two mercaptans in equivalent amounts: β-mercaptopropionic acid and α-hydroxy-β-mercaptopropionic acid.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY, TEMPE, ARIZ.]

Potential Purine Antagonists. XXVI. Preparation of Certain 8-Triazenopurine Nitrogen Mustards[1]

BY GERHARD A. USBECK,[2] JESSE W. JONES AND ROLAND K. ROBINS

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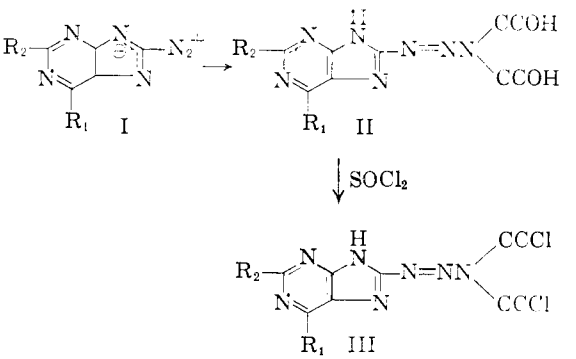
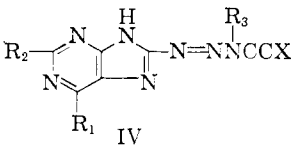
A number of 8-diazopurines has been coupled with diethanolamine and the resulting compounds treated with thionyl chloride to give the novel 8-triazenopurine nitrogen mustards (III). Several of these new derivatives have been prepared by coupling the appropriate 8-diazopurine directly with β,β-dichlorodiethylamine hydrochloride under carefully controlled conditions.

Bergel[3] first introduced the concept that several alkylating agents which are active in cancer chemotherapy can be considered as composed of an alkylating function and a "carrier" moiety. Recent synthesis of 5-bis-(2-chloroethyl)-aminouracil[4,5] and a study of the antitumor properties of this compound[4-7] have focused attention on this idea. The possibility that a certain biologically important carrier might provide nitrogen mustards of high specificity and less gross toxicity to the host provides an interesting area for synthetic study. Our current interest in purine antagonists has prompted an investigation which would utilize certain purine derivatives as carriers of the nitrogen mustard grouping because of the known biological importance of various purines in nucleic acid biosynthesis.

A literature survey revealed that DiPaco and Tauro[8] have previously reported the synthesis of three purines containing nitrogen mustard groupings, 6-bis-(β-chloroethyl)-aminopurine, 6-bis-(β-chloroethyl)-amino-2,8-dichloropurine and 7-bis-(β-chloroethyl)-amino-1,3-dimethylethylpurine-2,6-dione. However, to date no biological activity has been reported for these derivatives. In a preliminary study, position 8 was selected as the best position for substitution of the nitrogen mustard function since this would then allow the functional groups of the naturally occurring purines in positions 2 and 6 and allow position 9 to be free for possible ribosidation. It was decided that the nitrogen mustard function should be slightly removed from the 8-position to prevent possible cyclization with the imidazole nitrogens. Recent studies of various 8-diazopurines (I)[9] suggested that coupling might occur with diethanolamine. This reaction indeed proceeded readily to give the corresponding 8-[bis-(2-hydroxyethyl)-triazeno]-purine (II) which was then treated with thionyl chloride to give the desired 8-[bis-(2-chloroethyl)-triazeno]-purine (III).



Several secondary alkylamines containing a β-hydroxyethyl group were likewise coupled to the appropriate 8-diazopurine followed by treatment with thionyl chloride to give purines containing a "one-arm" mustard of the type IV, X = Cl. The mustards of this type and their hydroxy intermediates are listed in Table I. These compounds were



essentially prepared by the procedure described in the Experimental section for the appropriate corresponding 8-[bis-(2-chloroethyl)-triazeno]-purine. The 8-diazopurines chosen for study were 8-diazoadenine,[9] 8-diazoguanine,[9] 8-diazohypoxanthine,[9] 8-diazoxanthine[9] and 8-diazotheophylline.[9] It was discovered during the course of investigation that under certain conditions coupling could be effected directly with bis-(2-chloroethyl)-amine hydrochloride to give the desired 8-[bis-(2-chloroethyl) triazeno]-purine. Thus, in the case of 8-[bis-(2-chloroethyl)-triazeno]-theophylline (VI), this compound was prepared both by coupling 8-diazothe-

(1) Supported in part by a research grant from Parke, Davis and Co. and in part by research grant CY-4008(C1) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) C. H. Boehringer and Sohn, Ingelheim/Rhein, Germany.

