[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

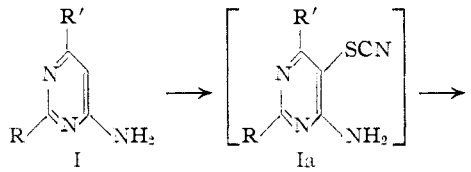
Studies on Condensed Pyrimidine Systems. VI. Some 2-Aminothiazolo[4,5-d]-pyrimidines[1,2]

BY ALLISON MAGGIOLO[3] AND GEORGE H. HITCHINGS

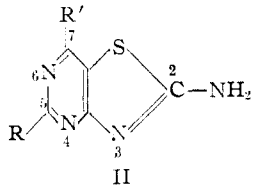
Thiocyanation in the 5-position occurs readily with pyrimidines bearing at least two hydroxyl or amino groups in positions 2, 4 and 6. The 4-amino-5-thiocyanopyrimidines are readily cyclized to 2-aminothiazolo[4,5-d]pyrimidines. An alternate synthesis of thiazolo[4,5-d]pyrimidines bearing non-functional substituents in the 2- and 4-position also was developed. In the latter method a 4-thiocyanopyrimidine is formed by the reaction of a 4-chloropyrimidine with potassium thiocyanate and after isomerization is eventually converted to a thioureidopyrimidine which is cyclized by treatment with bromine.

The synthesis of amino-, diamino- and dimercaptothiazolo[5,4-d]pyrimidines for study as purine antagonists[4] was reported recently.[5] The isomeric thiazolo[4,5-d]pyrimidines were desired for comparison. Representatives of this ring system had been reported.[6,7] Attempts to repeat the work in this laboratory failed, and recently a retraction has been published.[8]

Studies on the thiocyanation of N-heterocycles[9] indicated the feasibility of 5-thiocyanation of suitably substituted pyrimidines (I) as a route to 2-amino[4,5-d]thiazolopyrimidines (II) bearing the desired substituents in the pyrimidine moiety. In agreement with the findings with other N-heterocyclic systems,[9] disubstitution of the pyrimidine by hydroxyl or amino groups (I, R or R′ = OH or NH_2) is a prerequisite to successful thiocyanation.



I Ia



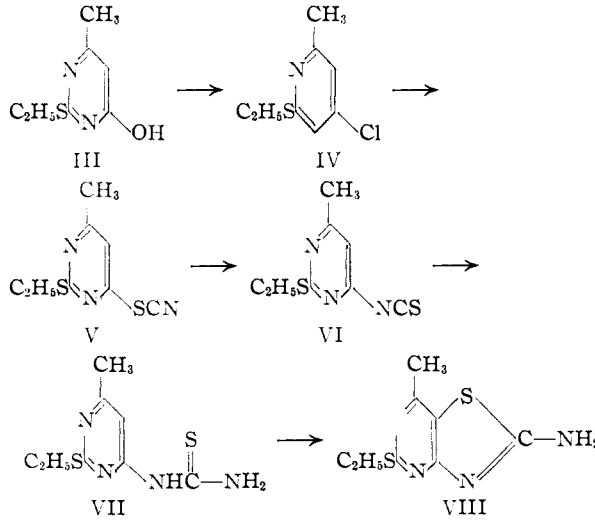
II

The limitations of the method with respect to the substituents of the pyrimidine moiety of the condensed system are thus apparent. However, an alternate synthesis (described below) with different limitations allows the preparation of a different group of derivatives, and between the two methods a considerable variety can be attained.

Thiocyanation of anilines gives rise to benzothiazoles[10] while thiocyanation of phenols results in the formation of benzthioxoles.[10] It was of interest therefore to determine, in an instance when either thioxole or thiazole formation would be possible (Ia, R′ = OH) whether one type of condensed pyrimidine system would be preferentially formed. In two such instances the thiazolopyrimidine (Table I, No. 4 and 6) was the sole product. Thiocyanation of three other 4-hydroxypyrimidines could be demonstrated: 2-amino-4-hydroxy-6-methylpyrimidine,[9] 2,4-dihydroxy-6-methylpyrimidine[9] and 2-amino-4-hydroxy-6-phenylpyrimidine. In none of these instances was any thioxolo derivative demonstrable. In the case of the 6-phenyl derivative 2-amino-4-hydroxy-6-phenyl-5-thiocyanopyrimidine was isolated, while the other two derivatives were isolated as 5,5′-disulfides[9] (*cf.* X, below).

