one sulfide sulfur to triphenylphosphine forming the phosphine sulfide.[8,9] Other disulfides are reactive under conditions of photolysis or free radicals.[7]

Recently Scott[10] has observed the reaction of triethyl phosphite with epoxides forming the corresponding olefin and triethyl phosphate. The reaction conditions were much more severe (three hours at 150–175°) than those needed for the sulfur compounds (one to three days at 25°). Scott suggested a mechanism involving ring opening by the phosphite at the carbon atom and then formation of a four membered 1-oxa-2-phosphacyclobutane ring which then decomposes to the phosphate and the olefin. Wittig[11] studied the reaction of triphenylphosphine with epoxides at 160–180°. A four center transition state was postulated and the relationship to the Wittig reaction is obvious. Discussions concerning the reaction mechanism are speculative.

Phosphines and phosphites appear to be well suited as reagents in the structure determination of various sulfur compounds. The conditions are mild; polysulfides, disulfides, thiiranes, thiols, and other functional groups can be easily degraded. Investigation of other functional groups containing sulfur is continuing with phosphorus-III compounds.

CAMBRIDGE 38, MASS.

(8) A. Schönberg and M. Z. Barakat, *J. Chem. Soc.*, 892 (1949).
(9) A. Schönberg, *Ber.*, **68**, 163 (1935).
(10) C. B. Scott, *J. Org. Chem.*, **22**, 1118 (1957).
(11) G. Wittig and W. Haag, *Ber.*, **88**, 1654 (1955).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY, KALAMAZOO, MICHIGAN]

Carbonic Anhydrase Inhibitors. I. Benzothiazole Derivatives

JEROME KORMAN

Received May 21, 1958

A number of aryl-substituted benzothiazole-2-sulfonamides have been prepared, all of which are potent carbonic anhydrase inhibitors. One of these, the 6-ethoxybenzothiazole-2-sulfonamide, produces a clinically useful diuresis.

Carbonic anhydrase inhibitors have become important drugs for the treatment of conditions which are evidenced by edema.[1] The more important substances are diuretics, which produce a beneficial loss of sodium and water with concurrent body weight loss, and are characterized by a sulfonamide group in which the sulfonamide nitrogen is unsubstituted.[2] Research in this field was prompted by the experiments of Schwartz[3] with sulfanilamide in cases of congestive heart failure. Although diuresis occurred, low potency and the recognized toxic effects of sulfanilamide rendered it valueless for this purpose.

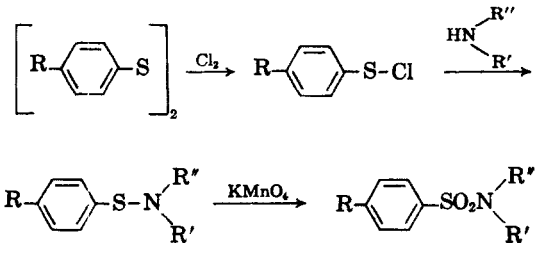
In the search for a more active replacement, Roblin and Clapp[4] prepared a large number of heterocyclic sulfonamides, many of which were indeed powerful carbonic anhydrase inhibitors. Among these, benzothiazole-2-sulfonamide, which appeared to be one of the most potent enzyme inhibitors, was subsequently reported[5] to be devoid of diuretic activity, a fact which was confirmed in these laboratories. This apparent anomaly can be reasonably explained on the basis of insolubility or an unfavorable rate of metabolism. Since diuretic activity in these compounds had been attributed to carbonic anhydrase inhibition,[3] it seemed of interest to prepare a series of substituted benzothiazole-2-sulfonamides and determine, firstly, the effect of the substituent and its position on carbonic anhydrase activity and, secondly, what effects, if any, these substituents might have upon such factors as solubility and metabolism.

