyrimidines (I and VII) were prepared by the method of Hilbert and Johnson.[3] Compound I has been reported before[3] and readily can be isolated from the reaction mixture in crystalline form.

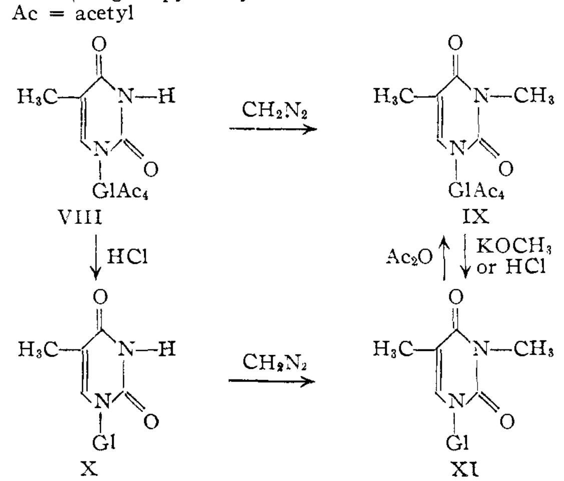


VII

The corresponding thymine compound VII, however, has not been isolated by previous workers[4,5] though it obviously has been present in reac-



Gl = β-D-glucopyranosyl
Ac = acetyl



(1) H. T. Miles, *Biochim. Biophys. Acta*, **22**, 247 (1956).
(2) P. A. S. Levene and R. S. Tipson, *J. Biol. Chem.*, **104**, 385 (1934).
(3) G. E. Hilbert and T. B. Johnson, THIS JOURNAL, **52**, 4489 (1930).
(4) W. Schmidt-Nickles and T. B. Johnson, *ibid.*, **52**, 4511 (1930).
(5) D. W. Visser, I. Goodman and K. Dittmer, *ibid.*, **70**, 1926 (1948).

tion mixtures. Since the O-ether was required as such and not merely as an intermediate, attempts were made to prepare it by varying the pyrimidine and the glucosyl halide reactants, the temperature and the pressure; but from none of the reactions could the desired product be isolated. Finally a chromatographic method was found which permitted isolation of the pure material from the reaction mixture.

Deacetylation of I and VII was accomplished by treatment with a catalytic amount of potassium methoxide, followed by removal of the potassium ion by titration with 60% perchloric acid to a *p*H meter reading of 8.1.[6] In each case the product was a glass, which could be reacetylated to the original O-alkyl material.

Synthesis of 1-Glucosyl-4-dimethylamino-2-pyrimidone (V).—In order to have a model compound for cytidine which would be incapable of tautomerizing, it was desired to prepare a compound of structure V. For this purpose it was found that when the enol ether I or its ethoxy analog was treated with dimethylamine at room temperature V was produced in almost quantitative yield. Acetylation with acetic anhydride in pyridine gave the tetraacetate of V.

Conversion of O-Methyl- to N-Methylnucleoside.—This transformation was carried out by two methods. The first was a thermal lactim–lactam rearrangement in which the enol ether I was heated at 230° to give the N-methyl compound II.

Although the formation of methyl bromide as a by-product of the glycosylation reaction of Hilbert and Johnson[3] results in the formation of N-methylpyrimidines, no formation of N-methylnucleosides has been reported among the reaction products. It was found, however, that heating the enol ether with methyl iodide at 100° resulted in the smooth conversion of I to II. When the analogous O-ethyl compound was heated with methyl iodide, the product has a methyl rather than an ethyl group on the nitrogen, as would be expected from direct N-methylation followed by thermal elimination of ethyl iodide.

Compound II also was prepared by methylating 1-β-D-glucopyranosyluracil with diazomethane, as described above, and acetylating the product III to give II.

Since the water-soluble compounds are to be used as model compounds for spectral study, it is necessary that their structures be proved by other means. The availability of the same compound by independent synthetic routes does much to establish a structure as well as to provide desirable flexibility in choice of starting materials. Such independent evidence was sought originally in the present case because the N-methyl determinations carried out in the usual way gave erratic results, the value found generally being about an eighth of the calculated amount. It has since been found possible to obtain good N-methyl values, though both analysts have reported that the methyl groups are very difficult to split off.[7]

Paper Chromatography.—The R_f values of the water-soluble members of the present series as well as some of the natural nucleosides were determined in two solvents and are presented in Table I. While these values are not precise physical constants they are reasonably reproducible even when no elaborate precautions are taken; they indicate the relative mobilities of the compounds and show which separations can be achieved readily. The compounds were detected by quenching of the fluorescence caused in the paper by a Mineralite ultraviolet lamp. Ascending chromatograms were used.

