Synthesis and Antimalarial Activity of Heterocyclic Alkyl Disulfides, Thiosulfates, and Dithio Acid Derivatives

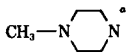
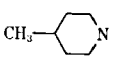
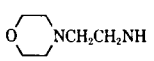
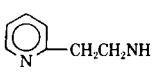
WILLIAM O. FOYE ˣ, JOSEPH J. LANZILLO, YOUNG HEE LOWE, and JOEL M. KAUFFMAN

Abstract □ Based on the antimalarial activity in mice of bis(4-*p*-acetamidobenzenesulfonamidophenyl) disulfide, a series of *N*-heterocyclic alkyl disulfides and thiosulfates was synthesized and screened for antimalarial activity. Several related dithio acid dianions and *S*-blocked derivatives were also screened to provide an indication of the possible role that thiol anions might play in malaria chemotherapy. Activity was limited by toxicity with these compounds, and none of those tested, with the exception of bis(4-*p*-acetamidobenzenesulfonamidophenyl) disulfide, showed curative activity in either a mouse or chick test.

Keyphrases □ Heterocyclic alkyl disulfides, thiosulfates, and dithio acid derivatives—synthesis and antimalarial activity □ Disulfides, heterocyclic alkyl—synthesis and antimalarial activity □ Thiosulfates, heterocyclic alkyl—synthesis and antimalarial activity □ Dithio acid derivatives—synthesis and antimalarial activity □ Antimalarial agents, potential—synthesis, screening of heterocyclic alkyl disulfides, thiosulfates, dithio acid derivatives

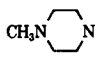
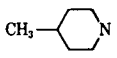
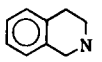
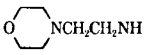
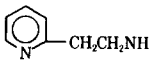
The ability of the disulfides of aminoalkylthiols to bind reversibly to DNA, RNA, and other nucleoproteins has been known for some years (1). The discovery of this ability led to a postulation (2), in regard to radiation protection, that many radioprotective bis(aminoalkyl) disulfides would fit those areas of the DNA helix uncovered by histones and undergo complex formation. The resulting complexes were believed to aid in restorative effects, one being a decrease in rate or temporary cessation of DNA replica-

Table I—*N*-Heterocyclylethanethiols $R-CH_2-CH_2-SH$

R	Boiling Point (mm)	n_D^{25}	Yield, %	Formula	Analysis, % Calc.	Found
	59–60° (1)	1.5061	78	$C_7H_{16}N_2S$	—	—
	45–46° (1)	1.5015	83	$C_7H_{15}NS$	—	—
	73–75° (4)	1.5053	82	$C_6H_{13}NOS$	—	—
	127–129° (1.5)	1.5783	73	$C_{11}H_{15}NS$	C 68.29 H 7.76 N 7.25 S 16.59	68.62 7.51 7.40 16.32
	35–36° (1.5)	1.5033	90	$C_6H_{13}NS$	C 54.89 H 10.00 N 10.67 S 24.43	55.05 9.97 10.16 24.16
	73° (3.5)	1.5010	78	$C_8H_{17}NS$	C 60.31 H 10.79 N 8.79	60.00 10.50 8.35
	58–59° (1.5)	1.4938	80	$C_8H_{17}NS$	C 60.31 H 10.79 N 8.79	59.90 10.60 9.20
	67–71° (3.5)	1.4957	50	$C_8H_{17}NS$	C 60.31 H 10.79 N 8.79	60.50 10.90 8.46
	122–124° (1.5)	1.5130	71	$C_8H_{18}N_2OS$	C 50.48 H 9.55 N 14.72	50.37 9.91 14.71
	130–132° (0.3)	1.5676	63	$C_9H_{14}N_2S$	C 59.89 H 7.76 N 15.37	60.14 7.85 15.59

[a] Reference 5.

