Antiradiation Compounds XVI: *N*-Heterocyclic Aminoethyl Disulfides and Aminoethanethiosulfuric Acids

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

Abstract □ A series of *N*-heterocyclic and *N*-heterocyclic alkyl derivatives of bis(2-aminoethyl) disulfide and aminoethanethiosulfuric acid was synthesized as potential antiradiation and anticancer agents. The compounds were prepared by the reactions of the heterocyclic halides with bis(2-aminoethyl) disulfide and aminoethanethiosulfuric acid. A dithio acid derivative, 3,3-dimercapto-2-cyanoacryloylpyrrolidide, was also prepared. Several compounds, including the dithio acid derivative, provided good radiation protection to mice. None of the compounds screened showed appreciable anticancer activities in two leukemia systems.

Keyphrases □ Antiradiation compounds, potential—series of *N*-heterocyclic aminoethyl disulfides and aminoethanethiosulfuric acids synthesized and screened □ Nitrogen heterocycles—series of *N*-heterocyclic aminoethyl disulfides synthesized and screened for antiradiation activity □ Disulfides, *N*-heterocyclic aminoethyl—series synthesized and screened for antiradiation activity □ Thiosulfuric acids, aminoethane—series synthesized and screened for antiradiation activity □ Radioprotective agents, potential—series of *N*-heterocyclic aminoethyl disulfides and aminoethanethiosulfuric acids synthesized and screened

Perhaps the most important single cellular event leading to radiation protection is the repair of DNA along with prevention of its breakdown. In the case of the radioprotective aminothiols, the ability of their disulfides to bind reversibly to DNA is known (1). This ability, according to Brown (2), can result in two restorative effects:

1. The loose ends of the helix resulting from single-strand rupture are held in place so that shortening or alteration of the chain is prevented.
2. The replication rate of DNA is diminished or halted so that repair can take place before radiation-induced alterations are replicated.

With the possibility that the presence of nitrogen heterocycles in the aminoalkylthiol structure might provide stronger binding to DNA, and hence increase radiation protective ability, a series of heterocyclic aminoethyl disulfides $(HetNHCH_2CH_2S)_2$ and thiosulfates $(HetNHCH_2CH_2S_2O_3H)$ was synthesized as potential radiation protective agents.

It is also possible that such compounds, with sufficiently strong binding to DNA, may have anticancer or antimalarial activities. Some evidence of the latter activity from compounds of this general type has been found (3).

DISCUSSION

Chemistry—Mercaptoethylation of the aromatic heterocyclic amines employed, using ethylene sulfide or ethylene monothiocarbonate, as a means of obtaining the desired heterocyclic aminoethyl disulfides and thiosulfates was unsuccessful. The disulfides were obtained by heating the heterocyclic halides with cystamine [bis(2-aminoethyl)disulfide, free base] in 1-propanol with a slight excess of base until the reaction was complete (one spot on TLC) or showed evidence of disulfide cleavage by a positive nitroprusside test for thiol. This procedure was superior to the use of cystamine dihydrochloride in the presence of a base, such as potassium carbonate or triethylamine, where appreciable cleavage of the disulfide was observed. However, the latter procedure was successful in several instances. Reaction of a chloromethyl heterocycle, 2-chloromethylpyridine, gave the bis(*N,N*-disubstituted) cystamine derivative. The synthesis of 2-(2-quinolylmethylamino)ethyl disulfide could not be obtained by direct reaction with cystamine.

Synthesis of the heterocyclic aminoethanethiosulfuric acids was accomplished by reaction of the heterocyclic halides with 2-aminoethanethiosulfuric acid in the presence of alkali. Yields ranged from 30 to 57%. Reaction with 2-chloromethyl-1,4-benzodioxane gave the bis(*N,N*-disubstituted) aminoethanethiosulfuric acid. One thiosulfuric acid derivative, 2-(2-quinoxalinyl)aminoethanethiosulfuric acid, was prepared from the corresponding disulfide by the method of Lecher and Hardy (4), using potassium metabisulfite and dimethyl sulfoxide. Characteristic IR peaks for $S_2O_3^-$ absorption near 1180 and 1240 cm^{-1} were shown by all of the thiosulfuric acids.

