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Alkylation of 2-Amino-4-hydroxypyrimidines with Acrylonitrile and with Dimethyl Sulfate: Two Pyrimido[1,2-*a*]pyrimidinediones

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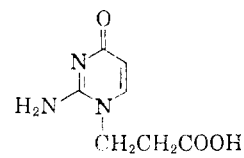
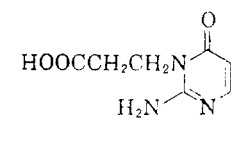
The reaction between 2-amino-4-hydroxypyrimidine (I) and acrylonitrile gave 2-amino-1-(2-carboxyethyl)-4(1H)-pyrimidinone (II) and none of the isomeric 3-substituted derivative (III) while the methylation of I gave a mixture of 2-amino-1-methyl-4(1H)-pyrimidinone (V) and 2-amino-3-methyl-4(3H)-pyrimidinone (VI). In contrast, 2-amino-4-hydroxypyrimidine-6-carboxylic acid (VIII) treated with acrylonitrile and with dimethyl sulfate gave, respectively, 2-amino-3(2-carboxyethyl)-4-oxo-3,4-dihydropyrimidine-6-carboxylic acid (IX) and 2-amino-3-methyl-4-oxo-3,4-dihydropyrimidine-6-carboxylic acid (XII) with none of the 1-substituted derivatives being formed. Compounds II and IX were successfully cyclized to 6,7-dihydro-2H-pyrimido[1,2-a]pyrimidine-2,8(9H)-dione (VII) and 6,7-dihydro-4H-pyrimido[1,2-a]pyrimidine-4,8(9H)-dione (X).

The reaction between 2-amino-4-hydroxypteridines and acrylonitrile has been shown to produce 8,9-dihydro-11H-pyrimido(2,1-b)pteridines.[1] This reaction involved a cyanoethylation of the 3-nitrogen of the pteridine ring followed by ring closure to produce the dihydropyrimidine ring. It was also shown that careful alkaline hydrolysis of the pyrimido(2,1-b)pteridines gave 2-amino-3-(2-carboxyethyl)-4(3H)-pteridinones.[1] As part of a program to explore the generality of this reaction several 2-amino-4-hydroxypyrimidines have been examined.

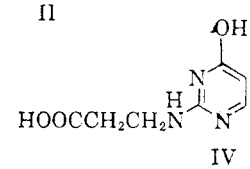
The reaction between isocytosine (2-amino-4-hydroxypyrimidine) (I) and an excess of acrylonitrile in a refluxing dilute sodium hydroxide solution produced, after acidification with acetic acid, a 66% yield of a chromatographically pure compound. Elemental analyses indicated the addition of a $C_3H_4O_2$ residue to I while the infrared spectra indicated the presence of a carboxyl group. On the basis of the corresponding reaction in the pteridine series[1] it seemed probable that the product was one of the two ring-alkylated compounds, 2-amino-1-(2-carboxyethyl)-4(1H)-pyrimidinone (II) or 2-amino-3-(2-carboxyethyl)-4-(3H)-pyrimidinone (III).[2]



II



III



IV

Compound IV was also considered since we had shown in the pteridine system that 2-amino-3-alkyl-4-pteridinones were converted to 2-alkylamino-4-hydroxypteridines by dilute base.[3] However, IV was eliminated since it should have ultraviolet absorption spectra similar to the spectra of isocytosine whereas the spectra of the product were entirely different. An examination of the literature revealed that 2-amino-3,6-dimethyl-4-(3H)-pyrimidinone[4] and 2-amino-1,6-dimethyl-4-(1H)-pyrimidinone[5] had been prepared but no ultraviolet absorption spectra were available. Todd, *et al;* have recently reported the synthesis of the two isocytosine nucleosides, 2-amino-1-(β-D-ribofuranosyl)-4(1H)-pyrimidinone[6] and 2-amino-1-(β-D-arabofuranosyl)-4(1H)-pyrimidinone.[7] Comparison of the ultraviolet absorption spectra of these isocytosine nucleosides and the isocytosine-acrylonitrile reaction product showed many similarities indicating that II was probably the correct structure. Preliminary attempts to convert II to a uracil derivative were not promising. However, structure II was corroborated by the synthesis and characterization of the 1- and 3-methyl derivatives of isocytosine.

