of Fig. 2 indicate that mercury is bound, at pH 9, to the amino group of adenosine and cytidine. The spectra of Fig. 3 are in accord with the data of Fig. 1 and 2.

The data revealing the attachment of mercury to the N_1O^6 site have been deliberately interpreted as such, without any attempt to specify that the mercury must be on either the nitrogen or oxygen atom. It has been suggested elsewhere that, because of the generally greater tendency of mercury to coordinate with nitrogen rather than oxygen atoms, the mercury should not be expected to complex with the hydroxyl group, but should prefer the nitrogen donors instead.[7] However, the nitrogen atoms in the purine and pyrimidine rings and the oxygen atoms on these rings do not have the electronic structure of amino or alcoholic nitrogen and oxygen atoms, and it is believed that there is no *a priori* basis for ruling out binding of mercury to these oxygen atoms on such a basis. Neither are the structures of the uncoordinated nucleosides any help in this matter. Uridine[15] and guanosine[16] do, indeed, have ketonic structures, thus placing the protons on the N_1 atoms. Removal of the proton at high pH, however, brings about conversion to the enolate ion. Whether mercuration results in retention of the protonated structure (nitrogen binding) or addition to the unprotonated structure (oxygen binding) appears to remain an open question.

Previous discussions of the probable orientation of the mercury bonds in these complexes have stressed the tendency of mercury to form two colinear sp-bonds and the relatively low degree of chelate stabilization of mercury complexes compared to some other metal complexes.[7] We should like to point out, nevertheless, that the tetrahedral configuration has been established for many mercury complexes, and that chelate stabilization does occur with mercury. Thus there is a possibility that mercury bound to the amino and hydroxyl groups, respectively, in adenosine and guanosine is also bound to the N_7 position. Attempts to prove or disprove such chelated structures by spectrophotometry and conductometric techniques were fruitless.

(15) H. T. Miles, *Biochim. Biophys. Acta*, **22**, 247 (1956).
(16) H. T. Miles, private communication.

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[CONTRIBUTION FROM THE DIVISION OF NUCLEOPROTEIN CHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH, SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK 21, N. Y.]

Spectrophotometric Studies of Nucleic Acid Derivatives and Related Compounds. V. On the Structure of 3-Methylcytosine[1]

BY TOHRU UEDA AND JACK J. FOX

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Several 2,3-dihydroimidazo[1,2-*c*]pyrimidines were synthesized by reaction of 4-thiouracil or 4-methylthio-2-pyrimidinone or 1-methyl-4-methylthio-2-pyrimidinone with amino alcohols followed by chlorination and ring closure to condensed ring systems. The absorption spectra of these compounds were determined and their dissociation constants measured spectrally. Spectral comparisons of appropriate molecular species showed that the structure of 3-methylcytosine (neutral species) is of the 4-amino-2-oxo form. 3-Methylcytosine exhibits a hitherto unreported second dissociation (as demonstrated spectrally) in the high alkaline region attributable to proton removal from the 4-amino group. The difference in pK_{a1} values between 1-alkylated and 3-alkylated cytosines is explained by the difference in basicity of their site of protonation. A 1,2,3,4-tetrahydropyrimido[1,2-*c*]pyrimidine (XVI), a new ring system, was also synthesized.

Introduction

The structure of cytosine in aqueous solution has been established as 4-amino-2(1H)-oxopyrimidine (I, R = H).[2,3] The structure of cytidine and 2′-deoxycytidine has also been assigned structure I (R = β-D-ribofuranosyl or 2′-deoxy-β-D-ribofuranosyl) by several investigators[4-6] on the basis of ultraviolet, infrared, and n.m.r. spectral studies.

Brookes and Lawley[7] have recently reported the synthesis of 3-methylcytosine and 3-methylcytidine. They compared the ultraviolet spectrum and pK_a of 3-methylcytosine (7.4)[7] and 3-methylcytidine (8.7)[7] with those for cytosine (4.61)[8] and 1,3-dimethylcytosine (9.3)[9] (see Table I). From these comparisons they implied the 4-amino-2-oxo structure to 3-methylcytosine (II, see Fig. 1).

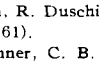
TABLE I
APPARENT pK_a VALUES OF VARIOUS CYTOSINES[a]

	pK_{a1}	pK_{a2}
Cytosine	4.61[8]	12.2[14]
1-Methylcytosine	4.55[4]	..
Cytidine	4.1[4]	..[d]
N,N-Dimethylcytosine	4.25[8]	12.3[8]
3-Methylcytosine	7.38[b]	13–14
3-Methylcytidine	8.73[c]	..[d]
1,3-Dimethylcytosine	9.3,[9] 9.4[7]	..
VIa, R = H	6.99	12.6
VIb, R = CH_3	7.01	..
VII	8.08	..
XVI	7.97	~14

[a] The pK_a values are spectrophotometrically determined and are accurate to ±0.05 pH unit unless otherwise indicated. [b] Brookes and Lawley[7] find 7.4. [c] Ref. 7 gives 8.7. [d] A dissociation is spectrophotometrically evident in the pH 12–14 region owing to ionization of the sugar moiety (see text).

