unequivocal demonstration of the occurrence of free radicals in the oxidation of aniline by means of lead peroxide.[8]

The intention of making a more thorough investigation of the reaction of primary aromatic amines with LTA has been repeatedly postponed due to the over-riding interest in other investigations. However, it seemed advisable to record briefly our results since they offer the promise of a simple procedure for the synthesis of symmetrically substituted azo compounds.[9]

Experimental Part

4,4'-Dibromoazobenzene.—To a solution of 5.0 g. (29.0 mmoles) of *p*-bromoaniline in 1.2 l. of anhydrous benzene was added with stirring 25.8 g. (58.2 mmoles) of finely powdered lead tetraacetate in the course of 1 hr. At the end of this period the lead diacetate was filtered off and the filtrate was washed thoroughly with 300 ml. of water. After separating the benzene and aqueous layers, the benzene solution was concentrated to a volume of 15 ml. The concentrate on cooling in ice yielded 3.1 g. of a solid material that on sublimation[10] *in vacuo* (0.001 mm.) within the temperature range of 200–250° (air-bath) gave 1.8 g. (36% of the theory) of 4,4'-dibromoazobenzene. After recrystallization from chloroform, its m.p. was 206.5–207.5°; reported m.p. for 4,4'-dibromoazobenzene 205°.[11] Its absorption spectrum in toluene from 400–680 mμ was found to be identical with that of an authentic sample of 4,4'-dibromoazobenzene.

Anal. Calcd. for $C_{12}H_8N_2Br_2$ (340.0): C, 42.38; H, 2.37; Br, 47.0. Found: C, 42.58; H, 2.46; Br, 47.2.

2,2',4,4',6,6'-Hexabromoazobenzene.—To a solution of 5.0 g. (15.1 mmoles) of pure 2,4,6-tribromoaniline[12] in 150 ml. of anhydrous benzene was added with stirring 7.0 g. (15.8 mmoles) of lead tetraacetate. After 90 minutes the benzene solution was washed with water, and brought to dryness under reduced pressure. The residue was taken up in cold ethyl acetate and filtered. The solid material on recrystallization from 200 ml. of ethyl acetate yielded 1.55 g. (31% of the theory) of 2,2',4,4',6,6'-hexabromoazobenzene, m.p. 217–218°, reported[13,14] m.p. 213°.

Anal. Calcd. for $C_{12}H_4N_2Br_6$ (655.7): C, 21.95; H, 0.61. Found: C, 21.95; H, 0.62.

2,2',4,4'-Tetrachloroazobenzene.—To a solution of 4.86 g. (30 mmoles) of 2,4-dichloroaniline in 250 ml. of anhydrous benzene were added 3 g. of magnesium oxide, 10 g. of anhydrous sodium sulfate and 13.3 g. (30 mmoles) of finely powdered lead tetraacetate, and the mixture was shaken for two hours. After removal of the solid material, the solution was washed with water and brought to dryness under reduced pressure. The residue, weighing 1.65 g., on recrystallization from chloroform or 96% ethanol yielded 1.3 g. (27% of theory) of 2,2',4,4'-tetrachloroazobenzene, m.p. 164–166°, reported[15,16] m.p. 161–162°.

Anal. Calcd. for $C_{12}H_6N_2Cl_4$ (320.0): C, 45.04; H, 1.89. Found: C, 45.01; H, 2.09.

(8) St. Goldschmidt and B. Wurzschmitt, *Ber. deut. chem. Ges.*, **55**, 3216 (1922).

(9) After this paper was accepted the authors became aware of a publication by K. H. Pausacker and J. G. Scroggie (*J. Chem. Soc.*, 4003 (1954)) describing the oxidation of primary aromatic amines by lead tetraacetate. These authors used substituted amines which differed from ours. The results of both investigations are in agreement.

(10) To obtain all 4,4'-dibromoazobenzene it was found necessary to interrupt the sublimation occasionally and to pulverize the residue.

(11) A. Werigo, *Ann.*, **165**, 189 (1873).

(12) H. Silberstein, *J. prakt. Chem.*, **27**, 98 (1883).

(13) H. v. Pechmann and A. Nold, *Ber.*, **31**, 557 (1898).

(14) F. D. Chattaway and K. J. P. Orton, *J. Chem. Soc.*, **79**, 467 (1901).

(15) Th. Zinke and A. Kuchenbecker, *Ann.*, **330**, 9, 53 (1904).

(16) Th. Zinke, *Ber. deut. chem. Ges.*, **34**, 2853 (1901).

TORONTO 5, CANADA

[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Studies on Condensed Pyrimidine Systems. XVI. Purines and Thiazolo[5,4-d]pyrimidines from 4-Amino-5-formamido-6-mercaptopyrimidines

BY GERTRUDE B. ELION, WILLIAM H. LANGE AND GEORGE H. HITCHINGS

RECEIVED NOVEMBER 8, 1955

When 4,5-diamino-6-mercaptopyrimidines are treated with aqueous formic acid at room temperature the 5-formamido derivatives can be isolated. The sodium salts of these, on heating, yield 6-mercaptopurines. With stronger formic acid and higher temperatures thiazolo[5,4-d]pyrimidines are produced. This method has been employed for the synthesis of thiazolo[5,4-d]pyrimidine analogs of adenine, hypoxanthine, 2,6-diaminopurine and 6-mercaptopurine.