Table II—*N*-Heterocyclylethyl Disulfides R–CH_2–CH_2–S–S–CH_2–CH_2–R

R	Salt	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
	4HCl	254–255°	24	$C_{14}H_{30}N_4S_2 \cdot 4HCl$	C 36.21 H 7.41 N 12.06 S 13.79	35.99 7.46 12.22 14.14
	2HCl	270–271°	54	$C_{14}H_{28}N_2S_2 \cdot 2HCl$	C 46.53 H 8.37 N 7.75 S 17.73	46.53 8.18 7.92 17.42
	2HCl	229–230°	60	$C_{12}H_{24}N_2O_2S_2 \cdot 2HCl$	C 39.44 H 7.19 N 7.66 S 17.55	39.54 7.17 7.42 17.48
	2HCl	227–228°	56	$C_{12}H_{24}N_2S_2 \cdot 2HCl$	C 43.22 H 7.88 N 8.40 S 19.23	42.88 7.62 8.20 19.45
	2HCl	235–237°	59	$C_{16}H_{32}N_2S_2 \cdot 2HCl$	C 49.31 H 8.81 N 7.19	49.58 8.71 7.46
	2HCl	239–240°	73	$C_{16}H_{32}N_2S_2 \cdot 2HCl$	C 49.31 H 8.81 N 7.19	49.66 8.59 7.34
		n_D^{25} 1.6064	92	$C_{22}H_{28}N_2S_2$	C 68.59 H 7.54 N 7.54 S 16.67	68.26 7.39 7.49 16.88
		1.5365	64	$C_{16}H_{32}N_2S_2$	C 60.69 H 10.21 N 8.85 S 20.25	60.4 10.5 9.05 19.59
		1.5376	62	$C_{16}H_{34}N_4O_2S_2$	C 50.75 H 9.07 N 14.80 S 16.93	50.3 9.3 14.7 16.5
		1.5918	43	$C_{18}H_{26}N_4S_2$	C 59.62 H 7.24 N 15.46 S 17.68	59.5 7.02 15.09 17.99

tion, allowing repair to be effected before alterations were replicated. It is possible that this postulation may be of value in designing agents effective against malaria parasites, particularly where such compounds may bind more strongly to DNA and halt the replication process.

DISCUSSION

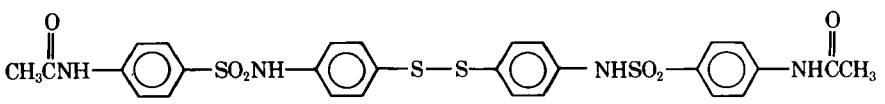
One compound, bis(4-*p*-acetamidobenzenesulfonamidophenyl) disulfide (I), has already been reported (3) that might act through such a mechanism. This compound effected a cure in one of five mice infected with *Plasmodium berghei* when administered at a toxic level (640 mg/kg); subsequent testing data are reported here. To test this idea more thoroughly, a series of *N*-substituted bis(aminoalkyl) disulfides was synthesized. These compounds carry heterocyclic rings that should increase binding ability to nucleic acids.

In addition, to determine whether compounds of this type act through the sulfur function as a thiolating agent, similar structures having thiosulfate groups in place of disulfide, as well as a few dithio acid dianions, were prepared for comparison of antimalarial activity. A thiosulfuric acid derivative of quinine, in which the thiosulfate group is located a distance of two carbons from the basic nitrogen, was recently synthesized and found to have greater activity against *P. berghei* KBG 173 in mice than did quinine (4).

Compounds of the type Het—CH_2CH_2SX were synthesized, where Het refers to a reduced nitrogen-containing heterocyclic ring and X to either hydrogen, a disulfide, or thiosulfate function. The method employed for the mercaptans was mercaptoethylation of the heterocyclic nitrogen using ethylene monothiocarbonate (5). Use of an excess of amine minimized polymerization of ethylene sulfide. All mercaptans obtained showed the loss of NH-stretching peaks and the presence of the weak SH-stretching peak at 2550–2600 cm^{-1}. Yields ranged from 70 to 90%. Physical data are listed in Table I.

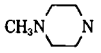
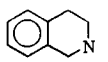
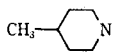
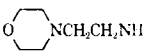
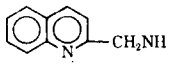
Disulfides were prepared by oxidation of the mercaptans with dimethyl sulfoxide and were generally isolated as the dihydrochlorides. The disulfide dihydrochlorides were obtained as oils, which were crystallized by trituration under anhydrous ether or ethanol. In a few cases the oils could not be crystallized, and the compounds were isolated as the free amines. All disulfides showed loss of the SH-stretching peak in the IR and gave positive nitroprusside tests for a disulfide. Yields ranged from 24 to 92%. Physical data are listed in Table II.