(3) F. Bergel, *N. Y. Acad. Sci.*, **68**, 1238 (1958).

(4) D. A. Lyttle and H. G. Petering, *J. Am. Chem. Soc.*, **80**, 6459 (1958).

(5) D. A. Lyttle and H. G. Petering, *J. Natl. Cancer Inst.*, **23**, 153 (1960).

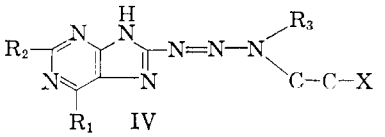
(6) M. Lane and M. G. Kelly, *Cancer Res.*, **20**, 511 (1960).

(7) J. S. Evans and G. D. Mengel, *Proc. Soc. Exptl. Biol. & Med.*, **99**, 620 (1958).

(8) G. DiPaco and C. S. Tauro, *Ann. chim. (Rome)*, **47**, 698 (1957).

(9) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **82**, 3773 (1960).

TABLE I

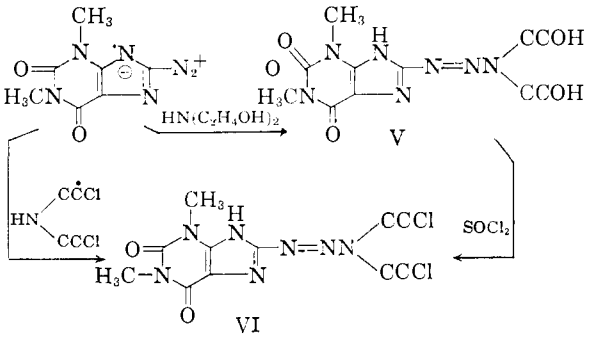


IV

R_1	R_2	R_3	X	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Recrystn. solvent	λ_{max}, mμ pH 1	ε	λ_{max}, mμ methanol	ε	Yield, %
OH	H	C_2H_5	OH	195–200 d.	37.5[a]	37.9	4.9	4.9	34.0	34.3	Methanolic HCl + acetone	245 337	9,200 22,200			98
OH	H	C_2H_5	Cl	>300	40.1	40.1	4.5	4.4	36.4	36.0	EtOH + petr. ether	244 354	8,900 11,900	255 380	12,000 12,400	42
OH	H	Iso-C_3H_7	OH	>300	39.9[a]	40.2	5.3	5.6	32.5	32.0	Methanolic HCl + acetone	244 338	10,200 20,800			61
OH	H	Iso-C_3H_7	Cl	>300	42.2	42.3	4.9	4.8	34.5	34.2	Methanol–benzene	245 354	9,100 12,500	255 380	11,300 12,200	58
OH	OH	CH_3	Cl	>250	35.4	35.2	3.7	4.0	36.2	35.8	Methanol–acetone	264 370	11,500 10,000	270 405	7,300 9,500	49
OH	NH_2	CH_3	OH	218 d.	33.3[a]	33.0	4.5	4.4	38.8	38.4	Methanolic HCl + acetone	250 285 350	14,100 10,100 7,200			68
OH	NH_2	CH_3	Cl	Dec. >250	31.3[a]	31.8	3.9	4.3	36.2	35.8	Methanol	248 285 364	11,900 7,300 4,800	250 287 415	8,100 8,600 4,000	47
OH	NH_2	C_2H_5	Cl	Dec. >250	33.7[a]	33.6	4.4	5.0	35.0	35.0	Methanol–acetone	248 287 350	21,200 13,400 5,300	252 290 415	9,100 11,000 3,100	81
OH	NH_2	Iso-C_3H_7	Cl	Dec. >210	35.9[a]	35.7	4.8	5.2	33.5	33.4	Methanol–acetone			278 415	10,700 9,300	66
NH_2	H	CCOH	OH	Dec. 185	33.2[b]	33.0	4.9	4.8			Methanolic HCl	253 340	8,800 21,700			82
NH_2	H	C_2H_5	OH	Dec. 215	43.2	43.1	5.6	5.9	44.8	45.0	Methanol	252 339	11,000 25,300			73
NH_2	H	CH_3	OH	Dec. >240	40.6	40.5	5.1	5.1	47.5	47.5	Ethanol	249 336	11,300 25,200			85
NH_2	H	Iso-C_3H_7	OH	Dec. 220	45.4	45.6	6.1	6.3	42.4	41.9	Water	253 341	12,200 25,900			87
NH_2	H	CH_3	Cl	Dec. >170	30.3[c]	30.3	4.7	4.5			Methanol–benzene	252 338	6,100 12,700	255 388	7,100 9,400	54
Theophylline		CH_3	OH	198–200	42.7	42.7	5.3	5.7	34.7	34.5	Methanol	255 346	11,800 19,700			83
Theophylline		C_2H_5	OH	193–195	44.7	44.8	5.8	5.9	33.2	33.2	EtAc–methanol	258 350	12,400 21,200			80
Theophylline		C_2H_5	Cl	218–220 d.	42.0	42.3	5.1	4.9	31.2	30.8	Ethanol–petr. ether	268 376	11,300 8,800			89
Theophylline		Iso-C_3H_7	OH	195 d.	46.6	46.8	6.4	6.4	31.7	31.8	Benzene–methanol	258 350	12,000 20,600			88
Theophylline		Iso-C_3H_7	Cl	227–230	43.9	43.8	5.5	5.7	29.9	30.1	Ethanol–petr. ether	268 375	11,200 8,500			89