Brookes and Lawley explained the rather high pK of 7.4 for II as *vs.* 4.61 for cytosine by the absence of

the acidic (–N–C–) grouping in II. This argument is not convincing, since 1-methylcytosine (I, R = CH_3),



in which the –N–C– acidic grouping is also absent,

(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). For part IV in this series see J. J. Fox, N. Yung, and I. Wempen, *Biochim. Biophys. Acta*, **23**, 295 (1957).

(2) A. R. Katritzky and A. J. Waring, *Chem. Ind.* (London), 695 (1962).
(3) D. J. Brown and J. M. Lyall, *Australian J. Chem.*, **15**, 851 (1962).
(4) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).
(5) H. T. Miles, *J. Am. Chem. Soc.*, **85**, 1007 (1963).
(6) O. Jardetzky, P. Pappas, and N. G. Wade, *ibid.*, **85**, 1657 (1963)
(7) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 1348 (1962).
(8) I. Wempen, R. Duschinsky, L. Kaplan, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4755 (1961).
(9) G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 855 (1955).

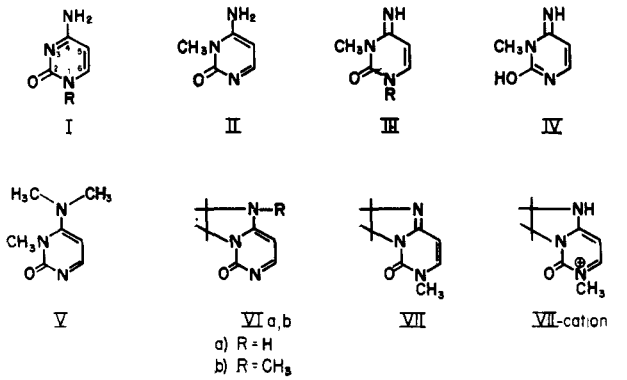


Figure 1.

shows a pK of 4.55,[4] quite similar to that for cytosine (see Table I).

Furthermore, 3-methylcytosine possesses a second pK_a of ~13 (apparently missed by the previous authors[7]). This pK_{a2} for 3-methylcytosine is attributable to proton removal from the neutral species. It is noteworthy that 1-methylcytosine does not possess a second dissociation up to pH 14.[4,10] Moreover, cytosine, which exists as I (R = H) in the neutral species, shows a pK_{a2} of 12.2 which has been attributed to

dissociation of the –N–C– grouping.[4] Thus the high pK_{a1} of 3-methylcytosine and the presence of a second dissociation (pK_{a2} = ~13) might reasonably be explained by assigning to 3-methylcytosine the imino structure III (R = H) or IV which possesses an acidic grouping in the 1,2-positions. The fact that the spectrum of cytosine and 3-methylcytosine are similar in acid solution (cationic species), as Brookes and Lawley[7] observed, is indicative, as they suggest, of protonation of cytosine at N^3. However, these data do not elucidate the structure of the neutral species of II. The fact that the spectrum of the neutral species of 3-methylcytosine differs from that for 1,3-dimethylcytosine (III, R = CH_3) (the latter must be in the 4-imino form) may suggest that 3-methylcytosine exists as II. However, here, too, there is an ambiguity since 3-methylcytosine may have structure IV, the 2-hydroxy-4-imino form. This latter possibility (among others) might account for the high pK_{a1} of 7.4 (due to the imino group) and the pK_{a2} of *ca.* 13 for the 2-enol.

Most important would be a comparison of the spectrum of the neutral and cationic species of 3-methylcytosine to that for the fully-methylated analog V. This compound (V) is fixed in the 2-oxo-4-amino form.

Syntheses of Reference Compounds.—Attempts to synthesize V from 3-methyluracil were unsuccessful. Thiation of 3-methyluracil afforded 3-methyl-4-thiouracil in good yield. Treatment of this thiopyrimidine with dimethylamine gave only sulfur-containing products which were not investigated further.

Another approach to compounds which should serve for spectral comparison to V is to be found in the 2,3-dihydro-5-oxoimidazo[1,2-*c*]pyrimidines, which, in the form of the methylated derivative VIb, present the fixed 4-amino-2-oxo structure. The 4-imino-2-oxo form would be presented by compound VII.

Compound VIa (R = H) was prepared by Martin and Mathieu[11] from 2,4-dithiouracil. The ready availability of 4-thiouracil directly from uracil[12] made possible a simpler approach to VIa as well as to VIb (R = CH_3). 4-Thiouracil was converted to 4-methylthio-2-pyrimidinone[13] (VIII) and then treated with ethanolamine to afford N^4-β-hydroxyethylcytosine (IX) (see Fig. 2). Treatment of IX with thionyl chloride gave X (R = H) (not isolated) which after refluxing in pyridine for 5 min. afforded VIa, identical with the product obtained by Martin and Mathieu.[11] Compound VIb was synthesized by treatment of VIII with 2-methylaminoethanol to form IX (R = CH_3). When IX (R = CH_3) was refluxed with thionyl chloride, VIb was obtained directly. The synthesis of VII was effected by treatment of 1-methyl-4-methylthio-2-pyrimidinone[13] (XI) with ethanolamine to afford XII which, upon reaction with thionyl chloride, yielded the chloro derivative XIII. This chloro compound (XIII) was cyclized to VII in refluxing pyridine; XIII was also converted to VII simply by heating at ~165°.