The present studies originated in an investigation of the synthesis of 6-mercaptopurine[1,2] (VII) from 4,5-diamino-6-mercaptopyrimidine (I) *via* 4-amino-5-formamido-6-mercaptopyrimidine (V). In early work this synthesis was complicated by erratic yields and the presence in the product of an alkali-insoluble fraction. The identification of the latter as 7-aminothiazolo[5,4-d]pyrimidine (VIII) revealed the source of these difficulties. Further investigation has allowed the definition of the conditions conducive to the isolation of the formamido derivative V and the thiazolopyrimidine (VIII), respectively, both in these and in the related reactions leading to thioguanine (XIX) and 5,7-diaminothiazolo[5,4-d]pyrimidine (XVIII).

Several thiazolo[5,4-d]pyrimidines have been synthesized by treatment of mercaptoaminopyrimidines with formic acid.[3–5] However, in none of these instances was an alternative ring closure possible.

The facile cyclization of 4-amino-5-formamido-6-mercaptopyrimidine to 7-aminothiazolo[5,4-d]pyrimidine provided a route to an adenine analog which had been sought unsuccessfully in earlier studies. Previously, as part of a broad program dealing with condensed pyrimidine systems as antagonists of nucleic acid derivatives[6–9] some thi-

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

(2) G. B. Elion and G. H. Hitchings, *ibid.*, **76**, 4027 (1954).

(3) S. J. Childress and R. L. McKee, *ibid.*, **73**, 3862 (1951).

(4) F. L. Rose, *J. Chem. Soc.*, 3448 (1952).

(5) G. P. Hager and C. Kaiser, *J. Amer. Pharm. Assn.*, **44**, 193 (1955).

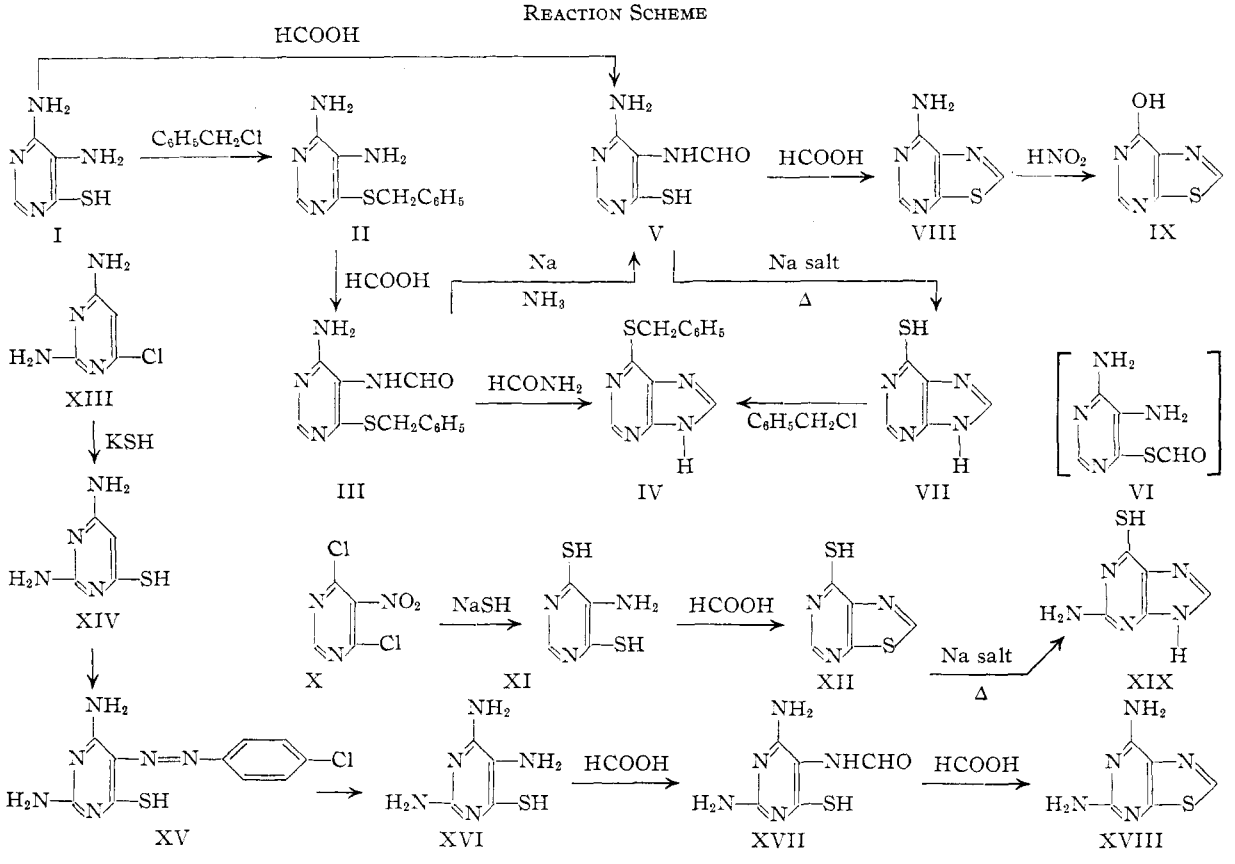
(6) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood and H. VanderWerff, *J. Biol. Chem.*, **183**, 1 (1950).

(7) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell and H. VanderWerff, *Ann. N. Y. Acad. Sci.*, **52**, 1318 (1950).

(8) G. H. Hitchings and G. B. Elion, *ibid.*, **60**, 195 (1954).

(9) G. H. Hitchings and G. B. Elion, 3^eme Congres International de Biochimie, *Rapports*, 185 (1955).

REACTION SCHEME



azolo[5,4-d]pyrimidines were prepared.[10] These were synthesized by the cyclization of 5-acetamido- and benzamido-6-mercaptopyrimidines and, therefore, were substituted in the 2-position. Since microbiological studies had indicated a greater potency for analogs unsubstituted in this position[9,11] the synthesis of a number of such substances with a variety of functional groups in the pyrimidine moiety was undertaken.

