TABLE III

DEPENDENCE OF RATE ON THE CONCENTRATION OF BROMOTRICHLOROMETHANE[a]

Diene	Mole ratio $BrCCl_3$:diene	$[BrCCl_3]$, moles $kg.^{-1}$	Rate $\times 10^3$, moles $kg.^{-1}$ $min.^{-1}$
Isoprene	1.03:1	2.98	2.84
	2.05:1	4.30	2.74
	2.98:1	4.54	3.09
	6.19:1	4.78	3.58
	8.36:1	4.84	3.66
	17.95:1	4.96	3.34
2,3-Dimethyl-butadiene	1:2.06	2.79	5.74
	0.99:1	3.56	12.29
	2.99:1	4.43	13.10
	4.25:1	4.60	12.70
	6.08:1	4.72	13.38
	10.63:1	4.85	14.88
	11.06:1	4.86	15.95
	11.22:1	4.86	15.83
	18.72:1	4.93	14.97

[a]Dose rate: isoprene, 5860 rads $min.^{-1}$; 2,3-dimethylbutadiene, 5920 rads $min.^{-1}$.

pendence of rate on the concentration of bromotrichloromethane when the rate in each case has been corrected to a common dose rate. It is seen that in the cases of both isoprene and 2,3-dimethylbutadiene, in the mole-ratio range of 1:1 to 18:1, the rates of disappearance of the dienes are essentially constant with variation in the mole ratio (within 25% variation), the maximum rates occurring at a mole ratio of 8:1 for isoprene and 11:1 for 2,3-dimethylbutadiene. However, the rate is much lower when a mole ratio of 1:2 is employed as indicated in the case of 2,3-dimethylbutadiene.

Acknowledgment.—The authors wish to express their appreciation to Mr. Stanley E. Polchlopek for obtaining the infrared and Raman spectra, to Dr. Robert F. Stamm and Mr. N. B. Colthup for advice on the interpretation of the Raman and infrared spectra, to Dr. Raymond Feinland for gas chromatographic analyses, and to Dr. John Lancaster for obtaining the n.m.r. spectra and interpreting them.

Potential Radiation-Protective Compounds. Synthesis of the Three Isomeric Three-Carbon Aminohydroxy Bunte Salts and Related Compounds[1]

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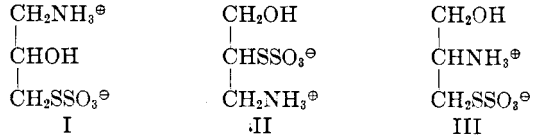
Received January 7, 1963

The isomeric internal Bunte salts, *S*-3-amino-2-hydroxypropylthiosulfuric acid (I), *S*-2-amino-1-(hydroxymethyl)ethylthiosulfuric acid (II), and *S*-2-amino-3-hydroxypropylthiosulfuric acid (III), have been prepared as the racemic forms. The last named has also been prepared in a levorotatory form. 3-Amino-2-bromo-1-propanol hydrobromide has been synthesized by the addition of hypobromous acid to the allylammonium ion; the isomeric 1-amino-3-bromo-2-propanol hydrobromide was a minor product of the addition. The acid hydrolysis of *N,O,S*-triacetyl-L-cysteinol probably proceeds *via* a thiazoline intermediate.

In recent years, Bunte salts,[2] *S*-alkyl thiosulfates, have been the subject of investigation in many laboratories. A Bunte salt related to glutathione, "S-sulfoglutathione", has been isolated from calf lens extracts.[3] Sulfite has been used to obtain soluble protein or peptide fractions from wool,[4] flour,[5] ribonuclease and insulin,[6] and from trypsinogen and α-chymotrypsinogen,[7] the cystine disulfide bonds are cleaved under mild conditions to form "S-sulfocysteinyl residues". The properties and potential uses of the "S-sulfocysteinyl residues" have been discussed by Swan.[8]

Several examples of Bunte salts containing amino or alkylamino groups were prepared by Bretschneider.[9] These, like the amino acids, are internal salts and the simplest compound of this type, *S*-2-aminoethylthiosulfuric acid, was found to have significant radiation-protective activity in mice. It was also 2.4 times less toxic than 2-aminoethanethiol (cysteamine) hydrochloride.[10] Other aminoalkyl thiosulfates have been prepared by Rosenthal and Citarel[11] who found that they were stable compounds which possessed significant anti-radiation activity. The low activity of 3-amino-1-propanethiol compared to the activity of *S*-3-aminopropylthiosulfuric acid[12] suggests that the protective activity of the Bunte salt is not, at least in this case, due to the formation of the thiol.

