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Synthesis of 3-β-D-Ribofuranosyluracil and 1-Methyl-3-β-D-ribofuranosyluracil[1]

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Two new analogs of nucleic acid constituents have been synthesized. 1-Methyl-3-β-D-ribofuranosyluracil was synthesized from 1-methyl-4-methoxyuracil and from silver and mercury derivatives of 1-methyluracil. The isomer of uridine, 3-β-D-ribofuranosyluracil was synthesized from 4-ethoxyuracil and from a mercury derivative of 2-methylthiouracil.

INTRODUCTION

The nucleoside component of a new nucleotide which was isolated from certain ribonucleic acids by Davis and Allen[2] was shown by Yu and Allen[3] to be an isomer of uridine. Scannell, Crestfield, and Allen[4] and Cohn[5] have presented evidence which indicates that the isomer is a C-5 ribonucleoside of uracil in which an unusual carbon to carbon linkage is found. At an early stage in the research the possibility that the isomer was 3-ribosyluracil was considered; hence it was decided to undertake the syntheses of *N*-3 ribonucleosides. An additional stimulus for the work was the possible use of such compounds as chemotherapeutic agents, metabolite antagonists, and substrates for enzyme specificity investigations.

RESULTS

Since the common methods[6,7] for the synthesis of pyrimidine *N*-nucleosides result in glyosidic linkage at the *N*-1 position rather than at the *N*-3 position, it was felt that the synthesis of an *N*-3 ribonucleoside could be accomplished most easily by blocking the more reactive *N*-1 position with a methyl group. There appeared to be two possible methods of approach to the problem. One involved the use of 1-methyl-4-methoxyuracil in a Hilbert-Johnson[6] type of synthesis. The other approach was by way of the mercuric salt of 1-methyluracil in a synthesis of the type introduced by Fox and co-workers.[7] The second approach was attempted first because yields are usually better in the mercuric salt reaction and 1-methyluracil was available in the laboratory while the preparation of 1-methyl-4-methoxyuracil required a three-step synthesis from uracil. 1-Methyluracil formed a salt with mercuric chloride of the type (1-methyluracil)$_2$ mercury. This compound was then condensed with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride to form an intermediate which was debenzoylated with sodium methoxide to produce a nucleoside, tentatively formulated as 1-methyl-3-β-D-ribofuranosyluracil, in 45% yield. This compound was, however, only slightly more stable to acid hydrolysis than the O^2 glycosides of uracil derivatives which were synthesized by Fischer[8] and by Levene.[9] Both Fischer and Levene had also synthesized compounds which they considered to be O^4 glycosides which were even less stable to acid hydrolysis than O^2 glycosides so that it was unlikely that the product of the mercuric salt condensation was 1-methyl-O^4-ribofuranosyluracil. However, the presence of a lactam-lactim system in 1-methyluracil indicated that this possibility could not be excluded. Consequently it was decided to employ the Hilbert-Johnson method in which an *O*-glycoside presumably cannot be formed in order to compare the properties of the products of the two types of reactions. To this end 1-methyl-4-methoxyuracil[10] was prepared. After condensation with the halogenose and debenzoylation of the intermediate a nucleoside which was identical with that formed from the mercuric salt of 1-methyluracil was obtained in 39% yield. It therefore seemed safe to conclude that the product was 1-methyl-3-ribofuranosyluracil. At the same time the silver salt of 1-methyluracil was prepared and condensed with the halogenose. Since the use of a great variety of silver salts of pyrimidine derivatives by previous workers[7-9,11] had invariably resulted in the formation of *O*-glycosides, it was expected that the O^4-ribonucleoside would be formed. Contrary to ex-

(1) This work was supported in part by Grant-in-aid RG 2496, U. S. Public Health Service, Grant G 8971 National Science Foundation and Cancer Research Funds of the University of California.

(2) F. F. Davis and F. W. Allen, *J. Biol. Chem.*, **227**, 907 (1957).

(3) C. T. Yu and F. W. Allen, *Biochim. et Biophys. Acta*, **32**, 393 (1959).

(4) J. P. Scannell, A. M. Crestfield, and F. W. Allen, *Biochim. et Biophys. Acta*, **32**, 412 (1959).

(5) W. E. Cohn, *Biochim. et Biophys. Acta*, **32**, 569 (1959).