Since the treatment of 4,5-diamino-6-mercaptopyrimidine (I) with formic acid can give rise to two products, V and VIII, a study was made of the conditions leading to ring closure. The formamidopyrimidine V can be differentiated easily from the thiazolopyrimidine VIII by its ultraviolet absorption spectrum; the former has a very high extinction value at 300 mμ at *p*H 1 (Table II) while the latter (VIII) has its maximum absorption at 265 mμ and negligible absorption at 300 mμ at *p*H 1. It is therefore possible to calculate from the ratio of the extinction values at 265 and 300 mμ how much VIII has been formed; for V, $E\ 265/E\ 300 = 0.24$ while for VIII, $E\ 265/E\ 300 = 7.0$. No ring closure to 6-mercaptopurine (VII) occurred in any of the reaction mixtures reported in Table I as evidenced by the fact that there was no absorption at 325 mμ, the wave length for maximum absorption of VII at *p*H 1.[1] It is apparent from Table I (expts. 1, 2, 3, 4, 8) that very little VIII was formed at room temperature unless the formic acid was very concentrated,

(10) E. A. Falco and G. H. Hitchings, THIS JOURNAL, **72**, 3203 (1950).

(11) G. B. Elion, G. H. Hitchings and H. VanderWerff, *J. Biol. Chem.*, **192**, 505 (1951).

TABLE I

FORMATION OF 7-AMINOTHIAZOLO(5,4-d)PYRIMIDINE FROM 4,5-DIAMINO-6-MERCAPTOPYRIMIDINE

Expt.	4,5-Diamino-6-mercaptopyrimidine, g.	HCOOH ml.	HCOOH %	Temp., °C.	Time, hr.	$E\ 265/E\ 300$ at *p*H 1	Thiazolopyrimidine %
1	0.1	0.9	55	25	20	0.20	0
2	0.1	0.7	71	25	20	.23	0
3	0.1	0.6	83	25	20	.34	15
4	1.0	1.5	98–100	25	170	.42	25
5	0.25	10.0	50	100	1	3.6	50
6	0.25	10.0	70	100	1	5.0	70
7	0.25	10.0	90	100	1	5.8	82
8	2.00	50.0	50	25	1	0.24	0
9	0.5	2.2	90	55–80	1	0.61	5
10	0.5	1.8	90	85–90	4	3.3	46
				25	20		
11	1.0	2.0	90	85–90	4	0.63	6
				25	70		
12	0.5	2.0	98–100	60–70	2	4.7	66
				25	24		
13	10.0	100.0	98–100	100	1	7.0	100

e.g., 98% (expt. 4). In a boiling water-bath, on the other hand, ring closure was appreciable even with 50% formic acid and was very marked with 90% formic acid (expts. 5, 6, 7). In the synthesis of 8-C^{14}-6-mercaptopurine previously reported,[2] the formylation of I was carried out with 90% formic acid. At this concentration of formic acid, both the temperature and the amount of acid can be critical (expts. 9, 10, 11) so that it is possible to get

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA

Pyrimidines	pH 1 λmax, mμ	E_m	λmin, mμ	E_m	pH 11 λmax, mμ	E_m	λmin, mμ	E_m
4-NH_2-5-NHCHO-6-SH	240	18,400	270	3,700	245	17,700	267	7,700
	306	21,600			292	14,300		
4-NH_2-5-NHCHO-6-$SCH_2C_6H_5$	248	15,100	270	6,300	282	8,600	265	7,100
	300	13,300						
4,5-$(NH_2)_2$-6-$SCH_2C_6H_5$	280[a]	5,930	255	4,800	305	9,200	275	4,300
	322	10,400						
4,6-$(SH)_2$-5-NH_2	265[a]	9,850	240	5,900	245	17,000	298	3,500
	276	10,500	312	2,900	342	18,100		
	375	15,600	388	14,300				
	405	16,700						
2,4-$(NH_2)_2$-6-SH	242	8,800	232	8,000	235[a]	15,300	270	3,700
	322	23,800	270	1,600	297	16,600		
2,4,5-$(NH_2)_3$-6-SH	310	21,800	270	1,300	240	13,600	280	2,650
					320	12,100		
2,4-$(NH_2)_2$-5-(N=NC$_6$H$_4$Cl-*p*)-6-SH	260	13,000	250	11,600	240	15,000	265	10,500
	277	14,600	266	12,900	298	17,900	340	6,800
	362	7,300	330	6,200	400	15,500		
	440	17,100	375	6,800				
2,4-$(NH_2)_2$-5-NHCHO-6-SH	315	21,100	273	2,400	240	13,400	274	4,000
					300	14,200		
Thiazolo(5,4-d)pyrimidines								
7-NH_2	265	9,850	242	4,200	261	10,200	238	3,700
					285[a]	4,300		
7-OH	252	5,770	235	3,820	265	7,200	240	4,600
	258	5,720	255	5,550	285	5,000	270	4,000
	276	4,500	267	4,000				
7-SH	235	10,600	265	2,200	240	11,800	265	3,000
	323	18,100			316	18,300		
5,7-$(NH_2)_2$	266	9,800	248	7,500	280	10,900	248	3,600
	310[a]	2,600						
5,7-$(OH)_2$	257	9,400			285	11,200	238	1,800

[a] Inflection.

as much as 46% ring closure (expt. 10) or as little as 6% (expt. 11) under almost the same conditions. Moreover, even a small increase in the concentration of the formic acid (expt. 12) can further increase the amount of VIII formed, even at lower temperatures, *e.g.* expt. 9. It is therefore better in this step to lower the temperature and to decrease the concentration of the formic acid to 50–70%. The two extremes in conditions suitable for the preparation of V and VIII are summarized in expts. 8 and 13, respectively, and are given in detail in the Experimental section.

