Pyrimidines. IV.[1] The Interconversion of N^4-Methylcytosine and 3-Methylcytosine

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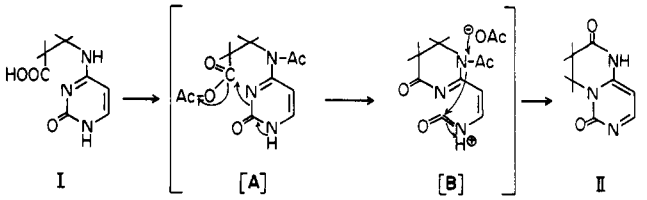
Received November 7, 1963

N^4-Methylcytosine (IIIb), when refluxed with acetic anhydride–acetic acid for prolonged periods, rearranges to 3-methylcytosine (IXb). The reversibility of this reaction is shown, and a mechanism for the rearrangement is given.

In a previous paper in this series,[2] we reported that N-(1H-2-oxo-4-pyrimidinyl)-β-alanine (I), when refluxed with acetic anhydride, underwent cyclization with rearrangement to form II (Chart I). The scope and limitations of this cyclization–rearrangement reaction with other pyrimidinyl amino acids was studied, and a plausible mechanism was presented.

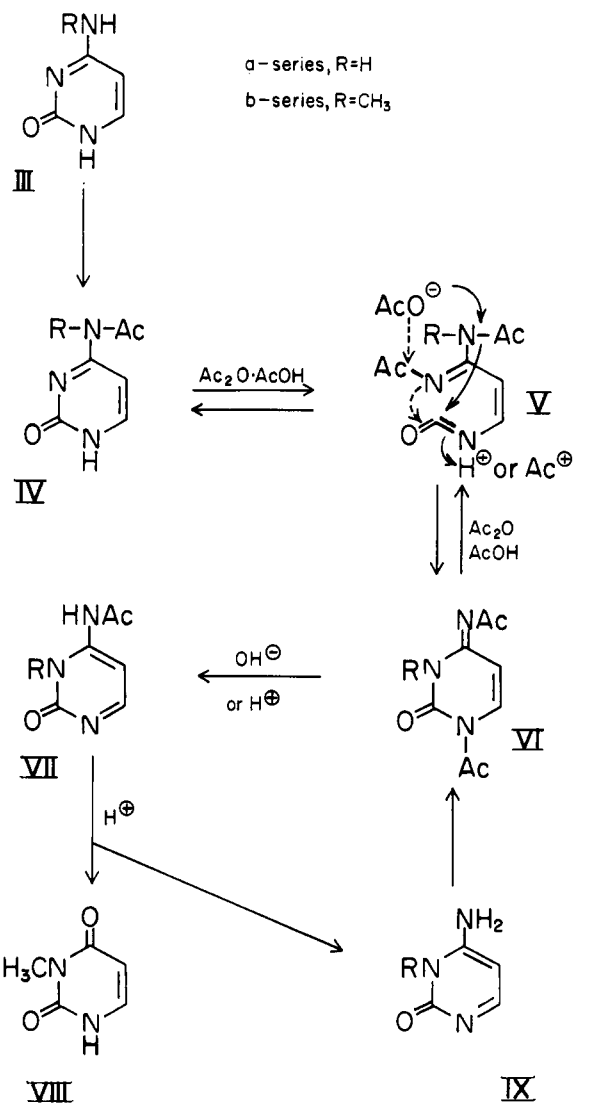
CHART I



This mechanism[2] is based essentially on the intramolecular attack by N^3 on the mixed anhydride group in A. If true, the rearrangement reaction should also occur when N^4-acetylcytosine (IVa, see Chart II) is treated with acetic anhydride. It would be expected that an intermolecular attack by N^3 of IVa on acetic anhydride would give intermediate Va which might cyclize as shown by the solid arrow to VIa and, after alkaline hydrolysis, regenerate cytosine (IXa). In the over-all reaction of IIIa to IXa, N^3 and N^4 should have been exchanged.[3]

To test this hypothesis, we employed N^4-methylcytosine[4] (IIIb) since, according to the above argument, the product of the reaction of IVb → IXb should be the easily identifiable isomer 3-methylcytosine (IXb). Compound IIIb was prepared easily by reaction of the readily available 4-thio-2-pyrimidinone[5] with methylamine. Treatment of IIIb with acetic anhydride or a mixture of acetic anhydride–acetic acid for 3 hr. under reflux gave IVb in good yield. When the reaction was carried out in acetic anhydride–acetic acid for 24 hr., the formation of a new compound was observed by paper chromatography although IVb (acetylated starting material) was still the predominant component. This new compound (sirup) was not isolated in pure form; however, it is most probably the diacetate VIb.[6] (This assignment is based on studies on the acetylation of 3-methylcytosine which will be described later.)