Aminoalkylthiosulfuric acids are, in general, stable, odorless, crystalline, water-soluble substances and are thus attractive potential anti-radiation drugs. This paper describes the synthesis of the three isomeric internal Bunte salts I, II, and III.



Bunte salts are prepared conveniently by the reaction of alkyl halides with thallous thiosulfate,[13] the insoluble thallous halide formed being removed easily from the reaction mixture. An aqueous solution of 1-

(1) This work was supported by a grant from the Surgeon General's Office, Medical Research and Development Command, U. S. Army.

(2) H. Bunte, *Ber.*, **7**, 646 (1874).

(3) S. G. Waley, *Biochem. J.*, **71**, 132 (1959).

(4) J. M. Swan, *Nature*, **180**, 643 (1947); *Australian J. Chem.*, **14**, 69 (1961).

(5) E. E. McDermott and J. Pace, *Nature*, **184**, 546 (1959).

(6) J. L. Bailey and R. D. Cole, *J. Biol. Chem.*, **234**, 1733 (1959).

(7) J. F. Pechère, G. H. Dixon, R. H. Maybury, and H. Neurath, *ibid.*, **233**, 1364 (1958).

(8) J. M. Swan, "Sulfur in Proteins," R. Benesch, *et al.*, Ed., Academic Press, New York, N. Y., 1959, p. 3.

(9) H. Bretschneider, *Monatsh. Chem.*, **81**, 372 (1950).

(10) B. Holmberg and B. Sörbo, *Nature*, **183**, 832 (1959).

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(13) H. Z. Lecher and E. M. Hardy, *J. Org. Chem.*, **20**, 475 (1955).

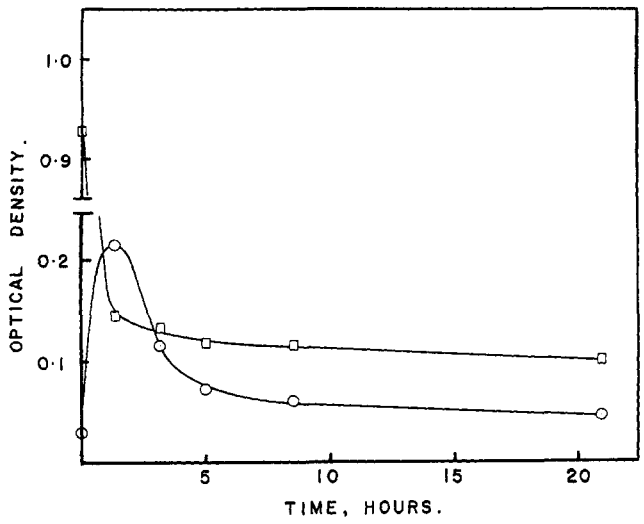


Fig. 1.—Hydrolysis of *N,O,S*-triacetyl-L-cysteinol (2.08×10^{-4} *M*) in hydrochloric acid (1.7 *N*) at 90°: □—optical density at 231.5 mμ; O—optical density at 261 mμ.

amino-3-chloro-2-propanol hydrochloride (IV),[14,15] unlike 2-chloroethylamine hydrochloride,[13] did not react readily with thallous thiosulfate at room temperature. Formation of the internal Bunte salt took place at higher temperatures but extensive decomposition also occurred. The corresponding bromo hydrobromide V

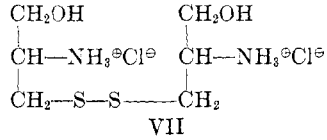
was originally prepared by condensation of potassium phthalimide with boiling epibromohydrin, followed by hydrolysis of the product with hydrobromic acid.[16] When *N,N*-dimethylformamide was added in the condensation step,[17] the reaction proceeded at 35–40° and the yield was improved slightly. Under these conditions, the epoxide ring is slowly opened by the potassium phthalimide. This was demonstrated by the formation of a small amount of a diaminopropanol (presumably 1,3-diamino-2-propanol) after hydrolysis of the condensation product. The bromo hydrobromide reacted more readily than IV with thallous thiosulfate and, after two days at 50°, the Bunte salt I, *S*-3-amino-2-hydroxypropylthiosulfuric acid, was isolated in 67% yield.

No suitable precursor for the Bunte salt II was known and attempts to prepare 3-amino-2-bromo-1-propanol hydrobromide (VI) from 2,3-dibromo-1-propanol and from 2,3-dibromopropylamine and their derivatives, by selective displacements of the primary bromine atoms, were unsuccessful. The hydrobromide VI was eventually obtained from the reaction of the allylammonium ion with hypobromous acid. The latter was conveniently generated *in situ* by the method of Leibman and Fellner[18] whereby a mixture of bromine vapor and air is passed into the reaction solution which contains one equivalent of silver nitrate. Both isomeric aminobromo alcohols were isolated, the desired 3-amino-2-bromo isomer VI being the major product. The hydrobromide VI did not react with thallous thiosulfate at 40° but, at 75–80°, conversion to the Bunte salt II was essentially complete after twelve hours. Amine impurities, which hindered crystallization, were removed by chromatography on silica gel. Crystallization of the purified material from methanol–ether gave the pure Bunte salt II, *S*-2-amino-1-(hydroxymethyl)-ethylthiosulfuric acid, in 51% yield.

