Deamination Studies on Pyrimidine and Condensed Pyrimidine Systems[1,2]

RICHARD B. TRATTNER, GERTRUDE B. ELION, GEORGE H. HITCHINGS, AND DAVID M. SHAREFKIN

Wellcome Research Laboratories, Burroughs Wellcome and Company (U. S. A.), Inc., Tuckahoe, New York, and Department of Chemistry, Brooklyn College, University of the City of New York, Brooklyn, New York

Received March 30, 1964

Five representative 2,4-diaminopyrimidines and condensed pyrimidine systems were subjected to several deamination procedures. The amino group in the 2-position of the pyrimidine ring can be removed by treatment with nitrous acid, but is resistant to acid or alkaline hydrolysis. The 4-amino group is unreactive toward nitrous acid, but is removed by acid or alkaline hydrolysis. The fused ring attached to the pyrimidine ring has a pronounced influence on the reactivity of each amino group. The results are consistent with the hypothesis that the reactive intermediates of the 2,4-diaminopyrimidine ring have a *para*-quinoid structure rather than an *ortho*-quinoid form.

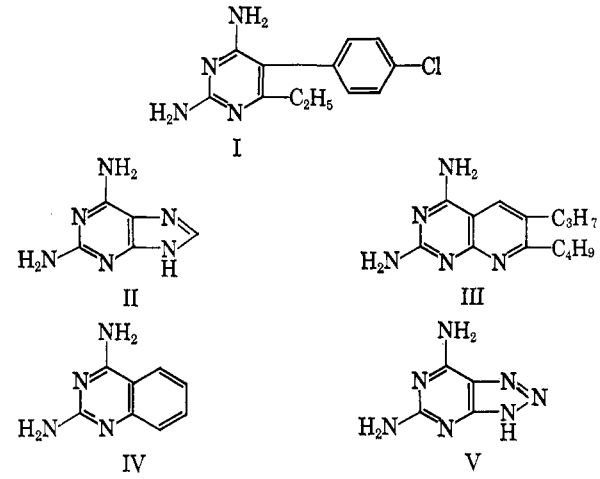
Although the literature contains a number of references to the deamination of individual pyrimidines, purines, and pteridines, only a few systematic studies have been done in which the reactivity of the amino groups in a related series has been studied under identical conditions. Only two of these studies have been concerned specifically with compounds containing the 2,4-diaminopyrimidine moiety.[3,4] Taylor and coworkers studied the acid hydrolysis of some aminopyrimidines and found that the presence of amino groups at positions 5 and 6 stabilized the 4-amino group as well as the pyrimidine ring itself, *e.g.*, in 2,4,5,6-tetraaminopyrimidine.[3] Nitrous acid has no effect on 2,4-diaminopteridines[4,5] or 4-amino-2-hydroxypteridines[4,6] (although 2-amino-4-hydroxypteridines are deaminated[4]) but the 4-amino group is readily removed by acid hydrolysis.[4] The deamination of 2,6-diaminopurine to isoguanine by nitrous acid has been reported,[7] but no studies have been done on the stability of 2,6-diaminopurine to acid or alkaline hydrolysis.

The biochemical significance of a number of pyrimidine and condensed pyrimidine systems and the biological activities of many of their 2,4-diamino derivatives has led to considerable interest in compounds of this type. The purpose of this investigation was to determine the effect of substitution in the 5- and 6-positions upon the reactivity of 2,4-diaminopyrimidines toward deamination procedures. The relative lability of the amino groups in the 2- and 4-position toward hydrolysis might be expected to give some information with regard to the nature of the predominant tautomeric form in the diaminopyrimidines. In addition, since many compounds of the diaminopyrimidine type are employed as chemotherapeutic agents,[8] such a study might be useful in the identification of possible metabolites as well as in affording a comparison between chemical and biochemical deamination.

Results and Discussion

For the present study, five representative diamino compounds were chosen, one pyrimidine and four condensed pyrimidine systems (Chart I). Each was subjected to acid hydrolysis, alkaline hydrolysis, and nitrous acid treatment under the conditions described in the Experimental section.