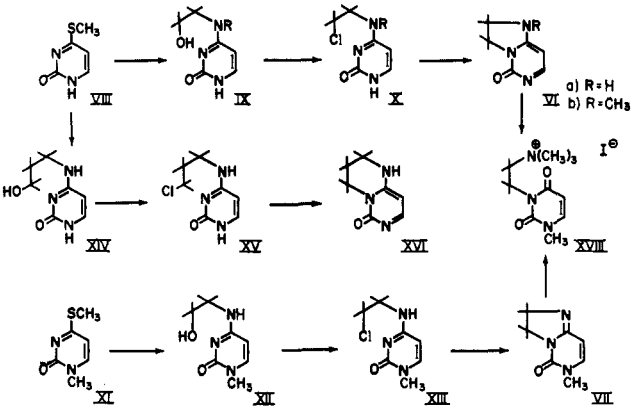


Figure 2.

Pyrimido[1,2-*c*]pyrimidine (a new ring system) may also be prepared by this procedure. Treatment of VIII with 3-aminopropanol afforded the N^4-hydroxypropylcytosine (XIV) which was converted to XVI *via* XV by treatment with thionyl chloride followed by refluxing in pyridine. It was of interest to compare the ultraviolet absorption spectra of XVI and VIa to ascertain the effect, if any, of ring size on spectra and pK values.

Proof of the fact that cyclization to VI, VII, and XVI had occurred at N^3 of X, XIII, and XV was obtained in the following manner: VI was subjected to alkaline hydrolysis. A solution was obtained which gave an absorption spectrum almost identical with that for 3-methyluracil. (The conversion of 3-methylcytosine to 3-methyluracil by alkaline hydrolysis has been reported[7]). Therefore cyclization must have occurred on N^3 of X to form VI. Similar results were obtained by alkaline hydrolysis of XVI. From VII, mild alkaline hydrolysis afforded a solution whose spectrum resembled that for 1,3-dimethyluracil (absence of spectral shifts between pH 7–11). In stronger alkali, the spectrum (optical density) slowly decreased. This characteristic of 1,3-dialkyl substituted uracils in strong alkali has already been described.[14] Furthermore, treatment of VIa, VIb, and VII with excess methyl iodide in alkali afforded the 1,3-disubstituted uracil (XVIII, a trimethylammonium salt) whose absorption spectrum was similar to that for 1,3-dimethyluracil.[14]

These data establish VI and VII as imidazo[1,2-*c*]-pyrimidines and XVI as a pyrimido[1,2-*c*]pyrimidine.

Spectral Comparisons and Discussion.—The spectra of 3-methylcytosine and 3-methylcytidine (curves for

(10) The spectral curve of 1-methylcytosine even in 6 *N* sodium hydroxide is identical with that for pH 7–14.

(11) R. H. Martin and J. Mathieu, *Tetrahedron*, **1**, 75 (1957).

(12) Y. Mizuno, M. Ikehara, a. d K. A. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **10**, 647 (1962).

(13) H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **42**, 30 (1909).

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

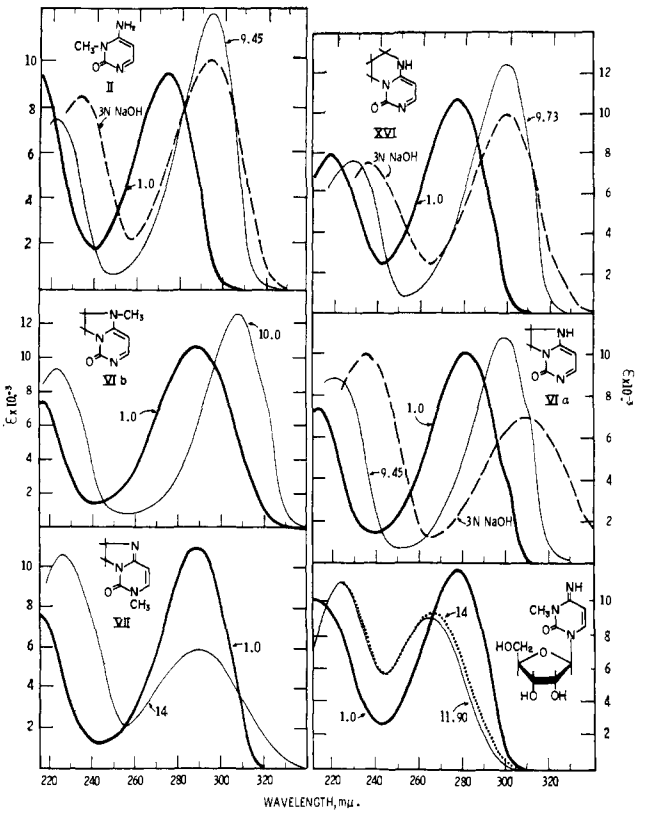


Fig. 3.—Absorption spectra of reference compounds in aqueous solution at pH or normality values indicated. All curves represent pure species with the exception of the 3 *N* NaOH curves for II and XVI which are almost completely representative of the anionic species (see text).