(6) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 4489 (1930).

(7) J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956).

(8) E. Fischer and B. Helferich, *Chem. Ber.*, **47**, 210 (1914).

(9) P. A. Levene and H. Sobotka, *J. Biol. Chem.*, **65**, 469 (1925).

(10) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 2001 (1930).

(11) A. Hahn, H. Fosold, and L. Schäfer, *Z. Biol.*, **84**, 35 (1926). A. Hahn, W. Laves, and L. Schafer, *Z. Biol.*, **84**, 411 (1926). A. Hahn and W. Laves, *Z. Biol.*, **85**, 280 (1926).

pectations a product was obtained in 35% yield which was identical with the products of the previous reactions.

In order to determine whether the silver salt of thymine could be used in place of the mercuric salt which Fox, *et al.*[7] had used to prepare thymine riboside a condensation reaction with the silver salt was carried out. A product which was stable to acid hydrolysis and which possessed the chromatographic and spectrophotometric properties of that synthesized from the mercuric salt was obtained. The yield was 23% compared to that of 50% reported for the product of the mercuric salt condensation.

The preparation of unsubstituted 3-β-D-ribofuranosyluracil was attempted next. Both Fischer[8] and Levene[9] had used the silver salt of 2-methylthiouracil to synthesize highly unstable nucleosides which they considered to be O^4 glycosides. It seemed worthwhile to prepare the mercuric salt of 2-methylthiouracil in order to determine whether it would react with a halogenose to form an *N*-3 glycoside. When this experiment was carried out a complex mixture of products was obtained. The major product was the unstable O^4 ribonucleoside but the *N*-3 riboside was also formed in 6% yield. In addition small amounts of uridine and an unidentified nucleoside were formed. The low yield and the extreme difficulty involved in the separation of the *N*-3 ribonucleoside from the other nucleosides made this method rather unsatisfactory so another approach was sought.

A method by which 4-ethoxyuracil [4-ethoxy-2-(1H)-pyrimidinone] can be obtained from the products of a partial alkaline hydrolysis of 2,4-diethoxyuracil was devised by Hilbert and Jansen.[12] When 4-ethoxyuracil was condensed with the halogenose in boiling xylene a 22% yield of 3-ribosyluracil was obtained from the product of the reaction. Purification was not difficult since no other nucleosides were formed.

EXPERIMENTAL[13]

Preparation of 1-methyl-3-β-D-ribofuranosyluracil from the mercuric salt of 1-methyluracil. 1-Methyluracil[10] (20.0 mmoles, 2.52 g.), was dissolved in 80 ml. of hot water which contained 0.8 g. of sodium hydroxide (20.0 mmoles). To this a saturated alcoholic solution of 2.70 g. of mercuric chloride (10.0 mmoles) was added whereupon a white precipitate was formed. The precipitate which was removed from the cooled supernatant solution by filtration was washed with cold water until the washings were free of chloride ion. The material was then washed with ethanol followed by ether. A yield of 4.2 g. (93%) of $(1\text{-methyluracil})_2$ mercury was obtained.

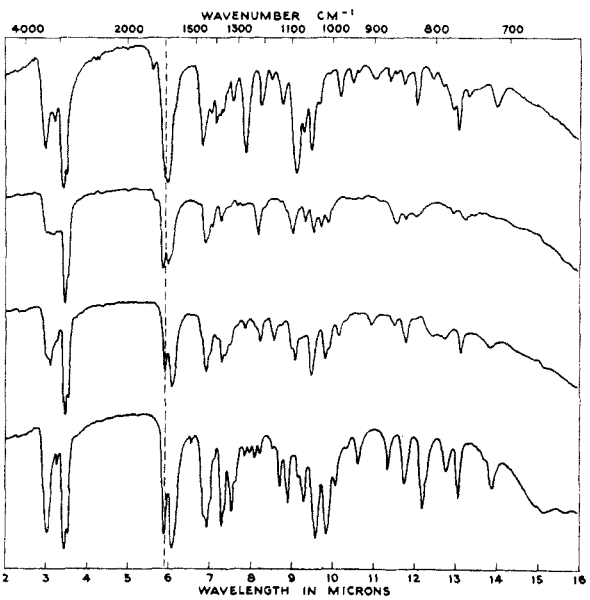

Fig. 1. Infrared spectra of uracil ribosides: (top to bottom) uridine; 5-ribosyluracil; 3-β-D-ribofuranosyluracil; 1-methyl-3-β-D-ribofuranosyluracil