Bunte salts have also been prepared by the action of sulfites on disulfides in the presence of an oxidizing agent. Air or oxygen are often sufficient but cupric ions,[19] iodosobenzoate, and tetrathionate[6] have also been used. The oxidant converts the thiol formed in the reversible reaction (1) to disulfide and eventually this is completely converted to the Bunte salt.

$$RSSR + SO_3^{-2} \rightleftharpoons RSSO_3^{\ominus} + RS^{\ominus} \qquad (1)$$

Cystinol dihydrochloride VII, 3,3′-dithiobis[2-amino-1-propanol]dihydrochloride, is, therefore, a suitable precursor for the internal Bunte salt III. It was



VII

prepared from 2-phenyl-2-thiazoline-4-methanol by Crawhall, *et al.*[20] These authors designated their product as the DL-form but the method of preparation would give a mixture of DL- and *meso*-isomers. We used the L-isomer of the thiazoline in an attempt to obtain the pure L-isomer of VII but hydrolysis of the thiazoline ring required much more vigorous conditions than those reported[20] and racemization occurred at this stage. The resultant DL-thiol ("cysteinol") was oxidized without isolation and a crystalline, optically inactive mixture of, presumably, the DL- and *meso*-forms of VII was obtained. No attempt was made to separate these isomers and the mixture was treated at room temperature with ammonium sulfite solution (pH 7) and oxygen. The reaction was followed by paper electrophoresis and was complete after six to seven hours. The product was freed from inorganic salts by fractionation on a column of a cation exchange resin in the lithium salt form[21] and subsequent recrystallization from methanol–ether gave *S*-2-amino-3-hydroxypropylthiosulfuric acid (III) in 65% yield.

L-Cysteinol, L-2-amino-3-mercapto-1-propanol (VIII), has been prepared recently by Enz and Cecchinato[22] by a method which should not cause appreciable racemization. L-Cysteine ethyl ester was reduced with lithium aluminum hydride and the L-cysteinol formed was isolated as the *N,O,S*-triacetate (IX) in 29% yield. These authors hydrolyzed the triacetate with dilute hydrochloric acid and obtained L-cysteinol as the crystalline hydrochloride, although they did not record the specific rotation. Thin layer chromatog-

(14) S. Gabriel and H. Ohle, *Ber.*, **50**, 819 (1917).

(15) Unless otherwise indicated, all compounds containing an asymmetric carbon atom are racemic mixtures.

(16) M. Weizmann and S. Malkowa, *Bull. soc. chim. France*, **47**, 356 (1930).

(17) J. C. Sheehan and W. A. Bolhoffer, *J. Am. Chem. Soc.*, **72**, 2786 (1950).

(18) K. C. Liebman and S. K. Fellner, *J. Org. Chem.*, **27**, 438 (1962).

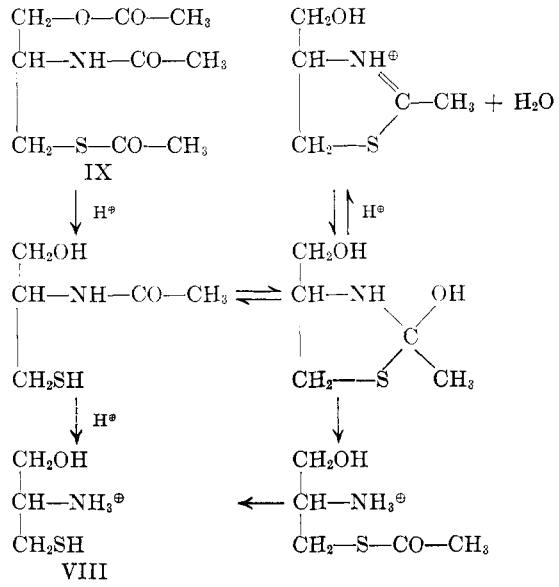
(19) I. M. Kolthoff and W. Stricks, *J. Am. Chem. Soc.*, **73**, 1728 (1951).

(20) J. C. Crawhall, D. F. Elliott, and K. C. Hooper, *J. Chem. Soc.*, 4066 (1956).

