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Synthesis of Potential Anticancer Agents. XXIII. 9-Aminohypoxanthine and Related Compounds

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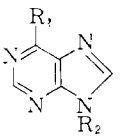
The reaction of 5-amino-4-hydrazinopyrimidines with formic acid has been found to give 9-aminopurines rather than the isomeric 1,2-dihydropyrimido[5,4-*e*]-*as*-triazines. Some 1-alkyl-1,2-dihydropyrimido[5,4-*e*]-*as*-triazines were prepared from 4-(1-alkylhydrazino)-5-aminopyrimidines.

The behavior of 5-amino-4-hydrazinopyrimidines when treated with reagents such as anhydrous formic acid[2] or ethyl orthoformate–acetic anhydride[3], normally used to prepare purines from 4,5-diaminopyrimidines, poses an interesting chemical problem, since one would assume that the reaction is equally likely to give 9-aminopurines[4] or 1,2-dihydropyrimido-[5,4-e]-*as*-triazines.[6] Either product could be utilized in the preparation of potential anticancer agents; the former compounds might interfere with purine metabolism and the latter with the one-carbon transfers involving folic or folinic acid.

To investigate the reaction, we prepared 5-amino-4-chloro-6-hydrazinopyrimidine (IV) from 5-amino-4,6-dichloropyrimidine (III) and anhydrous hydrazine.[8] Reaction of IV with anhydrous formic acid gave a product (XI) which on treatment with dilute acid or base yielded 9-aminohypoxanthine (XIX). The identity of XIX was established in a number of ways. First, treatment of XIX with aqueous nitrous acid gave a good yield of hypoxanthine (XIII), as did fusion with sulfur. Secondly, XIX was synthesized by an unequivocal route. 5-Amino-4-chloro-6-benzylidenehydrazinopyrimidine (VII) was prepared by the reaction of 5-amino-4,6-dichloropyrimidine (III) and benzaldehyde hydrazone and also by the reaction of 5-amino-4-chloro-6-hydrazinopyrimidine (IV) and benzaldehyde. Treatment of VII with formic acid gave 9-benzylideneaminohypoxanthine (X) which, on heating in dilute aqueous acid or base, was hydrolyzed to XIX. Compound XIX reacted with benzaldehyde to give X. Further, X was reduced catalytically to 9-benzylaminohypoxanthine (XII). In addition to these chemical proofs of structure, the infrared spectrum of XIX shows the carbonyl band[9] (1690 cm.$^{-1}$) and the N–H bands[9] (3450, 3250 and 1645) that would be expected from that structure, and the ultraviolet spectrum agrees well with that of 9-ethylhypoxanthine[10] (see Table I).

TABLE I



		—0.1 *N* HCl—		—*p*H 7—		—0.1 *N* NaOH—	
R_1	R_2	$\lambda_{max.}$ mμ	ε ×10^{-3}	$\lambda_{max.}$ mμ	ε ×10^{-3}	$\lambda_{max.}$ mμ	ε ×10^{-3}
H	C_2H_5	261.5	5.85	263	7.82	263	7.82
H	NH_2	262.5	...	264	5.05	265.5	...
OH	C_2H_5	250	10.8	250	11.7	250	12.4
OH	NH_2	250	10.4	249	11.0	254	12.0
OH	$N{=}CHC_6H_5$	250	...	254	21.1	254	...
		284	...	286	22.8	289	...
OH	$NHCH_2C_6H_5$	250	11.4	250	12.2	255	13.2
Cl	C_2H_5	265	9.6	266	9.4	266	9.4
Cl	$NHCOCH_3$	262	...	263	...	266	...
SH	C_2H_5	325	19.6	320	20.0	310	21.8
SH	$NHCOCH_3$	321	23.9	316	20.0	311	21.2

The hydrolysis of the chlorine atom of IV during ring closure was expected for this is a well documented reaction in the purine series.[10,11] Since the ring closure gave 9-formamidohypoxanthine (XI) instead of the isomeric 1,2-dihydropyrimido[5,4-*e*]-*as*-triazin-5-ol, it seems reasonable to assume that, although formylation of the terminal amino group of the hydrazino group of IV probably took place first, diformylation occurred in the large excess of anhydrous formic acid to give VIII, which would surely cyclize in the observed manner.

5-Amino-4-chloro-6-hydrazinopyrimidine (IV) was reduced catalytically[12] to 5-amino-4-hydrazinopyrimidine (VI), which on treatment with formic acid gave 9-aminopurine (IX).

Treatment of IV with ethyl orthoformate–acetic anhydride[3] gave crude 9-acetamido-6-chloropurine (I), which was hydrolyzed in acid to 9-aminohypoxanthine and treated with thiourea to give 9-acetamidopurine-6(1H)thione (II).

5-Amino-4,6-dichloropyrimidine was allowed to react with methylhydrazine and benzylhydrazine to give 5-amino-4-chloro-6-(1-methylhydrazino)-pyrimidine (XX, R = CH_3) and 5-amino-4-chloro-6-(1-benzylhydrazino)-pyrimidine (XX, R = $CH_2C_6H_5$), respectively. Catalytic reduction of XX (R = CH_3 and $CH_2C_6H_5$) using palladium-on-charcoal[12] gave

(1) Affiliated with the Sloan-Kettering Institute. This work was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-ph-1740. Part XXII. J. A. Montgomery and C. Temple, Jr., *J. Org. Chem.*, **25**, 395 (1960).