CHART I



The data, summarized in Table I, show that the groups or fused rings attached to the 5- and 6-positions of the pyrimidine ring greatly influence the reactivity of each amino group in deamination reactions.

TABLE I

DEAMINATION OF 2- AND 4-AMINO GROUPS BY VARIOUS REAGENTS[a]

	2-NH_2			4-NH_2		
	HNO_2	NaOH	HCl	HNO_2	NaOH	HCl
I	+	−	−	−	−	+
II	+	−	+[b]	−	−	+
III	+	−	+[b]	−	+	+
IV	+	−	−	−	+	+
V	+	−	+[b]	−	+	+

[a] +, reaction; −, no reaction. [b] After hydrolysis of the 4-amino group.

Treatment of the 2,4-diaminopyrimidines with boiling 2 *N* sodium hydroxide has no effect on the 2-amino group but removes the 4-amino group in some cases. The order of reactivity is pyridopyrimidine = quinazoline > triazolopyrimidine[9] >> pyrimidine,[10] purine.[10] For the pyridopyrimidine (III) and the quinazoline (IV), removal of the 4-amino group occurred in about 1

(1) Presented before the Organic Chemistry Section at the New York–New Jersey Regional Meeting of the American Chemical Society, New York, N. Y., Jan., 1962.

(2) This paper has been abstracted from the M.S. Thesis of R. B. Trattner presented to Brooklyn College in June, 1961, in partial fulfillment of the requirements for the M.S. degree.

(3) E. C. Taylor, Jr., and C. K. Cain, *J. Am. Chem. Soc.*, **71**, 2282 (1949).

(4) E. C. Taylor, Jr., and C. K. Cain, *ibid.*, **71**, 2538 (1949).

(5) R. G. W. Spickett and G. M. Timmis, *J. Chem. Soc.*, 2887 (1954).

(6) H. Wieland and R. Liebig, *Ann.*, **555**, 146 (1944).

(7) J. Davoll, *J. Am. Chem. Soc.*, **73**, 3174 (1951).

(8) G. H. Hitchings, *Trans. N. Y. Acad. Sci.*, **23**, 700 (1961).

(9) The order of reactivity of the triazolopyrimidine is based upon the yield of the dihydroxyl derivative obtained. This does not take into account the formation of side products resulting from the instability of the triazolopyrimidine in hot acid.

(10) No reaction.

hr. and the triazolopyrimidine (V) was converted to 8-azaguanine in about 6 hr., whereas I and II remained essentially unchanged even after 24 hr.

The progressive order of the ease of diazotization of the 2-amino group (5-amino in the triazolopyrimidines) of the five compounds studied (based on the yields of the 2-hydroxy-4-amino derivatives obtained) is quinazoline ≅ pyrimidine > pyridopyrimidine[11] ≅ purine > triazolopyrimidine. In no case was the 4-amino group removed by nitrous acid. There is a report[12] of the diazotization of cytosine in concentrated sulfuric acid; however, with the procedure employed in the present study, cytosine failed to deaminate.

For the 4-amino group, the ease of hydrolysis with 6 *N* hydrochloric acid followed the order: pyridopyrimidine (<0.5 hr.) > triazolopyrimidine = quinazoline (*ca.* 0.5 hr.) > pyrimidine (*ca.* 3 hr.) > purine (*ca.* 6 hr.). The hydrolysis of the 2-amino group (after hydrolysis of the 4-amino group) is represented by the order: purine > pyridopyrimidine > triazolopyrimidine >> quinazoline,[10] pyrimidine.[10] Both the pyridopyrimidine (III) and the triazolopyrimidine (V) gave good yields of the dihydroxyl derivatives after 24 hr. of heating, but the intermediate 2-amino-4-hydroxyl derivatives were identified spectrophotometrically after 0.5 hr. In the experiment with 2,6-diaminopurine, the hydrolysis of the 2-amino group of guanine was sufficiently rapid so that at the end of 6 hr., when only traces of diaminopurine were present according to chromatographic evidence, the reaction mixture contained both guanine and xanthine, but no isoguanine. The hydrolysis of the quinazoline (IV) and the pyrimidine (I) proceeded only as far as the 2-amino-4-hydroxyl derivatives in 24 hr.