(21) J. K. N. Jones, R. A. Wall, and A. O. Pittet, *Can. J. Chem.*, **38**, 2285 (1960).

(22) W. Enz and M. Cecchinato, *Helv. Chim. Acta*, **44**, 706 (1961).

raphy indicated that, during hydrolysis of the triacetate, an intermediate was rapidly formed and that this was slowly converted to cysteinol. In dilute hydrochloric acid at 90°, the absorption at 231.5 mμ (thiolacetate) decreased rapidly. A second absorption at 261 mμ (thiazolinium cation) appeared, increased to a maximum after *ca.* an hour and a half, and then decreased slowly (Fig. 1). It has been shown recently that thiazoline formation occurs in acid solutions of *N*-acetyl cysteine[23] and *N*-2-mercaptoethylacetamide[24] and hydrolysis of cysteinol triacetate (IX) probably proceeds *via* the same mechanism.



L-Cystinol dihydrochloride was prepared from L-cysteinol triacetate by acid hydrolysis followed by oxidation of the resultant thiol which was not isolated. The L-form of VII had a specific rotation of −108° and a much lower melting point than the values obtained for the mixtures of DL- and *meso*-forms.

The internal Bunte salt (III) prepared from this disulfide had a higher melting point than the DL-form and a specific rotation of −31° in water.

The three isomeric internal Bunte salts are stable crystalline solids, very soluble in water and slightly soluble in methanol. They decompose slowly in boiling water. They have been submitted to the Walter Reed Army Institute of Research for testing as radiation-protective agents.

Experimental

Solutions were concentrated under reduced pressure below 40°. Melting points were determined on a Kofler micro hot stage or in a Thomas–Hoover capillary melting point apparatus and are uncorrected, and optical rotations were measured at 5461 Å with an ETL–NPL automatic polarimeter (The Bendix Corporation, Cincinnati, Ohio). Molecular weights were determined in the solvents specified with a vapor pressure osmometer (Mechrolab, Inc., Mountain View, Calif.).

Paper electrophoresis was carried out on strips of Whatman no. 1 filter paper, 5 cm. wide, in 0.2 *M* acetate buffer, pH 5, and at a current of 5 ma. Whatman no. 1 filter paper was also used for paper chromatography by the descending method using the solvent systems (a) 1-butanol–ethanol–water (3:1:1) and (b) 1-butanol–pyridine–water (10:3:3). Ascending thin layer chromatography (t.l.c.) was performed on 0.25-mm. layers of "Silica Gel G acc. to Stahl" (distributed by Brinkmann Instruments, Inc., Great Neck, L. I., N. Y.). Compounds were located by the ninhydrin spray or with an alkaline permanganate spray. Silica gel, grade 950, 60–200 mesh from the Davison Co., Baltimore 3, Md., was used without pretreatment for column chromatography.

The microanalyses were done by Mr. C. DiPietro of this laboratory and by Dr. S. M. Nagy of the Massachusetts Institute of Technology.

1-Amino-3-chloro-2-propanol Hydrochloride (IV).—A suspension of phthalimide (147 g., 1 mole) in epichlorohydrin (300 ml.) was boiled under reflux for 10 hr. The mixture was allowed to cool and residual phthalimide (56 g.) was removed by filtration. The filtrate was concentrated to a yellow sirup which crystallized from benzene. After recrystallization, the product, *N*-(3-chloro-2-hydroxypropyl)phthalimide (77.6 g., 52% based on unrecovered phthalimide), had m.p. 93–97°.[14]

Hydrolysis of this product with 20% hydrochloric acid[14] gave crystalline 1-amino-3-chloro-2-propanol hydrochloride, m.p. 103–106°, in 73% yield. Gabriel and Ohle reported m.p. 103–104°.[14]

1-Amino-3-bromo-2-propanol Hydrobromide (V).—Potassium phthalimide (37.0 g., 0.2 mole) was added to a mixture of epibromohydrin (27.4 g., 0.2 mole) and *N*,*N*-dimethylformamide (100 ml.) and the suspension was stirred magnetically at 35–40° for 5 hr. Chloroform was added and the mixture was poured into stirred ice–water (*ca.* 600 ml.). The two layers were separated and the aqueous layer was extracted twice with chloroform. The combined chloroform extracts were washed with cold 0.1 *N* sodium hydroxide solution (100 ml.) and with water and were dried over sodium sulfate. Concentration afforded a white solid which was recrystallized twice from ethanol. The product, *N*-(2,3-epoxypropyl)phthalimide (32.1 g., 79%), had m.p. 100–102°. Weizmann and Malkowa reported m.p. 93–94°.[16]

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89. Found: C, 65.00; H, 4.68; N, 6.85.