(2) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952).

(3) E. Richter and E. C. Taylor, *Angew. Chem.*, **67**, 303 (1955); J. A. Montgomery, THIS JOURNAL, **78**, 1928 (1956).

(4) E. C. Taylor recently reported the synthesis of some derivatives of certain 9-aminopurines but was unable to convert these to the 9-aminopurines themselves.[5]

(5) E. C. Taylor, Abstr. of Papers, Am. Chem. Soc. Meeting, Boston, Mass., 1959, p. 8N.

(6) Two representatives of this heterocyclic system have recently been prepared by another method.[7]

(7) W. Pfleiderer, *Ann.*, **615**, 42 (1958).

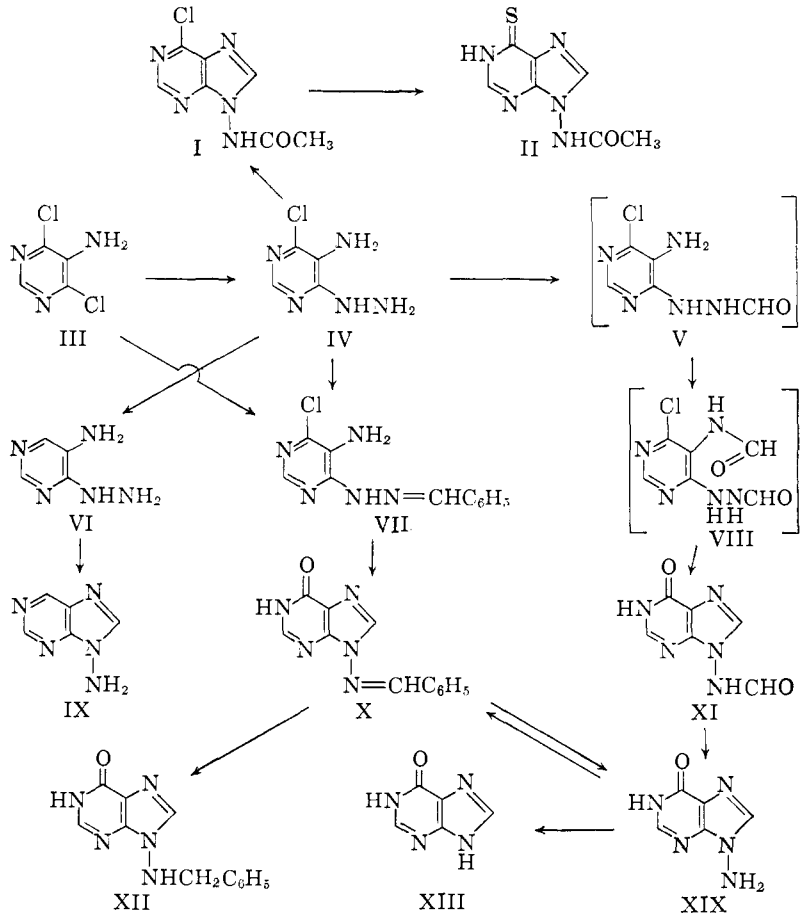
(8) J. A. Montgomery and L. B. Holum, THIS JOURNAL, **79**, 2185 (1957).

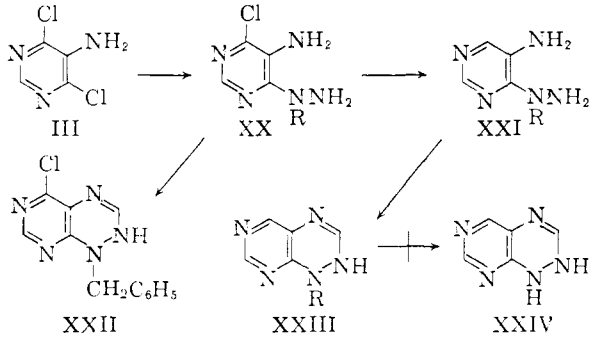
(9) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen and Co., Ltd., London, 1954.

(10) J. A. Montgomery and C. Temple, Jr., THIS JOURNAL, **79**, 5238 (1957).

(11) R. K. Robins, K. J. Dille and B. E. Christensen, *J. Org. Chem.*, **19**, 930 (1954); R. K. Robins and H. H. Lin, THIS JOURNAL, **79**, 490 (1957).

(12) D. J. Brown, *J. Appl. Chem.*, **4**, 72 (1954).