$(1\text{-Methyluracil})_2$ mercury, (1.0 mmole, 450 mg.), was added to 20 ml. of xylene. Traces of water were removed from the suspension by azeotropic distillation of about 5 ml. of the liquid. A slight excess (2.2 mmoles) of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[14] dissolved in benzene was added to the xylene suspension and the mixture was refluxed for 1 hr. During this time almost all of the suspended material dissolved and a white flocculent precipitate began to form. After the xylene solution was cooled 120 mg. of this precipitate was collected by filtration. The substance was identified as 1-methyluracil by butanol/water (86/14: v./v.) paper chromatography[15] and ultraviolet spectrophotometry. Thus, 48% of the starting material was accounted for. The volume of the filtered xylene solution was then reduced to about 5 ml. A pale yellow amorphous precipitate was obtained from this solution by the addition of 25 ml. of petroleum ether (b.p. 30–75°). This precipitate was dissolved in 50 ml. of chloroform and washed with a 30% aqueous solution of potassium iodide and then with water. After the chloroform solution was dried over sodium sulfate the solvent was removed *in vacuo*. Three 50-ml. portions of anhydrous methanol were then added and removed *in vacuo*. The hard glass which resulted was dissolved in 150 ml. of anhydrous methanol which contained 1.0 mmole of sodium methoxide. The solution was allowed to stand at room temperature overnight after which time it was saturated with carbon dioxide gas. The solvent was then removed under vacuum and 10 ml. of water was added to the residue. Methyl benzoate was removed by three 10-ml. washes with ether. The water soluble material was evaporated to dryness after which methanol was added and removed under vacuum until crystallization took place. In this manner 130 mg. of crystalline material was obtained. An additional 110 mg. was recovered by purification using a charcoal column[16] for an overall 45% yield. Recrystallization from

(12) G. E. Hilbert and E. F. Jansen, *J. Am. Chem. Soc.*, **57**, 552 (1935).

(13) Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Analyses were performed by M. V. Tashinian. Ultraviolet spectra were obtained by the use of a Beckman model DK-2 ratio recording spectrophotometer. A Beckman model IR-5 spectrophotometer was employed to measure infrared spectra. Thanks are due Dr. A. Shulgin for instruction in the use of the instrument and help in the interpretation of the results.

(14) H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, **77**, 18 (1955).

(15) R. Markham and J. D. Smith, *Biochem. J.*, **45**, 294 (1949).

(16) I. G. Walker and G. C. Butler, *Can. J. Chem.*, **34**, 1168 (1956).

ethanol produced pure 1-methyl-3-β-D-ribofuranosyluracil, m.p. 200–201°. The ultraviolet absorption spectrum shows a maximum at 270 mμ (ε, 10,000) and a minimum at 233 mμ (ε, 1,300λ).

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.84; H, 5.47; N, 10.67.

Preparation of 1-methyl-3-β-D-ribofuranosyluracil from the silver salt of 1-methyluracil. 1-Methyluracil (12.0 mmoles, 1.5 g.) was dissolved in 50 ml. of water. To this solution 50 ml. of water which contained 12 mmoles (2.04 g.) of silver nitrate was added. The solution was neutralized with 0.8 ml. of concd. ammonium hydroxide and a precipitate was formed which was collected by filtration and washed with water until the washings were free of chloride ion. The precipitate was then washed with ethanol followed by ether to obtain 2.36 g. of dry 1-methyluracil silver, a yield of 84%.

1-Methyluracil silver (2.0 mmoles, 466 mg.) was treated with 2.2 mmoles of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride. The condensation reaction, the removal of benzoyl groups and the purification of the product were performed in the manner described for the mercuric salt of 1-methyluracil. The product which was obtained in 35% yield was identical with 1-methyl-3-β-D-ribofuranosyluracil prepared from the mercuric salt with respect to chromatographic mobility, ultraviolet absorption spectrum, and melting point. No depression of the melting point of a mixture of the two products was observed.