CHART II



The sirup was treated with 1 *N* hydrochloric acid at room temperature overnight and the hydrolysate separated on a Dowex 50 (H^+) ion-exchange column. Elution of the column with water yielded 3-methyluracil (VIII), which was obtained in crystalline form, and whose identity was established by comparison of its melting point[7] and detailed ultraviolet absorption

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 03190-07).

(2) T. Ueda and J. J. Fox, *J. Org. Chem.*, **29**, 1762 (1964).

(3) It is understood, of course, that in intermediate Va, N^3 and N^4 are essentially equivalent; hence this conversion with cytosine isotopically labeled with N^{15} at N^4 should give a 50% loss of the label at N^4. Such an experiment is planned in our laboratory.

(4) (a) D. J. Brown, *J. Appl. Chem.*, **5**, 358 (1955). (b) C. O. Johns, *J. Biol. Chem.*, **9**, 161 (1911); F. H. Case and A. J. Hill, *J. Am. Chem. Soc.*, **52**, 1536 (1930); Y. Chi and S. Chen, *Sci. Sinica* (Peking), **6**, 111 (1957).

(5) H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **42**, 30 (1909); Y. Mizuno, M. Ikehara, and K. A. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **10**, 647 (1962).

(6) The position of the acetyl group which attaches on N^1 in VI is tentative. Other positions such as O^2 or N^4 are also possible.

(7) T. B. Johnson and F. W. Heyl, *Am. Chem. J.*, **37**, 628 (1907); C. W. Whitehead, *J. Am. Chem. Soc.*, **74**, 4267 (1952).

spectrum[8] with an authentic specimen. Further elution of the column with 1 *N* hydrochloric acid yielded the basic fraction which contained compound IIIb as the major component. Later eluates when examined spectrally, showed the presence of, 3-methylcytosine (IXb).[9]

Similar products (IXb and VIII) were obtained when IVb was refluxed with acetic anhydride–acetic acid solution. With acetic anhydride *or* acetic acid used as the reactant, IVb was recovered unchanged and no formation of VIb was detected either spectrally or by paper chromatography. This data showed that both reagents were required for the rearrangement reaction, as observed previously in some cases with certain pyrimidinylamino acids.[2]

The formation of 3-methyluracil and 3-methylcytosine from IVb shows that the rearrangement reaction must have occurred. This rearrangement could have occurred either in the step involving the acetic anhydride–acetic acid treatment of IVb, or in the hydrochloric acid treatment of the reaction mixture. Treatment of IVb directly with 1 *N* hydrochloric acid or with alkali afforded IIIb. It is almost certain that the rearrangement of IVb to VIII and IXb proceeded *via* such intermediates as Vb, VIb, and VIIb. Data supporting the presence of the rearranged intermediates VIIb or VIb in the reaction mixture was shown by an alternate approach.

When 3-methylcytosine[9a] was treated at room temperature with acetic anhydride, a sirupy product was obtained which showed the same ultraviolet absorption and chromatographic behavior as the minor component obtained previously by refluxing IVb (or IIIb) with acetic anhydride–acetic acid. Treatment of this sirup briefly in solution with 1 *N* sodium hydroxide or with boiling water produced a marked change in the ultraviolet spectrum which now resembled that for II (Chart I), a 3-alkyl-N^4-acylcytosine, and was dissimilar to that for IVb or IXb (Chart II). These data strongly indicate that a compound of structure VIIb had formed in the latter reaction. The spectral change also suggests the presence of a diacetate (VIb) containing one labile acetyl group in the sirupy product obtained either by acetylation of IXb or by acetic anhydride–acetic acid treatment of IVb. The lability of an acetyl group substituted on a ring nitrogen of pyrimidines has been observed previously.[10]