pure species) along with those for VI (R = H or CH_3), VII, and XVI are given in Fig. 3. It is clear from this figure that the spectra of the *cationic* species of all six compounds are similar (allowances made for small shifts in the maxima due to alkylation at N^1 or N^4).[15] The curve for the neutral species of 3-methylcytosine is very similar to that for VI (R = H *or* CH_3) and XVI but markedly dissimilar to that for 3-methylcytidine and VII. Since VIb (R = CH_3) is fixed in the 4-amino-2-oxo form and VII in the 4-imino-2-oxo form, *it must be concluded that in aqueous solution the neutral species of 3-methylcytosine, the imidazopyrimidines VIa and VIb, and the pyrimidopyrimidine XVI are all to be represented predominantly in the 4-amino-2-oxo structure.*[16]

This conclusion establishes the site of protonation of 3-methylcytosine (and VIa, VIb, and XVI) unequivocally on N^1 of the pyrimidine since their resonant cations are similar to those for VII and 3-methylcytidine. With 3-methylcytidine and VII protonation must occur on the imino nitrogen resulting in a resonant cation also similar to that for 3-methylcytosine, VIa, VIb, and XVI. The spectra for the cationic species of all six compounds in Fig. 3 are also similar to the cationic

(15) N^4 in I → V refers to the exocyclic nitrogen atom linked to C^4 of the pyrimidine. In the condensed ring compounds, this corresponding nitrogen atom should be referred to as N^1 (in VI, VII, and XVI). For the purposes of comparison, however, we call the exocyclic nitrogen atom of I → V and the corresponding nitrogen atom in VI, VII, and XVI as N^4. Similarly, the carbonyl groups in the condensed ring compounds are called "2-oxo" substituents. In VII the alkyl group is therefore called a 1-methyl substituent akin to 1 (R = CH_3). The correct nomenclature (*Chem. Abstr.*) of the condensed ring compounds is given in the Experimental section.

(16) The close spectral similarity of 3-methylcytosine to VIb also mitigates against structure IV (the 4-imino-2-hydroxy form) since it would be most unlikely that the spectrum of the neutral species of IV would be similar to VIb. Attempts to synthesize a 2-methoxy derivative from VIa (which would have the tautomeric structure of IV) were unsuccessful.

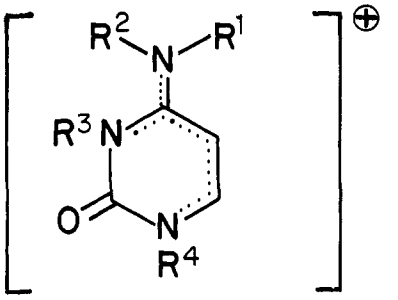


Figure 4.

curves for cytosine, 1-methylcytosine, and cytidine in accord with the cationic structure established for the latter three compounds.[2,3,6,7,17] A representation of these resonant cations is shown in Fig. 4.

3-Methylcytosine exhibits spectral shifts between pH 5–10 accounting for a spectrally-determined pK_{a1} of 7.38. Above pH 11, a new set of spectral shifts is observed which are almost completed in 3 *N* sodium hydroxide giving a pK_{a2} value of *ca.* 13–14. A similar phenomenon is exhibited by VIa and XVI, compounds which also possess a dissociable proton on N^4 of the pyrimidine. With VIa, the dissociation is completed in 3 *N* alkali giving a spectrally-determined pK_{a2} value of approximately 12.6 (see Table I). With XVI, the pK_{a2} is ~14. As expected, VIb and VII (where the dissociable proton is absent) do not exhibit the second pK_a. Since the tautomeric structure of 3-methylcytosine is now established as II, the second dissociation is obviously due to deprotonation at N^4. 3-Methylcytidine does show small shifts in the high alkaline region; however, these are readily attributed to dissociation of the hydroxy group(s) in sugar moiety. This latter phenomenon has been observed with pyrimidine nucleosides[4] and attributed to the cleavage of hydrogen bonds between the aglycon and the sugar moiety in this pH region.[18]

The spectrum of 3-methylcytidine is fairly similar to that for VII, as would be expected since both compounds possess the fixed 4-imino-2-oxo structure. The presence of a double bond in the imidazo ring of VII may cause strain which would affect the spectrum of the neutral species. Thus the comparison of the curve for the neutral species of VII to that for the neutral species of 3-methylcytidine is not as good. However, their cationic curves compare well. This may be due to appreciable delocalization of the double bond in the imidazoline ring when VII is protonated to give a resonant cation with the positive charge in the pyrimidine ring. One such resonance form is represented as VII-cation (Fig. 1).