Several dithio acid dianions were prepared (3) from the condensation of substituted acetonitriles with carbon disulfide and isolated as the dipotassium salts using a literature procedure (5). Compounds of this type have shown some radiation protective properties and could act similarly to the aminothiols or possibly as thiolating agents. These compounds are stable on storage when dry but cannot be recrystallized or even reprecipitated without decomposition. Reaction of carbon disulfide with cyanoacetylpyrrolidine gave a product for which a satisfactory carbon analysis could not be obtained, as was the case with previous compounds of this type (5). Conversion of the product to the 1,3-dithiolane and 1,3-dithiole derivatives, however, gave products with satisfactory analyses.

These dimercaptoacrylonitriles (see also Ref. 3) were converted to the corresponding 1,3-dithiole derivatives, using bromoacetophenone, according to a reported procedure (6). Attempts to oxidize the 1,3-dithioles to the corresponding dithiolium salts led to decomposition, apparently due to the presence of the cyano groups.

Biological Testing Results—Testing data[1] were reported for several dosage levels of the test compounds administered by intraperitoneal and oral routes in mice. Dosage levels giving some protection and the highest dosage levels for inactive compounds are reported here (Table I). The following compounds provided good protection against ionizing radiation, conferring better than 45% survival of treated mice over a 30-day observation period: 2-(4-methyl-1-piperazinyl)ethyl disulfide (V), 2-(*N*-morpholinyl)ethyl disulfide (VI), 2-(2-quinoxalinylamino)ethanethiosulfuric acid (IX), and 3,3-dimercapto-2-cyanoacryloylpyrrolidide (XVIII).

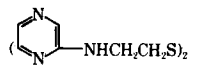
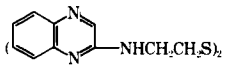
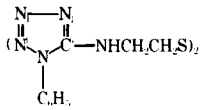
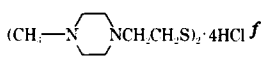
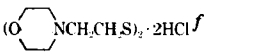
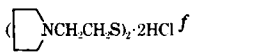
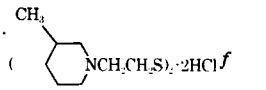
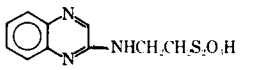
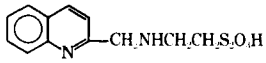
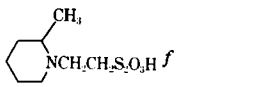
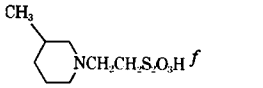
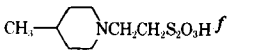
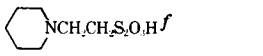
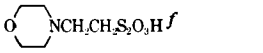
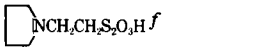
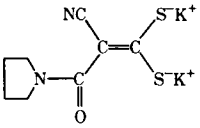
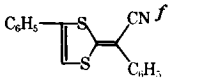
The disulfide of the quinoxalinylcysteamine (II) and the thiosulfate of the morpholinylcysteamine (XVI) gave either slight or no protection. This finding indicates that corresponding disulfides and thiosulfates do not necessarily protect against radiation damage in similar fashions, although thiosulfates are known to be readily converted to disulfides (7). One compound that gave good protection, XVIII, provides another example of a radiation protective compound lacking a basic nitrogen atom but containing a thiol anion in conjunction with an electron-rich heterocycle (8).

In antitumor tests[2], only small differences between mean survival times of treated and control animals or weight differences were found, none being large enough to warrant further testing (Table II).

[1] Tests of radiation protective properties of several of these and structurally related compounds, previously reported (3), were carried out at the Walter Reed Army Institute of Research.

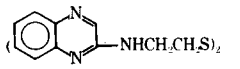
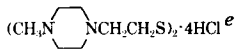
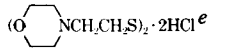
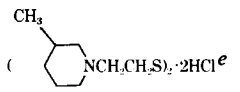
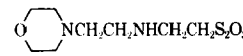
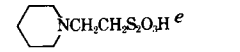
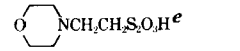
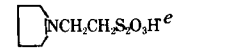
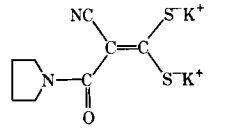
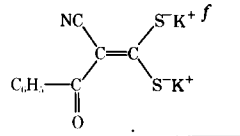
[2] Antitumor tests, using two leukemia systems, were carried out for some of these compounds by the Division of Cancer Treatment, National Cancer Institute, in accordance with their protocol (9).

