B. Intact Conjugates.—The conjugates of **9** and **10** were located as weakly fluorescent bands in the paper chromatograms (ten 46.4 × 53.3 cm. sheets) of concentrated (20-fold) human-benzquinamide urine (0.5 ml./sheet) in a butanol–acetic acid–water (5:1:4) system by sectioning each chromatogram, combining corresponding bands, eluting the latter with 0.01 *N* HCl, acid hydrolysis of each eluate, and detection of the O-demethyl metabolite formed (**10**). Relative R_f values (16-hr. run) are: **10**, 1.00; **9**-conjugate, 0.72; 8-hydroxyquinoline glucuronide, 0.63; **10**-conjugate, 0.53.

Unidentified Metabolites.—Evidence that three unidentified yellow-fluorescent zones, a, c, and e, observed in paper chromatograms of extracts of dog-benzquinamide urine (Fig. 1 and 4) are drug-related metabolites was furnished by their methanolysis (deacetylation) to the slower moving blue-fluorescent zones, b, d, and f, respectively. R_f values are: system 1—a, 0.38; b, 0.20; **1**, 0.18; **2**, 0.13. Relative R_f values are: system I (3 hr.)—a, off sheet; b, 1.00; **1**, 0.89; **2**, 0.63; system V (2.5 hr.)—**3**, 1.00; c, 0.85; **4**, 0.42; d, 0.26; system VI (18 hr.)—e, off sheet; **6**, 1.00; f, 0.83.

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Synthesis and Biological Evaluation of Water-Soluble 2-Boronoethylthio Compounds

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The radical-catalyzed addition of mercaptans to the double bond of dibutyl ethyleneboronate has been employed for the synthesis of several water-soluble boronic acids. Adducts have been obtained with mercaptoacetic acid, β-mercaptopropionic acid, mercaptosuccinic acid, mercaptoethylamine hydrochloride, cysteine, mercaptoethanol, and sodium bisulfite. The 2-mercaptopyrimidine adduct could not be obtained directly but was prepared from dibutyl mercaptoethaneboronate and 2-chloropyrimidine. The boronic acids have been tested in C_3H mice with subcutaneously implanted brain tumors to determine the ratio of boron in the tumor to that in brain, blood, and muscle, as a function of time. One of the more favorable compounds on this basis was S-boronoethylcysteine. High transient boron ratios were found to be inadequate, and the need for binding compounds to tumor with concomitantly low boron concentrations in blood and brain is discussed.

The possibility of treating intracranial tumors using neutron capture irradiation was first proposed by Sweet and Javid.[2] This type of therapy utilizes the fact that while thermal neutrons have insufficient energy to ionize tissue components, their absorption by certain nonradioactive nuclides with a high propensity for these neutrons, such as the boron isotope B^{10}, results in a fission reaction in which the emitted fragments possess considerable ionizing energy. In the case of B^{10} the following nuclear reaction occurs.

$$B^{10} + n \rightarrow (B^{11}) \rightarrow Li^7 + He^4 + 2.4 \text{ Mev.}$$

These two particles are of such size that they travel a maximum of 9 μ, thereby releasing this destructive energy only in the immediate vicinity of the site of the original B^{10} atom. The main problem in the successful utilization of this technique has been the attainment of high levels of boron in the neoplasm with concomitantly low concentrations in normal tissue surrounding this area and in areas which will be subjected to thermal neutron bombardment.

Evaluation of a variety of substituted benzeneboronic acids in C_3H mice with subcutaneously transplanted brain tumors had shown that the most favorable tumor–brain boron ratios, as well as compounds with low toxicity, were found among those boronic acids having a high water–lipid solvent partition coefficient.[3a,b] Thus a low lipid solubility seemed to be an important requirement for a boron compound. As clinical information became available, it was apparent that the problem was more involved than the mere attainment of a high tumor–brain boron ratio. However, the preparation and evaluation of a series of highly water-soluble boronic acids was chosen as the goal of the present work on the basis of these first results.

Synthetic methods have not been available previously for the general preparation of alkylboronic acids containing hydrophilic substituents. The facile radical-catalyzed addition of mercaptans to the double bond of dibutyl ethyleneboronate[4] has been exploited in this present work to synthesize several boronic acids containing other functional groups, many of which have imparted increased water solubility.