Having thus established the structure of 3-methylcytosine, VIa, VIb, and XVI as the 4-amino-2-oxo type (II) rather than the 4-imino types (III or IV, see Fig. 1), the high pK_{a1} values of the three substituted cytosines (as compared with cytosine, 1-methylcytosine, and cytidine) may be explained by differences in the sites of protonation. As stated previously, protonation of cytosine, 1-methylcytosine, and cytidine occurs at N^3 (see ref. 2, 3, 6, 7, 17); whereas it is now established (*vide supra*) that 3-methylcytosine, VIa, VIb, and XVI protonate at N^1. These facts suggest that N^1 in 3-methylcytosine is more basic than N^3 in cytosine and its 1-substituted derivatives.

(17) C. A. Dekker, *Ann. Rev. Biochem.*, **29**, 453 (1960).

(18) J. J. Fox, L. F. Cavalieri, and N. Chang, *J. Am. Chem. Soc.*, **75**, 4315 (1953).

This conclusion can be generalized further by stating that of the heteroatoms in the cytosine *anion*, N^1 is more basic than N^3. Thus, cytosine at pH ~14 exists in the enolate form.[14] In the conversion of the enolate species to the cation the sequence of protonations is *first* on N^1 (to give the neutral species I, R = H) and *then* on N^3. This sequence (anion to cation) of protonations establishes N^1 as the more basic heteroatom. A similar conclusion is derived from the methylation of 2-methoxy-4-aminopyrimidine[19] (which may be considered to be analogous to the anionic form of cytosine) which yields the 1-methiodide rather than the N^3-substituted derivative, again indicating that N^1 is more basic than N^3.

Therefore, 3-methylcytosine, VIa,b, and XVI, which protonate on N^1, should have a higher pK_{a1} than cytosine or its 1-substituted derivatives which protonate on N^3. It is of interest to note that 3-methylcytosine exhibits a pK_{a2} (13–14) in the high alkaline region, whereas its isomer 1-methylcytosine fails to exhibit an acidic dissociation *in this pH region*.[10] It is clear that the exocyclic amino group in II is less basic than that in 1-methylcytosine. This difference in basicity of the exocyclic amino functions is possibly related to the fact that N^1 in II is more basic (by ~3 pK units) than N^3 in 1-methylcytosine.

Experimental[20]

N^4-(β-Hydroxyethyl)cytosine (IX, R = H).—A mixture of 4-methylthio-2-pyrimidinone (VIII, 1.42 g.) and ethanolamine (1.0 g.) in 1-butanol (15 ml.) was refluxed for 3 hr. After cooling, the separated crystals were collected by filtration and recrystallized from aqueous methanol, 1.4 g., m.p. 203–204.5°; λ_{max} in mμ: 268 at pH 6.5, 280 in *N* hydrochloric acid, 283 at pH 14.

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found: C, 46.54; H, 5.81; N, 26.97.

2,3-Dihydro-1H-5-oxoimidazo[1,2-*c*]pyrimidine Hydrochloride (VIa). A. IX (R = H) with Methanesulfonyl Chloride.—A mixture of IX (R = H, 1.0 g.) and methanesulfonyl chloride (0.8 g., 1.1 equiv.) in anhydrous pyridine (18 ml.) was stirred for 18 hr. at room temperature. The precipitated crystals (0.4 g.) were collected (the filtrate which contains starting material was discarded) and washed with alcohol. This precipitate gives a positive test for nitrogen and sulfur. The crystals (0.3 g.) were dissolved in water and applied to a column of Dowex 50 (H^+, 2.4 × 15 cm.) and the column was washed with water, 0.1 *N* hydrochloric acid, and eluted with *N* hydrochloric acid. The fractions containing product (checked by the absorption at 280 mμ) were combined and concentrated to a sirup. The residual sirup was dissolved in ethanol from which the crystals precipitated. After trituration with cold ethanol, 0.2 g. was obtained as the hydrochloride salt, m.p. > 290° (picrate m.p. 230–232° dec.; lit.[11] reports 228–230° dec.); ultraviolet properties: λ_{max} (mμ) ε: 280, 10,100 at *p*H 1; 297, 10,800 at pH 9.45; 309, 7100 in 3 *N* sodium hydroxide.

Anal. Calcd. for $C_6H_7N_3O$·HCl: C, 41.51; H, 4.64; Cl, 20.43; N, 24.20. Found: C, 41.92; H, 4.36; Cl, 20.56; N, 24.21.

B. IX (R = H) with Thionyl Chloride.—Compound IX (R = H, 0.5 g.) in chloroform (5 ml.) was treated with thionyl chloride (3 g.) and refluxed for 1 hr. The gummy precipitate (X, R = H) was collected by decantation, washed with chloroform, then dissolved in pyridine (5 ml.) and refluxed for 30 min. A white crystalline precipitate formed which was separated by filtration and washed with ethanol and dried (0.4 g.). The identity of this substance (VIa, R = H) with that obtained by method A (*vide supra*) was established by spectral similarity and by paper chromatography.