5-amino-4-(1-methylhydrazino)-pyrimidine (XXI, R = CH_3) and 5-amino-4-(1-benzylhydrazino)-pyrimidine (XXI, R = $CH_2C_6H_5$). Reduction with Raney nickel resulted in dechlorination and cleavage of the nitrogen–nitrogen bond of the hydrazino group.[13] The resulting compounds, identified as 5-amino-4-methylaminopyrimidine[12] and 5-amino-4-benzylaminopyrimidine, serve to prove the structure of XX (R = CH_3 and $CH_2C_6H_5$). Reaction of XXI (R = CH_3 and $CH_2C_6H_5$) and XX (R = $CH_2C_6H_5$) with formic acid gave the corresponding 1,2-dihydropyrimido[5,4-*e*]-*as*-triazines (XXIII, R = CH_3 and $CH_2C_6H_5$, and XXII). It is significant that the chlorine atom of XX (R = $CH_2C_6H_5$) was not hydrolyzed during ring closure. A number of unsuccessful attempts were made to debenzylate XXIII (R = $C_6H_5CH_2$) to 1,2-dihydropyrimido-[5,4-*e*]-*as*-triazine (XXIV).

Table I shows the similarity of the ultraviolet spectra of the 9-aminopurines to the corresponding 9-ethylpurines. The spectrum of 9-benzylideneaminohypoxanthine shows a second maximum at 284 mμ which disappears on reduction to 9-benzylaminohypoxanthine.

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Experimental[14]

9-Acetamidopurine-6(1H)-thione (II).—A suspension of 5-amino-4-chloro-6-hydrazinopyrimidine (2.00 g.) in a mixture of ethyl orthoformate (20 ml.) and acetic anhydride (20 ml.) was stirred at room temperature until solution was effected (1 hr.). The solution was refluxed for 1 hr. and then evaporated to dryness *in vacuo*. The crude 9-acetamido-6-chloropurine thus obtained was dissolved in ethyl alcohol (50 ml.) containing thiourea (1.5 g.) and the resulting solution refluxed for 2 hr. The mixture was cooled and the precipitate which had formed was collected by

(13) C. Ainsworth, *J. Appl. Chem.*, **78**, 1636 (1956).

(14) All melting points are uncorrected. The ultraviolet spectra were determined in aqueous solution with a Beckman DK-2 spectrophotometer, but the optical densities at the maxima were determined with a Beckman DU. The infrared spectra were determined in pressed potassium bromide disks with a Perkin–Elmer model 21 spectrophotometer.

filtration, washed with ethanol and dried: yield 1.84 g. This material was dissolved in 2 *N* sodium hydroxide; the resulting solution was filtered and then neutralized with acetic acid. The solid that deposited was washed with water and dried *in vacuo* over phosphorus pentoxide; yield 1.51 g. (60% from IV), m.p. 312° (dec); spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 321 (23.9); *p*H 7, 316 (20.0); *p*H 13, 311 (21.2); $\bar{\nu}$ in cm.$^{-1}$: 3190 and 3150 (CH), 3920–2700 (acidic NH), 1680 (amide C=O), 1605, 1570 and 1550 (C=C, C=N), 1370 (–CH_3), 970 and 960 (ring CH).

Anal. Calcd. for $C_7H_7N_5OS$: C, 40.19; H, 3.37; N, 33.48; S, 15.30. Found: C, 39.73; H, 3.76; N, 33.04; S, 14.97.

5-Amino-4-chloro-6-hydrazinopyrimidine (IV).—To 63 ml. of hydrazine hydrate was added as rapidly as possible, with stirring, 5-amino-4,6-dichloropyrimidine (2.00 g.). The copious precipitate which formed was collected by filtration, washed thoroughly with propyl alcohol, then with ether, and dried *in vacuo* at room temperature over phosphorus pentoxide; yield 0.78 g. (40%), m.p. 184°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 225(5.95), 292(7.65); *p*H 7, 257 (5.65), 289 (5.25); *p*H 13, 240 (6.85); $\bar{\nu}$ in cm.$^{-1}$: 3412 (sharp)(*sec*-NH), 3550–3200 (NH), 1640 (NH), 1575, 1555 (shoulder), and 1540 (shoulder) (C=C, C=N).

Anal. Calcd. for $C_4H_6ClN_5$: C, 30.10; H, 3.79; N, 43.89. Found: C, 29.85; H, 3.69; N, 43.58.

5-Amino-4-hydrazinopyrimidine Hydrochloride (VI).—5-Amino-4-chloro-6-hydrazinopyrimidine (670 mg.) in alcohol (115 ml.) was reduced at atmospheric pressure and room temperature using 5% palladium-on-charcoal catalyst. After removal of the catalyst by filtration, the solution was taken to dryness *in vacuo*. The residue, 583 mg. (86%) of almost pure 5-amino-4-hydrazinopyrimidine hydrochloride, was recrystallized from 70% aqueous methyl alcohol with charcoal treatment, the recrystallization giving a light tan solid, m.p. 234° dec.; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 275 (7.7), 291 (7.9); *p*H 7, 252 (5.5), 285 (5.6); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3320 and 3185 (NH), 2890 (CH), 2900–2400 (H^+ of salt), 1660 (shoulder) and 1635 (NH), 1602, 1549 and 1530 (shoulder) (C=C, C=N), 1325 (CH).

Anal. Calcd. for $C_4H_7N_5 \cdot HCl$: C, 29.73; H, 4.99; N, 43.34. Found: C, 30.09; H, 4.82; N, 43.21.

The free base was prepared by dissolving the hydrochloride in water and treating the solution with portions of Dowex 1 ($CO_3^=$). The recovery was 40%, m.p. 159–161°.