Table I—Radiation Protective Activities in Mice[a]

Number	Compound	LD_{50}[b], mg/kg	Route[c]	Drug Dose, mg/kg	Minutes[d]	Survival, %[e]
I		150 600+	ip po	100 600	30 30	0 0
II		600+ 600+	ip ip po	200 600 600	30 30 30	20 10 10
III		125 600+	ip po	50 600	30 30	10 0
V	 [f]	140 600+	ip po	80 600	15 30	60 0
VI	 [f]	300 900+	ip ip po po	20 100 300 600	30 30 30 30	50 20 20 40
VII	 [f]	80 350	ip ip po po	20 20 100 200	15 30 30 30	0 10 10 20
VIII	 [f]	80 300+	ip ip po po po	30 60 50 100 300	30 30 30 30 30	10 10 10 20 22[g]
IX		400+ 600+	ip ip po	200 400 600	30 30 30	30 70 0
X	 [f]	130 200	ip ip po	50 100 100	30 30 30	20 0 0
XI	 [f]	80 75	ip po	50 100	30 30	0 0
XII	 [f]	80 300	ip ip po	50 50 200	15 30 30	0 0 10
XIII	 [f]	230 600	ip ip po po	50 100 150 300	30 30 30 30	20 10 20 10
XIV	 [f]	150 550	ip po	65 200	15 30	20 20
XV	 [f]	115 300+	ip po	60 180	15 30	20 0
XVI	 [f]	500 400+	ip po	100 400	15 30	0 0
XVII	 [f]	100 300	ip po	70 300	15 30	0 0
XVIII		600+ 600+	ip ip po	300 600 600	30 30 30	10 80 30
XIX	 [f]	280	ip ip	25 50	30 30	10 0

[a] Radiation dosage was 849 rads (a lethal dose to mice) from a cesium-137 γ-irradiator given at a rate of 141.5 rads/min. For each drug screened, 10 mice were used at each dosage level; 10 mice were used for the vehicle controls. [b] Mortality was determined 10 days after a single dose. [c] Compounds were administered by the indicated route in either water, physiological saline, or 0.3% methylcellulose–0.1% polysorbate (Tween 80) vehicle. Solutions ranged from 0.2 to 3.0%, and the pH was generally 3.5–5.5. [d] The number of minutes preirradiation at which the drug was administered. [e] Calculated from the number of surviving mice at 30 days postirradiation. Good protection was considered with >45% survival, fair protection with 25–44% survival, and slight protection at 1–24% survival. [f] Reference 3. [g] Nine mice were used.

Table II—Antitumor Activities in Mice

Number	Compound[a]	Tumor[b]	Dose, mg/kg	Animal Weight Difference (T − C), g[c]	Survival (T/C), %[d]
II		LE PS	400 200	−2.9 −1.4	87 86
IV		LE	200	−0.8	97
V	 [e]	LE PS	100 200	−0.9 −1.3	105 109
VI	 [e]	LE PS	200 100	−3.1 −0.3	104 113
VIII	 [e]	LE PS	12.5 25	−2.1 −0.7	104 113
XI	 [e]	LE PS	25 12.5	−0.2 −0.2	107 110
XV	 [e]	LE PS	50 12.5	−2.0 −0.8	104 110
XVI	 [e]	LE PS	200 200	−0.9 −0.9	106 115
XVII	 [e]	LE PS	12.5 50	−0.4 −0.4	107 110
XVIII		LE PS	400 200	−0.1 −0.2	103 100
XX	 [f]	LE PS	100 50	−0.5 −1.1	102 109

[a] Compounds were administered intraperitoneally every 4th day for the LE tumor system, for a total of three injections, and every day for the PS tumor system, for a total of nine injections, beginning on the 1st day after tumor implantation. The vehicle used was either saline or saline with polysorbate (Tween 80). Six mice were used at each dosage level. [b] LE refers to L-1210 lymphoid leukemia; PS refers to P-388 lymphocytic leukemia. [c] Average weight change of test group minus average weight change of control animals as determined on Day 5. [d] Ratio of mean survival time of treated animals to that of control animals. Observations were made for 20 days. [e] Reference 3. [f] Reference 5.