A solution of this product (10 g.) in 48% hydrobromic acid (100 g.) was boiled under reflux for 5 hr. Phthalic acid, which crystallized when the solution was allowed to cool, was removed by filtration and the filtrate was concentrated to a solid (10.1 g.). Paper electrophoresis showed that in addition to the main product, a small amount of a faster moving cation was present. The solid was dissolved in hot ethanol and most of the minor component crystallized when the solution was cooled. It was collected by filtration and, after drying, weighed 0.40 g. and had m.p. 197–200° dec. It analyzed as a diaminopropanol dihydrobromide.

Anal. Calcd. for $C_3H_{10}N_2O{\cdot}2HBr$: C, 14.30; H, 4.80; N, 11.12; Br, 63.43. Found: C, 14.25; H, 4.80; N, 10.80; Br, 62.98.

After removal of this compound, the solution was concentrated to a pale orange solid (9.7 g.) which was dissolved in 1-butanol. The solution was boiled with charcoal, filtered through Celite, and cooled. Crystalline 1-amino-3-bromo-2-propanol hydrobromide (V) was obtained with m.p. 115–117° (lit.[16] m.p. 113–113.5°). Paper electrophoresis indicated the presence of a trace only of the preceding diaminopropanol dihydrobromide.

Anal. Calcd. for $C_3H_8NOBr{\cdot}HBr$: C, 15.34; H, 3.86; N, 5.96; Br, 68.03. Found: C, 15.36; H, 3.94; N, 6.21; Br, 68.17.

Thallous Thiosulfate.—A solution of thallous acetate (52.7 g., 0.2 mole) in water (60 ml.) was clarified by filtration through Celite. To the vigorously stirred solution was added a solution of sodium thiosulfate pentahydrate (24.8 g., 0.1 mole) in water (50 ml.). The heavy white precipitate of thallous thiosulfate was collected by filtration, washed repeatedly with water, and dried. Yield: 50 g., 96%.

***S*-3-Amino-2-hydroxypropylthiosulfuric Acid (I). (A) Attempted Preparation from IV.**—The reaction of thallous thiosulfate with an aqueous solution of IV was followed by paper chromatography, paper electrophoresis, and t.l.c. (methanol). The internal Bunte salt I was formed in trace amounts after 1 day at 40° (compare ref. 13). The rate of reaction was increased by raising the temperature to 70, 85, or 100°, but the occurrence of side reactions at these temperatures led to the formation of by-products. In all experiments, the yield of I appeared to be less than 50%.

(B) Preparation from V.—Preliminary experiments indicated that the reaction of thallous thiosulfate with an aqueous solution of V gave I in good yield after 2 days at 50°. Accordingly, thallous thiosulfate (40 g.) was added to a magnetically stirred solu-

(23) H. A. Smith and G. Gorin, *J. Org. Chem.*, **26**, 820 (1961).

(24) R. B. Martin, S. Lowey, E. L. Elson, and J. T. Edsall, *J. Am. Chem. Soc.*, **81**, 5089 (1959).

tion of V (11.75 g.) in water (50 ml.). The temperature was maintained at 50° with an oil bath. After 1 day, an additional 20 g. of thallous thiosulfate was added and, after 2 days, the suspension was cooled to 0° and filtered. Concentration of the filtrate gave a sirup which was diluted with methanol, filtered through Celite, and cooled. *S*-3-Amino-2-hydroxypropylthiosulfuric acid (I) crystallized and was collected by filtration, washed with methanol, and dried. Total yield was 6.26 g. (67%), m.p. 164–167° dec., R_f 0.10 in solvent system A.

Anal. Calcd. for $C_3H_9NO_4S_2$: C, 19.24; H, 4.84; N, 7.48; S, 34.25; mol. wt., 187. Found: C, 19.34; H, 4.74; N, 7.50; S, 34.46; mol. wt., 187 (in water).