Anal. Calcd. for $C_4H_7N_5$: C, 38.39; H, 5.64. Found: C, 38.20; H, 5.63.

In later runs the free base was prepared directly in an average yield of 50% by reduction in the presence of magnesium oxide using sodium carbonate to destroy the magnesium complex which is formed.

5-Amino-4-benzylidenehydrazino-6-chloropyrimidine (VII). **A.**—A solution of 5-amino-4,6-dichloropyrimidine (850 mg.) in benzalhydrazine (8.5 ml.) was stirred at room temperature for 6 hours and the solid that deposited was collected by filtration and washed with ether (25 ml.). Recrystallization of this material from ethanol gave a light brown solid; yield 240 mg. The compound melts with decomposition at 180°. The ultraviolet and infrared spectra of this solid were practically identical with those of 5-amino-4-chloro-6-hydrazinopyrimidine.

After standing for 3 days, the benzalhydrazine filtrate deposited an additional 320 mg. of light brown material that was identified as 5-amino-4-benzylidenehydrazino-6-chloropyrimidine, m.p. 203–204° (when taken fast from 170°).

A small sample of this material was dissolved in ether and the solution concentrated in a stream of nitrogen. The white solid that deposited was collected by filtration and dried *in vacuo* over P_2O_5; m.p. 210° dec. The ultraviolet absorption of this sample changed rapidly in aqueous solution, but the change was much slower in methanol and acetic acid; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 284, 357; *p*H 7, 255, 340; *p*H 13, 288, 381; methanol, 259 (14.0), 348 (27.4); acetic acid, 259 (15.6), 355 (25.2); $\bar{\nu}$ in cm.$^{-1}$: 3395, 3295 and 3195 (NH); 3060 (aromatic CH); 1600, 1565, 1545 and 1510 (C=C, C=N); 1430 (unassigned); 760 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{11}H_{10}ClN_5$: C, 53.34; H, 4.07; N, 28.28. Found: C, 53.46; H, 4.01; N, 28.27.

B.—A solution of 5-amino-4-chloro-6-hydrazinopyrimidine (100 mg.) in ethanol (15 ml.) containing benzaldehyde (67.0 mg.) was allowed to stand overnight. The solid that deposited was collected by filtration and dried *in vacuo* over P_2O_5; yield 76.0 mg., m.p. 204–206° dec. The ultraviolet and infrared spectra were practically identical with those of 5-amino-4-benzylidenehydrazino-6-chloropyrimidine (see above).

Anal. Calcd. for $C_{11}H_{10}ClN_5$: C, 53.34; H, 4.07. Found: C, 53.06; H, 3.98.

9-Aminopurine (IX).—A solution of 5-amino-4-hydrazinopyrimidine (270 mg.) in formic acid (5 ml.) was refluxed for 30 minutes and then evaporated to dryness *in vacuo*. The excess formic acid was removed from the residue by repeated trituration with alcohol and removal of the volatiles *in vacuo*; the orange residue weighed 390 mg.

On attempted sublimation of the crude product at 150° (0.1 mm.) an unidentified orange-red solid (80 mg.) collected as the sublimate. The golden residue (160 mg.) did not melt but decomposed above 300°. Recrystallization of a small portion from water gave a light yellow solid, which was found to be unstable in either 0.1 *N* acid or 0.1 *N* base; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 262.5; *p*H 7, 264 (5.05); *p*H 13, 232, 266 (broad); $\bar{\nu}$ in cm.$^{-1}$: 3200 and 3130 (NH), 2950, 2875 and 2825 (CH); 1655 (NH); 1605, 1535 (shoulder) and 1515 (C=C, C=N); 1330 and 1270 (C=N); 960 and 860 (purine ring).

Anal. Calcd. for $C_5H_5N_5$: C, 44.44; H, 3.73; N, 51.83. Found: C, 44.13; H, 3.94; N, 52.01.

9-Benzylideneaminohypoxanthine (X). **A.**—Benzaldehyde (1 ml.) was added to a suspension of 9-aminohypoxanthine (1.00 g.) in methanol (50 ml.) containing concentrated hydrochloric acid (5 drops) and, after being stirred at room temperature for 3 hours, the mixture was refluxed for 1 hour. The insoluble solid was collected by filtration, washed with methanol (15 ml.) and dried *in vacuo* over P_2O_5: yield 1.42 g. (90%), m.p. >264°. The ultraviolet spectrum of this material indicated that it was unstable in both 0.1*N* hydrochloric acid and 0.1 *N* sodium hydroxide; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 7, 254 (21.1), 286 (22.8); $\bar{\nu}$ in cm.$^{-1}$: 3030 (aromatic CH); 2800–2400 (acidic H); 1710 (C=O); 1600, 1590, 1550, 1500 and 1490 (C=C, C=N); 1340, 1180 and 980 (strong unassigned bands).

Anal. Calcd. for $C_{12}H_9N_5O$: C, 60.24; H, 3.79; N, 29.28. Found: C, 60.35; H, 3.87; N, 29.34.