EXPERIMENTAL[3]

2-Aminoethyl Disulfide—2-Aminoethyl disulfide dihydrochloride was prepared by the procedure of Johnston and Gallagher (10). The free base was obtained by neutralization with 10% aqueous sodium hydroxide followed by evaporation to dryness *in vacuo* at 50°. The residue was extracted several times with chloroform, and the combined extracts were evaporated to dryness under reduced pressure, giving 92–95% of straw-colored oil; NMR (CF_3CO_2H): 2.45 (t, CH_2S) and 3.50 (t, CH_2N).

2-(2-Pyrazinylamino)ethyl Disulfide (I)—2-Chloropyrazine (17.2 g, 0.15 mole) in 1-propanol (25 ml) was added to a stirred mixture of 2-aminoethyl disulfide (14.1 g, 0.092 mole), anhydrous potassium carbonate (24.5 g, 0.17 mole), and 1-propanol (70 ml). The mixture was refluxed overnight and evaporated to dryness under reduced pressure. The residue was suspended in water (100 ml) and brought to pH 7 with 1 *N* hydrochloric acid. The oil that separated was extracted with benzene (4 × 50 ml), and the benzene extract was washed with water, treated with activated carbon[4], and dried over sodium sulfate.

The solution was evaporated to dryness under reduced pressure, the residue was triturated with small amounts of ethanol and water, and the triturate was chilled. A yellow crystalline product was obtained, and a second crop resulted on adding water to the concentrated filtrate, giving 9.65 g (31%), mp 154–155°; R_f [ethyl acetate–chloroform–ethanol (5:4:1)] 0.37; IR (KBr): 3290 (NH), 1585, 1340 (pyrazine ring), and 1220 (CH_2S) cm^{-1}; NMR (CH_3SOCH_2D): δ 2.55 (t, CH_2S) and 3.58 (t, CH_2N).

Anal.—Calc. for $C_{12}H_{16}N_6S_2$: C, 46.73; H, 5.23; N, 27.25; S, 20.79. Found: C, 47.00; H, 5.09; N, 26.93; S, 20.51.

2-(2-Quinoxalinylamino)ethyl Disulfide (II)—2-Chloroquinoxaline (24.7 g, 0.15 mole) in 1-propanol (20 ml) was added to a stirred mixture of 2-aminoethyl disulfide (14.5 g, 0.095 mole) and anhydrous potassium carbonate (24.5 g, 0.17 mole) in 1-propanol (80 ml). The reaction was carried out as in the preceding preparation, and 30.7 g (50%) of yellow crystals were obtained, mp 156–158°; R_f [chloroform–ethyl acetate (2:3)] 0.2; IR (KBr): 3300 (NH), 1590, 1240 (CH_2S), and 750–760 (quinoxaline ring) cm^{-1}.

Anal.—Calc. for $C_{20}H_{20}N_6S_2$: C, 58.80; H, 4.93; N, 20.57; S, 15.70. Found: C, 58.69; H, 4.80; N, 20.33; S, 16.20.

2-[5-(1-Phenyl-1*H*-tetrazolyl)amino]ethyl Disulfide (III)—To a stirred mixture of 2-aminoethyl disulfide (9.18 g, 0.06 mole) and anhydrous potassium carbonate (13.82 g, 0.1 mole) in 1-propanol (100 ml) was added 5-chloro-1-phenyl-1*H*-tetrazole (16.26 g, 0.09 mole) in 1-propanol (60 ml), and the resulting mixture was refluxed overnight. The cooled reaction mixture was diluted with water, and a crude product separated. The filtered product was dissolved in dimethyl sulfoxide and treated with activated carbon. Addition of water precipitated a white solid, mp 236–237°; IR (KBr): 3300 (NH), 1585, 1235 (CH_2S), and 1090 (tetrazole ring) cm^{-1}.