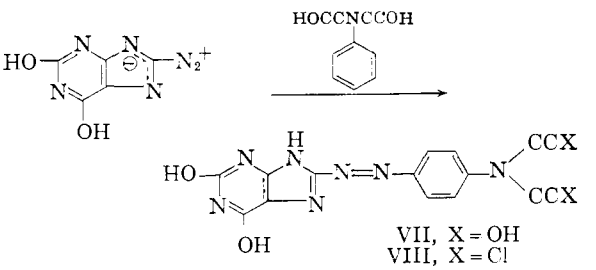
[a] Calcd. as monohydrochloride. [b] Calcd. as dihydrochloride. [c] Calcd. as $C_8H_{11}N_8Cl\cdot HCl\cdot 1^1/_2H_2O$.

ophylline directly with bis-(2-chloroethyl)-amine hydrochloride and also by coupling with diethanolamine followed by chlorination of V with thionyl chloride.



8 - [Bis - (2 - chloroethyl) - triazeno] - adenine (III, $R_1 = NH_2$, $R_2 = H$) was best prepared by the direct coupling of 8-diazoadenine and bis-(2-chloroethyl)-amine hydrochloride.

8-Diazoxanthine was coupled with 2,2′-(phenylimino) - diethanol to give *p* - diethanolamino - 8-phenylazoxanthine (VII) which was converted to VIII with thionyl chloride.

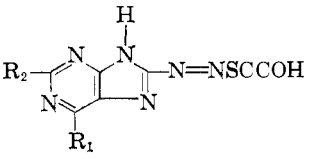


VII, X = OH
VIII, X = Cl

Ross and Warwick[10] have prepared and studied a number of *p*-bis-(2 - chloroethyl) - aminoazobenzenes and suggest the antitumor activity of these compounds is due to reduction of the azo group *in vivo*.

(10) W. C. J. Ross and G. P. Warwick, *J. Chem. Soc.*, 1364 (1956).

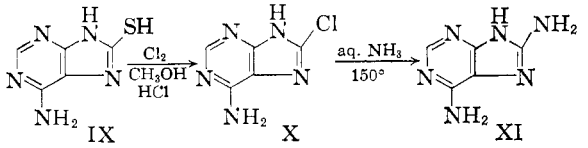
TABLE II



R_1	R_2	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Yield, %	λ_{max}, mμ pH 1	ε
NH_2	H	310 dec.	35.1	34.8	5.6	5.6			96	277	6,000
										360	11,700
OH	NH_2	Dec. >250	32.9	32.7	3.5	3.9			32	247	10,200
										280	7,400
										365	3,300
OH	H	>300	35.0	35.2	3.3	3.4	35.0	35.1	61	275	8,900
										358	7,600
Theophylline		158 dec.	37.9	37.9	4.2	4.1	29.5	30.0	83	293	10,300
										370	3,000

8-Diazotheophylline, 8-diazoguanine, 8-diazoadenine and 8-diazohypoxanthine were coupled with 2-mercaptoethanol to yield the corresponding 8-[(2-hydroxyethyl)-thioazo]-purine (see Table II). Efforts to convert these compounds to the corresponding sulfur mustard with thionyl chloride were unsuccessful.