The additions of mercaptoacetic and β-mercaptopropionic acid to dibutyl ethyleneboronate were easily accomplished. Either ultraviolet light or azobisisobutyronitrile could be used to initiate the reaction, but with additional experience it appeared that the azonitrile gave more consistent and better controlled results. The initially formed liquid adducts decomposed on attempted distillation and were therefore hydrolyzed directly to the corresponding boronic acids, 2-boronoethylthioacetic (**1a**), and 3-(2-borono-

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(2) W. H. Sweet and M. Javid, *J. Neurosurg.*, **9**, 200 (1952).

(3) (a) A. H. Soloway, *Science*, **128**, 1572 (1958); (b) A. H. Soloway, B. Whitman, and J. R. Messer, *J. Med. Pharm. Chem.*, **5**, 191 (1962).

(4) D. S. Matteson, *J. Am. Chem. Soc.*, **82**, 4228 (1960).

ethylthio)propanoic acid (**1b**), which were crystallized from water.

$$HO_2C(CH_2)_nSH + CH_2{=}CHB(OBu)_2 \rightarrow HO_2C(CH_2)_nSCH_2CH_2B(OBu)_2 \xrightarrow{H_2O} HO_2C(CH_2)_nSCH_2CH_2B(OH)_2$$

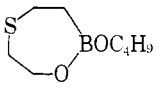
1a, $n = 1$; b, $n = 2$

Crystalline 3-(2-borono-1-propylthio)propanoic acid (**2**) was similarly obtained from dibutyl propene-2-boron-

$$HO_2CCH_2CH_2SCH_2\underset{\displaystyle CH_3}{\underset{|}{C}}HB(OH)_2 \quad \mathbf{2}$$

ate[4] and β-mercaptopropionic acid, but the corresponding product from mercaptoacetic acid failed to crystallize.

Mercaptoethanol added readily to dibutyl ethyleneboronate, but in this case the alcohol function in the adduct esterified the boron function to yield the distillable monobutyl 2-(2-hydroxyethylthio)ethaneboronate lactone (**3**) (a more systematic name would be 1-butoxy-2-oxa-5-thiaborepane). The boronic acid from **3** formed a sirup with water, which was used for the



3

biological tests without further purification.

Solubility problems were encountered with reactants substantially more polar than simple carboxylic acids or alcohols, since dibutyl ethyleneboronate itself is a relatively nonpolar solvent. It was found that the radical-catalyzed additions proceeded readily in methanol or a methanol–water solvent mixture. The rapid equilibration of hydroxy and alkoxy ligands on boron makes it impossible to say which of the vinylboron species is the principal reactant. However, the desired boronic acid was isolated readily upon the addition of water and subsequent concentration of the reaction mixture as a consequence of the volatility of methanol and the butanol–water azeotrope. Thus, for example, refluxing mercaptosuccinic acid and dibutyl ethyleneboronate with azobisisobutyronitrile as an initiator in methanol yielded, after hydrolysis, (2-boronoethylthio)-succinic acid. This formed a sirup with water but could be isolated as the crystalline calcium salt **4**. Un-

$$Ca^{+2}\ ^-O_2CCH_2\underset{\displaystyle CO_2^-}{\underset{|}{C}}HSCH_2CH_2B(OH)_2 \quad \mathbf{4}$$

der similar conditions, mercaptoethylamine hydrochloride led to 2-(2-boronoethylthio)ethylamine hydrochloride (**5**), and cysteine yielded S-(2-boronoethyl)-cysteine (**6**), both of which were crystallized from water.

$$Cl^-\ ^+H_3NCH_2CH_2SCH_2CH_2B(OH)_2 \quad \mathbf{5}$$

$$^-O_2C\underset{\displaystyle NH_3^+}{\underset{|}{C}}HCH_2SCH_2CH_2B(OH)_2 \quad \mathbf{6}$$

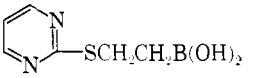
By extension of this technique it was also found possible to add sodium bisulfite to dibutyl ethyleneboronate. The resulting 2-boronoethanesulfonate ion was isolated as the potassium salt (**7**).

$$K^+\ ^-O_3SCH_2CH_2B(OH)_2 \quad \mathbf{7}$$

The isolation and purification of this compound required conversion of the initial sodium bisulfite adduct to the free sulfonic acid using an ion-exchange resin. Concentration of the acidic solution under vacuum removed residual bisulfite as sulfur dioxide. Boric acid was also a by-product of this reaction and had to be removed by the fractional distillation of the methanol–methyl borate azeotrope resulting from the addition of excess methanol. After conversion of the acid to its potassium salt with an ion-exchange resin, the exceedingly soluble salt **7** was crystallized from water. The structure of **7** was confirmed by the n.m.r. spectrum in water, which showed the expected pair of triplets due to the adjacent methylene groups.