When the sirupy product containing intermediate VIb obtained by acetylation of IXb at room temperature was treated with 1 *N* hydrochloric acid for several hours, crystalline 3-methyluracil (VIII) was obtained as the major product along with 3-methylcytosine as a minor component. Since IXb is known to be stable to acid,[9a] VIII must have been derived *via* VIIb by cleavage of the N^4–C^4 linkage. This hydrolysis (VIIb → VIII) is rather unusual, since most N^4-acetylcytosines or acylaminopyrimidines are cleaved under acidic conditions at the acyl–amide linkage to generate the parent aminopyrimidines.[11] However, there are some examples describing the cleavage at N^4–C^4, such as the conversion of N^4-acetylcytosine in boiling 80% acetic acid to uracil[10a] or of N^4-(*p*-toluoyl)-5-fluoro-2′-deoxycytidine in 0.1 *N* hydrochloric acid to 5-fluoro-2′-deoxyuridine.[12]

This rearrangement reaction (IVb → VIIb) differs in some details from that noted previously[2] in the conversion of I → II (Chart I). In the latter case, the reaction proceeded rapidly with acetic anhydride to II in high yields, leaving no detectable amount of starting material (I). In the present study, VIIb was formed from IVb as a minor component along with a considerable amount of starting material (IVb). These facts suggest that the conversion of IVb → VIIb is reversible through intermediate Vb. To test this hypothesis, IXb was *refluxed* with acetic anhydride–acetic acid for 20 hr., and the reaction mixture was hydrolyzed with 1 *N* hydrochloric acid. Paper ionophoretic examination of the hydrolysate showed three spots which were 3-methylcytosine (IXb), 3-methyluracil (VIII), and N^4-methylcytosine (IIIb). The conversion of IXb to IIIb was about 30%, whereas the conversion of IVb or IIIb to IXb and VIII was ~45%. Though these data attest to the reversibility of the rearrangement, they suggest that the conversions of VIb → Vb and/or of IVb → Vb were not complete.

Experimental[13]

N^4-Methylcytosine (IIIb).—Three grams of 4-thio-2-pyrimidinone[5] was dissolved in 100 ml. of methanol previously saturated with methylamine at 0°, and the solution was heated at 105° for 20 hr. in a sealed cylinder. After cooling, the crystals (2.7 g.) were separated by filtration, and recrystallized from water giving prisms, m.p. 277–280°, lit.[4a] 275–278°; ultraviolet absorption: λ_{max} 267 mμ in water (ϵ_{max} 8600), 277 in 1 *N* hydrochloric acid (11,700), 285 in 0.1 *N* sodium hydroxide (9250).

N^4-Acetyl-N^4-methylcytosine (IVb).—A solution of 0.6 g. of IIIb in 3.0 ml. of acetic anhydride and 1.0 ml. of acetic acid was refluxed for 3 hr. After concentration of the solution to dryness under reduced pressure, the resulting solid (0.8 g.) was washed with methanol and recrystallized from ethanol, m.p 193–194°; ultraviolet absorption properties: in water, maxima at 296, 256, and 212 mμ, (ϵ_{max} 6530, 9300, and 14,500, respectively); in 1 *N* hydrochloric acid, maxima at 309, 245, and 212.5 mμ (ϵ_{max} 12,900, 4670, and 10,500, respectively).

Anal. Calcd. for $C_7H_9N_3O_2$: C, 50.29; H, 5.43; N, 25.14. Found: C, 50.30; H, 5.32; N, 25.34.

When IVb was allowed to remain overnight in 1 *N* hydrochloric acid at room temperature, the ultraviolet absorption spectrum changed to that of IIIb in acid. In 1 *N* sodium hydroxide the hydrolysis was rapid and complete within 10 hr., giving a spectrum similar to that for IIIb in base. No formation of uracil was observed in either case.

Reaction of IIIb with Acetic Anhydride–Acetic Acid and Formation of 3-Methyluracil (VIII).—A solution of 1.0 g. of IIIb in 10 ml. of acetic anhydride and 2.0 ml. of acetic acid was refluxed for 24 hr. The resulting brown solution was concentrated *in vacuo* to a sirup, treated with ethanol and concentrated again to a sirup. This procedure was repeated twice, and the final amorphous solid was triturated with ethanol and filtered. The solid, 0.5 g., showed an absorption spectrum identical with that for IVb and, after one crystallization from ethanol, gave 0.4 g. of IVb (identified by absorption spectrum and mixture melting point with authentic material described above).