[3] Melting points were determined with a Mel-Temp capillary melting-point block and are uncorrected. IR spectra were measured using a Perkin-Elmer model 137B or 237B spectrophotometer. NMR spectra were obtained with a Varian A-60 spectrometer, using tetramethylsilane as the external or internal standard. TLC was carried out using silica gel, and products were detected by exposure to iodine vapor. Elemental analyses were done by F. B. Strauss, Oxford, England, or by Carol K. Fitz, Carlisle, Mass. Heterocyclic amines and other reagents were purchased from Aldrich Chemical Co., Eastman Organic Chemicals, and Fisher Scientific Co.

[4] Norite.

Anal.—Calc. for $C_{18}H_{20}N_{10}S_2$: C, 49.07; H, 4.58; N, 31.79; S, 14.56. Found: C, 48.8; H, 4.6; N, 31.5; S, 14.9.

2-(2-Pyrimidinylamino)ethyl Disulfide (IV)—To a stirred mixture of 2-aminoethyl disulfide (14.65 g, 0.096 mole), anhydrous potassium carbonate (24.5 g, 0.170 mole), and 1-propanol (75 ml) was added 2-bromopyrimidine (23.85 g, 0.15 mole), and the resulting suspension was refluxed for 6 hr. The reaction mixture was cooled and diluted with water (750 ml) to give a crude product which was vacuum dried over phosphorus pentoxide. Recrystallization from ethanol and activated carbon gave yellow crystals (71% yield), mp 166–167°; R_f [ethyl acetate–chloroform–methanol (5:4:1)] 0.45; IR (KBr): 3270 (NH), 1595, 1220 (CH_2S), 1010, and 800 (pyrimidine ring) cm^{-1}.

Anal.—Calc. for $C_{12}H_{16}N_6S_2$: C, 46.73; H, 5.23; N, 27.25; S, 20.79. Found: C, 46.48; H, 5.03; N, 26.98; S, 20.50.

2-[Bis(2-pyridylmethyl)amino]ethyl Disulfide—To a stirred mixture of 2-aminoethyl disulfide (1.5 g, 0.010 mole), anhydrous potassium carbonate (3.25 g, 0.020 mole), and 1-propanol (40 ml) was added 2-chloromethylpyridine (2.59 g, 0.020 mole), and the mixture was refluxed for 5 hr. The reaction mixture was cooled to 0° and filtered, and the filtrate was evaporated to 15 ml and diluted with water. A solid product resulted after standing at room temperature for 3 days. It was recrystallized from ethanol and water and dried *in vacuo* over phosphorus pentoxide, giving 0.94 g (9%) of tan solid, mp 76–77°; R_f [ethyl acetate–chloroform (3:2)] 0.1; IR (KBr): 1585, 1370, 1250 (CH_2S), and 780 (pyridine ring) cm^{-1}.

Anal.—Calc. for $C_{16}H_{22}N_4S_2$: C, 65.08; H, 6.25; N, 16.26. Found: C, 65.0; H, 6.6; N, 16.4.

2-Aminoethanethiosulfuric Acid—A mixture of 2-bromoethylamine hydrobromide (20.49 g, 0.1 mole) and thallous thiosulfate (11) (52.08 g, 0.1 mole) in water (100 ml) was stirred vigorously for 24 hr. The precipitated thallous bromide was filtered and washed with water, and the filtrate was evaporated under reduced pressure at 50°. The resulting syrup was dissolved in a small amount of water and crystallized by addition of methanol to give 12.5 g (80%) of white crystals, mp 196–198° [lit. (4) mp 195–196°]; R_f [1-butanol–ethanol–water (3:1:1)] 0.5; NMR (CH_3SOCH_2D): δ 3.33 (m, NCH_2CH_2S).

2-[Bis(2-benzimidazolylmethyl)amino]ethanethiosulfuric Acid—To a warm solution of sodium hydroxide (4 g, 0.1 mole) in 95% ethanol (50 ml) was added rapidly, with stirring, a hot solution of 2-aminoethanethiosulfuric acid (15.7 g, 0.1 mole) in water (10 ml). To the refluxing mixture was added dropwise 2-chloromethylbenzimidazole (13.7 g, 0.08 mole) in 60 ml of ethanol during 2 hr. Heating and stirring were continued for 48 hr, and the reaction mixture was evaporated. The residue was washed with ether and then acidified to pH 5 with acetic acid. The resulting organic layer was separated from the aqueous phase, and addition of a small amount of ethanol followed by water precipitated a solid.