Synthesis of the thiosulfuric acids was accomplished by reaction of the heterocyclic alkyl halides with either sodium or thallous



I

Table III—*N*-Heterocyclylethanethiosulfuric Acids $R-CH_2-CH_2-S_2O_3H$

R	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
	186–187.5°	24	$C_7H_{16}N_2O_3S_2$	C 34.98 H 6.68 N 11.66 S 26.68	34.51 7.44 11.16 26.25
	180–182°	40	$C_7H_{15}NO_3S_2$	C 37.31 H 6.72 N 6.21 S 28.41	37.32 6.70 6.29 28.20
	181–183°	61	$C_6H_{13}NO_4S_2$	C 31.70 H 5.78 N 6.16 S 28.21	32.01 5.70 6.18 28.42
	165–167°	28	$C_6H_{13}NO_3S_2$	C 34.10 H 6.21 N 6.63 S 30.34	34.24 6.19 6.60 30.40
	154–155°	54	$C_{11}H_{15}NO_3S_2$	C 48.32 H 5.54 N 5.12	48.05 5.53 4.92
	162.5–164°	35	$C_8H_{17}NO_3S_2$	C 40.14 H 7.17 N 5.81 S 26.79	40.50 7.31 6.06 26.49
	173–174.5°	62	$C_8H_{17}NO_3S_2$	C 40.14 H 7.17 N 5.81 S 26.79	40.42 7.20 6.04 26.58
	185–185.5°	31	$C_8H_{17}NO_3S_2$	C 40.14 H 7.17 N 5.81 S 26.79	39.89 7.43 5.65 26.82
	158–160°	30	$C_8H_{18}N_2O_4S_2$	C 35.52 H 6.71 N 10.36 S 23.71	35.4 6.8 10.2 23.87
	187–188°	57	$C_{12}H_{14}N_2O_3S_2$	C 48.30 H 4.73 N 9.39	48.3 4.8 9.3

thiosulfate or by reaction of either the mercaptan or disulfide with potassium metabisulfite. 2-(2-Quinolylmethylamino)ethanethiosulfuric acid and 2-(2-*N*-morpholinylethylamino)ethanethiosulfuric acid were prepared by treating 2-chloromethylquinoline and *N*-(2-chloroethyl)morpholine, respectively, with 2-aminoethanethiosulfuric acid. The morpholine derivative was also prepared from the thiol by reaction with potassium metabisulfite. The thiosulfates were generally water soluble and could be recrystallized. Characteristic peaks near 1020, 1170, and 1230 cm^{-1} (6) were exhibited by all thiosulfates; yields ranged from 24 to 63%. Physical data for the thiosulfuric acids are listed in Table III.

The dithio acid dianions were prepared as the dipotassium salts using the procedure of Foye and Kauffman (7), which employed the condensation of carbon disulfide with substituted acetonitriles with *tert*-butoxide as the condensing agent. None of these compounds could be recrystallized or even reprecipitated. One of the more stable of these dimercaptoacrylonitriles, the 2-phenyl derivative, was converted to the 4-phenyl-4-hydroxydithiolane-2-ylidene on condensation with α-bromoacetophenone, using the procedure of Campaigne and Haaf (8). This compound was dehydrated to the corresponding 1,3-dithiole in sulfuric acid at 0°, and the product provided an *S*-blocked derivative of a dithio acid dianion. Other derivatives of 2-cyano-3,3-dimercaptoacrylonitrile in which the sulfur function was blocked, *e.g.*, with methyls or a 2,3-pyrazinyl group, were prepared for comparison of antimalarial activities. The corresponding quinoxaline derivative was also prepared using 2,3-dichloroquinoxaline.

Antimalarial testing was carried out against *P. berghei* in mice according to the method of Osdene *et al.* (9). Results were supplied by the Walter Reed Army Institute of Research. The lead compound for this series, bis(4-*p*-acetamidobenzenesulfonamidophenyl) disulfide (I), was described as curative in this test. A sulfone which was submitted, 2-amino-5-thiazolyl-4-nitrophenylsulfone (II), was found to be active.