Preparation of 1-methyl-3-β-D-ribofuranosyluracil from 1-methyl-4-methoxyuracil. A benzene solution which contained 3.0 mmoles of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride was added to 2.0 mmoles (280 mg.) of 1-methyl-4-methoxyuracil. The solvent was removed *in vacuo* and the pasty residue was kept at 55° for one week. The reaction mixture was then extracted with benzene. The insoluble material was found to contain 105 mg. of 1-methyluracil which accounted for 47% of the starting material. The benzene solution was concentrated to 5 ml. prior to the addition of 30 ml. of petroleum ether. A precipitate formed which was dissolved in 50 ml. of methanol which contained 50 mg. of sodium methoxide and the solution was refluxed for 30 min. after which it was neutralized with carbon dioxide gas. The solvent was then evaporated under vacuum and methyl benzoate was removed by co-distillation with water. The residue which contained 190 mg. of 1-methyl-3-ribosyluracil (yield: 39%) was purified using a charcoal column and recrystallized from ethanol. The melting point, chromatographic mobility and ultraviolet absorption spectrum of the purified material were identical with those of the 1-methyl-3-ribosyluracil which was synthesized from the mercuric and silver salts of 1-methyluracil.

Preparation of 3-β-D-ribofuranosyluracil from 4-ethoxyuracil. 4-Ethoxyuracil (5.0 mmoles, 630 mg.) was dissolved in 35 ml. of hot xylene, and, after about 10 ml. of solvent was removed by distillation, 5 mmoles of a benzene solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride was added and the solution was refluxed for 30 min. Then another 5 mmoles of the halogenose was added and the solution was refluxed for 1 hr. A precipitate of 260 mg. of uracil which accounted for 56% of the starting material was removed from the cooled solution by filtration. After the solution had been evaporated *in vacuo* to a volume of 10 ml., 50 ml. of petroleum ether was added. The precipitate was dissolved in 250 ml. of absolute methanol which contained 2.0 mmoles of sodium methoxide. After the solution was allowed to stand overnight at room temperature it was saturated with carbon dioxide gas and the solvent was removed *in vacuo*. An investigation of the dissolved residue by means of paper chromatography in butanol-water solvent indicated that 1.3 mmoles of product was present along with 0.6 mmole of the starting material, 4-ethoxyuracil. In addition trace amounts of uracil and 3-ethyluracil were present. After methyl benzoate was extracted with ether the residue was dissolved in 50 ml. of 0.01*M* potassium borate

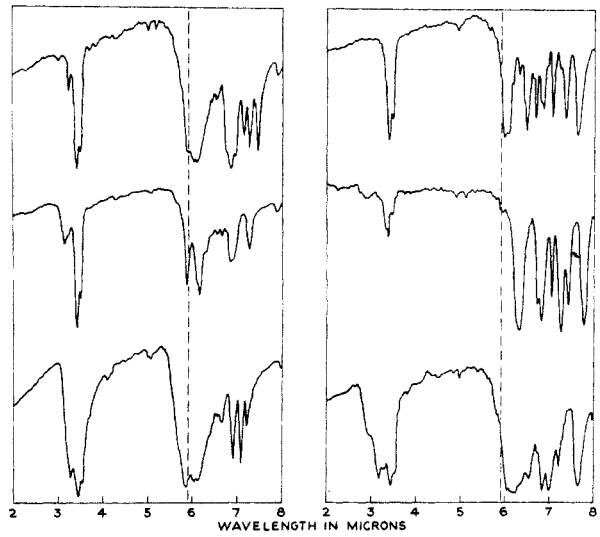

Fig. 2. Infrared spectra of uracil derivatives: (Left: top to bottom) 1,3-dimethyluracil; 3-methyluracil; uracil. (Right: top to bottom) 1-methyl-4-methoxyuracil; 2,4-dimethoxyuracil; 4-ethoxyuracil

and the solution was applied to a column which contained Dowex 1-X[2] resin (2.5 cm. diameter × 25 cm. length) in the borate form.[17] An additional 50 ml. of 0.01*M* borate solution was used to wash the material into the column. Elution was then begun with a 0.05*M* potassium borate solution which was 0.01*M* with respect to sodium chloride. After 2 l. of this solution was passed through the column all of the 4-ethoxyuracil present had been accounted for in the effluent. The eluent was then changed to a solution which was 0.1*M* with respect to sodium chloride.