Prior to the report of Roblin and Clapp, very few heterocyclic sulfonamides had been prepared. Chlorosulfonation, which works admirably with aromatic compounds to produce a sulfonyl chloride, fails generally with heterocyclic systems. Although a few such reactions have been reported,[6,7] the yields are poor, and the usual product is one in which nuclear chlorination has taken place. Similarly the reaction involving conversion of the salt of a sulfonic acid into the acid chloride results either in chlorination or replacement of the sulfonic acid

(1) C. K. Friedberg, R. Taylor, M. Halpern, *New Engl. J. Med.*, **248**, 883 (1953); W. M. Grant and R. R. Trotter, *Arch. Ophthalmol.*, **51**, 735 (1954); S. Merlis, *Neurology*, **4**, 863 (1954); E. M. Latts, *Minn. Med.*, **38**, 184 (1955); J. R. Ashe, B. Carter, W. L. Thomas, and W. R. Kerr, *Obstet. and Gynecol.*, **7**, 242 (1956).
(2) T. Mann and D. Keilin, *Nature*, **146**, 164 (1940), H. A. Krebs, *Biochem. J.*, **43**, 525 (1948).
(3) W. B. Schwartz, *New Engl. J. Med.*, **240**, 173 (1949).
(4) R. O. Roblin and J. W. Clapp, *J. Am. Chem. Soc.*, **72**, 4890 (1950).
(5) J. M. Sprague, *New York Academy of Sciences, Biology Section*, November 8, 1957.
(6) H. J. Backer and J. A. Keverling Buisman, *Rec. trav. chim.*, **63**, 228 (1944).
(7) G. R. Barker, N. G. Luthy, and M. M. Dhar, *J. Chem. Soc.*, 4206 (1954).

group by chlorine.[8-10] The oxidative chlorination of heterocyclic mercaptans to give the sulfonyl chloride, which was used so successfully in previous studies for the preparation of benzothiazole-2-sulfonamide, we found to be unsatisfactory with other members of this series. One experiment with 6-ethoxy-2-mercaptobenzothiazole gave a very low yield of impure sulfonamide, and another method was sought.

Among the processes reported for the preparation of N^1-substituted sulfanilamides is one involving an intermediate aryl sulfenamide, which is oxidized to a sulfonamide.[11] The sulfenamide was prepared from the sulfenyl chloride by treatment with the appropriate amine according to the scheme:



R = NO_2, CH_3CONH

This same procedure was found applicable to the preparation of benzothiazole-2-sulfenyl chloride[12] and benzothiazole-2-sulfenamides, references to which appear in the patent literature.[13] These latter compounds have found wide application as accelerators in the rubber processing industry. Alternate methods for the direct preparation of sulfenamides have also been described.[14] In these cases the sodium salt of a mercaptan is treated with a solution of chloramine, as such, or which is prepared *in situ*. Since we had found by experience that chlorine was an unsatisfactory reagent, we used the last method for the preparation of our sulfenamides. The scope of the reaction has been thoroughly covered in an excellent article by Carr, Smith, and Alliger.[15] Various substituted 2-mercaptobenzothiazoles in aqueous solution as the sodium salt were added simultaneously with a solution of sodium hydrochlorite to concentrated ammonium hydroxide. The resulting sulfenamides were oxidized to the sulfonamides with potassium permanganate in aqueous acetone solution (Table I):[16]

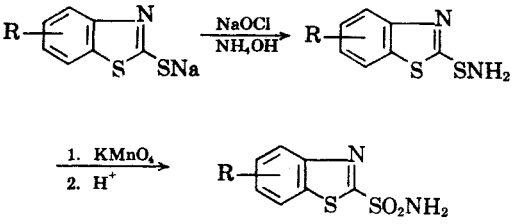


TABLE I

	Compound	M.p.	Analysis N Calcd.	N Found	S Calcd.	S Found
1.	Benzothiazole-2-sulfonamide	175.5–176°	[a]	[a]	[a]	[a]
2.	4-Methylbenzothiazole-2-sulfonamide[b]	182.5–184.5°	12.27	12.50	28.09	27.88
3.	6-Methylbenzothiazole-2-sulfonamide[b]	196–198.5°	12.27	12.64	28.09	28.13
4.	5-Chlorobenzothiazole-2-sulfonamide[c]	205.5–208°	11.27	11.94	25.78	25.68
5.	4-Methoxybenzothiazole-2-sulfonamide[d]	195–199°	11.47	11.70	26.26	26.52
6.	6-Ethoxybenzothiazole-2-sulfonamide[e]	188–190.5°	10.85	10.72	24.82	24.74
7.	6-Acetamidobenzothiazole-2-sulfonamide	233–235°	15.49	15.58	23.63	23.51