Among the disulfides tested, the *N*-pyrrolidylethyl (V) and *N*-3-methylpiperidylethyl (VII) disulfides showed differences in mean survival times from those of the controls (which survived 6.1 days) of 5.3 and 5.8 days, respectively. The corresponding thiosulfuric acids showed mean survival time differences of only 1.3 and 0.2 day, respectively. The other disulfides and thiosulfuric acids, as well as the dithio acid dianions and their *S*-blocked derivatives, showed very little difference in mean survival time over that of the controls (Table IV).

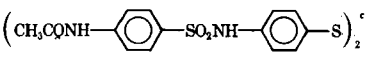
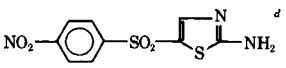
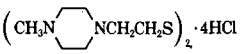
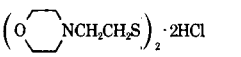
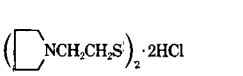
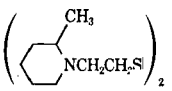
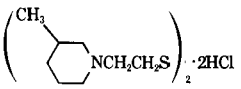
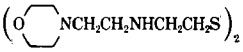
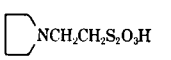
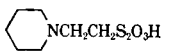
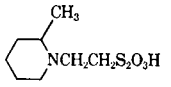
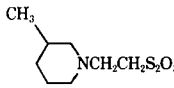
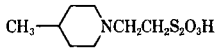
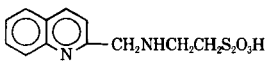
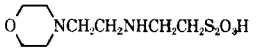
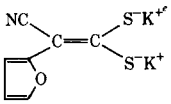
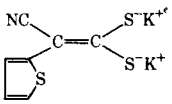
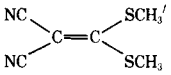
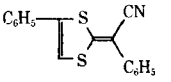
Several compounds were also evaluated for prophylactic action in chicks against *P. gallinaceum* according to the procedure of Rane and Rane (10). This test was uniformly fatal to untreated controls in 72–96 hr. In this test the disulfide (I), curative in the mouse test, showed a difference in mean survival time over controls of only 0.5 day, and the active sulfone (II) gave a mean survival time difference of only 0.4 day (Table V).

EXPERIMENTAL[1]

***N*-Heterocyclylethanethiols**—The cyclic amines were dried over calcium hydride and filtered prior to use. To a refluxing solution of the amine (0.3 mole) in 100 ml of anhydrous toluene was

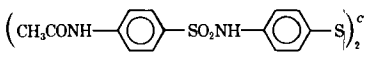
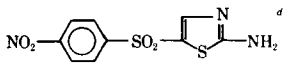
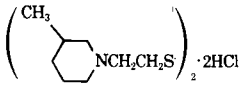
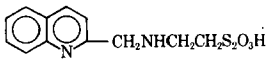
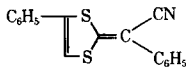
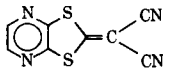
[1] Melting points were determined with a Mel-Temp capillary melting-point block and are uncorrected. IR data were obtained using a Perkin-Elmer model 137B spectrometer with sodium chloride optics. NMR spectra were obtained with a Varian A-60 spectrometer using tetramethylsilane as the external or internal standard. Elemental analyses were done by F. B. Strauss, Oxford, England; Carol K. Fitz, Carlisle, Mass.; or Russell J. Werby, Boston, Mass. TLC was carried out using silica gel, and products were detected by exposure to iodine vapor. The heterocyclic amines were obtained from Aldrich Chemical Co., Eastman Organic Chemicals, or Fisher Scientific Co.