Although the amino groups on positions 2 and 4 of the pyrimidine ring are potentially capable of existing in the amino or imino form, the amino form is overwhelmingly predominant according to physical measurements.[13] It is possible to rationalize the attack of a reagent at a given point if it is assumed that, other things being equal, a *para*-quinoid structure is preferred to an *ortho*-quinoid.

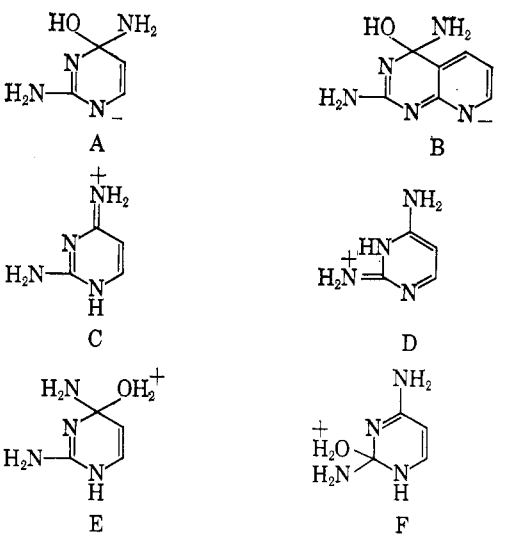
During alkaline hydrolysis an intermediate with a *para*-quinoid structure (A) accommodating a negative charge on ring nitrogen can be formed if OH^- attacks at C-4 but not if it attacks at C-2. (Structure A in Chart II is only one of the several canonical forms contributing to resonance of the intermediate. However, the various forms do not contribute equally to the over-all structure and no attempt will be made in this discussion to consider all of the other forms.) The experimental data show that alkaline hydrolysis does occur at position 4 but not at position 2. Substituents or fused rings which can share the negative charge of A, as in B, would be expected to accelerate hydrolysis. This is consonant with the higher reactivity of the pyridopyrimidine and quinazoline.

The reactive species of the 2,4-diaminopyrimidines for both reaction with nitrous acid and acid hydrolysis is the protonated one. Protonation at N-1 would be preferred over protonation at N-3 because *para*-quinoid forms in which the positive charge can be accommodated on one of the amino groups are possible for the N-1 protonated species, but not for the N-3 protonated one, *e.g.*, C *vs.* D.[14] The *para*-quinoid form C would be consistent with the relative susceptibility of the two amino groups to attack by nitrous acid, since in this form the 2-amino group would have greater nucleophilic reactivity. *ortho*-Quinoid forms, such as D, would be expected to react in a manner contrary to the observed results.

Acid hydrolysis may be considered to occur *via* addition of water to the protonated form of the pyrimidine or by simultaneous addition of a proton and water. In either case, attack of water at C-4 (intermediate form E) would be favored over attack at C-2 (F), since E is *para*-quinoid while F is *ortho*-quinoid. This agrees with the experimental finding that the 4-amino group is hydrolyzed faster than the 2-amino group in all cases. In the case of the bicyclic systems, *e.g.*, quinazoline, protonated forms resembling C would be favored over those resembling D. Accommodation of the positive charge of the latter on the 2-amino group would be impeded because aromatic resonance in the fused ring (*e.g.*, benzene) would be disrupted thereby. Delocalization of the positive charge on C would disfavor reaction by lowering the free energy of the protonated base. The facility of delocalization would be expected to be in the order: purine > triazolopyrimidine > quinazoline > pyrimidine > pyridopyrimidine. Except for the pyrimidine, which appears to be less reactive than would be predicted, the data are in good agreement with the proposals.