Somewhat unexpectedly, the general synthetic method failed with 2-mercaptopyrimidines. Starting materials were recovered when 2-mercaptoorotic acid and dibutyl ethyleneboronate were irradiated in dimethylformamide at 25°, or water at 100°, or when heated with azobisisobutyronitrile in dimethylformamide, pyridine, acetonitrile, or methanol–water. Attempted reaction of 2-mercaptopyrimidine in *t*-butyl alcohol or methanol–water and of β-(2-mercaptouracil-3)propionic acid in *t*-butyl alcohol with the azonitrile initiator also failed.

An alternative synthesis of 2-(2-boronoethylthio)-pyrimidine (**8**) was then devised. Dibutyl 2-mercaptoethaneboronate (**9a**) was prepared either by the light-initiated addition of hydrogen sulfide to dibutyl ethyleneboronate at −70° or, more conveniently, by



8

$XSCH_2CH_2B(OBu)_2$ a, X=H; b, X=CH_3CO

9

base hydrolysis of the thiolacetic acid–dibutyl ethyleneboronate adduct, dibutyl 2-(acetylthio)ethaneboronate (**9b**). In the presence of sodium butoxide, the mercaptan **9a** displaced chloride ion from 2-chloropyrimidine to yield, after hydrolysis, 2-(2-boronoethylthio)-pyrimidine (**8**). The possibility of extending this synthesis to other pyrimidines of greater potential biological interest is currently being explored.

Simple aliphatic boronic acids have been reported to be unstable to air oxidation and difficult to purify and characterize.[5] However, the crystalline boronic acids in the present series are stable in air at room temperature and, except for problems of high solubility, were easily purified.

Biological Evaluations.—The initial screening procedure used[3] with these compounds was to inject aqueous solutions, either intravenously or intraperitoneally, into C_3H mice bearing a subcutaneously transplanted brain tumor.[3] The animals were sacrificed at 15 min. to 3 hr. after injection and portions of excised tissues were analyzed for boron content. During the course of the biological evaluation of the compounds reported below it became apparent that this procedure was inadequate.

Therapeutic evaluation in man of two of the more promising compounds from the standpoint of tumor concentration, *p*-carboxybenzeneboronic acid and sodium perhydrodecaborate[6a,b] were singularly unsuc-

(5) H. R. Snyder, J. A. Kuck, and J. R. Johnson, *J. Am. Chem. Soc.*, **60**, 105 (1938).

(6) (a) A. H. Soloway, R. L. Wright, and J. R. Messer, *J. Pharmacol. Exptl. Therap.*, **134**, 117 (1961); (b) W. H. Sweet, A. H. Soloway, and R. L. Wright, *ibid.*, **137**, 263 (1962).

cessful. Tissue and vessel radiation damage was indicated[7] and this most probably resulted from the high levels of boron in the blood. Thus, though there was a favorable concentration ratio between brain and tumor,[6a] the levels in brain were concentrated in the blood vessels. Upon irradiation therefore the blood vessel walls would undoubtedly be damaged impairing the normal transport from blood to brain. On this basis the animal evaluation procedure for various compounds has been markedly altered.

Compounds have been injected on a daily basis and sacrifice has occurred several days following the last injection. The rationale for this screening procedure is that the type of compound which will be useful is one that will be bound to tumor but cleared from blood, brain, and other tissues subjected to thermal neutron irradiation. By such an evaluation, compounds may be discerned which gradually concentrate in the desired tissue.

In Table I are listed 10 of the compounds whose synthesis has been described. Several have been evaluated by both procedures, although many of the more recent ones have been examined only for their tumor-binding properties. The ratios listed are averages of up to 3 animals for each time sequence. For each animal 2–4 tumor, blood, and muscle samples were analyzed; in some instances the entire brain was used since its boron content was quite low. No correction was made for the blood content of these tissues.