Table IV—Antimalarial Activities in Mice[a]

Number	Compound	Dose, mg/kg	Difference in Mean Survival Time, days (Test Minus Control)[b]
I		40	0.5
		80	4.1
		160	8.4 (a) (t,1)
		320	9.9 (a) (t,1)
		640	20.9 (c,1) (t,3)
II		40	2.3
		80	4.9
		160	6.1 (a)
		320	9.5 (a)
		640	12.9 (a)
III		640	0.4
IV		160	0.8 (t,3)
V		20	0.6
		40	4.0
		80	4.4
		160	5.3 (t,3)
VI		160	0.0
VII		40	0.6
		80	1.2
		160	4.0
		320	5.8
VIII		160	0.0
IX		320	0.0
X		20	0.4
		40	0.4
		320	1.3 (t,3)
XI		160	0.8 (t,3)
XII		160	0.2
XIII		160	0.2
XIV		160	0.4
XV		160	0.4
		320	0.9 (t,3)
XVI		40	0.3
		80	0.9 (t,3)
XVII		320	0.1
XVIII		320	0.5
XIX		40	0.0 (t,5)
XX		160	0.3
		320	1.1

[a] The compounds were tested against *P. berghei* KBG 173 by administration to five male mice at a given concentration in a single subcutaneous dose 72 hr after infection. [b] The mean survival time of the controls was 6.1 days. Deaths during Days 2–5 after drug administration were attributed to toxicity (t, number that died). Compounds were classified as active (a) when the mean survival time of the treated mice was twice that of the controls, *i.e.*, test − control > 6.1 days, and curative (c, number of surviving mice) when test animals lived 60 days postinfection. [c] Reference 3. [d] Source: Aldrich Chemical Co. [e] Reference 7. [f] Reference 12.

Table V—Antimalarial Activities in Chicks[a]

Number	Compound	Dose, mg/kg	Difference in Mean Survival Time, days (Test Minus Control)[b]
I		100	0.5
II		30 60 120	0.4 0.4 0.4
VII		160	0.0
XV		240	0.0
XX		160	0.0
XXI		120	0.4

[a] The compounds were tested against *P. gallinaceum* Brumpt 8A injected into five chicks from heart blood of infected chicks. The compounds, suspended in peanut oil, were administered subcutaneously in a single dose immediately after infection. [b] The mean survival time of the control chicks was 72–96 hr. Deaths that occurred within 48 hr after infection were considered due to toxicity of the compound (t, number that died). An increase of 100% in survival time was recognized as the minimum effective response. Chicks with survival times of 30 days were recorded as cured (c, number of survivors). [c] Reference 3. [d] Source: Aldrich Chemical Co.

added 0.1 mole of ethylene monothiocarbonate dropwise over 20 min. The resulting solution was allowed to reflux overnight, and it was distilled *in vacuo* through a 17.7-cm (7-in.) vigreux column to yield a colorless oil. Physical constants of the mercaptans are listed in Table I.

***N*-Heterocyclylethyl Disulfide Dihydrochlorides**—In a representative procedure, 0.07 mole of mercaptan was dissolved in 25 ml of dimethyl sulfoxide, and the colorless solution was heated at 80–90° for 18–20 hr. The resulting solution was poured into 300 ml of ice water, and the mixture was stirred for 4 hr. The oily disulfide was extracted twice with ether, and the combined extracts were washed with water.

The ether layer was dried over magnesium sulfate, and the ether was removed by flash evaporation to give a clear oil, which was triturated with absolute ethanol to yield a partially solidified oil. To this was added concentrated hydrochloric acid in 95% ethanol, the solvent was removed by flash evaporation, and the resulting hydrochloride was recrystallized twice from 95% ethanol to yield colorless crystals. Physical constants of the disulfide dihydrochlorides are listed in Table II.

***N*-Heterocyclylethyl Disulfides**—In a representative procedure, 0.06 mole of mercaptan was dissolved in 25 ml of dimethyl sulfoxide, and the solution was heated at 85–90° overnight. The resulting solution was poured into 300 ml of ice water, the oily mixture was extracted twice with methylene chloride, and the combined extracts were washed twice with water. The methylene chloride layer was treated with charcoal, dried over magnesium sulfate, and filtered. The solution was flash evaporated to yield a yellow or brown oil. Physical constants of the disulfides are listed in Table II.

2-(*N*-4-Methylpiperazinyl)ethanethiosulfuric Acid—This procedure is representative of the conversion of mercaptans to thiosulfuric acids. 1-(2-Mercaptoethyl)-4-methylpiperazine (7.35 g, 0.046 mole) was dissolved in 60 ml of methanol, and potassium metabisulfite (11.1 g, 0.050 mole) was added with 10 ml of water. The mixture was refluxed overnight and was filtered while hot to remove potassium thiosulfate. The filtrate was flash evaporated to a solid, and the solid was taken up in boiling methanol and filtered. A precipitate of 3.1 g (24% yield) of colorless crystals was obtained on cooling, mp 186–187.5°.