The hypothesis that the *para*-quinoid form of the reactive intermediates of the 2,4-diaminopyrimidine

CHART II

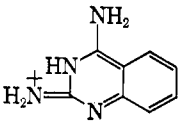


(11) Sulfuric acid (7 *N*) was used as the solvent. Acid hydrolysis of the 4-amino group preceded deamination by nitrous acid.

(12) A. Kossel and F. Steudel, *Z. Physiol. Chem.*, **38**, 49 (1903).

(13) Advances in Heterocyclic Chemistry," Vol. 1, A. R. Karitzky, Ed., Academic Press, New York, N. Y., 1963, p. 312.

(14) In the cases of pyrimidines with fused-on rings (*e.g.*, quinazoline), a further factor favors protonation at N-1 over N-3. If N-1 is protonated, the positive charge is easily delocalized into either the 2-NH_2 or the 4-NH_2 group. However, if N-3 is protonated, delocalization of the positive charge into the 2-NH_2 group is impeded because it involves disruption of resonance



in the fused-on ring. The situation is analogous to that concerning interaction of naphthalene 2-substituents with the 3-position.

ring is favored over the *ortho*-quinoid form is in agreement with both the experimental results and the theoretical considerations advanced by Waters.[15]

Experimental

General Methods.—For deamination by nitrous acid the conditions used were essentially those of Kossel.[16] The products were examined after the addition of both one and two molecular equivalents of sodium nitrite. For acid hydrolysis, 6 *N* hydrochloric acid was used, and 2 *N* sodium hydroxide was used for alkaline hydrolysis. The reactions were carried out on a 10-mmole scale and the mixtures were heated for the times indicated below; aliquots were removed periodically for examination by ultraviolet spectrophotometry and paper chromatography. All paper chromatograms were run employing an ascending method. All ultraviolet spectra were determined using either a Beckman DK-2 or DU spectrophotometer. Except when noted, 0.1 *N* hydrochloric acid was used for readings at pH 1 and a Sørenson glycine–sodium hydroxide buffer for pH 11. Melting points were taken on a copper block and are corrected.

Deamination of 2,4-Diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (I). A. Nitrous Acid.—A solution of 2.25 g. (0.0091 mole) of 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (I)[17] in 100 ml. of 1 *N* sulfuric acid was brought to a boil with stirring, and the ultraviolet absorption spectrum was determined: at pH 1, λ_{max} 272 mμ (ε 7750); at pH 11, λ_{max} 286 mμ (ε 9240). At this time the spectrum was that of the starting material; no reaction with the solvent had occurred. An aqueous solution (20 ml.) of 0.782 g. (0.0099 mole) of sodium nitrite was dropped slowly into the reaction mixture over a period of 10 min. with heating and stirring. After the final addition of sodium nitrite, the solution was boiled for 5 min., and the ultraviolet spectrum was determined again. The spectrum was that of 4-amino-5-*p*-chlorophenyl-6-ethyl-2-hydroxypyrimidine (VI, *vide infra*). The solution was then divided equally into two parts, A and B.

To portion A was added 0.392 g. of sodium nitrite as in the above procedure. The spectrum of this solution was identical with that of VI. The solution was then permitted to cool to room temperature and made alkaline by the addition of 2 *N* sodium hydroxide. The mixture was chilled thoroughly and filtered by suction. The white precipitate obtained was dried at 50° *in vacuo* (0.94 g., 80%): at pH 1, λ_{max} 284 mμ (ε 12,000); at pH 11, λ_{max} 275 mμ (ε 7950).

Anal. Calcd. for $C_{12}H_{12}ClN_3O \cdot 0.5H_2O$; C, 55.66; H, 5.07; N, 16.24; H_2O, 3.5. Found: C, 55.14; H, 5.05; N, 15.90; H_2O, 3.6.

Portion B was made alkaline directly. The mixture was chilled in an ice bath and filtered by suction. The spectrum of the material isolated was identical with that of VI.

The identity of VI as the 4-amino-2-hydroxyl derivative was established by its unequivocal synthesis from 4-amino-5-*p*-chlorophenyl-6-ethyl-2-mercaptopyrimidine *via* the 2-carboxymethylthio derivative (*vide infra*).