Though there are normally large variations in the ratios for these highly inbred mice, certain generalizations are possible. These compounds, as measured by boron content, attain at least the same but normally greater concentrations in tumor than in normal brain. The levels in blood, on the other hand, are invariably higher than tumor with the possible exceptions of S-(2-boronoethyl)cysteine and 2-(acetylthio)ethylboronic acid. The former compound was evaluated as well by as many as 4 daily injections with sacrifice occurring on the third day after the last dose. Boron tissue concentrations were too low for our analytical technique. This also applied to the other compounds in this series which were evaluated by repetitive injections. In no instance was there selective binding of these compounds by tumor or by any other tissue, nor is there any rationale why selective incorporation should occur since these compounds would not be considered antimetabolites. However, the mechanisms by which compounds are bound to tumors and other tissues are unknown and consequently a pragmatic approach is warranted.

Other compounds are now being considered which may have alkylating moieties or other biologically active functional groups containing a boron atom. In this manner, boron may become incorporated into the desired target tissues. Such an approach seems required if neutron capture therapy is to be rendered practicable.

Table I
Tissue Distribution Studies[a]

Time in min.	Tissue distribution ratio: Tumor–brain	Tumor–muscle	Tumor–blood
2-Boronoethylthioacetic Acid			
15	7.9	1.2	0.4
30	6.7	1.2	0.5
60	2.4	1.2	0.6
120	1.9	1.2	0.4
180	1.4	1.2	0.2
3-(2-Boronoethylthio)propanoic Acid			
15	5.6	1.0	0.2
30	5.9	1.5	0.6
60	4.1	...	0.5
120	2.5	1.5	0.8
180	2.0	1.4	0.9
3-(2-Borono-1-propylthio)propanoic Acid			
15	4.6	1.2	...
30	6.5	1.2	...
60	4.4	1.3	...
180	2.6	1.2	...
Lactone of 2-(2-Hydroxyethylthio)ethaneboronic Acid[b]			
15	1.1	0.6	0.5
30	1.4	1.2	0.8
60	1.6	0.9	0.6
120	3.0	1.2	0.7
180	4.5	1.5	0.8
Calcium (2-Boronoethylthio)succinate[c] **2-(2-Boronoethylthio)ethylamine Hydrochloride[d]** **S-(2-Boronoethyl)cysteine[b]**			
15	6.8	2.0	0.4
30	6.7	1.8	0.9
60	4.7	1.4	1.2
120	2.8	1.3	0.9
180	2.4	1.6	0.9
Potassium 2-Boronoethanesulfonate[b]			
15	5.3	1.5	0.2
30	4.3	2.3	0.3
2-(2-Boronoethylthio)pyrimidine[e] **2-(Acetylthio)ethaneboronic Acid**			
15	0.9	0.9	0.7
30	1.3	1.0	0.9
60	1.6	1.2	0.8
120	2.0	1.2	0.9
180	2.8	1.2	1.2

[a] The method for obtaining the animal data is discussed in the biological part of the Experimental procedure. In most cases 2 to 3 animals were used at each time interval and the values listed are averages of these ratios. Doses ranged from 35 to 140 γ of boron/g. of body weight. [b] Boron levels in tissues were extremely low if the last injection occurred several days before sacrifice. [c] Boron levels in tissues were extremely low. The compound was administered in daily doses (3–5 days) followed by sacrifice 2 days after the last injection. [d] This is an extremely toxic compound. Animals died at doses in excess of 9 γ of boron/g. Levels in tissue were uniformly low both in animals receiving repetitive injections with sacrifice occurring several minutes after the last injection and with those killed min. and hr. after a single dose. [e] This is an extremely toxic compound. Animals died at doses of 9 γ of boron/g. or greater. Boron levels in tissue were uniformly too low to be meaningful at lower doses.

Experimental

A. Synthesis.—Microanalyses are by Galbraith Laboratories, Knoxville, Tennessee. Analytical samples of crystalline boronic acids were dried briefly at 25° (0.1 mm.). All melting points were taken in capillary tubes and are corrected.

2-Boronoethylthioacetic Acid (1a).—A mixture of 5.25 g. of mercaptoacetic acid and 10 g. of dibutyl ethyleneboronate was kept at 80° under nitrogen for 4 hr. and 0.05-g. portions of azobisisobutyronitrile were added after 0 and 2 hr. Stirring of a 11.1-g. portion of the crude adduct with 3 ml. of hot water and cooling yielded 3.9 g. of **1a**; after recrystallization, m.p. 104–108°.