The methylation of isocytosine (I) with dimethyl sulfate in dilute sodium hydroxide gave two products as shown by paper chromatography. The similar solubilities of the two pyrimidines and the by-product, sodium methyl sulfate, made it necessary to resort to partition chromatography in order

(1) R. B. Angier and W. V. Curran, *J. Am. Chem. Soc.*, 81, 5650 (1959).

(2) When this reaction involved the use of 2-amino-4-hydroxypteridines the products were usually isolated as the cyclic derivatives, *i.e.* pyrimido(2,1-b)pteridines.[1] However, the reaction of 2-(2-carboxyethylamino)-4-hydroxypteridine-6-carboxylic acid with acrylonitrile gave a crude product which was a mixture of the pyrimido(2,1-b)pteridine and the 3-(2-carboxyethyl)-4-pteridinone derivatives.[1]

(3) W. V. Curran and R. B. Angier, *J. Am. Chem. Soc.*, 80, 6095 (1958).

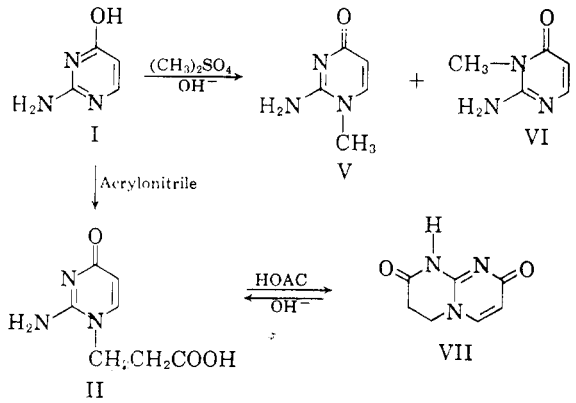
(4) R. Majima, *Chem. Ber.*, 41, 176 (1908).

(5) A. D. Ainley *et al.*, *J. Chem. Soc.*, 59 (1953).

(6) D. M. Brown, A. R. Todd and S. Varadarajan, *J. Chem. Soc.*, 868 (1957).

(7) D. M. Brown, D. B. Parihar, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 3028 (1958).

to obtain pure compounds.[8] The purified compounds, 2-amino-3-methyl-4(3H)-pyrimidinone (3-methylisocytosine) (VI) and 2-amino-1-methyl-4-(1H)-pyrimidinone (1-methylisocytosine) (V) were characterized by hydrolysis in hot 1*N* sodium hydroxide to the known uracil derivatives, 3-methyl-2,4-(1,3H)pyrimidinedione and 1-methyl-2,4(1, 3H) pyrimidinedione, respectively.[9,10] Examination of the data given in the experimental section shows that in this methylation the yield of 1-methylisocytosine (V) greatly exceeded the yield of the isomeric 3-methylisocytosine (VI).



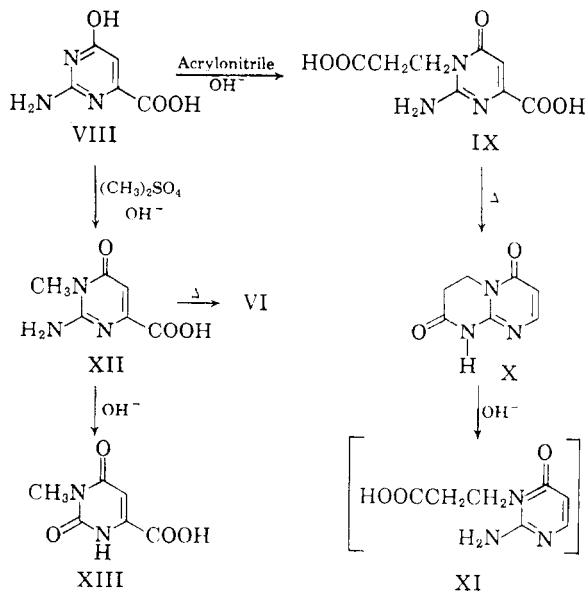
Comparison of the ultraviolet absorption spectra (Table I) of the isomeric methylisocytosines, V and VI, with the spectra of the acrylonitrile-isocytosine reaction product demonstrated conclusively that the latter compound was 2-amino-1-(2-carboxyethyl)-4(1H)-pyrimidinone (II). Attempts to cyclize II in boiling water or dilute acid, in a manner analogous to that used with the corresponding pteridines,[1] were only partially successful. However, II heated in glacial acetic acid for one hour produced 6,7-dihydro-2H-pyrimido[1,2-a]pyrimidine-2,8(9H)-dione (VII) in a 92% yield.[11] Upon standing in dilute alkali VII again reverted to II.[12]