3-Ribosyluracil began to appear in the effluent after 170 ml. of solution had passed through the column. Elution was virtually complete after 1450 ml. of effluent had been collected. Yellow colored polymers of ribose remained on the column under these conditions. Those fractions which contained significant amounts of 3-ribosyluracil were pooled and acidified with hydrochloric acid to *p*H 4.0, after which they were applied to a column which contained 3.0 g. of charcoal. The column was washed with five 50-ml. portions of water and elution was accomplished with 100 ml. of ammoniacal ethanol. After the solvent was removed under vacuum 160 mg. of 3-ribosyluracil was obtained (Yield: 22% based on 4-ethoxyuracil, 11% based on halogenose). The product was crystallized from ethanol-ether and recrystallized from ethanol; m.p. 202.5–203.5°.

Anal. Calcd. for $C_9H_{12}N_2O_6$: C, 44.26; H, 4.95; N, 11.47. Found: C, 44.35; H, 4.80; N, 11.25.

The *p*K of the enol dissociation, computed by the spectrophotometric method of Edwards[18] is 9.8.

Preparation of 3-β-D-ribofuranosyluracil from the mercuric salt of 2-methylthiouracil.[19] 2-Methylthiouracil (22.0 mmoles, 3.12 g.) was dissolved in 100 ml. of water at 55°. The solution was then made alkaline by the addition of 2.20 ml. of 10*M* sodium hydroxide and 11.0 mmoles of an alcoholic solution of mercuric chloride was immediately added. The precipitate which formed was filtered from the cooled solution and washed with water until the washings were free of chloride ion. After washing with ethanol followed by ether

(17) L. Jaenicke and K. von Dahl, *Naturwissenschaften*, **39**, 87 (1952).

(18) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950).

(19) H. L. Wheeler and H. F. Merriam, *Am. Chem. J.*, **29**, 478 (1903).

4.69 g. of dry material was obtained for a yield of 89% based on a formulation of the product as (2-methylthiouracil)$_2$ mercury.

A suspension of 4.0 mmoles (1.94 g.) of (2-methylthiouracil)$_2$ mercury in 70 ml. of xylene was dried by azeotropic distillation of 20 ml. of solvent. A benzene solution of 8.0 mmoles of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride was added and the suspension was refluxed for 30 min. During this process almost all of the suspended material became soluble. The xylene solution was then reduced to a volume of 15 ml. by distillation of 35 ml. of solvent and the cooled solution was treated with 50 ml. of petroleum ether. The solvent was decanted from the amorphous precipitate which formed and reduced to a volume of 5 ml. Addition of 30 ml. of petroleum ether to this solution resulted in further precipitation. The combined precipitates were dissolved in 100 ml. of chloroform, the solution was then washed with water and dried over sodium sulfate. After the solvent was removed under vacuum three 100-ml. portions of absolute methanol were added to the residue and removed *in vacuo*. This process converted the amorphous material to a dry powder. This material which moved as a compact spot when subjected to paper chromatography in butanol-water solvent was dissolved in 150 ml. of absolute methanol which contained 4.0 mmoles of sodium methoxide and allowed to stand overnight. The solution was then saturated with carbon dioxide gas and the solvent was removed under vacuum. The residue was dissolved in 100 ml. of water and methyl benzoate was removed by treatment with three 25-ml. portions of ether. The aqueous solution was then made 0.2*M* with respect to monochloroacetic acid. This solution was maintained at a temperature of 80° for 8 hr. after which time hydrolytic removal of the methylthio group was complete. Paper chromatography in butanol-water solvent followed by ultraviolet spectrophotometry indicated that 2.7 mmoles of uracil was present in the hydrolyzate along with 0.86 mmole of nucleoside. Further hydrolysis under the same conditions revealed that the nucleoside did not give rise to the uracil. The products of the hydrolysis were partially purified by the use of a charcoal column and were then separated by borate column chromatography. Uracil was quantitatively eluted by the use of a solvent which was 0.05*M* with respect to potassium borate and 0.01*M* with respect to sodium chloride. When the sodium chloride concentration was increased to 0.1*M* two peaks of substances absorbing ultraviolet light (260 mμ) were obtained. The first peak which appeared after 250 ml. of solution had passed through the column contained 0.06 mmole of uridine (yield: 0.8%). The second peak, which overlapped the first peak slightly, began to be eluted when the effluent volume reached 550 ml. and was contained in 500 ml. of solution. A charcoal column was used to isolate 0.70 mmole of nucleoside from this solution. Of this material 0.48 mmole (yield 6%) was 3-ribosyluracil. The nature of the remaining material (yield 4%) was not determined.