N^4-(β-Hydroxyethyl)-1-methylcytosine (XII).—The solution of 1-methyl-4-methylthio-2-pyrimidinone (XI, 1.4 g.) and ethanolamine (1.2 g.) in 1-butanol (10 ml.) was refluxed for 3 hr. After cooling, the separated crystals were collected by filtration (1.3 g., m.p. 161–163°), and recrystallized from ethanol (1.1 g., m.p. 161–163°; picrate m.p. 166–167°); ultraviolet properties: maximum at 275 mμ at pH 6–14; maximum at 288 mμ at pH 1.

Anal. Calcd. for $C_7H_{11}N_3O_2$: C, 49.69; H, 6.55; N, 24.84. Found: C, 49.77; H, 6.47; N, 24.68.

N^4-(β-Chloroethyl)-1-methylcytosine Hydrochloride (XIII).—Compound XII obtained above (0.5 g.) was treated with thionyl chloride (2 ml.) and refluxed for 15 min. The solvents were removed from the solid by decantation and the residue recrystallized from ethanol. White crystals (0.4 g.) were obtained, m.p. 163–164.5°. Ultraviolet properties were similar to those for XII.

Anal. Calcd. for $C_7H_{10}ClN_3O$·HCl: C, 37.52; H, 4.95; Cl, 31.64; N, 18.75. Found: C, 37.35; H, 5.23; Cl, 31.49; N, 18.77.

A picrate salt of XIII was prepared by dissolving the hydrochloride in aqueous alcohol and adding picric acid. The melting point of the picrate was 167–168.5° (similar to XII picrate). However, a mixture melting point with the picrate of XII gave a depression (155–162°).

When the hydrochloride XIII was heated above its melting point, resolidification occurred with subsequent melting at 271–273°. The ultraviolet absorption properties of this compound were similar to VII (*vide infra*) showing that cyclization occurred.

2,3-Dihydro-6-methyl-5-oxoimidazo[1,2-*c*]pyrimidine Hydrochloride (VII).—The above compound (XIII, 0.1 g.) in pyridine (3 ml.) was refluxed for 10 min. and the precipitated white crystals were collected by filtration, washed with alcohol, and dried; 0.07 g., m.p. 274–276°; ultraviolet properties: in 0.1 *N* HCl, maximum at 287.5 mμ (ϵ_{max} 10,900); in 1 *N* sodium hydroxide, max. at 289 mμ (ϵ_{max} 5900).

Anal. Calcd. for $C_7H_9N_3O$·HCl: C, 44.81; H, 5.37; Cl, 18.90; N, 22.40. Found: C, 44.71; H, 5.30; Cl, 18.73; N, 22.29.

N^4-(β-Hydroxyethyl)-N^4-(methyl)cytosine (IX, R = CH_3).—A mixture of 4-thiouracil (10 g.) and 2-methylaminoethanol (11.7 g.) in 1-butanol (85 ml.) was refluxed for 7 hr. After cooling, the separated crystals were collected by filtration and washed with ethanol (7.4 g., m.p. 172–173°). A small portion was recrystallized from ethanol; m.p. 173–175°; spectral properties: λ_{max} 276 mμ at pH 7; λ_{max} 285 mμ at pH 1.

Anal. Calcd. for $C_7H_{11}N_3O_2$: C, 49.69; H, 6.55; N, 24.84. Found: C, 49.63; H, 6.57; N, 25.03.

2,3-Dihydro-1-methyl-5-oxoimidazo[1,2-*c*]pyrimidine Hydrochloride (VIb).—The above compound (IX, R = CH_3, 1.8 g.) in chloroform (10 ml.) was treated with thionyl chloride (10 ml.) and refluxed for 30 min. The separated yellow sirup (lower layer) was freed from the upper layer by decantation, washed with chloroform, dissolved in ethanol, and concentrated to a sirup. On addition of ethanol to the sirup, white crystals separated (1.5 g.) which were washed with cold ethanol and recrystallized from hot ethanol; m.p. 281–283° dec., 0.9 g.; λ_{max} 287 mμ (ϵ_{max} 10,700) at pH 1; λ_{max} 307 and 223 mμ (ϵ_{max} 12,600 and 9400, respectively) at pH 10.

Anal. Calcd. for $C_7H_9N_3O$·HCl: C, 44.81; H, 5.37; Cl, 18.90; N, 22.40. Found: C, 44.77; H, 5.36; Cl, 18.75; N, 22.36.

N^4-(3-Hydroxypropyl)cytosine (XIV).—A mixture of 4-thiouracil (20 g.) and 3-aminopropanol (24.5 g.) in 1-butanol (200 ml.) was refluxed for 7 hr. After concentrating the solution to a sirup the residue was treated with hot ethanol. White crystals were obtained from the cooled solution; 20 g., m.p. 166–168°. One recrystallization from ethanol gave pure product, m.p. 173–175°; ultraviolet maxima: 267 mμ at pH 7, 280 mμ at pH 1.