The dark brown filtrate was concentrated *in vacuo* to a sirup. Paper chromatography of this sirup showed two spots, R_f

(8) D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

(9) (a) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 1348 (1962); (b) T. Ueda and J. J. Fox, *J. Am. Chem. Soc.*, **85**, 4024 (1963).

(10) (a) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2384 (1956). (b) M. Flysten and T. B. Johnson, *J. Am. Chem. Soc.*, **64**, 306 (1942); L. B. Spencer and E. B. Keller, *J. Biol. Chem.*, **232**, 185 (1958).

(11) D. J. Brown in "The Pyrimidines," Interscience Publishers, Inc., New York, N. Y., 1962, p. 329.

(12) R. Duschinsky, T. Gabriel, J. J. Fox, and M. Hoffer, Abstracts, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963, p. 18O.

(13) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Ultraviolet absorption spectra were measured with a Cary recording spectrophotometer, Model 15. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

= 0.60 and 0.90, in *n*-butyl-alcohol-water (86:14). Compound IVb had R_f 0.60 in this solvent system. The substance (VIb) having R_f 0.90 showed an ultraviolet absorption maximum at 275 mμ in water, which shifted to 318 mμ on addition of alkali. Acidification gave a maximum at 308 mμ. The sirup was taken up in 50 ml. of 1 *N* hydrochloric acid and allowed to stand overnight at room temperature. The solution was concentrated *in vacuo* to dryness, the residue was taken up in 30 ml. of water and applied to a column of Dowex 50 (H^+ 2.2 × 15 cm.) resin, and washed with water. The washings were collected in 70-ml. fractions. Fractions 2 and 3, having an absorption maximum at 260 mμ were combined and concentrated *in vacuo* to dryness. The residual semisolid was taken up in ethanol, the insoluble material was removed by filtration, and the filtrate was concentrated *in vacuo*, whereupon crystals formed, m.p. 163–169°. Recrystallization from ethyl acetate gave 0.1 g. of 3-methyluracil (VIII), m.p. 177–179° (lit.[6] m.p. 174–175°); mixture melting point with authentic material (m.p. 177–178°) was 177–179°. The ultraviolet absorption properties are identical to those reported.[7] The infrared spectrum was also identical with that of an authentic sample.

The column was then eluted with 1 *N* hydrochloric acid and fractions containing IIIb were obtained. The presence of trace amounts of 3-methylcytosine (IXb) was observed in the fractions collected after IIIb was eluted, although the separation was not complete.

The Reaction of IVb in Acetic Anhydride and/or Acetic Acid.—Compound IVb (*ca.* 30 mg. each) was dissolved in 5 ml. of acetic anhydride, or acetic acid, or acetic anhydride–acetic acid (2:1) and refluxed for 20 hr. An aliquot of each reaction solution was applied to paper chromatography (*n*-butyl alcohol–water, 86:14). Only the reaction of IVb in acetic acid–acetic anhydride showed the presence of a spot at R_f 0.9, along with the R_f 0.60 spot of the starting material. This reaction solution was concentrated to dryness and the residue was dissolved in 3 ml. of 1 *N* hydrochloric acid and allowed to stand for 2 hr. at 45°. An aliquot of the solution was examined by paper electrophoresis (pH 5.0, 0.1 *M* ammonium acetate, 800 v. for 2 hr.). Three spots were obtained migrating at −1.0, −5.9, and −13.5 cm. (VIII, IIIb, and IXb, respectively, as identified by spectral determination). From the spectral calculations *ca.* 45% of IVb was shown to be converted to VIII and IXb in the ratio of 3:1.