The initial formylation presumably occurs on the 5-amino group to give V, but the isomeric thioester VI could not be excluded *a priori*. However barring a very unlikely migration of the formyl group, the cyclization of the intermediate to 6-mercaptopurine would appear to eliminate this possibility. Furthermore, V could be synthesized by formylation of 4,5-diamino-6-benzylmercaptopyrimidine (II) followed by debenzylation of the product III to yield 4-amino-5-formamido-6-mercaptopyrimidine (V) by an unequivocal route. The structure of III was confirmed by conversion to 6-benzylmercaptopurine (IV) which was also obtainable by direct benzylation of 6-mercaptopurine.

The adenine analog VIII was converted to the hypoxanthine analog IX by deamination with nitrous acid. Reaction of the pyrimidine X with sodium hydrosulfide led to XI which, on treatment with formic acid, yielded the analog of 6-mercaptopurine (XII). This compound is of particular interest because of the demonstrated activity of 6-mercaptopurine in microbiological, embryonic, tumor and leukemic systems.[11–15]

Since 2,6-diaminopurine behaves as an adenine antagonist,[16] the preparation of its analog XVIII was undertaken. For this purpose it was necessary to synthesize 6-mercapto-2,4,5-triaminopyrimidine (XVI). This pyrimidine was also desired for the preparation of a number of 2-amino-6-mercaptopurines, since 2-amino-6-mercaptopurine (thioguanine)[17] itself had proved to be an active purine antagonist.[11,12,18,19] The synthesis was accomplished by converting XIII to XIV with sodium

(12) G. B. Elion, S. Singer and G. H. Hitchings, *J. Biol. Chem.*, **204**, 35 (1953).

(13) S. Bieber, R. F. Nigrelli and G. H. Hitchings, *Proc. Soc. Exptl. Biol. Med.*, **79**, 430 (1952).

(14) D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock, G. B. Elion and G. H. Hitchings, *Cancer Research*, **13**, 593 (1953).

(15) J. H. Burchenal, L. Murphy, R. R. Ellison, D. A. Karnofsky, M. P. Sykes, T. C. Tan, L. S. Leone, L. F. Craver, H. D. Dargeon and C. P. Rhoads, *Blood*, **8**, 965 (1953).

(16) G. B. Elion and G. H. Hitchings, *J. Biol. Chem.*, **187**, 511 (1950).

(17) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **77**, 1676 (1955).

(18) G. B. Elion, S. Singer, G. H. Hitchings, M. E. Balis and G. B. Brown, *J. Biol. Chem.*, **202**, 647 (1953).

(19) L. W. Law, *Proc. Soc. Exptl. Biol. Med.*, **84**, 109 (1953).

hydrosulfide, coupling to give XV and reducing with zinc and hydrochloric acid. The resultant pyrimidine XVI is readily formylated to XVII or converted to XVIII. The conditions for the formation of XVII and XVIII are similar to those for the analogous V and VIII except that the rate of formylation appears to be somewhat slower.

The ultraviolet absorption spectra of the intermediate pyrimidines and of the thiazolo(5,4-d)pyrimidines are given in Table II. It will be observed that the strong absorption band due to the 6-mercapto group in the 300 mμ region is not appreciably affected by formylation of the 5-amino group but is decreased in intensity and shifted to the shorter wave lengths by benzylation of the mercapto group. Ring closure of the 5-formamido-6-mercaptopyrimidines to the thiazolo(5,4-d)pyrimidines obliterates this absorption band almost completely. It is interesting that the spectra of 7-monosubstituted thiazolopyrimidines resemble closely those of the corresponding purines. In the case of the 5,7-diamino derivative, the resemblance to the purine is apparent in alkaline solution but not at *p*H 1.[20,21]

Experimental

6-Benzylmercapto-4,5-diaminopyrimidine (II).—To a solution of 10 g. (0.07 mole) of 4,5-diamino-6-mercaptopyrimidine[2] in 85 ml. of 2 *N* sodium hydroxide was added slowly, with stirring, 11.4 ml. (0.1 mole) of benzyl chloride. The stirring was continued for three hours after all the benzyl chloride had been added. The mixture was adjusted to *p*H 7 with acetic acid and the precipitate collected, washed with very dilute sodium hydroxide solution (*ca.* *p*H 9) followed by ether to remove some excess benzyl chloride. The product, recrystallized from one liter of water and dried in a vacuum desiccator (10.5 g., 60%), was a hydrate melting at 104–106°.

Anal. Calcd. for $C_{11}H_{14}N_4S \cdot H_2O$: C, 52.8; H, 5.6; N, 22.4. Found: C, 52.8; H, 5.6; N, 22.7.

4-Amino-6-benzylmercapto-5-formamidopyrimidine (III).—A mixture of 1 g. of 6-benzylmercapto-4,5-diaminopyrimidine hydrate and 20 ml. of 98–100% formic acid was heated on the steam-bath for 2 hours and then taken to dryness under reduced pressure. The residue, after being leached with 20 ml. of absolute ethanol and washed with ether, melted at 202–203° dec. (0.7 g., 62%).

Anal. Calcd. for $C_{12}H_{12}N_4OS$: C, 55.4; H, 4.6; N, 21.5. Found: C, 55.4; H, 4.8; N, 21.2.