The large quantities of 8-diazoadenine[9] required for these studies led to a new synthesis for the intermediate 6,8-diaminopurine (XI) beginning with 6-amino-8-purinethiol (IX).[11] Chlorine gas in methanolic hydrochloric acid converted IX smoothly to 6-amino-8-chloropurine (X) in nearly quantitative yield. The replacement of a mercapto group by chlorine in this manner has recently been shown by Robins[12] to be a general reaction which readily yields 2,6,8-trichloropurine from 2,6,8-purinetrithiol. Aqueous ammonia and X at 150–170° readily provides 6,8-diaminopurine previously available only from 6,8-dichloropurine.[11]



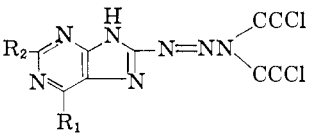
The 8-triazenopurines are in general rather stable compounds. An attempt, however, to purify 8-[(2-hydroxyethyl)-ethyltriazeno]-guanine (VI, R_1 = OH, R_2 = NH_2, R = C_2H_5, X = OH) by recrystallization from boiling methanolic hydrogen chloride resulted in hydrolysis of the 8-triazeno group to give a substantial quantity of 2-amino-6,8-dihydroxypurine which was identified by its ultraviolet absorption spectra. The ultraviolet absorption spectra of the 8-[bis-(2-chloroethyl)-triazeno]-purines prepared are listed in Table III. These compounds which are usually yellow-orange in color absorb in absolute methanol in the range of 390–425 mμ.

Several of the 8-triazenopurine bis-nitrogen mustards (III) have been found to exhibit significant antitumor activity against various animal tumors. These results will be made the subject of a future communication.

(11) R. K. Robins, *J. Am. Chem. Soc.*, **80**, 6671 (1958).
(12) R. K. Robins, *J. Org. Chem.*, **26**, 447 (1961).

TABLE III

ULTRAVIOLET ABSORPTION SPECTRA OF CERTAIN 8-[BIS-(2-CHLOROETHYL)-TRIAZENO]-PURINES



R_1	R_2	λ_{max}, mμ pH 1	ε	λ_{max}, mμ methanol	ε
OH	H	244	8,200	255	11,500
		360	12,000	390	12,800
OH	OH	264	11,500	270	13,100
		380	11,500	415	13,200
OH	NH_2	253	13,200		
		280	11,200		
		354	9,200		
NH_2	H	252	9,200	265	10,100
		352	14,700	374	14,200
Theophylline		268	12,900	270	10,100
		390	10,400	425	14,000

Experimental[13]

Preparation of 8-[Bis-(2-hydroxyethyl)-triazeno]-theophylline (V).—Five grams of 8-diazotheophylline[9] was suspended in 250 ml. of acetone containing 5 g. of diethanolamine, and the mixture was stirred for 6 hr. at room temperature. The yellow color of the diazo compound disappeared during this time, and a colorless precipitate formed. The product was filtered, washed with a little acetone, and recrystallized from a mixture of methanol and dimethylformamide. The yield was 6 g. (96%), m.p. 187–190°. The compound exhibited: $\lambda_{max}^{pH\ 1}$ 259, 350 mμ, ε 12,400, 21,200; $\lambda_{max}^{pH\ 11}$ 248, 347 mμ, ε 9,300, 20,900.

Anal. Calcd. for $C_{11}H_{17}N_7O_4$: C, 42.5; H, 5.4. Found: C, 42.4; H, 5.2.

8-[Bis-(2-chloroethyl)-triazeno]-theophylline (VI). Method 1.—Five grams of 8-[bis-(2-hydroxyethyl)-triazeno]-theophylline (V) was added to 30 ml. of thionyl chloride and the solution gently refluxed for 5 min. and then kept 24 hr. at room temperature. Benzene (150 ml.) was added, and the precipitate was filtered, washed with 50 ml. of benzene, and recrystallized from methanol and petroleum ether. The yield of orange needles was 5.0 g., dec. > 220°.