Since 2-amino-4-hydroxypteridines are alkylated exclusively in the 3-position by acrylonitrile[1]

TABLE I

Substituent	λmax., mμ(ε) 0.1*N* NaOH	0.1*N* HCl
2-Amino-4-pyrimidinones		
Isocytosine (I)	225(7,780)	215(9,400)
	273(6,500)	257(6,970)
3-Methyl (VI)	283(9,120)	256(5,940)
1-Methyl (V)	260(5,500)	217(9,250)
		260(7,620)
1-(2-Carboxyethyl) (II)	260(5,850)	217(9,780)
		260(7,880)
3-(2-Carboxyethyl) (XI)	286(8,150)	258(5,680)
6-Carboxylic acid (VIII)	292(5,230)	214(12,550)
		276(6,900)
3-Methyl-6-carboxylic acid (XII)	300(6,060)	215(10,400)
		278(5,630)
3-(2-Carboxyethyl)-6-carboxylic acid (IX)	302(6,680)	220(11,100)
		278(5,450)
Pyrimido (1,2-a) pyrimidinediones		
VII	233(32,400)	220(24,600)
	260(15,000)Sh	260(5,940)Sh
X	249(11,900)	212(9,350)
	304(14,600)	231(9,800)
		282(8,000)

it seemed plausible that an isomer of VII might be produced by introducing a bulky group in the 6-position of the pyrimidine ring in order to hinder attack at the 1-nitrogen. Therefore 2-amino-4-hydroxypyrimidine-6-carboxylic acid[13] (VIII) was prepared by a modification of a method previously described for the preparation of 2-thio-4-hydroxypyrimidine-6-carboxylic acid[14] and related compounds. When VIII was treated with excess acrylonitrile as previously described for isocytosine (I), the product had elemental analyses which indicated



(8) Mr. Charles Pidacks and staff carried out the chromatographic separation of these compounds, details of which are reported in the experimental section.

(9) D. J. Brown, E. Hoerger, and S. F. Mason, *J. Chem. Soc.*, 211 (1955).

(10) The simple hydrolysis of 3-methylisocytosine (VI) to 3-methyluracil is of some interest since in the pteridine series the corresponding 2-amino-3,6,7-trimethyl-4(3H)-pteridinone does not hydrolyze but undergoes a rearrangement to 2-methylamino-4-hydroxy-6,7-dimethylpteridine.[3]

(11) A 7% yield of the cyclic compound (VII) was also obtained from the mother liquor of the original acrylonitrile-isocytosine reaction.

(12) It is apparent that in neutral or moderately acidic aqueous solutions an equilibrium is set up between II and VII. With the corresponding compounds in the pteridine series under similar conditions this equilibrium was displaced toward the cyclic derivatives which were therefore readily isolated.[1] However, with the pyrimidine compounds the equilibrium seems to have favored isolation of the non-cyclic compounds, II and IX.

(13) S. Ruhemann and H. E. Stapleton, *J. Chem. Soc.*, **77**, 808 (1900).

(14) M. Bachstez, *Chem. Ber.*, **64B**, 322 (1931).

the addition of a $C_3H_4O_2$ residue. Fusion of this compound at 310° gave in low yield, a new compound with elemental analysis which indicated the loss of a mole of water and a mole of carbon dioxide. Basic hydrolysis then produced a compound which was assigned structure XI since its ultraviolet absorption spectra were very similar to the spectra of 3-methylisocytosine (VI) (Table I).

Hence the precursor of XI was 6,7-dihydro-4H-pyrimido[1,2-a]pyrimidine-4,8-(9H)-dione (X) and the product of the reaction of acrylonitrile with VIII must have been 2-amino-3-(2-carboxyethyl)-4-oxo-3,4-dihydropyrimidine-6-carboxylic acid (IX).