2-(*N*-Piperidyl)ethanethiosulfuric Acid—This procedure is representative of the conversion of halides to thiosulfuric acids. To a solution of *N*-(2-chloroethyl)piperidine hydrochloride (18.4 g, 0.1 mole) in 50 ml of water were added sodium thiosulfate pentahydrate (24.8 g, 0.1 mole) and an additional 50 ml of water. The solution was refluxed for 1 hr and was flash evaporated to give an oil; this oil was taken up in methanol and filtered. The methanol was removed by flash evaporation to give an oil, which was crystallized from 95% ethanol to yield 8.9 g (39.5%) of colorless crystals, mp 180–182°.

2-(2-Quinolylmethylamino)ethanethiosulfuric Acid—To a warm solution of sodium hydroxide in 95% ethanol (2 g in 70 ml) was added rapidly, with stirring, a hot solution of 2-aminoethanethiosulfuric acid (11) (7.85 g, 0.05 mole) in 5 ml of water. To this mixture under reflux was added dropwise 2-chloromethylquinoline (4.44 g, 0.025 mole) in 95% ethanol (50 ml). Heating and stirring were continued for 17 hr, and the mixture was concentrated to about 20 ml and was washed with ether to remove unreacted 2-chloromethylquinoline.

The solution was neutralized with acetic acid and allowed to stand at 0° for 2 days. The resulting semisolid was filtered to give 4.2 g (57%) of product, mp 183–185° (after recrystallization from ethanol–water, mp 187–188°); R_f 0.6 [silica gel plates with *n*-butanol–ethanol–water (3:1:1)]; IR (KBr): 1020, 1185, and 1240 ($S_2O_3^-$) and 3500 (NH) cm^{-1}.

2-(1-Cyanobenzylidene)-4-phenyl-1,3-dithiole—A mixture of 2-phenyl-3,3-dimercaptoacrylonitrile dipotassium salt (7) (23.7 g, 0.082 mole), sodium bicarbonate (16.4 g), and bromoacetophenone (19.9 g, 0.10 mole) was stirred at room temperature for 24 hr. The resulting solid was filtered, and concentrated sulfuric acid (50 ml) was added to the cooled filtrate. This mixture was cooled at 0° for 5 hr, and dilution with water precipitated a yellow solid. The product was filtered, washed with water, and recrystallized from chloroform, giving 5.3 g (22% yield), mp 162–163°; NMR (trifluoroacetic acid): δ 7.38 (5H).

Anal.—Calc. for $C_{17}H_{11}NS_2$: C, 69.60; H, 3.78; N, 4.77. Found: C, 69.85; H, 4.20; N, 4.57.

2-Dicyanomethylenepyrazino[2,3-*d*]dithiole—The dipotassium salt of 2-cyano-3,3-dimercaptoacrylonitrile (7) (7.8 g, 0.033 mole) was dissolved in 50 ml of dimethylformamide, and 2,3-dichloropyrazine (4.44 g, 0.03 mole) was added. The solution was heated at 85° for 16 hr, becoming black. It was diluted with 100 ml of water, cooled to 5°, and filtered. The dried brown powder (2.0 g) was sublimed at 0.5 mm with a free flame, giving 1.1 g (17% yield) of yellow powder, mp 175–205°, and 0.25 g (4%) of yellow needles, mp 212–213°; IR: 2210 (C≡N), 1490, 1342, 1148, 1075 (pyrazine ring), and 850 (CH) cm^{-1}.

Anal.—Calc. for $C_8H_2N_4S_2$: C, 44.02; H, 0 92; N, 25.70; S, 29.38. Found: C, 43.68; H, 0.96; N, 25.41; S, 28.98.

2-Dicyanomethylenequinoxalino[2,3-*d*]dithiole—The dipotassium salt of 2-cyano-3,3-dimercaptoacrylonitrile (7) (7.8 g,

0.033 mole), 2,3-dichloroquinoxaline (6.0 g, 0.03 mole), methanol (200 ml), and water (20 ml) were heated under reflux for 2 hr, diluted with 150 ml of water, and filtered. The dried solid was recrystallized from 250 ml of dimethylformamide and dried at 140° (0.2 mm) for 2 hr to give 3.2 g (25%) of bright-yellow crystals, mp 298–320° dec.; IR: 2210 (C≡N), 1505, 1180, and 1120 (quinoxaline ring) cm^{-1}.