B.—A solution of 5-amino-4-benzylidenehydrazino-6-chloropyrimidine (50 mg.) in formic acid (2 ml.) was refluxed for one hour. The solution was evaporated to dryness *in vacuo*. The solid residue was triturated with water and collected by filtration. The solid was washed with more water, alcohol and then ether, and dried *in vacuo* at room temperature over phosphorus pentoxide. The ultraviolet and infrared spectra of this material were practically identical with those of the sample prepared in A above.

Hydrolysis of 9-Benzylideneaminohypoxanthine. **A.**—Steam distillation of a suspension of 9-benzylideneaminohypoxanthine (200 mg.) in 1 *N* hydrochloric acid (24 ml.) and neutralization of the resulting solution with 1 *N* sodium hydroxide deposited 9-aminohypoxanthine: yield 95 mg. (75%). The ultraviolet and infrared spectra of this sample as well as the paper chromatograms developed in four systems were practically identical with those of an authentic sample of 9-aminohypoxanthine.

B.—A suspension of 9-benzylideneaminohypoxanthine (100 mg.) in 0.1 *N* sodium hydroxide (10 ml.) was refluxed for 1 hour and the resulting solution concentrated to one-half of the original volume. Neutralization of the cooled solution with 1 *N* hydrochloric acid deposited 9-aminohypoxanthine; yield 50 mg. (79%). This sample was practically identical with the sample isolated in A.

9-Benzylaminohypoxanthine (XII).—A suspension of 9-benzylideneaminohypoxanthine (300 mg.) in ethanol (25 ml.) containing 30% palladium-on-charcoal catalyst (300 mg.) was hydrogenated at room temperature and atmospheric pressure for 1 hour. The mixture was heated to boiling, the insoluble residue removed by filtration and washed with hot ethanol (25 ml.). Evaporation of the filtrate *in vacuo* gave 35 mg. of impure 9-benzylaminohypoxanthine.

Next, the insoluble residue was washed with 1 *N* hydrochloric acid (10 ml.), then water (10 ml.) and the washings

discarded. The residue was then triturated with 1 *N* sodium hydroxide (10 ml.), the insoluble material removed by filtration, and the filtrate acidified to *p*H 1 with 2 *N* hydrochloric acid. The solid that deposited was combined with the solid isolated from the ethanol and the whole heated in boiling water (50 ml.) containing one drop of concentrated ammonium hydroxide. After removing a small amount of insoluble solid, the filtrate was concentrated in a stream of nitrogen and the 9-benzylaminohypoxanthine that deposited was collected by filtration and dried *in vacuo* over P_2O_5; yield 60 mg. (20%), m.p. >264°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 5, 250 (12.2); *p*H 13, 255 (13.2); $\bar{\nu}$ in cm.$^{-1}$: 3320 (NH); 3070 (aromatic CH); 2920 and 2860 (aliphatic CH); 1680 (C=O); 1580, 1550, 1510 and 1500 (C=C, C=N); 720 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{12}H_{11}N_5O$: C, 59.74; H, 4.60; N, 29.03. Found: C, 60.09; H, 4.48; N, 29.26.

9-Aminohypoxanthine (XIX).—A solution of crude 5-amino-4-chloro-6-hydrazinopyrimidine (7.46 g.) in 98–100% formic acid (150 ml.) was refluxed for 4 hours, evaporated to a small volume *in vacuo*, and the residue triturated with methanol (50 ml.). Removal of the methanol *in vacuo* left a brown residue which was treated with warm 2 *N* hydrochloric acid (100 ml.). A small amount of insoluble material was removed by filtration (80 mg.), the filtrate treated with Norit (gave a reddish-purple solution), and the solution neutralized to *p*H 5 with 10 *N* sodium hydroxide. The brown solid (5.22 g.) that deposited was collected and recrystallized from water (800 ml.). The tan solid was collected by filtration and dried *in vacuo* at room temperature over phosphorus pentoxide; yield 3.30 g. (47%), m.p. >260°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 249 (10.6); *p*H 7, 249 (11.0); *p*H 13, 253 (12.0); $\bar{\nu}$ in cm.$^{-1}$: 3410 and 3250 (NH), 3100, 3040 and 2830 (CH); 1690 (C=O); 1645 (NH); 1595, 1555 and 1480 (C=C, C=N); 920 and 890 (purine ring).

Anal. Calcd. for $C_5H_5N_5O$: C, 39.73; H, 3.33; N, 46.34. Found: C, 40.00; H, 3.62; N, 46.51.

A second crop (0.98 g.) of almost pure material was obtained by concentration of the filtrate from the recrystallization; total yield 4.28 g. (61%).

Reactions of 9-Aminohypoxanthine (XIX). A. Nitrous Acid.—A solution of 9-aminohypoxanthine (500 mg.) in concentrated hydrochloric acid (5 ml.) was refluxed for 1 hour. The ultraviolet spectrum of an aliquot portion from the solution indicated that no reaction had taken place.