Anal. Calcd. for $C_7H_{11}N_3O_2$: C, 49.69; H, 6.55; N, 24.84. Found: C, 49.58; H, 6.40; N, 24.90.

1,2,3,4-Tetrahydro-6-oxopyrimido[1,2-*c*]pyrimidine Hydrochloride (XVI).—The above compound (XIV, 4 g.) in chloroform (50 ml.) was treated with thionyl chloride (25 ml.) and refluxed for 1 hr. After cooling, the precipitate (XV) was collected by filtration, washed with chloroform, dissolved in pyridine (50 ml.), and refluxed for 20 min. White crystals separated which were filtered, washed with alcohol, and recrystallized from aqueous ethanol. White prisms were obtained; 3.5 g., m.p. > 280°; ultraviolet properties: λ_{max} 275 mμ (ε 10,600) at pH 1; 298 mμ (ε 12,400) at pH 9.73; 299 mμ (ε 9800) in 3 *N* NaOH.

Anal. Calcd. for $C_7H_9N_3O$·HCl: C, 44.81; H, 5.37; Cl, 18.90; N, 22.39. Found: C, 44.59; H, 5.60; Cl, 18.98; N, 22.27.

Synthesis of XVIII from VIa, VIb, and VII. A.—Compound VIa or VIb (500 mg.), potassium hydroxide (5 equiv.), and methyl iodide (6 equiv.) in methanol (10 ml.) were refluxed for 4 hr. After concentrating the solution to dryness, the residue was taken up in methanol, the insoluble material discarded, and the filtrate evaporated to dryness. The residue was recrystallized from ethanol (450 mg.); m.p. > 280°; ultraviolet properties: λ_{max} 268 mμ at pH 1–14.

Anal. Calcd. for $C_{10}H_{18}IN_3O_2$: C, 35.41; H, 5.35; I, 37.41; N, 12.39. Found: C, 35.69; H, 5.20; I, 37.68; N, 12.17.

B.—Compound VII (0.44 g.), potassium hydroxide (5 equiv.), and methyl iodide (10 equiv.) in methanol (10 ml.) were refluxed

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(20) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

for 4 hr. After cooling, the crystals were filtered and recrystallized from methanol to give white needles, m.p. > 280°, 440 mg. Spectral properties were identical with XVIII.

Anal. Calcd. for $C_{10}H_{18}IN_3O_2$: C, 35.41; H, 5.35; I, 37.41; N, 12.39. Found: C, 35.29; H, 5.29; I, 37.30; N, 12.26.

Paper electrophoresis at pH 10.58 (0.05 *N* sodium carbonate), 700 v., 100 min., gave the following migrations: XVIII obtained from VIa, VIb, and VII showed −16.4 cm.; VII, VIa, and VIb showed −3.5 cm.

Alkaline Hydrolysis of VIa.—A small amount of VIa hydrochloride was dissolved in *N* sodium hydroxide and heated at ~90° for 20 hr. After neutralization to pH 7, the solution was analyzed by paper electrophoresis (pH 10.58, 700 v., 70 min.). Two ultraviolet-absorbing spots were obtained migrating at −2.3 and +5.5 cm. (VIa migrates at −2.3 cm. in this system). The +5.5 cm. spot was excised and eluted with water; ultraviolet absorption properties: at pH 1, maximum at 260 mμ; at pH 14, λ_{max} at 285 mμ; ratio of absorbancies $A_{260\ m\mu,\ pH\ 1}/A_{285\ m\mu,\ pH\ 14} = 0.69$ (3-methyluracil gives 0.68).[14]

The excised 5.5-cm. spot was run in paper electrophoresis at pH 5.2 (0.1 *N* ammonium acetate) (70 min., 700 v.) where it showed a migration of −10.6 cm.; VIa which also has a basic amine showed a migration of −14.0. Uracil, 1-methyluracil, and 3-methyluracil showed migrations of −0.5 to −2 cm. These data attest to the presence of a basic amino function in the alkaline hydrolysate product consistent with 3-(β-aminoethyl)uracil.

Alkaline Hydrolysis of VIb.—A small sample of VIb was treated with *N* alkali for 20 hr. at 60°. The results were similar; ultraviolet properties: λ_{max} 260 mμ at pH 1; λ_{max} 285 mμ at pH 14; $A_{260\ m\mu,\ pH\ 1}/A_{285\ m\mu,\ pH\ 14} = 0.68$.

Alkaline Hydrolysis of XVI.—After 10-hr. reflux in alkali, XVI was converted to the 3-(aminopropyl)uracil as shown by the ultraviolet properties: λ_{max} at 260 mμ (pH 1); λ_{max} at 284 mμ (pH 14); ratio of $A_{max\ pH\ 1}/A_{max\ pH\ 14} = 0.66$.