Ring closure of 4-amino-5-thiocyanopyrimidines to the 2-aminothiazolo[4,5-d]pyrimidines occurs readily, in general during the isolation procedure.

An alternative route to thiazolo[4,5-d]pyrimidines has been explored in one instance (Table I, No. 7). Chlorination of 2-ethylmercapto-6-ethyl-4-hydroxypyrimidine (III) gave a chloropyrimidine (IV) which was converted to the thiocyano derivative (V) by reaction with potassium thiocyanate. Isomerization (VI) followed by reaction with ammonia resulted in the thioureidopyrimidine (VII). Cyclization of the latter by treatment with bromine resulted in the thiazolopyrimidine (VIII). This procedure involves six steps, but was accomplished with an over-all yield of 38%. It is restricted essentially to non-functional derivatives since 2- and 6-amino-4-chloropyrimidines fail to react with sodium or potassium thiocyanate.



III IV V VI VII VIII

The structure of the thiazolo[4,5-d]pyrimidines follows from the analytical data, the ultraviolet

(1) Presented before the American Chemical Society, April, 1951, at Boston, Mass.

(2) Part V., A. Schrage and G. H. Hitchings, *J. Org. Chem.*, **16**, 207 (1951).

(3) This work was supported by grant from the Charles F. Kettering Foundation to The Wellcome Research Laboratories.

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

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

(6) H. Erlenmeyer and H. P. Furger, *Helv. Chim. Acta*, **26**, 366 (1943).

(7) H. Erlenmeyer and H. P. Furger, *ibid.*, **30**, 582 (1947).

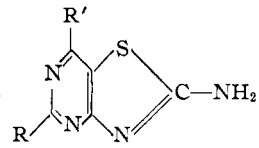
(8) H. P. Furger, *ibid.*, **33**, 1689 (1950).

(9) A. Maggiolo, THIS JOURNAL, in press.

(10) H. P. Kaufmann and E. Weber, *Arch. Pharm.*, **267**, 192 (1929).

TABLE I

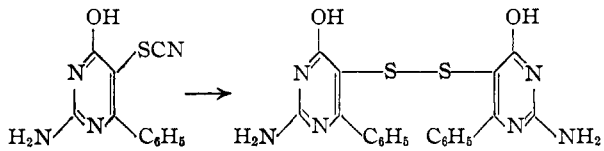
2-AMINO-5,7-DISUBSTITUTED-THIAZOLO[4,5-d]PYRIMIDINES



No.	R	R′	M.p., °C.	Yield,* %	Empirical formula	Analyses, % Nitrogen Calcd.	Nitrogen Found	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
1	NH_2	CH_3	249–250	52[a]	$C_6H_7N_5S\cdot H_2O$[e,h]	35.2	35.2	36.2	36.5	4.5	4.3
2	CH_3	NH_2	>300	63[a]	$C_6H_7N_5S\cdot{}^1/_3H_2O$[f]	37.4	37.8	38.5	38.8	3.9	4.0
3	NH_2	NH_2	>300	75[b]	$C_5H_6N_6S$	47.6	47.2	32.9	32.6	3.3	3.5
4	OH	OH	>310	45[b]	$C_5H_4O_2N_4S\cdot H_2O$	27.8	28.0	35.6	35.7	3.0	3.0
5	CH_3S	NH_2	208–209	79[c]	$C_6H_7N_5S_2$	32.9	32.8 32.6	33.8	33.9	3.3	3.3
6	NH_2	OH	>310	72[b]	$C_5H_5ON_5S$	38.2	38.3	27.3	27.5	2.7	2.9
7	C_2H_5S	CH_3	187–189	87[c,d]	$C_8H_{10}N_4S_2\cdot H_2O$[g,i]	22.9	22.8				
8	H	NH_2	245–247	54[b]	$C_5H_5N_5S\cdot H_2O$[j]	37.8	37.3				