In all cases the 3-N-methyl compound had a higher R_f than the parent nucleoside, presumably because of its greater relative solubility in the nonaqueous phase. Comparison of IV with X, of IV with uridine, and of uridine with thymidine suggest that with analogous compounds the R_f generally decreases with the number of hydroxyl groups on the sugar and increases with the number of methyl groups on the ring, especially if a labile hydrogen has been replaced by methyl.

A useful application of paper chromatography of these compounds is in following the diazomethane methylation. It is easy to tell whether the reaction has gone to completion or not and even, roughly, the ratio of reactant to product.

A Convenient Micro Method for Distinguishing O-Methyl from N-Methyl.—The greatest usefulness of paper chromatograms in the present study was in demonstrating with a few micrograms of material whether a methyl or ethyl group was attached to oxygen or nitrogen. When little material is available it is often impractical to carry out an acid hydrolysis of the enol ether and isolate the crystalline nucleoside.

A few micrograms of the methyl nucleoside were added to concentrated hydrochloric acid. After the solution had stood overnight the acid was evaporated and the reaction product was chromatographed. The compounds which were derived from acetates of nucleoside enol ethers (the structures of the acetates have been demonstrated by acid hydrolysis of larger quantities to known nucleosides[3,5]) by potassium methoxide deacetylation gave products which had the same R_f values as the corresponding nucleosides.

N-Methyluridine, whose structure was proved by Levene and Tipson,[2] and the other compounds prepared by diazomethane methylation in this study were unaffected by acid treatment. Since both N-methyl and O-methyl compounds move well above the parent nucleoside in all solvents, there is no difficulty distinguishing the two by acid treatment even when they have the same R_f.

The R_f's after acid treatment of the seven compounds tested are given for two solvents in Table I.

Experimental[7,8]

3-Methylthymidine.—To a solution of 800 mg. of thymi-

(6) W. Bonner and W. L. Koehler, THIS JOURNAL, **70**, 314 (1948.)

(7) "Microanalyses," by J. F. Alicino, Metuchen, New Jersey, with exception of O-CH_3 and N–CH_3 determinations of compounds II, IX(M) and XI, which were done by W. Manser of Zurich, Switzerland. The second N–CH_3 determination of IX(A) was done by Alicino by running the same sample through the procedure four times and using longer heating periods. The first three titrations gave roughly a third each of the theoretical value, and the fourth titration a negligible amount. A repeated N–CH_3 analysis of 3-methylthymidine has not yet been obtained.

(8) Melting points were measured on a Kofler apparatus and are corrected.

TABLE I

Schleicher & Schuell no. 598 paper was used for the chromatograms.

Compound	Solvent A[a]	Solvent A[a] After acid treatment	Solvent B[b]	Solvent B[b] After acid treatment
Uridine	0.29	..	0.26	..
3-Methyluridine	.69	0.68	.44	0.42
Thymidine	.62	..	.47	..
3-Methylthymidine	.84	.83	.72	.74
Cytidine	.28	..	.20	..
1-β-D-Glucopyranosyluracil	.19	..	.15	..
1-β-D-Glucopyranosyl-3-methyluracil (III)	.60	.60	.31	.30
1-β-D-Glucopyranosylthymine	.34	..	.24	..
1-β-D-Glucopyranosyl-3-methylthymine (XI)	.68	.70	.40	.39
1-β-D-Glucopyranosyl-4-ethoxy-2-pyrimidone[c]	.63	.20	.43	.13
1-β-D-Glucopyranosyl-4-methoxy-2-pyrimidone[c]	.50	.20	.28	.14
1-β-D-Glucopyranosyl-4-ethoxy-5-methyl-2-pyrimidone[c]	.70	.33	.40	.21
1-β-D-Glucopyranosyl-4-dimethylamino-2-pyrimidone (V)	.29	..	.17	..