In order to verify the above structural assignments VIII was methylated in the usual manner with dimethyl sulfate to give a good yield of a single monomethyl derivative. This compound was converted to 3-methylorotic acid (XIII) by alkaline hydrolysis and was also decarboxylated to 3-methylisocytosine (VI) in refluxing quinoline, establishing the structure to be 2-amino-3-methyl-4-oxo-3,4-dihydropyrimidine-6-carboxylic acid (XII). The ultraviolet absorption spectra of XII, both in 0.1*N* sodium hydroxide and 0.1*N* hydrochloric acid, were identical with those of IX, thus confirming the structure of the latter compound and providing additional evidence for X and XI.

Since the yield of 3-methylisocytosine (VI) from the methylation of isocytosine was quite low, the action of alcoholic-ammonia on 2-methylthio-3-methyl-4(3H)pyrimidinone[9] was investigated as a preparative procedure. This did not prove to be a satisfactory method. 3-Methylisocytosine (VI) was obtained but the yield was low (about 15%) and 3-methyluracil was also formed as a major by-product.

EXPERIMENTAL[15]

Paper chromatography. The descending technique was employed using Whatman No. 1 paper with the following solvent systems: solvent A, 1-butanol-5*N* acetic acid (7–3); solvent B, ethanol-water-concentrated ammonium hydroxide (80–16–4); solvent C, 1-butanol-pyridine-water (1-1-1); solvent D, acetone-water (4-1); solvent E, isopropyl alcohol-1*N* ammonium hydroxide (7–3). The compounds were detected as absorption spots using an ultraviolet lamp provided with a filter to give primarily light of 254 mμ and a zinc silicate plate coated with Du Pont phosphor No. 609 235.[16]

Methylation of isocytosine. To 11.1 g. (0.1 mole) of isocytosine (I), dissolved in 20 ml. of 5*N* sodium hydroxide, was added 12 ml. (0.12 mole) of dimethyl sulfate over a 2.5-hr. period. The temperature remained at 35–40° during this process. After chilling the solution overnight the solid was collected and dried; yield 2.2 g. (Fraction A). The mother liquor was evaporated to an oil, dissolved in absolute alcohol and again evaporated *in vacuo*. The solid residue was extracted with about 150 ml. of *n*-propyl alcohol; yield of residue 15.0 g. (Fraction B). The filtrate from Fraction B on cooling gave 5.5 g. of solid (Fraction C). The mother liquor was concentrated to about 35 ml. and ether added to give a slightly turbid mixture. This was cooled and the product was collected; yield 1.4 g. (Fraction D). The total of 24.1 g. of product represents an 86.5% yield assuming complete reaction to the methylisocytosines and sodium methyl sulfate. The following table gives sulfur analyses and R_f values in solvent A for the four fractions with R_f 0.51 corresponding to starting material. A value of 12% S corresponds to about 50% sodium methyl sulfate:

Fraction	R_f in Solvent A	% S
A (2.2 g.)	0.38, 0.51, 0.62	—
B (15.0 g.)	0.38	11.1
C (5.5 g.)	0.38, 0.62	13.2
D (1.4 g.)	0.38, 0.51, 0.62	9.9

Five hundred grams of Celite was mixed with 250 ml. of the stationary phase of the solvent system ethyl acetate:methanol:water (4:1:2, v./v.), then packed in uniform increments about equal to the diameter of the column (column = 5.5 cm. in diameter × 90 cm. in length). Fraction C was dissolved in 25 ml. of the stationary phase plus 25 ml. of the upper phase and mixed with 50 g. of Celite. This was packed into the column which was then developed with the mobile phase. The effluent from the column was run through a flow cell with a light path of 0.5 cm. and the ultraviolet absorption at 255 mμ recorded automatically. There was a peak at 650 ml. (1 hold-back volume) and a minimum after 1300 ml. Elution of the column with an additional 4 hold-back volumes of the mobile phase did not produce any more material. The column was then washed with methanol.[8] Evaporation of the first 1300 ml. of eluate left 1.25 g. of product; m.p. 254–264°. This product was shown to be 3-methylisocytosine (VI) by hydrolysis to 3-methyluracil. For analysis a portion of VI was recrystallized from absolute ethanol; m.p. 262–266°, R_f 0.62 (solvent A).