* Crystallized from: [a] 95% ethanol, [b] water, [c] 50% alcohol. [d] Prepared by Method B; the others by Method A. [e] Calcd. for $C_6H_7N_5S$: S, 17.7. Found: S, 17.6. [f] Calcd.: S, 17.1. Found: S, 17.3. [g] Calcd.: S, 26.2 Found: S, 25.8. [h] Calcd. H_2O, 9.0. Found: H_2O, 8.9. [i] Calcd.: H_2O, 7.4. Found: H_2O, 7.2. [j] Calcd.: H_2O, 9.7. Found: H_2O, 9.5.

absorption spectra and the demonstrable absence of thiocyano groups. Treatment of a thiocyano derivative with alkali results in the cleavage of the thiocyano group[9,10] with formation of a disulfide, cyanide and cyanate. The thiazolo[4,5-d]pyrimidines listed in Table I were recovered unchanged after such treatment while 2-amino-4-hydroxy-6-phenyl-5-thiocyanopyrimidine (IX) was converted to bis-(2-amino-4-hydroxy-6-phenylpyrimidyl-5)-1-disulfide (X).



IX X

The ultraviolet absorption spectra of the thiazolo-[4,5-d]pyrimidines resemble closely those of the isomeric thiazolo[5,4-d]pyrimidines,[5] *p*-oxazino-[2,3-d]pyrimidines[11] and dihydroxanthopterine[12] (Table II).

TABLE II

ULTRAVIOLET SPECTRA OF SUBSTITUTED THIAZOLO[4,5-d]-PYRIMIDINES

	*p*H 1 Maxima		*p*H 1 Minima		*p*H 11 Maxima		*p*H 11 Minima	
No.[a]	λ, mμ	E_m	λ, mμ	E_m	λ, mμ	E_m	λ, mμ	E_m
1	275	8,500	257	7,800	289	9,000	261	6000
2	265	9,000	250	7,500	285	5,000		
3	265	15,000	242	8,000	265	9,500	290	1000
	330	10,000	300	500	335	1,500		
4	262	15,200	300	4,000	269	12,500	305	3000
	310	4,500			330	4,000		
5	245	16,500	260	7,750	240	12,500		
	278	10,500						
6	262	11,500	298	2,500	265	8,000	310	2000
	313	3,250			330	2,500		
7	262	16,000	282	12,000	261	15,800	325	3500
	308	13,500			299	9,500		
8	268	9,000	256	7,500	240	19,000		

[a] Compounds numbered as in Table I.

The results of biological studies will be published elsewhere. However, it may be stated here that several resemblances to other purine analogs have been found in studies with *Lactobacillus casei*. It is also of interest that 2,7-diamino-5-methylmercapto-thiazolo[4,5-d]pyrimidine resembles 6-amino-2-methylmercaptopurine in its inhibitory effects on *Salmonella typhosa*.[13]

(11) P. B. Russell, G. B. Elion and G. H. Hitchings, THIS JOURNAL, **71**, 474 (1949).

(12) G. H. Hitchings and G. B. Elion, *ibid.*, **71**, 467 (1949).

Experimental

Two general methods of preparation of thiazolopyrimidines were employed. These will be illustrated with a specific example in each instance.

Method A. 2,5-Diamino-7-methylthiazolo[4,5-d]pyrimidine.—To a solution of 12.4 g. (0.1 mole) of 2,4-diamino-6-methylpyrimidine and 40 g. (0.41 mole) of potassium thiocyanate in 200 ml. of 96% acetic acid, cooled in an ice-bath in a darkened room, was added with mechanical stirring a solution of 6 ml. (0.12 mole) of bromine in 100 ml. of glacial acetic acid over a period of one-half hour. After the ice-bath was removed, the contents were stirred for an additional 40 minutes and then heated on a hot-plate to 70°. Hot water (50 ml.) was added and the lights turned on to increase polymerization of the excess thiocyanogen. The hot solution was filtered by suction from the insoluble orange polymer. To the filtrate, cooled in an ice-bath, concentrated ammonia was slowly added until the solution was alkaline. The resulting yellow crystalline solid was allowed to stand overnight at 5° and filtered off. There was obtained 10.1 g. (52%) of 2,5-diamino-7-methylthiazolo[4,-5-d]pyrimidine hydrate, m.p. 249–250° (recrystallized from 95% ethanol) (Table I, No. 3).