(7) A. K. Asbury and R. G. Ojemann, unpublished.

Anal. Calcd. for $C_4H_9BO_4S$: C, 29.29; H, 5.53; B, 6.59; S, 19.55. Found: C, 29.50; H, 5.66; B, 6.74; S, 19.64.

3-(2-Boronoethylthio)propanoic Acid (1b).—A mixture of 5 g. of dibutyl ethyleneboronate and 2.6 g. of β-mercaptopropionic acid at room temperature was irradiated in a quartz flask under nitrogen with an AH-4 mercury vapor lamp for 6–7 hr. Water (10 ml.) was added, and the water and butanol were distilled at 20–30 mm., leaving a residue which on recrystallization from water yielded 1.5 g. of **1b**, m.p. 118–121°.

Anal. Calcd. for $C_5H_{11}BO_4S$: C, 33.73; H, 6.22; B, 6.08; S, 18.01. Found: C, 33.53; H, 6.28; B, 6.20; S, 18.25.

3-(2-Borono-1-propylthio)propanoic Acid (2).—Substituting 5 g. of dibutyl propene-2-boronate for the ethyleneboronate in the procedure described for **1b** yielded 3.1 g. of crude **2**. It was recrystallized from water (0.5 ml./g.) repeatedly with considerable loss, m.p. 83–84°.

Anal. Calcd. for $C_6H_{13}BO_4S$: C, 37.5; H, 6.8; B, 5.6; S, 16.8. Found: C, 39.3; H, 6.8; B, 5.8; S, 17.3.

Monobutyl 2-(2-Hydroxyethylthio)ethaneboronate Lactone (3).—A mixture of 3.3 g. of mercaptoethanol and 7.4 g. of dibutyl ethyleneboronate kept at 80° for 4 hr. was treated with 0.08-g. portions of azobisisobutyronitrile after 0 and 2 hr. Distillation yielded 5.8 g. (77%) of **3**, b.p. 56–61° (0.1 mm.); fractionated, b.p. 60° (0.1 mm.).

Anal. Calcd. for $C_8H_{17}BO_2S$: C, 51.08; H, 9.11; B, 5.75; S, 17.05. Found: C, 50.99; H, 9.19; B, 5.91; S, 17.14.

An aqueous solution of 2-(2-hydroxyethylthio)ethaneboronic acid was prepared by adding excess water to **3** and distilling the butanol–water azeotrope out under vacuum.

Calcium (2-Boronoethylthio)succinate (4).—A solution of 7.9 g. of mercaptosuccinic acid and 9.2 g. of dibutyl ethyleneboronate in 25 ml. of methanol was refluxed for 16 hr. under nitrogen and treated with 0.1-g. portions of azobisisobutyronitrile after 0 and 6 hr. The methanol was distilled under vacuum; the residue was treated with 20 ml. of water and concentrated to a waxy residue at 20 mm. Treatment with calcium hydroxide and water followed by concentration yielded 53% of the calcium salt **4**, recrystallized from water with a loss of about 50%.

Anal. Calcd. for $C_6H_9BCaO_6S$: C, 27.71; H, 3.49; B, 4.16; Ca, 15.41; S, 12.33. Found: C, 27.65; H, 3.61; B, 3.90; Ca, 15.20; S, 12.49.

2-(2-Boronoethylthio)ethylamine Hydrochloride (5).—A solution of 6.0 g. of mercaptoethylamine hydrochloride and 9.2 g. of dibutyl ethyleneboronate in 30 ml. of methanol was refluxed under nitrogen for 18 hr. and 0.08-g. portions of azobisisobutyronitrile were added after 0 and 2 hr. The solvent was removed with a water pump, and the residue was stirred in 25 ml. of water for 1 hr. After washing with three 25-ml. portions of ether, the aqueous solution was cooled and the product (**5**) crystallized; yield, 6.5 g. (two crops) (70%); recrystallized, m.p. 106–109°.

Anal. Calcd. for $C_4H_{13}BClNO_2S$: C, 25.90; H, 7.06; B, 5.83; Cl, 19.11; N, 7.55; S, 17.29. Found: C, 25.71; H, 7.01; B, 5.95; Cl, 19.14; N, 7.50; S, 17.24.