Anal. Calcd. for $C_5H_7N_3O$ (125): C, 48.0; H, 5.6; N, 33.6. Found: C, 47.7; H, 5.6; N, 33.8.

Evaporation of the methanol wash from the column gave 2.5 g. of solid; m.p. 140–165° (12.9% S). Paper chromatography in solvent A showed the other isomer (Rf 0.38) was in this fraction. However, fraction B contained most of the R_f 0.38 isomer and it was purified in the following manner:

Three grams of fraction B was dissolved in 20 ml. of water and heated on a steam bath. To this was added 20 ml. of hot, saturated ethanolic picric acid and the picrate crystallized immediately. The mixture was cooled and the solid was collected. The damp picrate was dissolved in a solution of 300 ml. of water and 50 ml. of ethanol by heating. The solution was cooled to 50° and treated with Dowex-1(CO_3) until the supernatant was colorless. The resin was filtered off and the filtrate evaporated to dryness *in vacuo* with the aid of absolute ethanol. The residue was recrystallized from 60 ml. of absolute ethanol; yield 0.40 g. m.p. 272–278° dec. Concentration of the filtrate to 10 ml. gave an additional 0.17 g., m.p. 273–278° dec. For analysis a portion of the first crop was recrystallized from absolute ethanol; m.p. 275–280°; R_f 0.38 (solvent A). This product was shown to be 1-methylisocytosine (V) by hydrolysis to 1-methyluracil.

Anal. Calcd. for $C_5H_7N_3O$ (125): C, 48.0; H, 5.6; N, 33.6. Found: C, 48.4; H, 6.0; N, 33.3.

Alkaline hydrolysis of VI. Five hundred milligrams of the lower melting methylisocytosine (VI) was heated for 1.5 hr. on a steam bath in 5 ml. of 1*N* sodium hydroxide. The solution was evaporated to about 2.5 ml. and acidified hot to *p*H 5 with concentrated hydrochloric acid. The precipitated glass was removed by filtration and the filtrate evaporated to dryness *in vacuo* with the aid of absolute alcohol. The residue was extracted with 25 ml. of absolute alcohol and the extract was evaporated to 10 ml. and cooled; yield of crystalline product 124 mg.; m.p. 179–181°. The infrared spectrum of this material was identical with the spectrum of

(15) The melting points have been corrected for the exposed stem of the thermometer.

(16) Commercially available from E. I. du Pont de Nemours & Co., Inc., Polychemicals Dept., 350 5th Ave., New York, N. Y.

an authentic sample of 3-methyluracil.[9] Paper chromatography of the mother liquor in solvents A, B, and C showed that 3-methyluracil was the only detectable product.

Alkaline hydrolysis of V. Ninety-five milligrams of the higher melting isomer (V) obtained from the methylation of isocytosine was dissolved in 2 ml. of 1*N* sodium hydroxide and heated on a steam bath for 1.5 hr. keeping the volume constant by the addition of water. The solution was acidified hot to *p*H 5 with glacial acetic acid and filtered from some precipitated glass. The filtrate deposited needles when cooled; yield 40 mg.; m.p. 230–235°. This compound was identical with an authentic specimen of 1-methyluracil[9] (mixed melting point and infrared spectra).

3-Methylisocytosine (VI) *via the amination of 2-methylthio-3-methyl-4(3H)-pyrimidinone.* One and a half grams (9.6 mmoles) of 2-methylthio-3-methyl-4(3H)-pyrimidinone[9] was added to 50 ml. of absolute methanol, cooled to 5°, and saturated with anhydrous ammonia. The resulting solution was heated in a bomb at 155° overnight, then filtered to remove some black, amorphous material. The filtrate was evaporated to dryness *in vacuo* and the residue dissolved in absolute ethanol and again evaporated to dryness. The residue was then dissolved in 15 ml. of absolute ethanol, treated with Norit, and filtered. After chilling the solution overnight, the crystals were collected; yield 0.50 g., melts over long range starting at 135°. Paper chromatography in solvents A, B, and C revealed that this was a mixture of 3-methylisocytosine and 3-methyluracil. Recrystallization of the mixture from 5 ml. of ethanol (Norit) gave 0.20 g. (16%), m.p. 253–260° of 3-methylisocytosine. This material was chromatographically homogeneous and traveled side-by-side in solvents A, B and C with one of the isomers (VI) obtained from the methylation experiment.