The above solution was cooled to room temperature, and a solution of sodium nitrite (300 mg.) in water (5 ml.) was added slowly. During the addition a solid deposited from the solution. After the mixture was stirred at room temperature for 1 hour, the solid was collected and suspended in water (10 ml.). The acidic suspension was neutralized with 5 *N* sodium hydroxide, the insoluble hypoxanthine collected by filtration, washed with water (10 ml.), and dried *in vacuo* over P_2O_5; yield 295 mg. (65.5%). Paper chromatographs of this sample, developed in four systems, gave the same R_{hl} values as an authentic sample of hypoxanthine; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 248 (12.1); *p*H 7, 249 (10.3); *p*H 13, 261 (11.5); $\bar{\nu}$ in cm.$^{-1}$: 3200–2200 (OH and acidic H); 1670 (C=O); 1580 and 1515 (C=C, C=N); 1220, 970 and 895 (strong unassigned bands).

Anal. Calcd. for $C_5H_4N_4O$: C, 44.12; H, 2.96; N, 41.17. Found: C, 44.09; H, 2.94; N, 41.31.

B. Sulfur Fusion.—A melt of 9-aminohypoxanthine (500 mg.) and sulfur (320 mg.) was heated for 6 minutes at 290–300°. The residue was boiled in water (125 ml.), the insoluble material removed by filtration, and the filtrate identified by its chromatograms in four solvents and its ultraviolet spectrum as hypoxanthine.

5-Amino-4-chloro-6-(1-methylhydrazino)-pyrimidine (XX, R = CH_3). A.—To methylhydrazine (100 ml.) was added slowly, with stirring, 5-amino-4,6-dichloropyrimidine (5.00 g.). The warm solution was stirred for 0.5 hour, evaporated to dryness *in vacuo*, and the residue triturated with water, collected by filtration, and dried; yield 3.8 g. Recrystallization of the residue from water gave pure material; yield 2.05 g., m.p. 203–204°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 249.5 (7.0), 303 (7.3); *p*H 7, 273.5 (7.1), 304 (9.07); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3390 and 3285 (NH); 2940, 2900 and 2835 (aliphatic CH); 1620 (NH); 1555 and 1525 (C=C, C=N); 1450 (N—CH_3); 1400 (unassigned).

Anal. Calcd. for $C_5H_8ClN_5$: C, 34.55; H, 4.61; N, 40.30. Found: C, 34.55; H, 4.73; N, 40.55.

Concentration of the filtrate from the recrystallization gave a solid that was identified as **5-amino-4,6-bis-(1-methylhydrazino)-pyrimidine**; yield 780 mg., m.p. 117–179°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 234 (10.9), 285 (9.35); *p*H 7, 298.5 (9.5); *p*H 13, 295 (9.8); $\bar{\nu}$ in cm.$^{-1}$: 3420, 3280 and 3170 (NH); 2960 and 2900 (aliphatic CH); 1655 and 1620 (NH); 1600 and 1560 (C=C, C=N); 1410 (unassigned).

Anal. Calcd. for $C_6H_{13}N_7$: C, 39.33; H, 7.15; N, 53.52. Found: C, 38.95; H, 6.62; N, 54.05.

B.—To a pre-heated (50°) suspension of 5-amino-4,6-dichloropyrimidine (500 mg.) in water (20 ml.) was added, with stirring, methylhydrazine (0.5 ml.). After being heated at this temperature for 1 hour, the mixture was cooled, the insoluble material collected by filtration, washed with water (5 ml.) and dried *in vacuo* over P_2O_5; yield 415 mg., m.p. 201–203° (with sublimation).

The filtrate was evaporated to dryness, the residue triturated with water (5 ml.), and the insoluble solid collected by filtration and dried *in vacuo* over P_2O_5; yield 60 mg., m.p. 201–202° (with sublimation). The total yield was 475 mg. (90%).

Evaporation of the washings from the first solid gave a trace of 5-amino-4,6-bis-(1-methylhydrazino)-pyrimidine, m.p. 180°.

5-Amino-4-(1-benzylhydrazino)-6-chloropyrimidine (XX, R = $CH_2C_6H_5$).—Benzylhydrazine (2.98 g.) was added to a solution of 5-amino-4,6-chloropyrimidine (2.00 g.) in benzene (20 ml.) and the mixture refluxed for 2 hours. The benzylhydrazine hydrochloride (1.22 g.) that deposited from the solution was removed by filtration, and the filtrate was decanted from a small amount of oil. The benzene solution was evaporated to dryness and the residue washed with Skellysolve C (3 × 20 ml.) and dried *in vacuo* over P_2O_5; yield 2.93 g., m.p. 100° with softening from 90°. Recrystallization of this solid from Skellysolve C, then cyclohexane, gave a white solid; yield 1.53 g. (50%), m.p. 110° with softening from 105°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 250 (7.65), 308 (7.0); *p*H 7, 271 (7.0), 306 (9.1); *p*H 13, 271 (6.7), 306 (8.2); $\bar{\nu}$ in cm.$^{-1}$: 3290, 3260 and 3170 (NH); (=CH); 2920 and 2900 (aliphatic CH); 1640 (NH); 1590, 1545, 1520 and 1500 (C=C, C=N); 1460 (aliphatic CH); 750 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{11}H_{12}ClN_5$: C, 52.91; H, 4.81; N, 28.05. Found: C, 53.27; H, 4.90; N, 28.33.