3-Amino-2-bromo-1-propanol Hydrobromide (VI).—A solution of freshly distilled allylamine (19 g., 0.33 mole) in water (350 ml.) was neutralized (to pH 6) with concentrated nitric acid (*ca.* 22 ml.). Silver nitrate (56.7 g., 0.33 mole) dissolved in water (150 ml.) was added and a mixture of bromine vapor and air was drawn into the well stirred solution which was cooled to 0–10° in an ice bath. Total weight of bromine added was 53.3 g., 0.33 mole. When the addition of bromine was completed (2.5 hr.), the solution was stirred for an additional hour at room temperature, silver bromide was then removed by filtration and the filtrate was neutralized with the weakly basic Amberlite ion-exchange resin IR 45 (OH). Concentration afforded a sirupy residue which was diluted with water. The solution was applied to a column of Dowex ion-exchange resin 50W-X2 (H) (450 g.), nitrate ions were eluted with water, and the amines were then desorbed with 9.7% hydrobromic acid. The acidic effluent was collected in 400–500-ml. fractions, which were separately concentrated with repeated additions of methanol. The following fractions were obtained: (1) 23.2 g., (2) 15.7 g., (3) 2.0 g. Total yield of mixed bromohydrins was 40.9 g. (52%). Fractions 1 and 3 crystallized on standing and fraction 2 crystallized in part. After treatment with charcoal and recrystallization from 1-butanol, fractions 1 and 2 yielded large colorless prisms of 3-amino-2-bromo-1-propanol hydrobromide (VI) (15.6 g., 20%), m.p. 107.5–109.5°, depressed to 75–100° when admixed with 1-amino-3-bromo-2-propanol hydrobromide (V). The infrared spectrum (in potassium bromide) was similar to, but not identical with, that of V.

Anal. Calcd. for $C_3H_8NOBr \cdot HBr$: C, 15.34; H, 3.86; N, 5.96; Br, 68.03. Found: C, 15.28; H, 3.95; N, 6.09; Br, 68.14.

After treatment with charcoal and recrystallization from 1-butanol, fraction 3 yielded pure 1-amino-3-bromo-2-propanol hydrobromide (V) (0.91 g., 1%), m.p. 114–116°, undepressed by admixture with authentic material.

***S*-2-Amino-1-(hydroxymethyl)ethylthiosulfuric Acid (II).**—A solution of VI (7.05 g., 0.03 mole) in water (90 ml.) was stirred magnetically with thallous thiosulfate (15.6 g., 0.03 mole) at 75–80° (oil bath). The reaction was followed by paper electrophoresis and, after 2 hr., an additional portion of thallous thiosulfate (15.6 g., 0.03 mole) was added. After 12 hr., only a trace of starting material remained and the reaction mixture was cooled and filtered. Concentration of the filtrate gave a sirup which was extracted with methanol; the extracts were filtered and concentrated to a sirup (5.83 g.), which slowly deposited crystals when diluted with methanol (10 ml.). The crystals (0.95 g.) were collected by filtration and the residual sirup which contained small amounts of amine impurities (which probably hindered crystallization) was fractionated on a column of silica gel (300 g.) with methanol as solvent. The sirupy product (4.63 g.) still contained one impurity (which appeared on paper chromatograms to be the isomeric Bunte salt I, probably arising from traces of the bromide V in the starting material) but crystallization from methanol–ether gave a further 1.90 g. of crystalline material. Total yield of *S*-2-amino-1-(hydroxymethyl)ethylthiosulfuric acid (II) was 2.85 g. (51%), m.p. 164–172° dec., R_f 0.15 in solvent system A.

Anal. Calcd. for $C_3H_9NO_4S_2$: C, 19.24; H, 4.84; N, 7.48; S, 34.25. Found: C, 19.14; H, 4.95; N, 7.40; S, 34.63.

Preparation of Optically Inactive (DL- + *meso*-) Cystinol Dihydrochloride (VII).—L-2-Phenyl-2-thiazoline-4-methanol was prepared from L-cysteine hydrochloride in an over-all yield of 25% according to Crawhall, *et al.*[20] The thiazoline (m.p. 75.5–76°) was dissolved in concentrated hydrochloric acid (sp. gr. 1.191 at 60°F) and the solution was boiled under reflux in a stream of nitrogen for 6 hr. Fifty per cent of the starting material was recovered as its crystalline hydrochloride (cubes from ethanol), m.p. 135–139°.

Anal. Calcd. for $C_{10}H_{11}NOS \cdot HCl$: C, 52.27; H, 5.26; N, 6.10; S, 13.95; Cl, 15.43. Found: C, 52.06; H, 5.20; N, 6.01; S, 14.07; Cl, 15.48.

Under these conditions, the above workers reportedly obtained a 90% yield of thiol, determined by iodine titration.[20] In trial experiments, we determined the extent of hydrolysis under these conditions by iodine titration and by the weight of benzoic acid liberated. It was found to be 59% and 82% after 7 and 14 hr. respectively. A solution of the thiazoline (9.55 g.) in concentrated hydrochloric acid (190 ml.) was boiled under reflux in a stream of nitrogen for 17 hr. The cooled solution was extracted with ether (two 100-ml. portions); benzoic acid (5.35 g., 88%) was recovered from dried extracts. The aqueous layer was concentrated to a sirup (7.6 g.) which was taken up in water (90 ml.). The pH was adjusted to 8–9 with ammonia and the small amount of unchanged thiazoline which precipitated was removed by filtration. A small crystal of ferrous sulfate was added to the filtrate and air was drawn through the solution until the mauve color was discharged (*ca.* 8 hr.). The solution was then concentrated to half its volume, filtered to remove a little more thiazoline, and evaporated to dryness. The residue was crystallized from a mixture of ethanol and concentrated hydrochloric acid (3:2) giving an optically inactive mixture (DL- and *meso*-) of cystinol dihydrochlorides (VII) (5.71 g., 81%). This preparation had m.p. 199–202°. The so-called "DL-cystinol" previously reported[20] with m.p. 183° was very probably a mixture of DL- and *meso*- forms and the difference in melting points probably reflects a difference in the relative amounts of these isomers in the two preparations.