The product was collected after standing at room temperature for a day, yielding 6.4 g (46%) of crude material. This material was dissolved in ethanol and treated with activated carbon. Addition of water and chilling overnight gave white crystals which were dried over phosphorus pentoxide, mp 128–129°; R_f [1-butanol–ethanol–water (3:1:1)] 0.6; IR (KBr): 3400 (NH), 1620, 1235, 1175 (S_2O_3), 1020, and 750 (benzimidazole ring) cm^{-1}.

Anal.—Calc. for $C_{18}H_{19}N_5O_3S_2$: C, 51.78; H, 4.59; N, 16.77. Found: C, 51.6; H, 5.0; N, 16.3.

2-[2-(1,4-Benzodioxanyl)methylamino]ethanethiosulfuric Acid—To a warm solution of sodium hydroxide (1 g, 0.025 mole) in 95% ethanol (30 ml) was added rapidly, with stirring, a hot solution of 2-aminoethanethiosulfuric acid (3.8 g, 0.025 mole) in water (3 ml). To the refluxing mixture was added dropwise 2-chloromethyl-1,4-benzodioxane (2.8 g, 0.015 mole) in ethanol (15 ml), and heating and stirring were continued for 24 hr. After removal of the solvent, 30 ml of water was added; this solution was neutralized with acetic acid and extracted with ether to remove unreacted starting material.

The resulting mixture was stored at 0° for 2 days, and a solid was collected. A second crop was obtained from the mother liquor by evaporation, addition of methanol to the residue, removal of unreacted 2-aminoethanethiosulfuric acid, concentration of the solution, and addition of water. A combined yield of 1.35 g (30%) was obtained which was recrystallized from aqueous ethanol, mp 227–229°; R_f [1-butanol–ethanol–water (3:1:1)] 0.6; IR (KBr): 3450 (NH), 1575, 1180–1240 (S_2O_3), 1025, and 755 (benzodioxane ring) cm^{-1}.

Anal.—Calc. for $C_{11}H_{15}NO_5S_2$: C, 43.26; H, 4.95; N, 4.58. Found: C, 43.2; H, 4.9; N, 4.5.

2-(2-Quinoxalinylamino)ethanethiosulfuric Acid (IX)—A solution of potassium metabisulfite (4 g, 0.036 mole) in hot water (3 ml) was added to a solution of 2-(2-quinoxalinylamino)ethyl disulfide (3.68 g, 0.009 mole) in methanol (75 ml) and dimethyl sulfoxide (15 ml). The mixture was refluxed overnight under nitrogen, and potassium hydroxide (0.51 g, 0.009 mole) in methanol (5 ml) was added to convert excess metabisulfite to sulfite. The mixture was filtered while hot, and the residue was washed with hot methanol. The filtrate was evaporated under reduced pressure and neutralized with sulfuric acid.

After removal of potassium sulfate, the methanol solution was concentrated *in vacuo*, and addition of water gave a solid product. Recrystallization from methanol–water produced yellow crystals, 0.85 g (35%), mp 192–194°; R_f [ethyl acetate–chloroform–ethanol (5:4:1)] 0.75; IR (KBr): 3500 (NH), 1600, 1160–1210 (S_2O_3), 1025, and 765 (quinoxaline ring) cm^{-1}.

Anal.—Calc. for $C_{10}H_{11}N_3O_3S_2$: C, 42.09; H, 3.89; N, 14.73. Found: C, 42.0; H, 4.2; N, 14.4.

3,3-Dimercapto-2-cyanoacryloylpyrrolidide (XVIII)—Metallic potassium (3.9 g, 0.1 mole) was dissolved in 2-methyl-2-propanol (85 ml) under dry nitrogen, with stirring, at 100° during 4 hr. 1-Cyanoacetylpyrrolidine (6.9 g, 0.05 mole) in absolute ethanol (30 ml) was added in one portion at 25°, and carbon disulfide (3.8 g, 0.05 mole) in anhydrous ether (30 ml) was added dropwise at 5–7° during 30 min. Stirring was continued for 15 hr at room temperature, and the yellow precipitate was filtered, washed with anhydrous ether, and dried over phosphorus pentoxide to give 14.7 g (90%), mp ~140°.