[a] Identical with that prepared by the method of Roblin and Clapp (*cf.* ref. 4). [b] The starting mercaptan was prepared by the method of L. B. Sebrell and C. E. Boord, *J. Am. Chem. Soc.*, **45**, 2390 (1923). [c] The starting mercaptan was prepared by the method of J. Teppema and L. B. Sebrell, *J. Am. Chem. Soc.*, **49**, 1748 (1927). [d] The starting mercaptan was prepared by a modification of the method of H. Erlenmeyer, H. Ueberwasser, and H. M. Weber, *Helv. Chim. Acta*, **21**, 709 (1938). [e] The starting mercaptan was obtained from duPont. It was purified by precipitation from a solution of the sodium salt with excess acid followed by recrystallization from 95% ethyl alcohol.

When one considers the number of reactions which may occur in a mixture of mercaptan, hypochlorite, and ammonium hydroxide, it is surpris-

(8) C. M. Suter, *The Organic Chemistry of Sulfur*, John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 459, 500.

(9) R. Forsyth, J. A. Moore, and F. L. Pyman, *J. Chem. Soc.*, 919 (1924); G. R. Barnes and F. L. Pyman, *J. Chem. Soc.*, 2711 (1927).

(10) S. B. Greenbaum, *J. Am. Chem. Soc.*, **76**, 6052 (1954).

(11) E. H. Northey, *The Sulfonamides and Related Compounds*, Reinhold Publishing Corp., New York, N. Y., 1948, pp. 258, 309, and 310.

(12) W. E. Messer, U. S. Patent **2,257,974** (1941).

(13) W. H. Ebelke, U. S. Patents **2,343,538** (1944) and **2,351,496** (1944).

(14) Ger. Patent **586,351** (1933); R. S. Hanslick, U. S. Patent **2,304,568** (1942); R. H. Cooper, U. S. Patent (1944); **2,339,002** G. E. P. Smith, Jr., U. S. Patent **2,560,021** (1951).

(15) E. L. Carr, G. E. P. Smith, Jr., and G. Alliger, *J. Org. Chem.*, **14**, 921 (1949).

(16) Shortly after the culmination of our work the preparation of 6-Uracilsulfonamide by this procedure was reported (*cf.* ref. 10).

ing that any of the desired product can be isolated. For example, hypochlorite and ammonia may react in the following ways:[17]

(a) $NH_3 + NaOCl \longrightarrow NH_2Cl + NaOH$

(b) $2NH_3 + 3NaOCl \longrightarrow N_2 + 3H_2O + 3NaCl$

Any free chlorine in the hypochlorite may also react with excess ammonia to produce nitrogen and ammonium chloride.[17] Reaction (b) is an accepted analytical method for the quantitative determination of ammonia. In addition the hypochlorite may react with the mercaptan to produce a disulfide or the salt of a sulfonic acid:[15]

(c) $2RSNa \longrightarrow RSSR$

(d) $RSNa \longrightarrow RSO_2Na$

Despite the fact that some of these side reactions are most certainly taking place, we have been able to prepare sulfenamides of high purity in satisfactory yields. Although they appear to be stable when dry and free from alkali, it has been found advantageous to convert the crude material directly into the sulfonamide without further treatment.