Alkaline Hydrolysis of VII.—A dilute solution of VII in 0.01 *N* sodium hydroxide was heated at 85° for 48 hr. The ultraviolet absorption maximum of the hydrolysate was 268 mμ at pH 1-14. (Appreciable loss of absorption was noted during the hydrolysis characteristic of 1,3-dialkylated uracils.[14]) Another sample of VII was refluxed with 0.1 *N* sodium hydroxide for 2 hr. and applied to paper electrophoresis (pH 10.58, 800 v., 1 hr.). The ultraviolet-absorbing spots were obtained with migrations of −2.5 and +1.0 cm. (Starting material migrates at −2.5 cm. and uracil gives +6.7 cm.) The 1.0-cm. spot was excised and eluted with water and showed a maximum at 268 mμ, minimum at 237 mμ between pH 1-14. (1,3-Dimethyluracil gives a maximum at 266 and a minimum at 234 mμ in the same pH range.[14]) Paper electrophoresis at pH 3.75 (0.1 *N* NH_4OAc, 800 v. 1 hr.) also gave two spots (−11.5 and −8.0 cm.). The −11.5-cm. spot was starting material VII. These data attest to the formation of 1-methyl-3-(β-aminoethyl)uracil.

Synthesis of 3-Methyl-4-thio-2-pyrimidinone.—A suspension of 5.0 g. of 3-methyluracil[21] and 7.0 g. of phosphorus pentasulfide in 100 ml. of pyridine was heated to reflux with efficient stirring. A few drops of water was added so that the reaction mixture assumed an orange-turbid appearance. After 5 hr. the stirred, refluxing solution was cooled and allowed to remain at room temperature overnight. The mixture was decanted from a dark oil and the decantate concentrated *in vacuo* to dryness. The residue was treated with benzene and the benzene removed *in vacuo*. This process was repeated several times. The residual sirup was triturated five times with 50-ml. portions of absolute ethanol. The combined triturates were concentrated under vacuum to a solid mass which was dissolved in ~80 ml. of boiling water, treated with charcoal, and filtered hot. The filtrate was allowed to cool slowly. Yellow feathered clusters were obtained; 2.34 g., m.p. 181–182°. Recrystallization from water gave m.p. 183–184°. Ultraviolet properties agree with those for 4-thio-2-pyrimidinones[22]: pH 2–7, λ_{max} at 322 mμ and 260 mμ; ratio: max. at 322 mμ/max. at 260 mμ = 5.0. At pH 14: max. at 333 mμ, shoulder at 250 mμ, min. at 280 mμ; ratio: max./min. = 15.0.

Anal. Calcd. for $C_5H_6N_2OS$: N, 19.72; S, 22.54. Found: N, 19.42; S, 22.43.

3-Methylcytosine (II) and 3-methylcytidine were prepared according to Brookes and Lawley.[7]

Spectrophotometric Studies.—Ultraviolet absorption data were determined with a Cary recording spectrophotometer, Model 15, using buffer and techniques previously described.[14] The apparent pK_a values are accurate to ±0.05 pH unit and were determined spectrophotometrically by methods previously employed.[14,23]

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[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE, PITTSBURGH, PENNA.]

Insulin Peptides. VIII. A Synthetic Heptadecapeptide Derivative Corresponding to the C-Terminal Sequence of the B-Chain of Insulin[1,2]

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A synthesis is described of the protected heptadecapeptide N-carbobenzoxy-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-γ-benzyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester and the partially protected octapeptide N-carbobenzoxy-γ-benzyl-L-glutamyl-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine. The former peptide derivative corresponds to the arrangement of the carboxyl terminal 17 amino acid residues, positions 14 to 30, of the B-chain of insulin, and the latter to positions 13 to 20 of the same chain.

The amino acid sequence glutamyl-alanyl-leucyl-tyrosyl-leucyl-valyl-cysteinyl-glycyl-glutamyl-arginyl-glycyl-phenylalanyl-phenylalanyl-tyrosyl-threonyl-prolyl-lysyl-alanine represents the carboxyl terminal portion of the B-chain of insulin from several species.[3] In connection with our studies[4] directed toward the synthesis of the insulin molecule we have reported, in previous communications, the preparation of a protected nonapeptide and of two decapeptide derivatives containing the C-terminal portion of the aforementioned sequence.[5] In the present communication we report detailed experimental procedures for the preparation of N-carbobenzoxy-γ-benzyl-L-glutamyl-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine (VII) and N-carbobenzoxy-L-alanyl-L-leuycl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-γ-benzyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester (VIII). The partially protected octapeptide VII occupies the N-terminal position of the aforementioned segment of the B-chain of insulin. The fully protected heptadecapeptide VIII contains the C-terminal sequence of that fragment.

The synthesis of the octapeptide derivative VII was accomplished by the stepwise elongation approach which we now use routinely in our studies. Starting

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(2) A preliminary report of portions of the work described in this paper has been presented (P.G.K.) in the Eighth National Medicinal Chemistry Symposium of the American Chemical Society held in Boulder, Colo., June 18–20, 1962.

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