Anal.—Calc. for $C_8H_8K_2N_2OS_2 \cdot \frac{1}{2}C_4H_9OH$: N, 8.52; S, 19.58. Found: N, 8.5; S, 19.98.

2 - (2 - Cyanoacetopyrrolidide - 2 - ylidene)-4-phenyl-4-hydroxy-1,3-dithiolane—To a solution of XVIII (3.25 g, 0.010 mole) in ethanol (100 ml) and dimethylformamide (10 ml), a solution of sodium bicarbonate (1.6 g) and bromoacetophenone (1.99 g, 0.010 mole) in ethanol (20 ml) was added. After the mixture was stirred for 24 hr at room temperature, a precipitate was removed. The filtrate was evaporated to dryness under reduced pressure, and the gummy residue was triturated with ether to remove unreacted bromoacetophenone. The residue was dissolved in a small amount of ethanol, and addition of water precipitated a bright-yellow solid (1.3 g). An additional 0.2 g was obtained from the mother liquor, giving a yield of 45%. Recrystallization was accomplished from 1-propanol and 2-propanol after treatment with activated carbon, and the product was dried *in vacuo* over phosphorus pentoxide, mp 217–219°; IR (KBr): 3400–3500 (OH), 2200 (C≡N), and 1590 (C=O) cm^{-1}.

Anal.—Calc. for $C_{16}H_{16}N_2O_2S_2$: C, 57.81; H, 4.85; N, 8.46; S, 19.25. Found: C, 57.6; H, 5.0; N, 8.70; S, 19.41.

2-(2-Cyanoacetopyrrolidide-2-ylidene)-4-phenyl-1,3-dithiole—A solution of 2-(2-cyanoacetopyrrolidide-2-ylidene)-4-phenyl-4-hydroxy-1,3-dithiolane (1 g) in concentrated sulfuric acid (15 ml) was poured into chilled ethanol (50 ml) at a rate that did not allow the temperature to exceed 20°. After the solution stood at room temperature for a few hours, water was added and a yellow solid precipitated. The crude product was filtered, washed with water, and dried *in vacuo* over phosphorus pentoxide. It was dissolved in 1-propanol, treated with activated carbon, and concentrated to give yellow plates (0.54 g, 55%), mp 217–218°; R_f [ethyl acetate–chloroform–ethanol (6:3:1)] 0.7; NMR (CH_3SOCH_2D): δ 7.5 (m, 5H, aromatic H).

Anal.—Calc. for $C_{16}H_{14}N_2OS_2$: C, 61.12; H, 4.49; N, 8.91. Found: C, 60.7; H, 4.9; N, 8.6.

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[x] To whom inquiries should be directed.

Gravimetric Determination of Chlorhexidine Using Tetraphenylborate Ion

SERGIO PINZAUTI [x], VITTORIO DAL PIAZ, and ENZO LA PORTA

Abstract □ A precise and accurate gravimetric procedure was developed for the determination of chlorhexidine diacetate, digluconate, or dihydrochloride. Sodium tetraphenylborate solution was the precipitant in an acidic medium (pH 1). Tablets containing both chlorhexidine diacetate and benzocaine also were assayed.

Keyphrases □ Chlorhexidine—gravimetric analysis, tetraphenylborate as precipitant, pharmaceutical formulations □ Gravimetry—analysis, chlorhexidine in pharmaceutical formulations, tetraphenylborate as precipitant □ Tetraphenylborate—use as precipitant in gravimetric analysis of chlorhexidine □ Bactericidals, topical—chlorhexidine, gravimetric analysis, pharmaceutical formulations

Chlorhexidine is a potent topical bactericidal, effective in high dilutions (1). Presently, it is the most efficient drug for the inhibition of dental bacterial plaque (2–4). Chlorhexidine is employed as the digluconate (5), dihydrochloride (6), and diacetate (7), alone and in combinations with neomycin, cetrimide, or benzocaine in liquid and solid pharmaceutical dosage forms.