B. Alkaline Hydrolysis.—A suspension of 1.125 g. of I in 100 ml. of 2 *N* sodium hydroxide was heated under reflux conditions for 24 hr. The spectrum remained unchanged after 24 hr. and 1.02 g. of the starting material was recovered. The filtrate contained a maximum of 20 mg. of the ultraviolet-absorbing material.

C. Acid Hydrolysis.—A solution of 1.125 g. of I in 100 ml. of 6 *N* hydrochloric acid was heated under reflux conditions for 24 hr. After about 3 hr. the reaction appeared complete, but heating was continued for another 18 hr. The solution was chilled and the pH value was adjusted to 7 with concentrated sodium hydroxide solution. The white precipitate was filtered, washed with water, and dried at 50° *in vacuo* (1.08 g., 95%). A 100-mg. sample was recrystallized for analysis from absolute ethanol (55 mg., 53%). The ultraviolet absorption spectrum showed at pH 1, λ_{max} 278 mμ (ε 9550); at pH 11, λ_{max} 223 (ε 15,650) and 276 mμ (ε 9550).

Anal. Calcd. for $C_{12}H_{12}ClN_3O \cdot 0.75H_2O$: C, 55.0; H, 5.17; N, 15.97; H_2O, 5.1. Found: C, 55.40; H, 5.10; N, 16.20; H_2O; 5.3.

The analysis indicated that one amino group had been removed by the action of the acid. Since the ultraviolet absorption spectrum of the product differed considerably from that of the 4-amino-2-hydroxyl compound (VI), the product was identified as 2-amino-5-*p*-chlorophenyl-6-ethyl-4-hydroxypyrimidine (VII).

4-Amino-2-carboxymethylthio-5-*p*-chlorophenyl-6-ethylpyrimidine.—To a solution of 4.5 g. (0.05 mole) of chloroacetic acid in 50 ml. of water was added 45 ml. of 2 *N* sodium hydroxide (0.09 mole) and 10.6 g. (0.04 mole) of 4-amino-5-*p*-chlorophenyl-6-ethyl-2-mercaptopyrimidine. The solution was boiled for 5 min. and the pH value was adjusted to 7 by the addition of 4 g. of chloroacetic acid. There was no drift in pH over a 35-min. period. The solution was acidified with hydrochloric acid, and the precipitate was collected and washed with water. After recrystallization from ethanol the colorless prisms (6.5 g., 50.5%) melted at 203–205°.

Anal. Calcd. for $C_{14}H_{14}ClN_3O_2S$: C, 51.9; H, 4.33. Found: C, 52.4; H, 4.50.

4-Amino-5-*p*-chlorophenyl-6-ethyl-2-hydroxypyrimidine (VI).—A mixture of 4.2 g. of 4-amino-2-carboxymethylthio-5-*p*-chlorophenyl-6-ethylpyrimidine and 16 ml. of concentrated hydrochloric acid was heated for 2 hr. on the steam bath. (Solution occurred within 15 min. with the evolution of thioglycolic acid.) The mixture was diluted to 600 ml. with water and heated to dissolve the precipitate which had formed. The hot solution was filtered. Upon cooling, a crystalline precipitate formed. It was collected, washed with water, and dried at 50° *in vacuo* (2.2 g., 66%). This compound was identical in its ultraviolet absorption spectrum with the compound prepared by treatment of I with nitrous acid.

Anal. Calcd. for $C_{12}H_{12}ClN_3O \cdot 0.5H_2O$: C, 55.66; H, 5.07; N, 16.24. Found: C, 56.0; H, 5.30; N, 16.7.

Deamination of 2,6-Diaminopurine (II). A. Nitrous Acid.—The procedure was the same for the diazotization of I except that 1 *N* hydrochloric acid was employed as the solvent. The solvent did not react with the starting material before the addition of sodium nitrite, as evidenced by the ultraviolet absorption spectrum.[18] The reaction with one equivalent of nitrous acid gave isoguanine in 56% yield after recrystallization from 1 *N* sulfuric acid. The addition of another equivalent of nitrous acid did not deaminate isoguanine further.