This as well as the other crystalline monohydrates in Table I lose their water of crystallization after three hours at 140° *in vacuo*, but they tend to regain it on exposure to air.

Method B. 2-Amino-5-ethylmercapto-7-methylthiazolo-[4,5-d]pyrimidine.—To 30 g. of 2-ethylmercapto-4-hydroxy-6-methylpyrimidine was added 100 ml. of phosphoryl chloride and the mixture was heated on the steam-bath for one hour. Most of the phosphoryl chloride was removed at steam-bath temperature under reduced pressure. To the remaining thick oil were added 200 g. of cracked ice, 200 ml. of ether and while cold, ammonium hydroxide solution to a *p*H value of 8–9. The ether layer was separated and added to 100 ml. of 95% ethanol containing 17 g. of potassium thiocyanate. The reaction mixture was warmed on the steam-bath for one-half hour to remove the ether, and 100 ml. of toluene was added. The solution was concentrated to 75 ml. to remove the alcohol and water. After the addition of 100 ml. of xylene, the reaction mixture was heated under a reflux condenser for two hours and filtered while still warm. To the warm filtrate 20 ml. of concentrated ammonia was carefully added with shaking. The tan crystalline precipitate of 2-ethylmercapto-6-methyl-4-thioureidopyrimidine was filtered off and dried. The yield was 18 g. (45%), m.p. 235–236°.

(13) G. H. Hitchings, G. B. Elion and M. B. Sherwood, *Fed. Proc.*, **9**, 185 (1950).

Anal. Calcd. for $C_8H_{12}N_4S_2$: C, 42.1; H, 5.3; N, 24.6. Found: C, 42.2; H, 5.4; N, 24.5.

To 4.4 g. of the above finely divided pyrimidyl thiourea in 20 ml. of dry chloroform was slowly added with shaking a solution of 1 ml. of bromine in 10 ml. of chloroform over a period of 15 minutes, the temperature being maintained below 30°. The solution was evaporated to dryness on the steam-bath. The dry, orange, solid residue was treated with a solution of 2 g. of sodium hydrosulfite in 50 ml. of water for ten minutes to remove excess bromine. The tan oil which formed was treated with excess ammonia and it crystallized upon scratching. After two hours the crystalline solid was filtered off and recrystallized from 50% alcohol to yield 4 g. (87%) of 2-amino-5-ethylmercapto-7-methylthiazolo[4,5-d]pyrimidine, m.p. 187–189 (Table I, No. 7).

Failure of 2-Amino-4-chloro-6-methylpyrimidine to React with Potassium Thiocyanate.—The refluxing of molecular equivalents of 2-amino-4-chloro-6-methylpyrimidine and potassium thiocyanate for six hours in either 95% ethanol, toluene or xylene gave only a quantitative recovery of the original chloropyrimidine.

2-Amino-4-hydroxy-6-phenyl-5-thiocyanopyrimidine.—A warm solution of 4.7 g. (0.025 mole) of 2-amino-4-hydroxy-6-phenylpyrimidine and 6.2 g. (0.076 mole) of sodium thiocyanate in 200 ml. of methanol (saturated with sodium bromide) and 50 ml. of glacial acetic acid was cooled to 5°. While stirring a solution of 1.8 ml. (0.035 mole) of bromine in 50 ml. of glacial acetic acid was added over a period of 20 minutes. The ice-bath was removed and stirring was continued for one hour. The solution was heated to boiling and 20 ml. of hot water was added. The hot solution was filtered, made to *p*H 6 with 10% ammonia, and most of the alcohol removed under vacuum. The yellow crystalline deposit was dried, yield 4.1 g. (66%). For purification the product was crystallized from alcohol, m.p. 287–289° with decomposition.

Anal. Calcd. for $C_{11}H_8ON_4S$: N, 23.0, S, 13.1. Found: N, 22.7; S, 13.2.