A literature review showed that few analytical methods (colorimetry and high-pressure liquid chromatography) were used to determine chlorhexidine quantitatively (8–10). The British Pharmacopoeia offers a nonaqueous method, with glacial acetic acid as the solvent system for the analysis of chlorhexidine digluconate aqueous solution (20% w/v) and chlorhexidine dihydrochloride (5, 6). The procedure for the digluconate involves preliminary evaporation to low bulk of a weighed aliquot of the solution; no monograph is provided for pharmaceutical dosage forms.

This report describes the gravimetric analysis of chlorhexidine salts (gluconate, hydrochloride, and acetate) in aqueous acid solution. Sodium tetraphenylborate, a compound that has found extensive application as a reagent for potassium as well as for the identification and determination of organic bases (11), is used as a precipitant. The method also was applied to tablets containing chlorhexidine diacetate and benzocaine in combination.

EXPERIMENTAL

Reagents—The chlorhexidine salt samples were the highest grades of commercially available materials and were used without further purification. Tablets were prepared in house, and their composition was similar to a product[1] marketed in Italy.

For the sodium tetraphenylborate[2] solution (0.6% w/v), an appropriate amount (purity of 99.5%) was dissolved in water and stabilized according to Cooper (12) at pH 8–9.

Procedures—*Chlorhexidine Diacetate or Digluconate*—Weigh accurately about 30–60 mg of chlorhexidine diacetate, or pipet 2 ml of 2% (w/v) chlorhexidine digluconate solution. Transfer into a 150-ml beaker and dissolve with 20 ml of 0.2 *N* HCl. Slowly add, with stirring, 20 ml of 0.6% sodium tetraphenylborate solution and then allow the mixture to stand for 10–15 min.

Filter the precipitate under suction through a previously dried and tared sintered-glass crucible (porosity 4); then wash the residue with three 5-ml portions of water. Dry the crucible and contents to constant weight (4 hr) at 40–45° at a pressure not exceeding 0.2 mm Hg. Each milligram of the dried chlorhexidine tetraphenylborate is equivalent to 0.5459 mg of chlorhexidine diacetate or 0.7834 mg of chlorhexidine digluconate.

Anal.—Calc. for $C_{70}H_{72}B_2Cl_2N_{10}$: C, 73.37; H, 6.33; N, 12.22. Found: C, 73.69; H, 6.46; N, 11.95.

Chlorhexidine Dihydrochloride—Weigh accurately about 20–60 mg of chlorhexidine dihydrochloride and transfer it into a 150-ml beaker. Dissolve with 20 ml of water by warming gently at 80–85° and cool; then add 0.3 ml of 37% (w/w) HCl. Complete the assay as described for chlorhexidine diacetate or digluconate, beginning with: "Slowly add, ..." Each milligram of the dried chlorhexidine tetraphenylborate is equivalent to 0.5047 mg of chlorhexidine dihydrochloride.

Tablets—The declared amounts, in milligrams per tablet, were: chlorhexidine diacetate, 5; benzocaine, 2; magnesium stearate, 15; mannitol, 300; lactose, 100; and sucrose, 578.

Weigh and powder 25 tablets. Weigh accurately, into a previously tared sintered-glass crucible (porosity 4), a quantity of the powder calculated to contain approximately 100 mg of chlorhexidine diacetate. Then add, under suction, three 20-ml portions of ether and discard the filtrate. Dry the crucible and contents at 50° for 10 min and then dissolve, under suction, chlorhexidine diacetate with 70 ml of 0.2 *N* HCl.

Transfer the combined filtrate and washings of the büchner flask (20 ml of water) into a 100-ml volumetric flask and dilute to volume with water. Pipet an accurately measured aliquot (50 ml) of the solution into a 150-ml beaker. Complete the assay as described for chlorhexidine diacetate or digluconate, beginning with: "Slowly add, ..." Each milligram of the dried chlorhexidine tetraphenylborate is equivalent to 0.5459 mg of chlorhexidine diacetate.

Estimation of Maximum Value for K_{sp}—The apparent solubility product value of chlorhexidine tetraphenylborate was determined in an aqueous hydrochloric acid solution (pH 1) by measurement of the molar detection limit for chlorhexidinium ion in the presence of

[1] Visan, Angiolini S.p.A., Milan, Italy.
[2] E. Merck, Darmstadt, Germany.