4-Amino-5-formamido-6-mercaptopyrimidine (V). A. From 4-Amino-6-benzylmercapto-5-formamidopyrimidine.—To a mixture of 5.55 g. (0.0214 mole) of 4-amino-6-benzylmercapto-5-formamidopyrimidine in 250 ml. of liquid ammonia was added, with mechanical stirring, small pieces of sodium (1.2 g., 0.052 mole), each piece being allowed to react before the next was added. The pyrimidine went into solution slowly during the course of the reaction; at the end, a precipitate formed. The ammonia was allowed to evaporate and the residue was dissolved in 50 ml. of water and filtered to remove a small amount of insoluble oil. The filtrate was adjusted to *p*H 5 by the addition of glacial acetic acid and the precipitate of colorless, fluffy needles filtered off. After recrystallization from 350 ml. of boiling water, the product (2.2 g., 61%) melted at 255° dec.

Anal. Calcd. for $C_5H_6N_4OS$: C, 35.3; H, 3.5; N, 32.9. Found: C, 35.6; H, 3.5; N, 33.2.

B. From 4,5-Diamino-6-mercaptopyrimidine.—A solution of 2 g. of 4,5-diamino-6-mercaptopyrimidine[2] in 50 ml. of 50% aqueous formic acid was prepared by warming the mixture gently to 30°. The solution deposited a copious crystalline precipitate on standing at room temperature. After one hour, the precipitate (1 g.) was collected, washed with ice-water and dried in a vacuum desiccator. Another 0.9 g. of product was obtained by adjusting the filtrate to *p*H 3 by the addition of concentrated ammonium hydroxide and chilling. The product (1.9 g., 80%) was identical in m.p. and ultraviolet absorption spectrum with the 4-amino-5-formamido-6-mercaptopyrimidine prepared by procedure A above.

6-Mercaptopurine from 4-Amino-5-formamido-6-mercaptopyrimidine. A. *Via* the Sodium Salt.—(This ring closure was used in the synthesis of 8-C^{14}-6-mercaptopurine[2] but the formamidopyrimidine (V) was not isolated.) A solution of 100 mg. (0.59 millimole) of V in 0.295 ml. of 2 *N* sodium hydroxide was evaporated to dryness under reduced pressure and the residue was heated gradually to 240° and kept at this temperature for 45 minutes. The melt was cooled, dissolved in 15 ml. of water, filtered and the solution acidified to *p*H 5 with acetic acid. The yellow crystalline precipitate (79 mg., 79%) was identical with 6-mercaptopurine hydrate in all respects.

B. Ring Closure with Formamide.—A solution of 110 mg. of V in 5 ml. of formamide was heated at 200° for 15 minutes, cooled and diluted with an equal volume of water. The pale yellow crystalline precipitate (44 mg., 40%) was identical with 6-mercaptopurine hydrate in all respects. The ultraviolet absorption spectrum of the formamide filtrate indicated the presence of adenine, indicating that under the conditions of this experiment the mercapto group is partially replaced by an amino group.

6-Benzylmercaptopurine. (IV). A. From 6-Mercaptopurine.—To a solution of 17 g. (0.1 mole) of 6-mercaptopurine hydrate in 120 ml. of 2 *N* sodium hydroxide was added slowly, with stirring, 12.6 ml. (0.11 mole) of benzyl chloride. Stirring was continued for two hours after the addition was complete. The mixture was adjusted to *p*H 5 with glacial acetic acid and the precipitate of 6-benzylmercaptopurine hydrate (24.8 g., 95.5%) collected, washed with water and dried at room temperature. After recrystallization from 1000 parts of hot water, the colorless needles melted at 188–189° dec.; ultraviolet absorption spectrum: at *p*H 1, λ_{max} 295 mμ (E_m 16,600), λ_{min} 255 mμ (E_m 3,900); at *p*H 11, λ_{max} 292 mμ (E_m 16,000), λ_{min} 255 mμ (E_m 3,500).

Anal. Calcd. for $C_{12}H_{10}N_4S \cdot H_2O$: C, 55.4; H, 4.6; N, 21.5. Found: C, 55.3; H, 4.7; N, 21.2.

B. From 4-Amino-6-benzylmercapto-5-formamidopyrimidine.—A mixture of 350 mg. of III and 5 ml. of formamide was heated at 200° for 10 minutes, cooled, diluted with 20 ml. of water and chilled. The product which precipitated as the hydrate in the form of colorless needles was collected, washed with water and dried at room temperature (320 mg., 91%), m.p. 187–188° dec. The ultraviolet absorption spectrum was identical with that of the benzylmercaptopurine prepared by method A.

7-Aminothiazole(5,4-d)pyrimidine (VIII).—A mixture of 10 g. of 4,5-diamino-6-mercaptopyrimidine[2] and 100 ml. of 98–100% formic acid was heated under reflux conditions for one hour and then evaporated to dryness under reduced pressure. The residue was taken up in 250 ml. of water, ammonium hydroxide added until *p*H 7 was reached, and the mixture chilled. The colorless needles were collected, washed with water and dried in a vacuum desiccator (8.5 g., 80%), m.p. 211–212°. A small sample was recrystallized as the hydrochloride from 20 parts of *N* hydrochloric acid.

Anal. Calcd. for $C_5H_4N_4S \cdot HCl$: C, 31.8; H, 2.6; N, 29.7. Found: C, 32.0; H, 2.7; N, 30.0.