The real simplicity of the process lies in the fact that the course of both oxidative steps may be followed potentiometrically. In later experiments the end-point of sulfenamide formation was determined with an antimony *vs.* standard calomel electrode system,[18] and the permanganate oxidation with a platinum *vs.* calomel system. Excellent results were obtained with a Beckman "Model G" *p*H Meter which reads in millivolts. Since the potentiometer indicates the presence of excess oxidizing agents, exact concentrations of solutions are relatively unimportant. This is a decided advantage when one works with commercial sodium hypochlorite solution, the concentration of which varies from about 9 to 16 per cent during shipment and storage.

The crude sulfonamides were usually purified by recrystallization from a suitable solvent. In some cases, however, the product was always contaminated with a second substance which could best be separated by chromatography. This was subsequently shown by its infrared absorption spectrum and elementary analysis to be an aryl substituted 2-hydroxy-benzothiazole (I), which displayed a keto-enol system: The formation of this impurity

appeared to be dependent both upon the type of aryl substituent and its position. Generally the stronger electron donating groups stabilized the sulfonamide, whereas an increased amount of cleavage was observed with the weaker donors. The substituent at position 6 tended to impart greater stability than did that in position 4 or 5. Electron attracting groups, on the other hand, contributed to extensive decomposition. For example, 6-nitrobenzothiazole-2-sulfonamide was not isolated by this procedure. The only product obtained was 2-hydroxy-6-nitrobenzothiazole. The order of stability in the series under discussion, then, appeared to be as follows: H > C_2H_5O > CH_3CONH > CH_3 > Cl > NO_2.

The acid-catalyzed nucleophilic displacement of 6-uracilsulfonic acid to give barbituric acid was recently reported by Greenbaum and Holmes.[19] Similarly Roblin and Clapp[4] described the cleavage of a number of their heterocyclic sulfonamides including benzothiazole-2-sulfonamide, which took place upon warming with 6*N* hydrochloric acid. The products obtained were the hydroxy compounds. Except for the acidification of the final solution of the potassium salt of the sulfonamide, the conditions for our procedure dictate basic solutions. We believe that a base-catalyzed nucleophilic displacement of the sulfonamide group adequately accounts for the observed results. In those cases where hydrolysis occurred a noticeable odor of sulfur dioxide was detected when the final solution was acidified. It does not seem reasonable to expect the cleavage to be instantaneous, and we conclude that the by-product is formed during the permanganate oxidation, when the *p*H increases to approximately 10.

Biological activity. (Table II). All of the compounds prepared were found to be potent carbonic anhydrase inhibitors when tested by the method of Philpot and Philpot.[20] Of the three compounds which possessed significant diuretic activity in the rat,[21] the most potent, 6-ethoxybenzothiazole-2-sulfonamide (Cardrase),[22] has received extensive clinical study in cases of congestive heart failure and glaucoma.[23] Published clinical studies showed this compound to possess two to four times the diuretic activity of 2-acetamido-1,3-4-thiadiazole-5-sulfonamide (Diamox)[24] on a milligram basis. Pharmacological activities are shown in Table II. More complete pharmacological data will be published elsewhere.

(17) C. A. Jacobson, *Encyclopedia of Chemical Reactions*, Reinhold Publishing Corp., New York, N. Y., 1955, pp. 143, 144.

(18) P. T. Paul and B. D. Hunter, U. S. Pat. 2,419,283 (1947).

(19) S. B. Greenbaum and W. L. Holmes, *J. Am. Chem. Soc.*, 76, 2899 (1954).

(20) F. J. Philpot and J. S. L. Philpot, *Biochem. J.*, 30, 2191 (1936).

(21) The assay method is a modification of the procedure of W. L. Lipschitz, Z. Hadidian, and A. Kerpcsar, *J. Pharmacol. and Exptl. Therap.*, 79, 97 (1943).

(22) CARDRASE is the Upjohn trademark for ethoxyolamide.

(23) (a) J. Moyer, S. Kinard, and R. Serscherger, *Antibiotic Med. & Clin. Therapy*, 3, 179 (1956). (b) H. Gold, T. H. Greiner, L. Warshaw, N. T. Kwit, and A. Ganz, *J. Am. Med. Assoc.*, 167, 814 (1958).

(24) DIAMOX is the trademark for acetazolamide.