Anal. Calcd. for $C_{11}H_{15}N_7O_2Cl_2$: C, 37.9; H, 4.3; N, 28.2; Cl, 20.4. Found: C, 38.1; H, 4.4; N, 28.5; Cl, 20.8.

(13) All melting points were taken on a Fisher–Johns melting point apparatus and are uncorrected.

Method 2.—Eighteen grams of 8-diazotheophylline[9] was added to 300 ml. of methanol containing 20 g. of β,β-dichlorodiethylamine hydrochloride. The reaction mixture was stirred and heated at 50–55° for 1 hr. Celite (3 g.) was added, and stirring was continued for 5 min. The red solution was filtered, and the filtrate was evaporated to a thin sirup in a rotary evaporator at 50° under reduced pressure. The residue was then triturated with 150 ml. of dimethoxyethane. The yellow precipitate that separated was filtered and washed first with dimethoxyethane and then cold methanol. The crude product was recrystallized from 250 ml. of methanol to yield 12 g. This product was identical to that prepared by method 1.

8-[Bis-(2-hydroxyethyl)-triazeno]-hypoxanthine (II, R_1 = OH, R_2 = H).—Ten grams of 8-amino-6-hydroxypurine[11] was diazotized according to the directions of Jones and Robins,[9] and the filtered 8-diazohypoxanthine was transferred while wet to a flask containing 100 ml. of methanol and 4 g. of diethanolamine. The solution was stirred for 4 hr. at room temperature then diluted with 100 ml. of benzene and refrigerated overnight. The colorless precipitate was filtered, washed with benzene, and dried. The yield was 9.5 g. For analysis a small amount was recrystallized twice from ethanolic hydrogen chloride followed by the addition of acetone. The hydrochloride obtained was dried at 80° *in vacuo* over phosphorus pentoxide. It decomposed at 185–190°.

Anal. Calcd. for $C_9H_{13}N_7O_3 \cdot HCl$: C, 35.6; H, 4.6; N, 32.2. Found: C, 35.5; H, 4.6; N, 31.8.

8-[Bis-(2-chloroethyl)-triazeno]-hypoxanthine (III, R_1 = OH, R_2 = H).—Six grams of crude 8-[bis-(2-hydroxyethyl)-triazeno]-hypoxanthine (II, R_1 = H, R_2 = OH) and 70 ml. of thionyl chloride were refluxed 70 min., then kept at room temperature overnight. Benzene (150 ml.) was then added and the precipitate filtered and washed with 50 ml. of benzene. The crude product was recrystallized from 200 ml. of absolute methanol with the addition of acetone and benzene. The yield was 3.2 g. (53%) of small orange needles. For analysis a small amount was recrystallized twice from a methanol and acetone mixture; m.p. >300°.

Anal. Calcd. for $C_9H_{11}N_7Cl_2O$: C, 35.5; H, 3.6; N, 32.2. Found: C, 35.3; H, 3.8; N, 31.8.

8-[Bis-(2-hydroxyethyl)-triazeno]-xanthine (II, R_1, R_2 = OH).—Twelve grams of 8-diazoxanthine[9] was suspended in 100 ml. of dimethylacetamide containing 12 g. of diethanolamine. After stirring for 15 min. at room temperature the suspended 8-diazoxanthine dissolved, and after 30 min. the product slowly began to precipitate. The stirring was continued for 6 hr.; then 400 ml. of acetone was added and the solution refrigerated overnight. The precipitate was then filtered, washed with acetone, and dried. The yield was 18.5 g. (96%). For analysis a small amount was reprecipitated twice from dilute potassium hydroxide with acetic acid.

Anal. Calcd. for $C_9H_{13}N_7O_4$: C, 38.2; H, 4.6; N, 34.6. Found: C, 38.3; H, 4.9; N, 34.3.