7-Hydroxythiazolo(5,4-d)pyrimidine (IX).—To a solution of 1.65 g. (0.0108 mole) of 7-aminothiazolo(5,4-d)pyrimidine in 40 ml. of 0.5 *N* sulfuric acid at 80–85° was added dropwise, over a 15-minute period, a solution of 1.65 g. (0.024 mole) of sodium nitrite in 10 ml. of water. The mixture was kept at 80° for another 15 minutes, by which time the evolution of gas had ceased and a precipitate had begun to form. After standing overnight at room temperature, the mixture was chilled and filtered. The precipitate was recrystallized from 150 ml. of boiling water with the addition of Darco. It was washed with water and acetone and dried at 100° (0.9 g., 54%).

Anal. Calcd. for $C_5H_3N_3OS$: C, 39.2; H, 2.0; N, 27.4. Found: C, 39.5; H, 2.2; N, 27.1.

(20) L. F. Cavalieri, A. Bendich, J. F. Tinker and G. B. Brown, THIS JOURNAL, **70**, 3875 (1948).

(21) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

5-Amino-4,6-dimercaptopyrimidine (XI).—To 1 liter of 2 *N* sodium hydrosulfide solution was added 18 g. of 4,6-dichloro-5-nitropyrimidine.[22] The mixture was allowed to stand at room temperature for one-half hour with stirring. At the end of this time essentially all the solid was in solution. The solution was heated on the steam-bath for three hours with intermittent introduction of hydrogen sulfide and then allowed to cool slowly. The *p*H value was adjusted to 6.5 with concentrated hydrochloric acid, the precipitate of sulfur was filtered off and the filtrate concentrated to 300 ml. under reduced pressure. The solution was acidified to *p*H 3 with hydrochloric acid and chilled, whereupon the product deposited as a yellow precipitate, which was filtered, washed with cold water and dried in a vacuum desiccator (11.85 g., 80%). The product was 90% pure according to ultraviolet absorption spectrum and was used for the following step without further purification. A small sample was purified by solution in dilute sodium hydroxide and precipitation at *p*H 4 with hydrochloric acid. The compound does not melt below 330°.

Anal. Calcd. for $C_4H_5N_3S_2$: C, 30.2; H, 3.1; N, 26.4. Found: C, 29.7; H, 2.9; N, 25.9.

7-Mercaptothiazolo(5,4-d)pyrimidine (XII).—A mixture of 3.7 g. of 5-amino-4,6-dimercaptopyrimidine and 300 ml. of 98–100% formic acid was heated on the steam-bath for 2 hours and then taken to dryness under reduced pressure. The residue was washed with ethanol and dried at room temperature (3.6 g., 94%). The ultraviolet absorption spectrum indicated this product was about 90% pure. For analysis a small sample was recrystallized from 2000 parts of hot water from which it precipitated as orange crystals which did not melt below 330°.

Anal. Calcd. for $C_5H_3N_3S_2$: C, 35.5; H, 1.8; N, 24.8; S, 37.9. Found: C, 35.9; H, 2.0; N, 24.8; S, 37.9.

5,7-Dihydroxythiazolo(5,4-d)pyrimidine.—This compound was prepared from thiouramil[23] essentially by the method of Childress and McKee[3] except that 98% formic acid was used instead of 85%.

Anal. Calcd. for $C_5H_3N_3O_2S$: C, 35.5; H, 1.8; N, 24.9. Found: C, 35.0; H, 2.0; N, 25.1.

2,4-Diamino-6-mercaptopyrimidine (XIV).—A mixture of 10 g. of 6-chloro-2,4-diaminopyrimidine[24] and 200 ml. of 1 *N* potassium hydrosulfide was heated in a sealed bomb at 150° for 20 hours. After cooling, the reaction mixture was filtered to remove a small amount of precipitate, and acidified to *p*H 5 with glacial acetic acid and chilled. The resultant precipitate (2.85 g.) was collected, washed with water and dried at 100°. The filtrate, after concentration to 50 ml. under reduced pressure, deposited an additional 2.85 g. of product. The ultraviolet absorption spectrum of the filtrate indicated the presence of a further 3 g. of product. This pyrimidine has a remarkable tendency to remain supersaturated for long periods of time. It was therefore found convenient to estimate the amount of compound in the filtrates spectrophotometrically and to use these solutions directly for the coupling reaction described below. A sample of 2,4-diamino-6-mercaptopyrimidine was recrystallized for analysis from 50 parts of hot water. It melts at 309–310°.

Anal. Calcd. for $C_4H_6N_4S$: C, 33.8; H, 4.2; N, 39.4. Found: C, 33.9; H, 4.6; N, 39.4.

5-*p*-Chlorobenzeneazo-2,4-diamino-6-mercaptopyrimidine (XV).—A solution of 8.4 g. (0.06 mole) of 2,4-diamino-6-mercaptopyrimidine in 40 ml. of 2 *N* sodium hydroxide and 700 ml. of water was chilled to 5° in an ice–salt-bath. To this was added a *p*-chlorobenzene diazonium chloride solution (prepared in the usual way from 7.7 g. (0.06 mole) of *p*-chloraniline, 130 ml. of 2.8 *N* hydrochloric acid and 4.4 g. of sodium nitrite) and an ice-cold solution of 15 g. of sodium bicarbonate in 125 ml. of water. The mixture was allowed to stand at 0° for 3 hours and the bright orange-red precipitate filtered off, washed with water and dried at 90° (14.4 g., 86%). This crude azo compound was used for the next step without further purification. A 1-g. sample was purified for analysis by solution in dilute sodium hydroxide and precipitation at *p*H 5 with acetic acid. It was dried at 100° and then re-exposed to the atmosphere until the weight was constant.