Acetylation of 3-Methylcytosine (IXb) Followed by Acid Hydrolysis.—3-Methylcytosine hydrochloride[8a] (0.5 g.) and anhydrous sodium acetate (0.2 g.) were suspended in 3.0 ml. of acetic anhydride and shaken for 4 hr. or refluxed for 1 hr. After cooling, the precipitate was removed by filtration, and the filtrate was concentrated *in vacuo* to a sirup (VIb). This sirup showed a single spot at R_f 0.9 in *n*-butyl alcohol–water (86:14) paper chromatography. The ultraviolet absorption maximum in water was at 275 mμ. On addition of 1 drop of 30% sodium hydroxide in the 3-ml. cuvette, the maximum shifted to 318 mμ, and acidification of the solution showed a new maximum at 308 mμ. The sirup was dissolved in 10 ml. of 1 *N* hydrochloric acid and the solution was allowed to stand overnight at room temperature. The solution was concentrated *in vacuo* to dryness, the residue was dissolved in ethanol at room temprature, the insoluble material was separated by filtration, and the filtrate was concentrated *in vacuo* to a solid mass (0.2 g.), which showed the characteristic ultraviolet absorption spectra for VIII.[7] The alcohol-insoluble material was treated with boiling ethanol and separated from a small amount of insoluble material. The ethanol solution was concentrated to dryness to give 0.4 g. of a solid. Paper electrophoretic examination of this solid showed the presence of 3-methylcytosine along with a large amount of 3-methyluracil. The ratio of IXb to VIII was 1:9.

Reaction of 3-Methylcytosine with Acetic Anhydride–Acetic Acid.—The hydrochloride salt of 3-methylcytosine (IXb, 0.1 g.) and anhydrous sodium acetate (0.5 g.) was suspended in acetic anhydride (3.0 ml.) and acetic acid (2.0 ml.), and refluxed for 20 hr. The solution was concentrated *in vacuo* to a small volume, treated with ethanol, and evaporated to a sirup. The sirup was dissolved in 5 ml. of 1 *N* hydrochloric acid and kept for 18 hr. at room temperature. After concentration *in vacuo* to a solid mass, this amorphous solid was dissolved in 25 ml. of water. An aliquot of the solution was examined by paper electrophoresis (pH 5.0, 0.1 *M* ammonium acetate, 800 v., 90 min.). Three spots were obtained migrating −0.2, −4.2, and −12.0 cm. Each spot was excised, eluted with 40 ml. of water, and examined spectrophotometrically. From the comparison of the migration of authentic materials and ultraviolet absorption spectra, the spots were characterized as 3-methyluracil (−0.2), N^4-methylcytosine (−4.2), and 3-methylcytosine (−12.0 cm.).

The ratio of formation of 3-methyluracil, 3-methylcytosine, and N^4-methylcytosine was approximately 1.6:1.0:1.0, respectively. These data show that ~30% of 3-methylcytosine was converted to N^4-methylcytosine (IIIb). The water solution of the acid hydrolysate was further applied to a column of Dowex 50 (H^+ form, 2.5 × 12 cm.), washed with water, and eluted with 0.5 *N* hydrochloric acid. From the water washings, fractions containing 3-methyluracil were obtained. With 0.5 *N* hydrochloric acid, fractions containing N^4-methylcytosine, which was eluted first, and 3-methylcytosine were obtained, although the separation of the latter two was not complete.

Acknowledgment.—The authors wish to thank Dr. George Bosworth Brown for his warm and continued interest.

Nucleosides. XXI. Synthesis of Some 3′-Substituted 2′,3′-Dideoxyribonucleosides of Thymine and 5-Methylcytosine[1]

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Received January 21, 1964

The disulfide of 3′-deoxy-3′-mercaptothymidine (VI) was synthesized by reaction of anhydronucleoside II with potassium thiobenzoate in dimethylformamide followed by removal of the protecting groups. Potassium phthalimide in dimethylformamide was shown to be a useful reagent for the conversion of a 3′-*O*-mesylthymidine (I, R = trityl) to the 3′-deoxy-3′-phthalimido derivative (VIII). This latter reaction also proceeds *via* anhydronucleoside II. Removal of the protecting groups from VIII yielded 3′-amino-3′-deoxythymidine (X). Detritylation of VIII followed by acetylation yielded XII which was thiated to the 4-thionucleoside and converted to the 3′-amino-3′-deoxy derivative (XV) of 5-methyl-2′-deoxycytidine. Under certain conditions, the 4-amino group of cytosine nucleosides was readily exchanged with *n*-butylamine to produce 4-*n*-butylamino nucleoside derivatives.

It was demonstrated in a previous study[2] that under acid-catalyzed conditions di-*O*-mesylthymidine (I, R = mesyl) is converted directly in refluxing *N*,*N*-dimethylformamide containing sodium benzoate to di-*O*-benzoylthymidine (III, R = benzoyl). This reaction was

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 03190-07).

(2) Paper XVI in this series: J. J. Fox and N. C. Miller, *J. Org. Chem.*, **28**, 936 (1963).