S-(2-Boronoethyl)cysteine (6).—A solution of 12.7 g. of cysteine and 18.4 g. of dibutyl ethyleneboronate in 150 ml. of methanol and 200 ml. of water was refluxed in an oil bath at 80° for 14 hr. under nitrogen and a 0.15-g. portion of azobisisobutyronitrile was added after 0 and 4 hr. The solvents were distilled at approximately 20 mm., care being taken not to overheat the residue, leaving 24.7 g. of a crude waxy product which was recrystallized from hot water to yield 15.0 g. (78%) of **6**; the analyzed sample had a decomposition range of 155–190°.

Anal. Calcd. for $C_5H_{12}BNO_4S$: C, 31.11; H, 6.27; B, 5.60; N, 7.26; S, 16.61. Found: C, 30.98; H, 6.29; B, 5.60; N, 7.30; S, 16.86.

Potassium 2-Boronoethanesulfonate (7).—A solution of 10.4 g. of sodium bisulfite and 18.4 g. of dibutyl ethyleneboronate in 100 ml. of methanol and 100 ml. of water was heated under nitrogen in an oil bath at 80° for 16 hr. and a 0.1-g. portion of azobisisobutyronitrile was added after 0, 4, and 8 hr. Most of the methanol was removed with a water aspirator and the aqueous solution was stirred with an excess (75 ml.) of a sulfonic acid ion-exchange resin for 1 hr. The mixture was then filtered and concentrated at 20 mm. The sirupy residue was dissolved in 25 ml. of water to which was added 175 ml. of methanol. The solution was fractionated at atmospheric pressure until all the methyl borate–methanol azeotrope had been distilled (negative flame test for boron in distillate). The solution was then treated with excess potassium sulfonate ion-exchange resin, filtered, and concentrated under the aspirator. A viscous sirup remained which crystallized to waxy solid in a few hr. at 5°. Recrystallization from a minimum of hot water (0.5 ml./g.) yielded approximately 11 g. (57%) of **7**. Recrystallization was carried out with about 50% loss of material each time. The n.m.r. spectrum of the salt in water showed the expected two triplets from the two sets of methylene hydrogens.

Anal. Calcd. for $C_2H_6BKO_5S$: C, 12.51; H, 3.15; B, 5.63; S, 16.70. Found: C, 12.58; H, 3.27; B, 5.35; S, 16.42.

Dibutyl 2-(Acetylthio)ethaneboronate (9b).—Heating 4.1 g. of thiolacetic acid and 9.2 g. of dibutyl ethyleneboronate at 80° for 4 hr. with the addition of 0.08 g. of azobisisobutyronitrile after 0 and 2 hr. followed by distillation yielded 8.4 g. (64%) of **9b**, b.p. 86–87° (0.2 mm.); fractionated, b.p. 84° (0.2 mm.).

Anal. Calcd. for $C_{12}H_{25}BO_3S$: C, 55.39; H, 9.68; B, 4.16; S, 12.32. Found: C, 55.75; H, 9.78; B, 4.38; S, 12.54.

2-(Acetylthio)ethaneboronic Acid.—Stirring 6.2 g. of **9b** with 25 ml. of water at 60–70° for 1 hr. and concentrating at 20 mm., followed by cooling to 5°, yielded 2.9 g. (82%) of product; twice recrystallized, m.p. 72–74.5°.

Anal. Calcd. for $C_4H_9BO_3S$: C, 32.46; H, 6.13; B, 7.31; S, 21.67. Found: C, 32.60; H, 6.38; B, 7.43; S, 21.66.

B. Biological.—In other studies[6a] it has been shown that C_3H mice bearing subcutaneously transplanted gliomas provide a useful means of assaying the tumor–brain ratios as well as other tissue concentrations of various boron compounds. The present variety of glioma, an ependymoma, was used throughout the investigations. The method used for transplanting the tumor has been described,[3b] and the general analytical technique for boron has also been recently presented.[8] Aqueous solutions of the drugs were prepared and their toxicity in mice determined. The tumor-bearing animals were injected intravenously or intraperitoneally, usually under light ether anesthesia, with doses of from 35 to 140 γ of boron per g. of body weight (γ B/g.). They were sacrificed by ether inhalation after the desired periods of time and various tissues were excised, weighed, and analyzed for boron content.[8] The tissue distribution ratios were then determined from the averages of the boron content in the tissue samples.

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(8) A. H. Soloway and J. R. Messer, *Anal. Chem.*, **36**, 433 (1964).