B. Alkaline Hydrolysis.—No reaction occurred after 24 hr. of heating under reflux conditions; the starting material was recovered.

C. Acid Hydrolysis.—At the end of 6 hr., the paper chromatogram (solvent: 5% disodium phosphate–isoamyl alcohol) showed spots corresponding to guanine (R_f 0), xanthine (R_f 0.41), and a small amount of starting material (R_f 0.20), but no isoguanine (R_f 0.36). At the end of 24 hr. the product isolated was xanthine (83% yield) judged by the spectrum[18] and the nitrogen analysis.

Anal. Calcd. for $C_5H_4N_4O_2$: N, 36.8. Found: N, 36.9.

Deamination of 2,4-Diamino-7-*n*-butyl-6-*n*-propylpyrido[2,3-*d*]pyrimidine (III). A. Nitrous Acid.—Because of the extreme insolubility of III[19] in 1 *N* sulfuric acid, 7 *N* sulfuric acid was used as the solvent. The spectrum of the solution taken just before the addition of nitrite indicated that the solvent was attacking the 4-position. Later studies (part B) confirmed this hydrolysis.

Treating this solution with one equivalent of nitrous acid gave a product identical in ultraviolet absorption spectrum with that of the product of 6 *N* hydrochloric acid hydrolysis of III (part D), identified as the 2,4-dihydroxyl derivative (yield 55%).

B. 7 *N* Sulfuric Acid Hydrolysis.[20]—III (2 g.) was heated under reflux conditions with 200 ml. of 7 *N* sulfuric acid for 1 hr. The solution was chilled and the pH value adjusted to 7 with concentrated sodium hydroxide solution. The white precipitate which formed was filtered by suction, triturated with water, and refiltered. This product was dried at 50° *in vacuo* (1.8 g., 90%): at pH 1, λ_{max} 278 (ε 13,300) and 352 mμ (ε 11,100); at pH 11, λ_{max} 268 (ε 8840), and 333 mμ (ε 8450).

Anal. Calcd. for $C_{14}H_{20}N_4O$: C, 64.4; H, 7.74; N, 21.5. Found: C, 64.3; H, 7.63; N, 21.6.

The compound obtained from this reaction was assigned the structure of 2-amino-7-*n*-butyl-4-hydroxy-6-*n*-propylpyrido[2,3-*d*]pyrimidine on the basis of the analytical data and the close resemblance of its ultraviolet absorption spectrum to that of authentic 2-amino-7-ethyl-4-hydroxy-6-methylpyrido[2,3-*d*]pyrimidine.[19]

(15) W. A. Waters, *J. Chem. Soc.*, 727 (1948).

(16) A. Kossel, *Z. Physiol. Chem.*, **10**, 248 (1886).

(17) P. B. Russell and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 3763 (1951).

(18) L. F. Cavalieri, A. Bendich, J. F. Tinker, and G. B. Brown, *ibid.*, **70**, 3875 (1948).

(19) R. K. Robins and G. H. Hitchings, *ibid.*, **80**, 3449 (1958).

(20) Because of the solvent reaction with III in part A, it was necessary to determine the reaction products at the end of 1 hr. of reflux (the over-all reaction time of the diazotization of III).

C. Alkaline Hydrolysis.—Because of the extreme insolubility of III in alkali, this reaction was carried out only on a microscale and followed spectrophotometrically. After about 1 hr. of heating, the solution exhibited the same spectrum as the product formed in B. The spectrum did not change upon continued heating for 24 hr.