Anal.—Calc. for $C_{12}H_4N_4S_2$: C, 53.71; H, 1.50; N, 20.88; S, 23.90. Found: C, 54.07; H, 1.74; N, 20.5; S, 23.6.

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Bisulfite-Ion-Catalyzed Degradation of Fluorouracil

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Abstract □ 5-Fluorouracil (I) reversibly adds bisulfite ion across its $>C_5{=}C_6<$ bond to form 5-fluoro-5,6-dihydrouracil-6-sulfonate (II). The pH-independent equilibrium constant for this reaction was calculated to be 560 M^{-1} at ionic strength 1.00 M at 25°. Compound II was observed to be unstable in alkaline solution and reacted to yield uracil-6-sulfonic acid, fluoride ion, and α-fluoro-β-ureidopropionic acid-β-sulfonate (III), along with I (*via* a loss of HSO_3^-). In strongly alkaline conditions, *i.e.*, 1 M NaOH, III was observed to undergo a subsequent reaction to produce a compound believed to be α-fluoro-β-ureidoacrylic acid (V) or fluoromalonaldehydic acid (VII). These irreversible degradation reactions of II lead to a complete degradation of I in sodium bisulfite solutions. The similarity of this bisulfite-ion-catalyzed degradation of I to its hydrolytic degradation is discussed.

Keyphrases □ Fluorouracil—bisulfite-ion-catalyzed degradation □ Sodium bisulfite—role in degradation of 5-fluorouracil, nucleophilic addition □ Equilibrium—bisulfite ion addition to fluorouracil

It has been reported (1) that the antimetabolite 5-fluorouracil (I) undergoes a reversible, covalent addition of bisulfite ion (HSO_3^-) across its 5,6-carbon–carbon double bond to yield 5-fluoro-5,6-dihydrouracil-6-sulfonate (II). Additional findings that II undergoes irreversible reactions in sodium bisulfite solutions leading to complete degradation of I (Scheme I) are now reported.

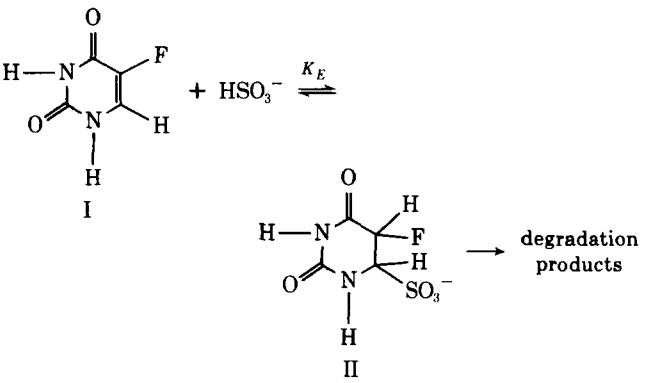
Although I is comparatively stable at room temperature in aqueous buffers that do not contain sodium bisulfite[1], it has been observed to degrade at higher temperatures and in alkaline conditions (2). It has been suggested that the first step in these reactions is covalent hydration of the 5,6-double bond of I to produce 5- or 6-hydroxy-5-fluoro-5,6-dihydrouracil, which then degrades to nonchromophoric products (*via* opening of the pyrimidine ring) (2).

The results of the present study are discussed in terms of similarities between the water-catalyzed and bisulfite-ion-catalyzed degradations of I.

EXPERIMENTAL

Materials and Equipment—Fluorouracil[2] was used as received. All water was redistilled from a Pyrex apparatus before use. All other chemicals were a reagent grade and were used without additional purification.

Potassium 5-fluoro-5,6-dihydrouracil-6-sulfonate monohydrate



Scheme I

[1] Fluorouracil is formulated for injection as a pH 9 solution (adjusted with sodium hydroxide) at a concentration of 50 mg/ml. Fluorouracil package insert, Roche Laboratories, Dec. 1972.

[2] Sigma Chemical Co.