Anal. Calcd. for $C_{10}H_9N_6ClS \cdot {}^1/_4H_2O$: C, 42.1; H, 3.3; H_2O, 1.58. Found: C, 42.2; H, 3.7; H_2O (120°), 1.52.

6-Mercapto-2,4,5-triaminopyrimidine Dihydrochloride (XVI).—To a suspension of 20 g. (0.0715 mole) of the crude azo compound XV and 20 g. of zinc dust[25] in 500 ml. of boiling 50% aqueous methanol was added slowly 30 ml. of concentrated hydrochloric acid and boiling was continued until the *p*H value of the mixture was between 4 and 5. The mixture was filtered hot, the filtrate concentrated to *ca.* 100 ml. under reduced pressure and chilled. The precipitate was filtered off, washed with cold water, and dried in a vacuum desiccator. The crude product (6 g.) was heated with 60 ml. of 2 *N* hydrochloric acid; a small insoluble residue was filtered off and the filtrate evaporated to dryness under reduced pressure. The acid-soluble residue was taken up in 75 ml. of methanol, 400 ml. of absolute ether added and the dihydrochloride filtered off and dried in a vacuum desiccator (4.3 g., 26%).

Anal. Calcd. for $C_4H_7N_5S \cdot 2HCl$: C, 20.9; H, 3.9; N, 30.4. Found: C, 21.1; H, 4.2; N, 30.6.

2,4-Diamino-5-formamido-6-mercaptopyrimidine (XVII).—To 1.15 g. (0.005 mole) of 6-mercapto-2,4,5-triaminopyrimidine dihydrochloride was added 10 ml. of 1 *N* sodium hydroxide, 5 ml. of water and 15 ml. of 98–100% formic acid. After the mixture had been allowed to stand at room temperature for six hours, 100 ml. of absolute ethanol was added and the reaction mixture was taken to dryness rapidly under reduced pressure. The residue was treated with 20 ml. of absolute ethanol and the alcohol again evaporated under reduced pressure. The solid residue was suspended in 25 ml. of water, adjusted to *p*H 5 by the addition of a few drops of sodium hydroxide, chilled, filtered, washed with water and dried in a vacuum desiccator (0.66 g., 71%). This crude material had a purity of 92% according to its ultraviolet absorption spectrum. The product is difficult to purify because of its tendency to form supersaturated solutions. A small sample was purified for analysis by solution in *ca.* 50 parts of dilute sodium hydroxide, adjustment to *p*H 5–6 with acetic acid, removal of the initial reddish precipitate and evaporation of the filtrate to dryness. The residue was leached with cold water followed by acetone and dried in a vacuum desiccator. The product darkens gradually above 260° and decomposes *ca.* 275°,

Anal. Calcd. for $C_5H_7N_5OS$: C, 32.4; H, 3.8. Found: C, 32.5; H, 3.9.

5,7-Diaminothiazolo(5,4-d)pyrimidine (XVIII).—A solution of 1.15 g. (0.005 mole) of 6-mercapto-2,4,5-triaminopyrimidine dihydrochloride, 5 ml. of 2 *N* sodium hydroxide and 75 ml. of 98–100% formic acid was heated on the steam-bath for four hours and then evaporated to dryness under reduced pressure. The residue was suspended in 40 ml. of water and adjusted to *p*H 8 by the addition of 2 *N* sodium hydroxide. After chilling, the precipitate was collected, washed with water and dried at 100° (0.6 g., 72%). Recrystallization from 350 parts of nitromethane gave tan crystals which did not melt below 300°.

Anal. Calcd. for $C_5H_7N_5S$: C, 35.9; H, 3.0; N, 42.0; S, 19.1. Found: C, 36.3; H, 3.3; N, 41.9; S, 19.0.

2-Amino-6-mercaptopurine (XIX).—To 185 mg. (0.001 mole) of 2,4-diamino-5-formamido-6-mercaptopyrimidine was added 0.5 ml. of 2 *N* sodium hydroxide and the solution was evaporated to dryness under reduced pressure. The residue was heated in an oil-bath at 220–240° for 1.5 hours with the resultant evolution of water vapor. The dark red brown residue was dissolved in 20 ml. of water, containing 0.3 ml. of 2 *N* sodium hydroxide, filtered and the filtrate adjusted to *p*H 5 with hydrochloric acid. A dark red precipitate was centrifuged off and washed with water, acetone and ether (80 mg.). This precipitate contained only 25% of thioguanine according to its ultraviolet absorption spectrum. The combined filtrates and washings were evaporated to dryness and the residue leached with 10 ml. of cold water. The insoluble residue (60 mg., 36%) had an ultraviolet absorption spectrum identical with that of an authentic sample of thioguanine.[16]

(22) W. R. Boon, W. G. M. Jones and G. R. Ramage, *J. Chem. Soc.*, 96 (1951).

(23) E. Fischer and L. Ach, *Ann.*, **288**, 157 (1895).

(24) E. Büttner, *Ber.*, **36**, 2227 (1903).

(25) L. F. Cavalieri, J. F. Tinker and A. Bendich, THIS JOURNAL, **71**, 533 (1949).

Ultraviolet Absorption Spectra.—The spectra were measured with a Beckman spectrophotometer, model DU, using solutions containing 10 mg. per liter. For solutions of *p*H 1, 0.1 *N* hydrochloric acid was used, for *p*H 11, a glycine–sodium hydroxide buffer.

Acknowledgment.—We are indebted to Samuel W. Blackman, Veronica Purdey and P. R. W. Baker for the microanalyses reported here.

TUCKAHOE 7, N. Y.