[a] Solvent A: triethylamine, *t*-butyl alcohol, water (2:10:3). [b] Solvent B: 88% formic acid, *n*-butyl alcohol, water (10:10:3); let stand 24 hr. and use upper layer. Fresh solvent was not used because of the rapid esterification, which caused a marked change in composition and deposition of an aqueous layer during the running of the chromatogram. A 24-hour period of storage was chosen for convenience, though it does not represent equilibrium. [c] The values are reported for compounds from which the potassium ion has been removed. When this has not been done, the R_f values are lower though still reproducible for a given sample.

dine (Schwarz) in 70 ml. of methanol was added 80 ml. of a solution of diazomethane[9] in ether. The loss of color and evolution of nitrogen were rapid during the early part of the reaction. After the addition the solution was allowed to stand several hours in an ice-bath. The product after evaporation of solvent was a sirup which could not be induced to crystallize.

The reaction mixture was then chromatographed on 100 g. of potato starch in butanol saturated with water. The progress of the chromatogram was followed by the optical density at 260 mμ. The peak of optical density came at about 80 ml. of effluent, and the fractions from 60 to 120 ml. of effluent, representing 540 mg., were combined and crystallized from a mixture of methanol, ethyl acetate and ether.[10]

Further crystallizations were from methanol and ether and gave a material melting at 132.5–134° (unless the material had been thoroughly dried, it melted at about 72°, then resolidified and melted at 130–131°).

Anal. Calcd. for $C_{11}H_{16}N_2O_6$: C, 51.56; H, 6.29; N, 10.93; OCH_3, 0.00; NCH_3, 5.85. Found: C, 51.51; H, 6.34; N, 10.46; OCH_3, 0.00; NCH_3, 0.89.[7]

1-Glucosyl-3-methyluracil (III).—To a suspension of 170 mg. of IV (prepared by the Hilbert and Johnson[3] method) in 15 ml. of methanol was added 8 ml. of ether solution of diazomethane.[9] The reaction was slower than the first one, so 1 ml. of water was added and the suspension refluxed to bring most of the material in solution and then cooled before the slow addition of another 7 ml. of diazomethane solution. The product was treated with charcoal, filtered, and most of the solvent evaporated.

The initial sirup was finally induced to crystallize from a mixture of methanol, ethyl acetate and ether.[10] After four more crystallizations and drying under vacuum, the m.p. was 247–248.5°.

Anal. Calcd. for $C_{11}H_{16}N_2O_7$: C, 45.83; H, 5.60; N, 9.72; NCH_3, 5.20. Found: C, 45.79; H, 5.56; N, 9.93; NCH_3, 5.09.

The same compound was prepared from a sample of II by deacetylation with potassium methoxide. After crystallization from methanol and ether the latter material had an infrared spectrum identical with III prepared by the diazomethane reaction, and a mixture of the two showed no melting point depression.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-3-methyluracil (II). A. Reaction with Methyl Iodide.—To a mixture of 7 ml. of methyl iodide and 4 ml. of acetone was added 400 mg. of I (in other preparations the ethoxy analog was used with equal success). The reaction tube was sealed and heated at 100° for about 60 hours. The tube was opened, the solvent evaporated, the residue taken up in methanol, treated with charcoal and filtered. The compound crystallized to give 320 mg. of m.p. 99–103°. The material was recrystallized to a constant m.p. of 105–108° after drying. A sample of narrower melting range was not obtained in any of these experiments.

Anal. Calcd. for $C_{19}H_{24}N_2O_{11}$: C, 50.00; H, 5.30; N, 6.14; OCH_3, 0.00; NCH_3, 3.29. Found: C, 49.09; H, 5.02; N, 5.98; OCH_3, 0.00; NCH_3, 2.98.

The low carbon value of the analysis can be attributed to inadequate purity of the compound, although it was recrystallized five times from methanol to a constant melting range. For some reason the compound appears to be unusually difficult to purify.

Since II could be formed by two methods from the known compound I and by simple acetylation from III (which gave a very good analysis) there is no reason to doubt that the compound has the indicated structure. The conversion of II back to III by deacetylation confirms that no unusual change had taken place on acetylation.

B. Pyrolysis of I.—A combustion tube containing 300 mg. of I was flushed out with helium, sealed and heated in a Carius furnace at 230° for 24 hr. A solution of the material in methanol deposited 200 mg. of crystals, m.p. 104–107°. The light tan material was treated with charcoal and recrystallized, the m.p. remaining the same. An infrared spectrum of a chloroform solution of this material was superimposable on that of II prepared by method A.