Hydrogenolysis of 5-Amino-4-chloro-6-(1-methylhydrazino)-pyrimidine. A. Raney Nickel.—A solution of 5-amino-4-chloro-6-(1-methylhydrazino)-pyrimidine (100 mg.) in 10 ml. of buffer solution (*p*H 7) containing 400 mg. of wet Raney nickel (washed with water and buffer solution) was refluxed for 1 hour. The catalyst was removed by filtration, and the filtrate evaporated to dryness *in vacuo*. Recrystallization of the residue from water gave white needles of **5-amino-4-chloro-6-methylaminopyrimidine**, m.p. 167° (lit.[12] m.p. 166–167°); spectral data: λ_{max} mμ ($\epsilon \times 10^{-3}$): *p*H 1, 275 (8.15), 301.5 (10.65); *p*H 7, 263 (8.22), 288.5 (8.4); *p*H 13, 264 (8.22), 288 (8.36).

B. Palladium.—A solution of 5-amino-4-chloro-6-(1-methylhydrazino)-pyrimidine (2.05 g.) in a 1:2 mixture of water–ethanol (200 ml.) was hydrogenated over a 5% palladium–charcoal catalyst (1 g.) in the presence of magnesium oxide (2 g.). After the mixture had absorbed 257 ml. of hydrogen (calcd. 290 ml.), the insoluble material was removed by filtration and washed with hot methanol (100 ml.). To the combined filtrate and washings was added a 10% sodium carbonate solution (100 ml.) and the solution evaporated to dryness *in vacuo*. This residue was extracted with benzene (3 × 75 ml.), the extracts combined and concentrated under reduced pressure. The solid that deposited was collected by filtration and dried *in vacuo* over P_2O_5; yield 900 mg. (55%), m.p. 136°.

Sublimation of a small sample (400 mg.) at 125° (0.2 mm.) gave 390 mg. of a white solid, m.p. 136°. This material was identified as **5-amino-4-(1-methylhydrazino)-pyrimidine (XXI, R = CH_3)**; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 288 (7.85); *p*H 7, 265 (6.4), 300 (7.2); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3380, 3270 and 3140 (NH);

3000 and 2960 (aliphatic CH); 1620 (NH); 1600, 1560 and 1545 (C=C, C=N); 1400 (unassigned).

Anal. Calcd. for $C_5H_9N_5$: C, 43.15; H, 6.52; N, 50.33. Found: C, 42.97; H, 7.01; N, 50.08.

Hydrogenolysis of 5-Amino-4-(1-benzylhydrazino)-6-chloropyrimidine. A. Raney Nickel.—Raney nickel (2.0 g. wet, washed twice with *p*H 7 buffer solution) was added to a mixture of 5-amino-4-(1-benzylhydrazino)-6-chloropyrimidine (200 mg.) in *p*H 7 buffer solution (25 ml.) and the resultant mixture refluxed for 2 hours. The mixture was filtered hot, the filtrate evaporated to dryness *in vacuo*, and the residue extracted with chloroform (2 × 10 ml.). Evaporation of the combined extracts and sublimation of the residue at 124° (0.1 mm.) gave a white solid; yield 75 mg., m.p. 130–132°. Recrystallization of this material from benzene gave a white solid, m.p. 136–137°. This material was identified as **5-amino-4-benzylaminopyrimidine** by elemental analysis and by comparison of its infrared and ultraviolet spectra with those of 5-amino-4-ethylaminopyrimidine[10]; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 290 (12.7); *p*H 7, 255 (9.1); 290 (8.3); *p*H 13, 255 (9.6), 290 (7.7); $\bar{\nu}$ in cm.$^{-1}$: 3380 and 3200 (NH); 3060 (aromatic CH); 2920 and 2880 (aliphatic CH); 1680 (NH); 1600, 1590, 1570 and 1510 (C=C, C=N); 1460 (aliphatic CH); 750 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{11}H_{12}N_4$: C, 65.98; H, 6.04; N, 27.98. Found: C, 66.09; H, 6.04; N, 28.20.

B. Palladium.—A solution of 5-amino-4-(1-benzylhydrazino)-6-chloropyrimidine (1.00 g.) in 1:1 ethanol–water (100 ml.) containing magnesium oxide (1.00 g.) was hydrogenated over a 5% palladium-on-charcoal catalyst. After the steady uptake of one equivalent of hydrogen, there was a further, but much slower, absorption of hydrogen. The catalyst was removed by filtration and the residue was washed with ethanol (25 ml.). A 5% solution of sodium carbonate (50 ml.) was added to the combined wash and filtrate, and the whole evaporated to dryness. Extraction of the residue with chloroform (2 × 50 ml.) and evaporation of the combined extracts gave 550 mg. of material, m.p. 101° with softening from 95°. Sublimation of this sample at 105° (0.1 mm.) gave pure **5-amino-4-(1-benzylhydrazino)-pyrimidine** (**XXI**, R = $CH_2C_6H_5$); yield 510 mg. (59%), m.p. 100–101°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 293 (shoulder) (8.3), 314 (9.3); *p*H 7–265 (7.6), 307 (8.4); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3410 and 3300 (NH); 3040 (aromatic CH); 1650 (NH); 1600, 1575, 1550 and 1490 (C=C, C=N); 1460 (aliphatic CH); 730 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{11}H_{13}N_5$: C, 61.37; H, 6.09; N, 32.54. Found: C, 60.93; H, 6.08; N, 32.29.