2-Amino-1-(2-carboxyethyl)-4(1H)-pyrimidinone (*II*). Isocytosine (20.0 g., 0.18 mole) was added to 180 ml. of 1*N* sodium hydroxide containing 40 ml. of acrylonitrile and refluxed for 2.5 hr. After the excess acrylonitrile was removed *in vacuo*, the solution was treated with Norit and filtered. The filtrate was acidified to *p*H 5 with 25 ml. of glacial acetic acid and cooled; yield of product 22.2 g. (66%). The mother liquor deposited a second crop (2.6 g.) after long standing in the cold. Paper chromatography in solvent D showed that the second crop was the pyrimido[1,2-a]pyrimidine (VII).

Two grams of the first crop was recrystallized from water for analytical purposes; R_f 0.44 (solvent D).

Anal. Calcd. for $C_7H_9N_3O_3$ (183): C, 45.9; H, 5.0; N, 22.9; Found: C, 45.6; H, 5.2; N, 23.1.

6,7-Dihydro-2H-pyrimido[1,2-a]pyrimidine-2,8-(9H)-dione (VII). Two grams (10.9 mmoles) of 2-amino-1-(2-carboxyethyl)-4(1H)-pyrimidinone (II) was refluxed for 1 hr. in 50 ml. of glacial acetic acid. The solution was evaporated *in vacuo* to an oil which crystallized after adding absolute ethanol. The alcohol was removed *in vacuo* and the crystalline product slurried in absolute alcohol and filtered; yield 1.65 g. (92%), m.p. 255–258° dec., R_f 0.60 (solvent D).

Anal. Calcd. for $C_7H_7N_3O_2$ (165): C, 50.9; H, 4.3; N, 25.5. Found: C, 50.6; H, 4.3; N, 25.7.

When VII was dissolved in 0.1*N* sodium hydroxide and allowed to stand for 24 hr. at room temperature, spectroscopic and chromatographic examination of the solution showed that VII had been converted back to the open chain compound II.

2-Amino-4-hydroxypyrimidine-6-carboxylic acid (VIII). Guanidine hydrochloride (9.5 g., 0.1 mole), 21.2 g. of diethyl oxalacetate sodium salt (0.1 mole) and 0.5 g. of ammonium thiocyanate were added to 15 ml. of ethanol and stirred for 10–15 min. Ten drops of concentrated hydrochloric acid was added and the stirring was continued for an additional 2.5 to 3.0 hr. After standing overnight at room temperature, a solution of 28 g. of potassium hydroxide in 100 ml. of water was added and refluxed for 1 hr. The solution was then treated with Norit and acidified with 30 ml. of concd. hydrochloric acid; yield of product 8.8 g. This product was dissolved in 120 ml. of 1*N* sodium hydroxide on a steam bath, treated with Norit and filtered. The filtrate was acidified with 10 ml. of concd. hydrochloric acid; yield 6.3 g. A second purification was carried out using 200 ml. of 0.5*N* sodium hydroxide and 8.3 ml. of concd. hydrochloric acid; yield 5.3 g. (34%); R_f 0.56 (solvent E).

Anal. Calcd. for $C_5H_5N_3O_3$ (155): C, 38.3; H, 3.6; N, 26.7. Found: C, 38.7; H, 3.3; N, 27.1.

2-Amino-3-(2-carboxyethyl)-4-oxo-3,4-dihydropyrimidine-6-carboxylic acid (IX). 2-Amino-4-hydroxypyrimidine-6-carboxylic acid (3.9 g., 0.025 mole) was added to 51 ml. of 1*N* sodium hydroxide containing 6 ml. of acrylonitrile and refluxed for 10 hr. Two 6-ml. portions of acrylonitrile were added after 6 and 9 hr. The excess acrylonitrile was removed *in vacuo*. The solution was acidified to *p*H 2.0 with 6*N* hydrochloric acid, cooled, and the product was collected and dried; yield 4.25 g. (75%), m.p. 294–295° (gas evolution with previous browning and wetting). For analysis a portion of this product was purified twice by dissolving in 2 equivalents of hot 0.3*N* sodium hydroxide and acidifying with concentrated hydrochloric acid; m.p. 299–302°; R_f 0.49 (solvent E).