Anal. Calcd. for $C_6H_{16}N_2O_2S_2 \cdot 2HCl$: C, 25.26; H, 6.36; N, 9.82; S, 22.49; Cl, 24.85. Found: C, 25.08; H, 6.17; N, 9.85; S, 22.36; Cl, 24.68.

***S*-2-Amino-3-hydroxypropylthiosulfuric Acid (III).**—Oxygen was passed through a solution of these cystinol dihydrochlorides, (2.85 g., 0.01 mole) in ammonium sulfite solution, pH 7 (110 ml.) (prepared by adding concentrated ammonia to a 6% aqueous solution of sulfur dioxide). The reaction, which was followed by paper electrophoresis, was complete in 6–7 hr. at room temperature. The solution was left overnight at room temperature and then concentrated to dryness. The residue (11.6 g.) was extracted with absolute methanol (110 ml.) and the filtered solution was concentrated to a solid (7.2 g.), which contained both Bunte salt and inorganic salts. A portion (1.0 g.) of the mixture was fractionated on a column (93 × 2.3 cm.) of Dowex cation-exchange resin, 50W-X2 (200–400 mesh) in the lithium salt form.[21] The column was eluted with water and 15-ml. fractions were collected and tested for sulfite and sulfate (with barium chloride solution) and for amino-Bunte salt (with ninhydrin). Inorganic salts were eluted first (fractions 7–13) and then the Bunte salt (fractions 18–22). Concentration of the latter fractions gave a sirup (0.4 g.) which crystallized slowly from methanol–ether. The remainder of the salt mixture was similarly fractionated (maximum load for the above column was about 1.5 g.) and 3.08 g. (82%) of crystalline *S*-2-amino-3-hydroxypropylthiosulfuric acid (III), m.p. 153–156° dec., was isolated. Recrystallization from methanol–ether gave the pure Bunte salt, m.p. 159–161° dec., R_f 0.16 in solvent system A. This preparation of III had no optical activity.

Anal. Calcd. for $C_3H_9NO_4S_2$: C, 19.24; H, 4.84; N, 7.48; S, 34.25; mol. wt., 187. Found: C, 19.17; H, 4.67; N, 7.31; S, 34.16; mol. wt., 184 (in water).

Preparation of L-Cystinol Dihydrochloride.—*N*,*O*,*S*-Triacetyl-L-cysteinol (IX) was prepared in 29% yield from L-cysteine ethyl ester hydrochloride. The original procedure[22] was modified as follows. Crude cysteinol was separated from inorganic salts remaining after the reduction step by ethanol extraction of the dried residue. After acetylation of this material with acetic anhydride–sodium acetate, the reaction mixture was concentrated to a solid which was extracted with benzene. Concentration of the benzene extracts afforded the crystalline triacetate (IX) which was recrystallized from methanol–ether. The product had m.p. 101–102°, $[\alpha]^{25}D$ −45° (*c* 1.92 in water), $\lambda_{max}^{H_2O}$ 231.5 mμ, ε 4300.

A solution of IX (2.08×10^{-4} *M*) in 1.7 *N* hydrochloric acid was heated at 90–92° under nitrogen and aliquots were removed at intervals for measurements of the absorptions at 231.5 mμ and at 261 mμ. The results are shown in Fig. 1.

In a second experiment, a solution of IX (4.66 g., 0.02 mole) in 1.7 *N* hydrocloric acid (200 ml.) was heated at 90° for 15 hr. The solution was then concentrated to a sirup which was taken up in water (25 ml.). The pH was adjusted to 7–8 with ammonia

and air was drawn through the solution. T.l.c. (1-propanol) indicated a slow conversion to the disulfide which was essentially complete after 2 days. The solution was concentrated to a sirup which was dissolved in methanol containing a little hydrochloric acid. Ether was added and L-cystinol dihydrochloride (VII) (1.91 g., 67%) crystallized. After recrystallization from methanol–ether it had m.p. 145–146°, $[\alpha]^{25}D$ −108° (*c* 1.0 in methanol).