C. Acetylation of III.—Fifty mg. of III (prepared by diazomethane methylation of IV) was added to 1.5 ml. of pyridine and 1.5 ml. of acetic anhydride and the mixture allowed to stand in the cold for a week. The solution was worked up to give 30 mg. of m.p. 100–105°, which had an infrared spectrum in chloroform solution identical with those of the samples prepared by methods A and B.

Preparation of 1-(Tetra-O-acetyl-β-D-glucopyranosyl)-4-ethoxy-5-methyl-2-pyrimidone (VII).—A mixture of 8.5 g. of 2,4-diethoxy-5-methylpyrimidine and 13.4 g. of freshly recrystallized tetra-O-acetyl-α-D-glucopyranosyl bromide was heated in a 50-ml. flask at 60°. Through a side arm protected by a Dry Ice trap the flask was connected to a house vacuum line of about 100 mm. pressure. The flask was heated for a week, the last day at 75°. From the reaction mixture there was obtained 19 g. of sirup which resisted all attempts at crystallization (small amounts of methylpyrimidines formed in this and similar reactions were filtered off from ether solution).

A successful chromatographic method was found and applied as follows to 14 g. of the sirup. A column of 350 g. of Magnesol[11] (Food Machinery and Chemical Co.; the material was previously washed with acetone and then dried) was prepared from a benzene slurry and a benzene solution of the 14 g. of reaction mixture poured on it.

(9) The diazomethane solutions used in these reactions were prepared by covering solid N-methyl-N-nitroso-N'-nitroguanidine with 10 ml. of ether per g. of solid and adding 40% potassium hydroxide solution slowly to the chilled mixture. At the completion of the reaction the diazomethane solution was decanted.

(10) In recrystallizing this and other water soluble members of the present series the compound was dissolved in a minimum quantity of methanol at the boiling point and either ethyl acetate (in which most of the compounds are only slightly soluble) or ether (in which they are insoluble) or both were added slowly to the point of cloudiness. Chilling at −10 to 0° then generally caused crystal formation.

(11) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

The eluting solution was ethanol in benzene, the volume used at each concentration being as follows: 500 ml., 100:1 benzene–ethanol (even with concentrated solutions there is very little volume change on mixing benzene and ethanol); 700 ml., 50:1; 700 ml., 20:1; 1000 ml., 10:1; 200 ml., 5:1; 300 ml., 2:1.

The effluent was tested for the desired material by placing a drop on filter paper and looking for quenching of fluorescence. As long as no quenching material came off, large fractions of 500–1000 ml. were collected and evaporated to confirm the absence of material; 100-ml. fractions were evaporated and weighed as the solute came off the column. The fractions from 1970 to 2000 ml. effluent were combined to give 4.2 g. of sirup, which crystallized spontaneously on standing two days. The fractions from 2000 to 2200 ml. effluent gave an additional 1.3 g. of sirup, which crystallized on seeding. The remainder of the effluent provided no crystalline material.

The substance obtained from the column was recrystallized to a m.p. of 132–133°.

Anal. Calcd. for $C_{21}H_{28}N_2O_{11}$: C, 52.07; H, 5.83; N, 5.97. Found: C, 52.00; H, 5.91; N, 5.87.

The enol ether obtained in this manner was deacetylated with potassium methoxide as described above to give a glass, which could be reacetylated to the original tetraacetate.

More careful chromatography of 5 g. of reaction sirup on 90 g. of Magnesol, using an automatic fraction collector and taking 7-ml. cuts, gave a 1.6-g. fraction which provided 1.3 g. of material melting at 131.5–133°. If the same per cent. yield of crystalline product had been obtained in the larger chromatogram, the yield of crystalline material for the reaction would have been about 31% instead of the observed 19%.

Although previous workers[5] failed to isolate the present compound they obtained its acid hydrolysis product, β-D-glucopyranosylthymine, in 43% yield.