Anal. Calcd. for $C_8H_9N_3O_5$ (227): C, 42.3; H, 4.0; N, 18.5 Found: C, 42.2; H, 4.1; N, 18.8.

6,7-Dihydro-4H-pyrimido[1,2-a]pyrimidine-4,8(9H)-dione (X). 2-Amino-3-(2-carboxyethyl)-4-oxo-3,4-dihydropyrimidine-6-carboxylic acid (1.0 g., 4.4 mmoles) (IX) was heated to 310° in a silicone bath for several minutes until gas evolution appeared to be complete. The cooled residue was extracted with two 80-ml. portions of hot 50% ethanol. The extract was evaporated to dryness *in vacuo* and the residue was taken up in 20 ml. of 50% ethanol. After standing overnight at room temperature a small amount of a gummy solid had precipitated. This was filtered off and discarded. The filtrate, on chilling, deposited crystals; yield 0.25 g. (34.5%). Paper chromatography in solvent E showed the primary spot at R_f 0.70 with some fluorescent impurities. Two recrystallizations from 50% ethanol gave 80 mg. (11%) of chromatographically pure material; m.p. 295–298° dec.

Anal. Calcd. for $C_7H_7N_3O_2$ (165): C, 50.9; H, 4.3; N, 25.5. Found: C, 51.1; H, 4.5; N, 25.6.

When a sample of X was dissolved in 0.1*N* sodium hydroxide and allowed to stand 24 hr. at room temperature its ultraviolet absorption spectra were essentially identical to the spectra of 3-methylisocytosine (VI). In a second experiment 73 mg. of X was dissolved in 1.0 ml. of 1.0*N* sodium hydroxide and allowed to stand 24 hr. at room temperature. The solution was then cooled and acidified, first to *p*H 5 with acetic acid and then to *p*H 2.5 with 6*N* hydrochloric acid; yield of crystalline product 12 mg. The ultraviolet absorption spectra of this product were again essentially identical to the spectra of VI; R_f 0.67 (solvent E), 0.52 (solvent A).

2-Amino-3-methyl-4-oxo-3,4-dihydropyrimidine-6-carboxylic Acid (XII). Five grams (0.032 mole) of 2-amino-4-hydroxypyrimidine-6-carboxylic acid was dissolved in 100 ml. of water by adding 21 ml. of 10*N* sodium hydroxide solution. Dimethyl sulfate (13.8 ml., 0.138 mole) was added dropwise over a 50-min. period with stirring. The temperature rose to 40° during this process. After stirring for an additional 1.5 hr., the solution was treated with Norit, filtered, and the filtrate acidified to *p*H 1 with 6 ml. of concd. hydrochloric acid. The crystals were collected and dried; yield 4.8 g. Paper chromatography of this material in solvent E revealed two spots R_f 0.56 and R_f 0.67, the slower-moving spot corresponding to the starting compound. The product was redissolved in 100 ml. of 1*N* sodium hydroxide and treated with 6.5 ml. (0.065 mole) of dimethyl sulfate, dropwise, over a 20-min. period with stirring. After stirring again for an additional 1.5 hr. the solution was treated with Norit and acidified to *p*H 1 with concentrated hydrochloric acid; yield 3.4 g. (63%), m.p. 290–292.5° with gas evolution; R_f 0.67 (solvent E) with only a trace of starting material.

Anal. Calcd. for $C_6H_7N_3O_3$ (169): C, 42.6; H, 4.2; N, 24.9. Found: C, 42.5; H, 4.7; N, 24.7.

3-Methylorotic acid (XIII). 2-Amino-3-methyl-4-oxo-3,4-dihydropyrimidine-6-carboxylic acid (100 mg., 0.59 mmole) (XII) was dissolved in 2 ml. of 1*N* sodium hydroxide and heated for 1.5 hr. on a steam bath during which time the volume decreased to 1 ml. The volume was adjusted to 2 ml. with water and the solution acidified to *p*H 5 with glacial acetic acid; yield 12 mg. Paper chromatography in solvent E showed this crop to be a mixture of starting material (R_f 0.54) and an unidentified, lower R_f product. The filtrate was further acidified with 6*N* hydrochloric acid to give 34 mg. (34%) of 3-methylorotic acid m.p. 313–316°. This was identical (melting point and chromatography in solvent E [R_f 0.66)] to an authentic specimen prepared by the method of Fox *et al.*[17]