TABLE II

Compound	Carbonic Anhydrase Inhibition[a]	Diuretic Activity[b]
1	3.2×10^{-6}	Inactive
2	6.5×10^{-6}	Inactive
3	2.8×10^{-6}	Active
4	4.8×10^{-6}	Inactive
5	1.04×10^{-7}	Inactive
6	2.7×10^{-8}	Active
7	7.3×10^{-8}	Active

[a] The concentration required to produce 50% inhibition. [b] Indicates at least a 25% increased urine excretion in rats in excess of the controls, at doses of 5, 10, and 20 mg./kg.

EXPERIMENTAL[25]

The procedure used was essentially the same in all cases and is illustrated by the preparation of 6-ethoxybenzothiazole-2-sulfonamide. The apparatus consisted of a small battery jar fitted with a clear plastic (Lucite) cover. Holes were drilled in the cover to accommodate a stirrer, thermometer, two dropping funnels, and the two electrodes (calomel and either antimony or platinum). An additional small opening served as a vent. The same container was used for both reactions.

A. *6-Ethoxybenzothiazole-2-sulfenamide.* A solution of 21 g. (0.1 mole) of 6-ethoxy-2-mercaptobenzothiazole and 4 g. of sodium hydroxide in 75 ml. of water, and a solution of sodium hypochlorite (approximately 10%) were added dropwise simultaneously to 300 ml. of concentrated ammonium hydroxide which was cooled to 7–10° and vigorously stirred. The initial reading of the potentiometer was −530 mv; the final reading, indicating a slight excess of hypochlorite, was −430 mv. The material, which began to precipitate almost immediately, was filtered and thoroughly washed with ice water to remove ammonia.

B. *6-Ethoxybenzothiazole-2-sulfonamide.* The crude, damp sulfenamide was suspended in 250 ml. of acetone and 450 ml. of water. With stirring there was added 440 ml. of 5% potassium permanganate solution at such a rate that the temperature was maintained at about 35° and the potentiometer showed no great excess of oxidizing agent at any time. The initial reading was usually less that +50 mv; the final potential was +440 mv. The mixture was filtered from the precipitated manganese dioxide, and the latter washed with 100 ml. of 5% sodium hydroxide solution and then with 200 ml. of water. The combined filtrates were concentrated under reduced pressure at 40° to remove most of the acetone. Acidification with concentrated hydrochloric acid gave the sulfonamide as a light tan solid. There was obtained 17.6 g. of material which melted at 175–188°. Recrystallization from ethyl acetate–petroleum ether gave 12.3 g. of colorless product, m.p. 188–190.5°.

When it was necessary to separate the sulfonamide from the 2-hydroxybenzothiazole, the crude mixture was dissolved in methylene chloride and chromatographed over Florisil (80 g. of adsorbent for each gram of material). Elution with 5% acetone in Skellysolve B gave the cleavage product. The sulfonamide could be recovered with 20% acetone in Skellysolve B. The compounds which were identified are: (a) 5-Chloro-2-hydroxybenzothiazole, m.p. 235–237°. *Anal.* Calcd. for C_7H_4ClNOS: N, 7.55; S, 17.27; Cl, 19.10. Found: N, 7.88; S, 17.36; Cl, 19.24.

(b) 6-Nitro-2-hydroxybenzothiazole, m.p. 252–256°. *Anal.* Calcd. for $C_7H_4N_2O_3S$: N, 14.28; S, 16.34. Found: N, 14.44; S, 16.88.

Acknowledgment. We wish to acknowledge the encouragement and support given by Dr. Robert H. Levin and Dr. Richard V. Heinzelman. The determinations of carbonic anhydrase inhibition were made by Dr. Margaret Greig and Miss Anna Gibbons. The diuretic and electrolyte studies were performed by Mr. Boyd E. Graham and associates. Microanalyses were prepared by Mr. William A. Struck and members of the Analytical Section of the Upjohn Company.

KALAMAZOO, MICH.

(25) Melting points are uncorrected.