D. Acid Hydrolysis.—After 0.5 hr. of boiling with 6 *N* hydrochloric acid the ultraviolet absorption spectrum was identical with that of the 2-amino-4-hydroxyl compound obtained in B. At the end of 24 hr. of heating under reflux conditions, the solution was permitted to cool to room temperature and the pH value was adjusted to 5 with concentrated ammonium hydroxide. After chilling, the white precipitate was collected, washed with water, and dried at 50° *in vacuo* (yield 80%). The product was 7-*n*-butyl-2,4-dihydroxy-6-*n*-propylpyrido[2,3-*d*]pyrimidine. The ultraviolet absorption spectra showed λ_{max} (EtOH) 248 (ϵ 9000) and 314 mμ (ϵ 8600); at pH 1, λ_{max} 246 (ϵ 7650) and 320 mμ (ϵ 11,000); at pH 11, λ_{max} 265 (ϵ 8350) and 318 mμ (ϵ 8870).

Anal. Calcd. for $C_{14}H_{19}N_3O_2$: C, 64.3; H, 7.33; N, 16.1. Found: C, 64.2; H, 7.25; N, 15.8.

Deamination of 2,4-Diaminoquinazoline (IV).[21] **A. Nitrous Acid.**—No reaction of IV with 1 *N* hydrochloric acid could be detected spectrophotometrically, prior to the addition of one molecular equivalent of sodium nitrite. After deamination an impure product was obtained, which could not be purified by recrystallization from 95% ethanol. A quantitative paper chromatogram was run in a 5% ammonium sulfate–isopropanol (19:1) solvent. One major fluorescent spot was observed: R_f 0.46 (light blue). Elution of this spot with 1.5 ml. of 0.1 *N* hydrochloric acid yielded the following spectral data: at pH 1, λ_{max} 328 mμ (optical density = 0.315); at pH 13, λ_{max} 322 mμ (optical density = 0.307). This spectrum is identical with the spectrum of 4-amino-2-hydroxyquinazoline, the synthesis of which is described below. From the optical density values, it was estimated that a yield of 86% of 4-amino-2-hydroxyquinazoline (VIII) was obtained. Treatment of VIII with another molecular equivalent of nitrous acid failed to deaminate it further.

B. Acid Hydrolysis.—After only 0.5 hr. of heating, the 4-amino group appeared to have been removed and the spectrum was similar to that reported for 2-amino-4-hydroxyquinazoline (IX): at pH 1 λ_{max}, 304 mμ; at pH 11, λ_{max} 262 and 314 mμ.[17] Further heating did not affect this compound. After 24 hr., IX was isolated in 88% yield, m.p. 312° dec.[22]: at pH 1, λ_{max} 305 mμ (ϵ 3620); at pH 11, λ_{max} 265 (ϵ 7730), 270 (ϵ 7410), and 314 mμ (ϵ 2900).

Anal. Calcd. for $C_8H_7N_3O$: N, 26.1. Found: N, 25.8.

C. Alkaline Hydrolysis.—Hydrolysis of the 4-amino group occurred in *ca.* 1 hr. The product (50% yield) was identical in melting point with that obtained in part B and the mixture melting point was undepressed.

Anal. Calcd. for $C_8H_7N_3O$: N, 26.1. Found: N, 25.9.

2-Hydroxy-4-quinazolinethione.—A mixture of 2 g. of 2,4-dihydroxyquinazoline, 7 g. of phosphorous pentasulfide, and 100 ml. of pyridine was heated under reflux conditions for 2 hr. The solvent was removed under reduced pressure. To the residue was added 100 ml. of water and the mixture was heated to 120° (oil-bath temperature) for 0.5 hr. The mixture was then diluted with 1300 ml. of water and heated to boiling. A small amount of sulfur was removed by filtration and the filtrate was permitted to cool to room temperature. The product was collected, dried on the filter, and then recrystallized from water to give 1.5 g. of feathery yellow crystals which melted at 270° (dried *in vacuo* at 100°): at pH 1, λ_{max} 240 (ϵ 17,300), 305 (ϵ 6070), 320 (ϵ 6230), and 358 mμ (ϵ 13,000); at pH 11, λ_{max} 242 (ϵ 20,800), 304 (ϵ 6500), 315 (ϵ 6550), and 350 mμ (ϵ 9520). The compound was identified as the 2-hydroxy-4-mercapto isomer since its absorption spectrum is completely different from that reported for the 4-hydroxy-2-mercapto isomer.[23]

Anal. Calcd. for $C_8H_6N_2OS$: C, 53.9; H, 3.37; N, 15.73. Found: C, 54.04; H, 2.96; N, 15.40.