8-[Bis-(2-chloroethyl)-triazeno]-xanthine (III, R_1, R_2 = OH).—8-[Bis-(2-hydroxy-ethyl)-triazeno]-xanthine (II, R_1, R_2 = OH) (5.4 g.) was powdered and slowly added in portions to 50 ml. of thionyl chloride. The reaction started slowly and was complete after 30 min. of refluxing. The mixture was then kept overnight at room temperature; 100 ml. of benzene was added and the precipitate filtered and washed with 50 ml. of benzene. The precipitate was extracted 5 times with 200 ml. of boiling absolute ethanol. The liquid was filtered, treated with charcoal, and concentrated to approximately 500 ml. Upon the careful addition of petroleum ether, 2.5 g. of the desired compound was obtained. For analysis a small amount was recrystallized twice from methanol and acetone.

Anal. Calcd. for $C_9H_{11}N_7O_2Cl_2$: C, 33.7; H, 3.4; N, 30.6. Found: C, 33.7; H, 3.7; N, 30.0.

***p*-Diethanolamino-8-phenylazoxanthine** (VII).—Five grams of 8-diazoxanthine[9] was suspended in 300 ml. of methanol. Then 5 g. of phenyliminodiethanol was added and the mixture stirred for 8 hr. at room temperature. The precipitate, a deep-red dye, was filtered, washed with a little methanol, and dried. The yield was 10.0 g. For analysis a small amount was reprecipitated from dilute potassium hydroxide with dilute acetic acid. At *p*H 1 the compound exhibited λ_{max} 288, 535 mμ, ε 7,900, 33,700.

Anal. Calcd. for $C_{15}H_{17}N_7O_4 \cdot H_2O$: C, 47.7; H, 5.0; N, 25.9. Found: C, 47.7; H, 5.0; N, 25.5.

***p*-Bis-(2-chloroethylamino)-8-phenylazoxanthine** (VIII).—Five grams of VII was added in small portions to 50 ml. of thionyl chloride and the solution refluxed for 30 min. The solution was cooled, allowed to stand overnight, and 100 ml. of benzene was added. The mixture was poured on 500 g. of ice, with stirring. The reddish-purple precipitate was filtered and slurried three times with petroleum ether, filtered, and washed repeatedly with petroleum ether. The product which was extremely difficult to recrystallize was analyzed directly. In methanol the compound exhibited λ_{max} 275, 475 mμ, ε 7,500, 21,400.

Anal. Calcd. for $C_{15}H_{15}N_7O_2Cl_2.HCl.2H_2O$: C, 41.7; H, 4.4; N, 22.7. Found: C, 41.7; H, 4.2; N, 22.8.

8-[Bis-(2-chloroethyl)-triazeno]-guanine (III, R_1 = OH, R_2 = NH_2).—To a solution of 100 ml. of dimethylacetamide and 11.5 g. of diethanolamine was added 11.5 g. of 8-diazoguanine.[9] The solution was stirred for 8 hr. at room temperature, then diluted with 400 ml. of acetone, and placed in the refrigerator overnight. The precipitate was filtered, washed with acetone, and dried to yield 13.6 g. of crude product. This product was finely powdered and added in small portions to 120 ml. of thionyl chloride. The solution was refluxed for 1 hr. on the steam-bath; then 100 ml. of benzene was added and the solution allowed to remain in the refrigerator for 4 days. The precipitate was filtered, washed with benzene, and dissolved in approximately 1 l. of boiling methanol to which had been added approximately 50 ml. of a solution of methanol saturated with dry hydrogen chloride. The solution was treated with charcoal, filtered, and concentrated to 500 ml. under vacuum. The addition of benzene induced crystallization to yield 7.87 g. of a yellow solid. A small sample was recrystallized from a methanol–acetone mixture for analysis.

Anal. Calcd. for $C_9H_{12}N_8OCl_2 \cdot HCl$: C, 30.4; H, 3.8; N, 31.6. Found: C, 30.7; H, 3.7; N, 31.6.

6-Amino-8-chloropurine.[11]—Forty grams of 6-amino-8-purinethiol,[11] 240 ml. of concentrated aqueous hydrochloric acid, and 80 ml. of methanol were cooled to −5 to 0° with an ice-salt-bath. Chlorine gas was bubbled into the stirred slurry for 3 hr. at such a rate that the temperature did not exceed the stated range. At the end of this time a drop of the mixture placed on *p*H paper bleached it within 20 to 30 sec. indicating that there was an excess of free chlorine in the solution and the reaction was complete. The colorless precipitate was filtered in a sintered glass funnel, washed with a little methanol, and transferred into a beaker containing 300–500 ml. of an ice–water mixture. The manually stirred slurry was treated with concentrated aqueous ammonia until the *p*H was 7–8. The precipitate was then filtered, washed with acetone, and air-dried. The yield was 39.5 g. (97%). The product was identified by its ultraviolet absorption spectrum.[11]