Anal. Calcd. for $C_6H_{16}N_2O_2S_2 \cdot 2HCl$: C, 25.26; H, 6.36; N, 9.82; S, 22.49; Cl, 24.85. Found: C, 25.09; H, 6.15; N, 9.80; S, 22.66; Cl, 24.57.

L-S-2-Amino-3-hydroxypropylthiosulfuric Acid (III).—The Bunte salt III was prepared from L-cystinol dihydrochloride as described previously for the DL-isomer except that sodium sulfite was used instead of ammonium sulfite. The product had m.p. 190–193° dec., $[\alpha]^{25}D$ −31° (*c* 0.62 in water).

Electronic Effects on the Stereochemistry of the Diels–Alder Reaction[1]

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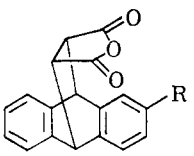
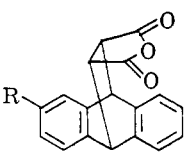
Received December 28, 1962

Various 2-substituted anthracenes have been synthesized and their reaction with maleic anhydride investigated. The amounts of the two possible isomers formed, *syn* and *anti*, afforded a medium for the evaluation of the electronic effect on the stereochemistry of the reaction. The results of these studies are discussed.

The Diels–Alder reaction, in which there is the possibility of the formation of more than one product, has been extensively investigated; however, the asymmetrical nature of reacting species has not permitted unequivocal evaluation of the electronic effect in determining the isomer formed.[3] The introduction of a group on the 2-position of the anthracene nucleus permits the formation of two isomeric products in the reaction with maleic anhydride. The amounts of *syn* (I) and *anti* (II) isomers formed would be a function of the



syn Adduct (I)
a. R = NO_2
b. R = NAc
c. R = $N(Me)_2$



anti Adduct (II)
a. R = NO_2
b. R = NAc
c. R = $N(Me)_2$

substituent. The symmetrical nature of the molecule, except for the substituent, would tend to minimize all other effects which determine the isomer ratio and thus reflect the importance of polar attractive forces in the two possible transition states. The substitution of anthracene in the 2-position rather than the 1-position further removes any steric effect the group may have on the reacting centers.

The dimethylamino, acetamido, and nitro groups were selected as substituents. The method of Hodgson and Marsden[4] for the replacement of a diazonium group by a nitro group was used to prepare the reported 2-nitroanthracene.[5] Excellent yields of 2-acetamidoanthracene were obtained by the treatment of 2-aminoanthracene with acetic anhydride[6] and lithium aluminum hydride reduction of 2-N,N-dimethylaminoanthracene methiodide in tetrahydrofuran gave 2-N,N-dimethylaminoanthracene.[7]

All Diels–Alder reactions were carried out in refluxing benzene with a ten- to twentyfold excess of freshly sublimed maleic anhydride present. 2-Dimethylaminoanthracene reacted with the appearance of a transient deep red color and the insoluble 2-acetamidoanthracene dissolved slowly as it reacted. The yellow color of the 2-nitroanthracene solution did not intensify as the reaction occurred and disappeared as the reaction approached completion. The relative rates of reaction of 2-nitroanthracene, anthracene, and 2-dimethylaminoanthracene were determined under pseudo first-order conditions similar to those used by Andrews and Keefer.[8]

Compound	*k*, l./mole sec.
2-Nitroanthracene	0.086×10^{-5}
Anthracene	$.014 \times 10^{-3}$
2-Dimethylaminoanthracene	$.055 \times 10^{-3}$

The spectra of the products exhibited typical succinic anhydride adsorption in the infrared at 5.34 and 5.60 μ.[9] At least one intense band appearing in the spectrum of starting material was completely absent in all cases. The infrared spectra of chloroform, methylene chloride, benzene, or dioxane solutions of pure *syn*- and *anti*-2-nitro-9,10-dihydroanthracene-9,10-*endo*-α,β-succinic anhydrides (Ia and IIa) differed mainly in the 10.0- to 11.0-μ region. Their ultraviolet spectra were similar and as expected for adducts.[10]

The theoretical dipole moments of the two adducts, which contain rigid ring systems free of rotation, were computed from three main components: 9,10-dihydroanthracene (0.4 Debye),[11] succinic anhydride (4.2 Debyes),[12] and nitrobenzene (3.9 Debyes).[13] A value of 7.11 Debyes was obtained for the *syn* adduct and 2.11 Debyes for the *anti* adduct. The measurement of the dielectric constant and refractive index of a series of

(1) Taken in part from the dissertation of Fred Kaplan presented to the Faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy, September, 1959.

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