[CONTRIBUTION FROM THE OAK RIDGE NATIONAL LABORATORY, BIOLOGY DIVISION]

The Catalytic Hydrogenation of Pyrimidine Nucleosides and Nucleotides and the Isolation of their Ribose and Respective Ribose Phosphates[1,2]

BY WALDO E. COHN AND DAVID G. DOHERTY

RECEIVED DECEMBER 1, 1955

The complete catalytic hydrogenation of the 4,5-double bond of pyrimidine nucleotides has been carried out under mild conditions with a rhodium catalyst. Dihydrouridylic acid, formed from either uridylic or cytidylic acid, is cleaved by dilute alkali at room temperature to give the N-ribosyl phosphate of β-ureidopropionic acid. Dilute acid at room temperature hydrolyzes this substance to ribose phosphate and β-ureidopropionic acid without appreciable isomerization of the phosphate group, thus making available the sugar phosphates of pyrimidine nucleotides. Similar reductions and degradations have been carried out on cytidine, thymidine and deoxycytidylic and thymidylic acids. From uridylic acids a and b, ribose 2- and 3-phosphates, respectively, were obtained, thus confirming the identity of the pyrimidine nucleotide isomers.

Introduction

The accessibility of the sugars of pyrimidine nucleosides, or of the sugar phosphates of pyrimidine nucleotides, is severely limited by the resistance to acid hydrolysis of the N-glycosidic linkage. It has long been known that this stability is dependent on the ethylenic unsaturation between the adjacent carbon atoms in the ring; reduction or bromination of the 4,5-double bond destroys the resonating structure and renders the N-glycosidic linkage susceptible to acid hydrolysis. In this manner Levene and LaForge[3] identified ribonic acid from cytidine, following bromination and oxidation, establishing ribose as the sugar in these substances as well as the purine nucleotides. Bromination has also been used to render the ribose susceptible in colorimetric procedures used to identify pentose reducing groups.[3–5] Hydrogenation of pyrimidine nucleoside, although as old as bromination, was originally used to identify only the dihydrouracil component[3,6] and subsequently to show that the dihydropyrimidine nucleotide had the same rate of acid hydrolysis as purine nucleotides as judged from the appearance of reducing groups.[7] A variety of procedures have been utilized to effect the reduction of pyrimidines, *e.g.*, hydrogenation under pressure with colloidal platinum[7–9] or palladium,[3,6] the dropping mercury cathode[10] and sodium and ethanol in liquid ammonia.[11–13] In only a few cases has reduction of nucleosides or of nucleotides been successful.[3,6,11,12]

Now the sugar moiety has been isolated as such, for the first time without oxidation or other significant change, from both natural and synthetic pentosides and from deoxypentosides reduced by sodium and ethanol in liquid ammonia[11] and cleaved with a sulfonic acid cation-exchange resin.[12] In the deoxyribosides, this marked the first chemical identification of deoxyribose in the pyrimidine compounds. In all other cases recorded, the sugar has been identified indirectly, and in no case has a sugar phosphate been isolated. The purpose of this investigation was to recover quantitatively the sugar phosphate component of pyrimidine nucleotides, under conditions mild enough to avoid phosphomigration, in order to identify it both as to sugar type and phosphate location. The availability of a new rhodium catalyst,[14] which is particularly suited for the reduction of heterocyclic compounds, made a reinvestigation of this route seem feasible. With it, it was possible to achieve complete reduction of the 4,5-double bond at 1–1.2 atmospheres of hydrogen at *p*H 2–5 and at room temperature. Disappearance of the ultraviolet absorption at 260 mμ was used as a criterion of reduction, for it has been shown that the same double bond that is critical for the integrity of the N-glycosidic linkage is also critical for the characteristic ultraviolet spectrum of the pyrimidine nucleotides.[15] It was found that the increase in pentose concentration as detected by conventional orcinol reaction could be directly correlated with the disappearance of ultraviolet spectrum, as expected. It

(1) This work was performed under USAEC Contract No. W-7405-eng-26.

(2) Presented in part at the Federation of American Societies for Experimental Biology, San Francisco, April 11–15, 1955.

(3) P. A. Levene and F. B. LaForge, *Ber.*, **45**, 608 (1912).

(4) L. Massart and J. Hoste, *Biochim. Biophys. Acta*, **1**, 83 (1947).

(5) T. G. Brady and F. McEvoy-Bowe, *Nature*, **168**, 299 (1951).

(6) P. A. Levene and F. B. LaForge, *J. Biol. Chem.*, **13**, 508 (1912).

(7) P. A. Levene and E. Jorpes, *ibid.*, **81**, 575 (1929).

(8) E. R. Brown and T. B. Johnson, THIS JOURNAL **45**, 2702 (1923).

(9) R. D. Batt, J. K. Martin, J. M. Ploeser and J. Murray, *ibid.*, **76**, 3663 (1954).

(10) L. F. Cavalieri and B. A. Lowy, *Arch. Biochem. Biophys.*, **35**, 83 (1952).

(11) D. C. Burke, *J. Org. Chem.*, **20**, 643 (1955).

(12) W. Bergmann and D. F. Burke, *Angew. Chem.*, **67**, 127 (1955).

(13) A. Bendich in "The Nucleic Acids," Vol. I, E. Chargaff and J. N. Davidson, Editors, Academic Press, New York, N. Y., 1955, p. 81.

(14) A 5% rhodium-on-alumina, procured from Baker & Co., Inc., Newark, N. J.

(15) L. F. Cavalieri and A. Bendich, THIS JOURNAL, **72**, 2587 (1950); L. F. Cavalieri, A. Bendich, J. F. Tinker and G. B. Brown, *ibid.*, **70**, 3875 (1948).