Bis-(2-amino-4-hydroxy-6-phenylpyrimidyl-5)-1-disulfide.—To 30 ml. of 10% sodium hydroxide solution was added 0.5 g. of 2-amino-4-hydroxy-6-phenyl-5-thiocyanopyrimidine. After standing overnight at room temperature the solution was made to 200 ml. with distilled water and filtered from a trace of residue. The filtrate was neutralized to *p*H 6 with 10% acetic acid. The pale yellow crystalline precipitate was filtered off, washed with water, then alcohol, and dried. The yield was quantitative, m.p. > 300°.

Anal. Calcd. for $(C_{10}H_8ON_3S-)_2$: N, 19.3; S, 14.7. Found: N, 19.3; S, 14.7.

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

Acknowledgments.—The authors are indebted to S. W. Blackman, N. Martinez, Jr., and J. Tillmans for microanalyses and Phoebe Lee Graham for the measurement of ultraviolet absorption spectra.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY]

Quinone–Hydroquinone Exchange Reactions. I. Non-Exchange in Duroquinhydrone[1]

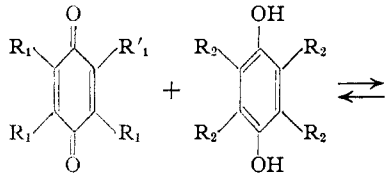
BY AKSEL A. BOTHNER-BY

Quinhydrone complexes have been prepared from duroquinone and durohydroquinone, one compound being labeled with carbon-14. Thermal decomposition *in vacuo* yielded the original compounds, no exchange of radioactive isotope having taken place after complex formation. The formulation of quinhydrones as symmetrical resonance hybrids is thus disproven.

It has been reported by Gragerov and Miklukhin[2] that the exchange reaction between benzoquinone (Ia) and hydroquinone-2,3,5,6-d_4 (IIa) in a quinhydrone complex does not occur to any appreciable extent in 24 hours at room temperature, or in 6 hours at 70°.

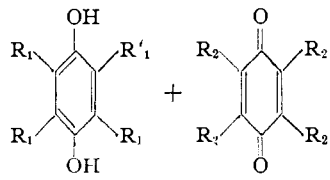
As part of a general program for the investigation of oxidation–reduction exchange reactions in organic systems, supplies of carbon-14 labeled duroquinone and durohydroquinone had been prepared, and it was therefore decided to determine whether this interesting observation could be repeated in the case of the quinhydrone complex between duroquinone and durohydroquinone. This complex, although unstable, can be prepared by the method of Michaelis and Granick.[3]

Durohydroquinone-α-C^{14} (IIIb) was prepared from trimethylhydroquinone diacetate (V) by chloromethylation according to the method of Smith and Carlin[4] with formaldehyde-C^{14} to give 2-chloromethyl-C^{14}-3,5,6-trimethyl-4-acetoxyphenol (VI) which was reduced with lithium aluminum hydride. The latter reagent simultaneously reduced the chloromethyl group to a methyl group and



Ia, $R_1 = R'_1 = H$
Ib, $R_1 = CH_3$, $R'_1 = C^{14}H_3$

IIa, $R_2 = D$
IIb, $R_2 = CH_3$



IIIa, $R_1 = R'_1 = H$
IIIb, $R_1 = CH_3$, $R'_1 = C^{14}H_3$

IVa, $R_2 = D$
IVb, $R_2 = CH_3$

cleaved the acetoxy ester linkage to give the desired compound in good yield. The easy reduction of the chloromethyl group may perhaps be attributed to an intramolecular reduction by a phenoxyaluminohydride of the type suggested in a previous communication.[5] Oxidation with ferric sulfate in the usual manner gave duroquinone-α-C^{14}.

Two experiments were conducted. In the first the quinhydrone was prepared from radioactive

(1) Work carried out under the auspices of the U. S. Atomic Energy Commission.

(2) I. P. Gragerov and G. P. Miklukhin, *Doklady Akad. Nauk., S. S. S. R.*, **62**, 79 (1948).

(3) L. Michaelis and S. Granick, THIS JOURNAL, **66**, 1023 (1944).

(4) L. I. Smith and R. B. Carlin, *ibid.*, **64**, 524 (1942).

(5) A. Bothner-By, *ibid.*, **73**, 846 (1951).