Decarboxylation of 2-amino-3-methyl-4-oxo-3,4-dihydropyrimidine-6-carboxylic acid (XII). Compound XII (0.75 g., 4.4 mmoles) was refluxed for 2 hr. in 100 ml. of quinoline. The quinoline solution was then extracted with three 100-ml. portions of water and the combined water washings were extracted with ether (to remove excess quinoline). The aqueous layer was evaporated to dryness *in vacuo* and the residue was taken up in about 50 ml. of absolute alcohol. After concentrating the solution to about 15–20 ml. and chilling there was obtained 137 mg. of product. Two further crops weighing 82 and 42 mg. (47%) total yield) were obtained by concentrating the mother liquor. All three crops traveled side-by-side with 3-methylisocytosine in solvents A (R_f 0.62) and E (R_f 0.77). The three crops were combined and recrystallized from 8 ml. of ethanol using Norit; yield 100 mg., m.p. 264–267°. The infrared spectrum of this product was identical with the spectrum of the 3-methylisocytosine obtained by methylation.

(17) J. J. Fox, N. Yung, and I. Wempen, *Biochim. et Biophys. Acta*, **23**, 295 (1957).

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Synthesis of Some Pyrimidylphosphonates

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Nine dialkyl pyrimidylphosphonates and six free phosphonic acids, corresponding to these esters, were synthesized for biological studies by modifications of the Arbuzov and the Michaelis reactions.

The present investigation was undertaken for the purpose of exploring the possibilities of biological activity among organophosphorus compounds containing a pyrimidyl radical joined directly to a phosphorus atom. Our particular attention was directed to the compounds of the phosphonic acid family, *i.e.*, $RPO(OH)_2$, in which the radical is a pyrimidyl group either with the most simple substituents or one that is unsubstituted otherwise. This selection was made in order that the behavior of the simplest members of the series could be evaluated. This line of approach appeared to be potentially useful in view of the existence of many biologically important organic compounds which contain the pyrimidyl radical in one form or another. The compounds constituted about a pyrimidyl radical joined directly to phosphorus could constitute a possible line of metabolic antagonists since it might be expected that the carbon-phosphorus bond would prove to be resistant to enzymatic cleavage and the substantially dipolar molecules could be expected to persist in a living cell.

It was shown by one of us some years ago that the conventional Arbuzov and Michaelis reactions may be applicable for the synthesis of phosphonates from nitrogen heterocycles containing a halogen atom at a carbon atom in the cycle.[2] For this reason, this specific approach was used for the synthesis of various pyrimidyl members. The very low reactivity of 5-halopyrimidine prevented the synthesis of the 5-pyrimidylphosphonate, but the analogous 2- and 4-isomers were prepared successfully, as were seven phosphonates with various substituents in the pyrimidine cycle.

It may be noted that several unsuccessful attempts to accomplish such syntheses may be found in the earlier literature.[3]

The phosphonates synthesized by us displayed a strong absorption band at 300–320 mμ in the ultraviolet. The infrared absorption spectra of these substances were also examined. The notable features of these spectra are as follows. The 2-pyrimidylphosphonate displays a sharp peak at 1225 cm.$^{-1}$ which is absent in the spectrum of the 4-isomer. The 2-chloro-4-pyrimidylphosphonate shows a band at 685 cm.$^{-1}$, probably caused by the C—Cl grouping, as this band does not appear in the substances devoid of chlorine in the 2-position. The normally expected absorption near 990 cm.$^{-1}$ for the pyrimidine ring is obscured by the prox-

(1) This material represents the dissertation submitted by C. H. Roy in partial fulfillment of the requirements for the Ph.D. degree at the Auburn University School of Graduate Studies in June 1960.

(2) G. M. Kosolapoff, *J. Am. Chem. Soc.*, **69**, 1002 (1947).

(3) B. A. Arbuzov and B. P. Lugovkin, *Zhur. Obshchei Khim.*, **22**, 1199 (1952); A. Burger, J. B. Clements, N. D. Dawson, and R. B. Henderson, *J. Org. Chem.*, **20**, 1383 (1955).