Methylation of 3-β-D-ribofuranosyluracil. Methylation of the compound with dimethylsulfate was carried out under conditions equivalent to those used by Scannell, Crestfield, and Allen[4] for the methylation of uridine and 5-ribosyluracil. The *N*-methylated product was isolated by paper chromatography in butanol-water solvent and was found to be identical with the riboside which was synthesized from 1-methyl-4-methoxyuracil and from the heavy metal salts of 1-methyluracil.

Preparation of 1-β-D-ribofuranosylthymine from the silver salt of thymine. A solution of 10.0 mmoles of thymine (1.26 g.) in 25 ml. of hot water was treated with 25 ml. of water which contained 10.0 mmoles of silver nitrate. The solution was neutralized with 10.0 mmoles of sodium hydroxide, the gel which formed was dispersed by boiling, and the precipitate was removed from the hot solution by filtration. The precipitate was dehydrated by treatment with five 25-ml. portions of absolute ethanol followed by two washes with 25 ml. of ether and dried in an oven at 80° for 2 days. The yield was 1.25 g. (55%).

A suspension of 1.0 mmole of the silver salt of thymine (232 mg.) in 30 ml. of xylene was dried by azeotropic distillation of one half of the solvent. A slight excess, 1.2 mmoles, of a benzene solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride was added and the solution was refluxed for 1 hr. The xylene soluble material was then treated in the manner described for condensation products of metal salts of 3-methyluracil and a 23% yield of product was obtained. This compound was chromatographically and spectrophotometrically identical with 3-β-D-ribofuranosylthymine[20] synthesized by the method of Fox, *et al.*[7] from the mercuric salt of thymine. Moreover the compound was not affected when treated with 1.0*M* hydrochloric acid at 100° for 3 hr.

DISCUSSION

The results of the elementary analysis of the compound, tentatively identified as 3-ribosyluracil, as well as consideration of the nature of the materials used in its synthesis, make it clear that the substance is a ribofuranoside of uracil. It remains only to determine the configuration of the glycosidic bond and the atom of the uracil molecule to which the ribose residue is attached.

Since methylation of the substance resulted in a compound which was spectrophotometrically and chromatographically identical with the nucleoside synthesized from the heavy metal salts of 1-methyluracil and from 1-methyl-4-methoxyuracil, conclusions regarding the structure of one compound are valid for the other. It is therefore clear since nucleosides were synthesized from 2-methylthiouracil, 1-methyluracil, 4-ethoxyuracil, and 1-methyl-4-methoxyuracil that the 1, 2, and 4 positions of uracil are not involved in the glycosidic linkage. Moreover if ribose were attached to either the 5 or the 6 positions of 1-methyluracil the nucleoside would still possess a dissociable hydrogen atom. Since no changes occur in the spectrum of 1-methyluracil riboside over the *p*H range 2 to 12 it is clear that no such dissociable hydrogen atom is present. The change in the spectrum at *p*H 14 is due to the dissociation of a hydrogen ion from one of the hydroxyl groups of the ribose residue.[21] Since all other linkages are excluded the ribose must be attached to the 3 nitrogen atom of uracil. This formulation is consistent with the mechanism of the Hilbert-Johnson condensation reaction which proceeds by way of a quaternary nitrogen intermediate. When 4-ethoxyuracil is treated with a halogenose it is conceivable that condensation could take place on the N^1 nitrogen atom but the subsequent expulsion of the O^4 ethyl group would require an unlikely tautomeric shift of the O^2 hydrogen atom to the *N*-3 position. Moreover in the case of 1-methyl-4-methoxyuracil the re-

(20) A sample of 1-β-D-ribofuranosylthymine synthesized by Fox, *et al.* was kindly supplied by Dr. M. Gordon.

(21) J. J. Fox and D. Shugar, *Biochim. et Biophys. Acta*, 9, 369 (1952).

action could not proceed beyond the stage of the quaternary nitrogen intermediate.