6,8-Diaminopurine.—Forty grams of 6-amino-8-chloropurine and 250 ml. of aqueous concentrated ammonia were heated in a pressure reaction vessel for 8 hr. between 150–170°. To the cooled solution was then added 15.0 g. of potassium hydroxide. The solution was gently boiled for 15 min. to remove most of the ammonia, treated with charcoal, and the warm solution adjusted to *p*H 2–3 with hydrochloric acid. On cooling, 23.5 g. of 6,8-diaminopurine monohydrochloride crystallized in sturdy colorless prisms (53%). This product was identified by its ultraviolet absorption spectrum.[11]

8-[Bis-(2-chloroethyl)-triazeno]-adenine (III, R_1 = NH_2, R_2 = H).—Nine grams of 6,8-diaminopurine monohydrochloride[11] was dissolved in 100 ml. of 5% hydrochloric acid and chilled to 5–10°. An aqueous solution of 5.0 g. of sodium nitrite was slowly added to the stirred solution. After 1.5 hr. the precipitate which separated was filtered and washed with 50 ml. of cold methanol. The crude product was then transferred to a solution of 3 ml. of concentrated hydrochloric acid, 150 ml. of water, 100 ml. methanol and 7 g. of β,β'-dichlorodiethylamine hydrochloride at room temperature. The reaction mixture was stirred for 4 hr. then filtered from a small amount of insoluble material. The filtrate was diluted with 1 l. of acetone. The precipitate that separated from the solution was collected after

10 hr. at 5°. After drying at room temperature for 6 hr. the orange precipitate (6 g.) was dissolved in 125 ml. of water at 29°, stirred for 10 min. with Celite, and filtered. The filtrate was again diluted to 1 l. with acetone and allowed to stand at 5° for 15 hr. The bright yellow precipitate was filtered, washed with acetone, and finally with ether. The product was dried and stored over solid sodium hydroxide in a vacuum desiccator.

Anal. Calcd. for $C_9H_{12}N_8Cl_2 \cdot {}^1/_2H_2O \cdot HCl$: C, 31.0; H, 4.0; N, 32.1. Found: C, 31.2; H, 4.5; N, 31.7.

8-[(2-Hydroxyethyl)-thioazo]-hypoxanthine.—The preparation of this compound is illustrative of the preparation of the compounds listed in Table II.

Ten grams of 8-amino-6-hydroxypurine[11] was diazotized[9] and the 8-diazohypoxanthine added to a flask containing 100 ml. of methanol and 5 g. of 2-mercaptoethanol. The solution was stirred at room temperature for 6 hr.; then 150 ml. of benzene was added and the reaction mixture cooled overnight. The precipitate was filtered, washed with benzene, and dried to yield 7.8 g. of yellow-green powder. For purification the product was reprecipitated from very dilute potassium hydroxide solution with acetic acid.

Propionic Acid(3)-[(6-hydroxypurine-8-yl)-azothio].—Ten grams of 8-amino-6-hydroxypurine[11] was diazotized[9] and the damp diazohypoxanthine transferred to a flask containing 100 ml. of methanol and 5.0 g. of 3-mercaptopropionic acid. The reaction mixture was stirred for 6 hr.; then 150 ml. of benzene was added and the solution cooled overnight. The precipitate was filtered, washed with acetone, and dried to give 9.5 g. of crude product. This compound was purified by dissolving in hot sodium bicarbonate solution followed by precipitation with hydrochloric acid. The compound exhibited at *p*H 1, λ_{max} 272, 358 mμ, ε 8,000, 8,000.

Anal. Calcd. for $C_8H_8N_6O_3S$: C, 35.8; H, 3.0. Found: C, 35.6; H, 3.0.

Propionic Acid(3)-[theophylline-8-yl-thioazo].—8-Diazotheophylline[9] (5 g.) was suspended in 250 ml. of acetone. Then 5 g. of 3-mercaptopropionic acid was added and the solution stirred for 3 hr. The yellow precipitate was filtered, washed with acetone, and dried to yield 6.7 g. The product was purified by reprecipitation from warm sodium bicarbonate solution with dilute hydrochloric acid; m.p. 162° dec. The compound exhibited at *p*H 1, λ_{max} 293, 370 mμ, ε 8,700, 7,200.