1-β-D-Glucopyranosyl-3-methylthymine (XI).—The most convenient preparation of 1-tetra-O-acetyl-β-D-glucopyranosylthymine (VIII) proved to be the method of condensing dithyminylmercury with tetra-O-acetyl-α-D-glucopyranosyl bromide reported recently by Fox, Yung, Davoll and Brown.[12]

Six g. of VIII was dissolved in 40 ml. of chloroform and 10 ml. of methanol. On chilling VIII came out of solution, but as the material reacted with the first 15 ml. of diazomethane solution (a total volume of 40 ml. was eventually used) it all went into solution. The solvent was evaporated, more chloroform added, the solution treated with charcoal and again evaporated. The sirupy product could not be induced to crystallize; after standing several days without solvent, it crystallized spontaneously. The yield of IX was 5 g., m.p. 112–116°. A mixture of ethanol, ether and a little hexane was found to give the best results of the solvents tried. The m.p. of the purified material was 120–122°.

Anal. Calcd. for $C_{20}H_{26}N_2O_{11}$: C, 51.06; H, 5.57; N, 5.96; OCH_3, 0.00; NCH_3, 3.19. Found: C, 51.23; H, 5.46; N, 5.89; OCH_3, 0.00; NCH_3, 3.13 (M); 3.57 (A).[7]

The above tetraacetate IX was converted to XI by treating a solution of 500 mg. of pure tetraacetate in 18 ml. of absolute methanol with several mg. of potassium, warming for 10 min. and leaving overnight. When ether was added to the solution, the product came out as a sirup. The potassium ion was then removed by titration with perchloric acid followed by centrifugation.[6] This time when ether was added to the point of turbidity and the solution cooled, crystals separated. The yield was 250 mg. of m.p. 197–200°. This material was treated with alumina and charcoal in methanol and crystallized 5 times to a constant melting range of 196–202°. (The upper limit is the point at which the last crystals visible under the polarizer disappear.) Despite the broad range, the analysis indicated that the material was pure and had the expected constitution.

Anal. Calcd. for $C_{12}H_{18}N_2O_7$: C, 47.68; H, 6.00; N, 9.27; OCH_3, 0.00; NCH_3, 4.96. Found: C, 47.79; H, 5.90; N, 9.33; OCH_3, 0.00; NCH_3, 3.85.

Alternate Preparation of IX.—In view of the originally poor NCH_3 analyses it was considered desirable to carry out the reverse of the above transformation, that is, the methylation of 1-β-D-glucopyranosylthymine, a known compound,[6] to give IX.

Accordingly, dry hydrogen chloride was passed into a cold solution of VIII in 200 ml. of methanol for 15 minutes and the stoppered solution allowed to stand a day. The solution was evaporated under vacuum and the sirup crystallized from methanol and ether. After one recrystallization the m.p. was 272–274.5°, in agreement with the reported value[5] of 271°.

The glucosylthymine was then treated in aqueous methanol (10% water to increase solubility) with ethereal diazomethane, as described above for IV. The product was a sirup that crystallized on seeding with a crystal of IX obtained by the potassium methoxide deacetylation described above. After recrystallization the present material, when mixed with XI, showed no melting point depression and had an identical infrared spectrum.

1-β-D-Glucopyranosyl-4-dimethylamino-2-pyrimidone (V).—To a mixture of 30 ml. of dimethylamine and 15 ml. of methanol in a pressure bottle was added 1.5 g. of the 4-ethoxy analog of I. The bottle was closed and allowed to stand at room temperature for two days. Most of the solvent was evaporated under air stream on the steam-bath and ethyl acetate and ether[10] then added to cause crystallization. The yield was 0.95 g. (99%). The material even after several crystallizations and drying for analysis exhibited a double melting point, the first at about 170–180°, with subsequent crystallization of the melt, the second at 271–273°.

In an earlier experiment the same reaction was carried out with I at 60° and the same product obtained in 82% yield.

Anal. Calcd. for $C_{12}H_{19}N_3O_6$: C, 47.83; H, 6.36; N, 13.95; NCH_3, 9.96. Found: C, 47.53; H, 6.59; N, 13.96; NCH_3, 6.75.

The tetraacetate of V was prepared by reaction with acetic anhydride in pyridine in the cold. The m.p. of the pure material was 281–282°.

Anal. Calcd. for $C_{20}H_{27}N_3O_{10}$: C, 51.17; H, 5.80; N, 8.95; NCH_3, 6.40. Found: C, 51.21; H, 5.87; N, 9.04; NCH_3, 6.74.

BETHESDA, MD.

(12) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, THIS JOURNAL, **78**, 2117 (1956).