4-Amino-2-hydroxyquinazoline (VIII).—A solution of 500 mg. of 2-hydroxy-4-quinazolinethione in 10 ml. of concentrated ammonium hydroxide was heated under reflux conditions in an oil bath for 1 hr. A precipitate began to form after *ca.* 20 min. The precipitate was collected, washed with concentrated ammonium hydroxide, ethanol, and ether, and dried *in vacuo* at 120° (350 mg.): at pH 1, λ_{max} 328 mμ (ϵ 4430); at pH 11, λ_{max} 320 mμ ϵ 4380).

Anal. Calcd. for $C_8H_7N_3O$: C, 59.63; H, 4.35. Found: C, 59.55; H, 4.21.

Deamination of 5,7-diamino-1-*v*-triazolo[*d*]pyrimidine (V).[18] **A. Nitrous Acid.**—There was no reaction of V with the solvent. Treatment of V with one molecular equivalent of nitrous acid gave 8-azaisoguanine (X).[18] After neutralization of the reaction solution with concentrated sodium hydroxide, the precipitate was collected and recrystallized from 6 *N* hydrochloric acid (43% yield): at pH 1, λ_{max} 276 mμ (ϵ 8300); at pH 11, λ_{max} 248 (ϵ 9240) and 277 mμ (ϵ 10,300). Treatment of X with a second molecular equivalent of nitrous acid failed to deaminate it further.

Anal. Calcd. for $C_4H_4N_6O\cdot HCl$: C, 25.47; H, 2.67. Found: C, 25.42; H, 2.72.

B. Alkaline Hydrolysis.—After approximately 6 hr. of heating, V was almost completely converted to 8-azaguanine (XI). At the end of the 24-hr. reflux period, the solution was cooled, neutralized with glacial acetic acid, and filtered by suction. The precipitate, after it was collected, washed with water, and dried at 50° *in vacuo*, contained inorganic material (probably silicates). Its ultraviolet absorption spectrum was qualitatively identical with that of 8-azaguanine. As calculated from the spectrum, a yield of 45% of 8-azaguanine was obtained.

C. Acid Hydrolysis.—After *ca.* 0.5 hr. of heating, V was deaminated to 8-azaguanine (XI) as was evidenced by the ultraviolet absorption spectrum of the reaction mixture. After 24 hr., the solvent was removed by distillation on the steam bath under reduced pressure. To the remaining residue was added a small amount of water and the solution was again evaporated to dryness. This was repeated twice more to remove the last traces of hydrochloric acid. A quantitative paper chromatogram of this residue was run with butanol–acetic acid–water (4:1:5) as the solvent. Three spots were detected by examination of the paper chromatogram under ultraviolet light: R_f 0.03, 0.35, and 0.43. The ultraviolet absorption spectrum of the material at R_f 0.43 as well as the R_f value were identical with the corresponding values for authentic 8-azaxanthine.[24] The other two spots exhibited ultraviolet spectra which were not similar to any of the possible deamination products. These could conceivably be decomposition products. It was estimated that 8-azaxanthine was present in the crude material to the extent of *ca.* 55%.

Acknowledgment.—Grateful acknowledgment is made to Dr. S. W. Blackman and V. Purdey for the microanalyses. We are indebted to Dr. Joseph Bunnett and Dr. Stuart Hurlbert for helpful discussions relating to this work.

(21) E. Vopicka and N. A. Lange, *J. Am. Chem. Soc.*, **57**, 1068 (1935).

(22) F. Kunckell, *Ber.*, **38**, 1212 (1905).

(23) P. Grammaticakis, *Compt. rend.*, **247**, 2013 (1958).