Additional evidence in support of the *N*-3 position is provided by hydrolytic studies. When the nucleoside formed from 4-ethoxyuracil was treated with 1.0*M* hydrochloric acid for three hours at 100° a 43% yield of uracil was obtained and the remainder of the material was recovered unchanged. The O^2 xyloside of 3-methyluracil which was synthesized by Levene and Sobotka[9] is 90% hydrolyzed under these conditions. Uridine is not appreciably hydrolyzed by boiling 1.0*M* hydrochloric acid.[22] If ribose were attached to the C-5 or C-6 position, as is the case for the naturally occurring 5-ribosyluracil, an even greater stability to acid hydrolysis would be expected.[3] An O^4 linkage, on the other hand, would be very unstable. Levene and Sobotka[9] found that the O^4 xyloside which they synthesized from the silver salt of 2-ethylthiouracil was completely hydrolyzed by treatment with 0.2*M* hydrochloric acid at 100° for two hours. It is almost certain that the xylosides of isocystosine derivatives which were synthesized by Hahn and coworkers[11] were also O^4 glycosides since these compounds exhibited a similar instability to boiling dilute acid. Although the *N*-3 riboside is less stable than the *N*-1 riboside it is considerably more stable than the *N*-9 glycosidic bond of purine nucleosides which is completely hydrolyzed by 1.0*M* hydrochloric acid at 100° for one hour.[23]

Finally the ultraviolet and infrared absorption spectra of the synthesized nucleosides are consistent with the formulation as *N*-3 ribosides. The ultraviolet absorption maxima of uracil derivatives which possess unsubstituted *N*-1, O^2 lactim-lactam systems shift to higher wave lengths at alkaline *p*H values;[24] a shift from λ_{max} 261 mμ (ε, 8000) at *p*H 2 to 292 mμ (ε, 11,400) at *p*H 12 for the *N*-3 riboside of uracil was observed.

The infrared absorption spectra of several ribosides of uracil are presented in Fig. 1. When these spectra are compared with those of a number of other uracil derivatives (Fig. 2) it is apparent that those compounds in which the *N*-3, O^4 system is of necessity in the lactam form such as 1,3-dimethyluracil, 3-methyluracil, 1-methyl-3-ribosyluracil and 3-ribosyluracil are characterized by a peak in the region of the spectrum from 5.8 to 5.9 μ while those compounds in which this system is of necessity in the lactim form such as 4-ethoxyuracil, 2,4-diethoxyuracil, and 1-methyl-4-methoxyuracil lack this peak. It is noteworthy that the spectra of uracil, uridine, and 5-ribosyluracil possess this peak. Uracil and uridine are believed to exist chiefly in the diketo form[21,24,25]; 5-ribosyluracil apparently does also.

It can be argued that an *N*-3 riboside was formed from the silver salt of 1-methyluracil because the presence of a methyl group in the 1-position destroys the aromatic resonance by forcing a double bond out of the ring into the O^2 carbonyl group; consequently neither O^4 nor *N*-3 substitution is stabilized by ring resonance. In the case of 2-methylthiouracil an O^4 riboside would be stabilized by ring resonance while an *N*-3 riboside would not and this could explain the formation of the O^4 glycoside from the silver and mercuric salts. Unfortunately this argument does not explain the formation of an O^2-riboside from the silver salt of 3-methyluracil.[9]

The glycosidic linkage can almost certainly be assigned the β configuration since the use of acyl esters of glycosides has invariably resulted in a glycosidic linkage which is *trans* to the 2′ substituent. The mechanism of this neighboring group effect is believed to involve the formation of an ortho ester ion which covers the *cis* side of the carbon atom 1.[26]

From the foregoing discussion it can be concluded that the nucleoside which was synthesized from 4-ethoxyuracil is 3-β-D-ribofuranosyluracil. Since the melting point, chromatographic mobility, ultraviolet spectrum, and infrared spectrum of this nucleoside differ from those of the nucleoside component[3] of the new nucleotide which was isolated from ribonucleic acid by Davis and Allen,[2] the naturally occurring nucleoside cannot be 3-β-D-ribofuranosyluracil. The biological effects of 3-β-D-ribofuranosyluracil are at present under investigation.

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(26) B. R. Baker, *Ciba Foundation Symposium on the Chemistry and Biology of Purines*, J. and A. Churchill Ltd., London, 1957, p. 120.