1-Benzyl-5-chloro-1,2-dihydropyrimido[5,4-*e*]-*as*-triazine (**XXII**).—A solution of 5-amino-4-(1-benzylhydrazino)-6-chloropyrimidine (320 mg.) in formic acid (10 ml.) was refluxed for 4 hours, evaporated to a small volume *in vacuo*, and the residue heated to boiling in 2 *N* hydrochloric acid (40 ml.). The acidic solution was treated with Norit and the filtrate neutralized to *p*H 6 with concentrated ammonium hydroxide. The solid that deposited was collected by filtration, dissolved in acetone (10 ml.), a small amount of insoluble material removed by filtration, and the filtrate evaporated to dryness *in vacuo*. The solid was dried *in vacuo* over P_2O_5 at 56° for 3 hours; yield 150 mg. (48.5%), m.p. 182–183° dec.; spectral data: λ_{max} in mμ: *p*H 1, 334 (3.98); *p*H 7, 226 (11.2); 244 (9.33), 346 (2.78); *p*H 13, 248 (8.46), 280 (4.97); $\bar{\nu}$ in cm.$^{-1}$: 3265 (NH); 2940 (aliphatic CH); 1655 (C=N); 1600, 1575, 1550 and 1480 (C=C, C=N); 740 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{12}H_{10}ClN_5$: C, 55.50; H, 3.85; N, 27.00; Cl, 13.67. Found: C, 55.06; H, 4.02; N, 27.01; Cl, 13.87.

1,2-Dihydro-1-methylpyrimido[5,4-*e*]-*as*-triazine (**XXIII**, R = CH_3).—A solution of 5-amino-4-(1-methylhydrazino)-pyrimidine (480 mg.) in formic acid (50 ml.) was refluxed for 0.5 hour and evaporated to dryness under reduced pressure. The reddish-brown residue was then heated on a water-bath under high vacuum until the color of the residue had changed to yellow; yield 580 mg. This material decomposes rapidly without melting above 175° and leaves a residue on combustion.

A portion (200 mg.) of this material was extracted with ether (3 × 100 ml.); the extracts were combined and evaporated to dryness in a stream of nitrogen to give a slightly brown solid; yield 100 mg., m.p. 202° dec. (when taken rapidly from 175°); spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 239 (shoulder, 5.1), 331 (4.55); *p*H 7, 239 (shoulder), 335 (unstable); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3430 and 3220 (NH); 3040 (=CH); 2910 and 2840 (aliphatic CH); 1660, 1600 and 1500 (C=C, C=N).

Anal. Calcd. for $C_6H_7N_5$: C, 48.31; H, 4.73; N, 46.96. Found: C, 47.84; H, 4.90; N, 47.13.

1-Benzyl-1,2-dihydropyrimido[5,4-*e*]-*as*-triazine (**XXIII**, R = $CH_2C_6H_5$).—A solution of 5-amino-4-(1-benzylhydrazino)-pyrimidine (460 mg.) in 98–100% formic acid (25 ml.) was refluxed for 30 minutes, evaporated to dryness, and the residue dissolved in 0.5 *N* hydrochloric acid (15 ml.). The solution was neutralized with 1 *N* sodium hydroxide, and the oil that deposited extracted with ether (3 × 50 ml.). Evaporation of the ether and trituration of the residue with a small amount of methanol gave a light yellow solid; yield 220 mg. (46%).

A small sample of this material was recrystallized from benzene by the addition of Skellysolve C; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 335 (4.3); *p*H 7, 341 (3.9); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3200 (NH); 2950 (aliphatic CH); 1660 (C=N); 1600, 1590, 1570 and 1490 (C=C, C=N); 775 and 700 (monosubstituted phenyl).

Anal. Calcd. for $C_{12}H_{11}N_5$: C, 63.98; H, 4.92; N, 31.09. Found: C, 63.91; H, 4.91; N, 30.96.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

N,N′-Carbonyldiimidazole, a New Peptide Forming Reagent[1]

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N,N′-Carbonyldiimidazole was shown to be a useful peptide forming reagent. Conditions were worked out for avoiding racemization in the formation of ethyl carbobenzoxyglycyl-L-phenylalanylglycinate, a sensitive case.

It has been demonstrated by Wieland and Schneider[2] that peptide derivatives can be synthesized through acylation of the imidazole ring of methyl N-benzoyl-L-histidinate followed by reaction with the appropriate amine. Their method, however, was not suitable for general use because of low yields. It occurred to us that a more direct agent for making acyl-imidazoles might be N,N′-carbonyldiimidazole. This would be a convenient reagent since the by-products, carbon dioxide and imidazole, are innocuous. The carbon dioxide evolution would provide a driving force for the reaction.

(1) Preliminary communication G. W. Anderson and R. Paul, THIS JOURNAL, **80**, 4423 (1958).

(2) T. Wieland and G. Schneider, *Ann.*, **580**, 159 (1953); see also M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **175**, 145 (1928).