Anal. Calcd. for $C_{10}H_{12}N_6O_4S$: C, 38.4; H, 3.8; N, 26.8. Found: C, 38.4; H, 4.0; N, 26.5.

[CONTRIBUTION FROM THE VENABLE CHEMICAL LABORATORY, UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL, N. C.]

Kinetics of the Acid-catalyzed Hydrolysis of 4-(*p*-Sulfophenylazo)-1-naphthyl Methyl Ether and 4-(*p*-Sulfophenylazo)-anisole[1]

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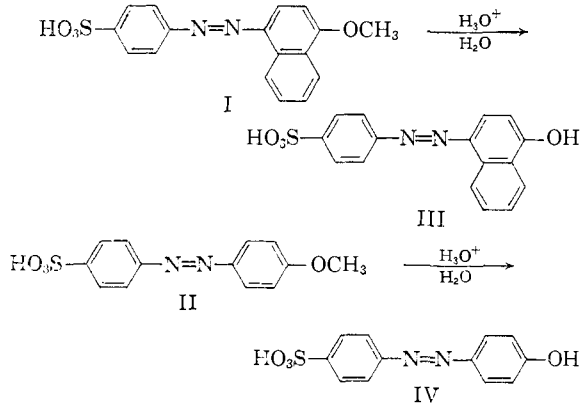
Azonaphthyl ether I is readily hydrolyzed to azonaphthol III. In dilute acid, the reaction is first order in oxonium ion concentration and is specific oxonium ion catalyzed. In concentrated acids, there is a rate maximum at about 2.5 *M* but suitable treatment of the data reveals correlation of rate with oxonium ion concentration. Alternatively, hydrolysis rate is related to the activity of water in concentrated acids by a new procedure. The resulting *w*-values indicate a mechanism in which the slow step is general acid-catalyzed detachment of a methoxy group from intermediate complex VIII. Azophenyl ether II is much less sensitive to hydrolysis. A rate maximum is again observed in concentrated acids, but rate cannot be correlated either with h_0 or with oxonium ion concentration. It is, however, easily related to the activity of water, the *w*-value being +3.1. This suggests a mechanism in which water effects an SN2 displacement on the methyl carbon of protonated II. Incident to the kinetics, pK_a values for I and II have been determined.

The facile acid-catalyzed hydrolysis of azonaphthyl ethers is described in an earlier paper.[3] A kinetic study of this reaction is now presented.

We have studied the kinetics of the hydrolytic cleavage of 4-(*p*-sulfophenylazo)-1-naphthyl methyl ether (I) and of its benzene-series analog, 4-(*p*-sulfophenylazo-anisole (II). These sulfo compounds were chosen because of their solubility in water.



The kinetics were followed by photometric measurements, advantage being taken (as previously[3]) of the great difference in spectra of the azo-ethers I and II and the corresponding azo-phenols III and IV in alkaline solution. In all cases, the acid catalyst was present in large excess over I or II and the kinetics were first order in the azo compound.

4-(*p*-Sulfophenylazo)-1-naphthyl Methyl Ether (I)

Dependence of Rate on Acid Concentration, Dilute Range.—First-order rate coefficients in hydrochloric acid solutions representing a thousand-fold variation in oxonium ion concentration, but in which ionic strength was maintained constant by compensation with potassium chloride, are displayed in Table I. The constancy of the second-order coefficients, $k_\psi/[H_3O^+]$, indicates that the reaction is first order in oxonium ion concentration within this range.

Specific Oxonium Ion *vs.* General Acid Catalysis.—The rate of cleavage was studied as a function of buffer concentration in two series of phosphate buffers. In one series (Table II, part A) the ratio $[H_3PO_4]:[NaH_2PO_4]$ was 1:3 and ionic strength was held constant by compensation with sodium perchlorate; *p*H was virtually constant within this series (the *p*H as measured by a glass electrode was constant, but one reckons a decrease in $[H_3O^+]$ of

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(3) J. F. Bunnett and G. B. Hoey, THIS JOURNAL, **80**, 3142 (1958).