Penetration of Brain and Brain Tumor by Aromatic Compounds as a Function of Molecular Substituents. III[1,2]

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To a great extent there is a correlation between high aqueous/lipid solvent partition coefficients of a series of substituted aromatic boronic acids and the high tumor/brain ratios of these compounds in mice with subcutaneously transplanted gliomas. There are, however, exceptions to this observation and the mechanism of transport into the central nervous system of compounds with low lipid solubility remains obscure. The position of groups on an aromatic nucleus is of importance in determining the degree of penetration of brain. An attempt is made to correlate chemical and physical properties with biologic attributes.

An understanding of the types of compounds which will penetrate brain tumors but not the brain is essential in the treatment of such neoplasms by neutron capture irradiation[3] and by chemotherapy.[4] Previous studies[5,6] have shown a definite correlation between brain tumor/brain ratio of various substituted benzeneboronic acids in C3H mice and their lipid solubility. A histologically-reproducible tumor, such as this ependymoma, was used throughout. By comparing the amount of a compound in this tissue with the amount in normal brain, the necessity of maintaining a constant blood level, in order to ascertain the permeation of the brain, becomes unnecessary. In this way both tissues are subjected to the same fluctuation in blood concentration and the ratio becomes a true measure of the

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(2) For Papers I and II: see references 5 and 6.

(3) W. H. Sweet, A. H. Soloway, and G. L. Brownell, *Acta Union Int. Contre le Cancer*, **16**, 1216 (1960).

(4) V. H. Mark, R. N. Kjellberg, R. G. Ojemann, and A. H. Soloway, *Neurol.*, **10**, 772 (1960).

(5) A. H. Soloway, *Science*, **128**, 1572 (1958).

(6) A. H. Soloway, B. Whitman, and J. R. Messer, *J. Pharmacol. Exptl. Therap.*, **129**, 310 (1960).

permeability of brain relative to this tissue by the compound under such conditions.

Substances with a high lipid solubility invariably penetrated the brain readily, were toxic, and gave poor tumor/brain ratios as measured by their boron content. Only among those substances with low lipid solubility were compounds obtained whose ability to penetrate the central nervous system was restricted. Many of these hydrophilic ones were relatively non-toxic and some did give high tumor/brain boron ratios.[7] However, two groups of compounds with low lipid solubility gave poor ratios. These were the amines, with the exception of those containing a carboxylic acid function, and the phenols. Such substances penetrated the brain nearly as well as the tumor and were quite toxic.

The purpose of this investigation in part was to determine whether the amines were an exception to the assumption that lipid solubility of a compound is one of the main factors in determining its ability to penetrate the central nervous system. Additional information was also sought relating to the effect produced by groups in an aromatic compound upon its ability to penetrate the brain. In particular, are position isomers handled in the same or in a different manner by the central nervous system? Such information would permit a correlation between the physical and chemical properties of drugs with their biological qualities.

Experimental Methods

The method for determining lipid solubility is essentially the same as previously described.[6] Approximately 10 mg. of each substance was distributed between 25 ml. of a phosphate-buffered aqueous medium of pH 7.2 and 25 ml. of a lipid solvent, chloroform or benzene. The mixing was carried out in a separatory funnel and the layers were separated. Aliquots of each phase then were analyzed for boron content.[8] The values in Table I are listed in μg. of boron per ml. of each solution.

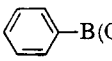
To determine tumor/brain ratios, C3H mice bearing subcutane-

(7) A. H. Soloway, R. W. Wright, and J. R. Messer, *J. Pharmacol. Exptl. Therap.*, **134**, 117 (1961).

(8) G. H. Ellis, E. G. Zook, and O. Baudisch, *Anal. Chem.*, **21**, 1345 (1949).

TABLE I

AQUEOUS/LIPID SOLVENT PARTITION COEFFICIENTS

	Aqueous[a]	Benzene[a]	Aqueous/ benzene	Aqueous[a]	Chloro- form[a]	Aqueous/ chloro- form
4-Cl[b]	6.9	5.2	1	11.8	10.8	1
4-H[b]	14.4	2.3	6	23.6	6.0	4
3-NO_2-4-NH_2[c]	20.9	0.73	29	22.0	2.2	10
3-NH_2-4-CH_3[b]	15.4	.23	67	29.2	1.9	15
4-COOH[b]	11.4	.17	67	23.3	0.32	73
3-NO_2-5-NH_2[c]	23.5	.14	170	25.4	.38	67
2-NO_2-4-NH_2[c]	22.8	.10	>200	25.2	.15	170
3-NH_2[b]	12.4	.06	>200	29.8	.44	68
3,5-$(NH_2)_2$[c]	26.0	0	>200	29.0	0	>200
2-CH_3-3,5-$(NH_2)_2$[c]	25.2	0	>200	30.0	0.04	>200

[a] Values listed are in μg. of boron/ml. [b] For aqueous/benzene values, see ref. 6. [c] The authors wish to thank Dr. Kurt Torssell of the Biokemiska Institutet in Stockholm, who very kindly supplied us with these compounds.

ously transplanted gliomas[9] were used. Fresh tumor tissue was ground in normal saline and a cellular suspension was injected in the region of the left scapula in 6-to 8-week old C3H mice. Within 7 to 10 days the tumors were large enough for use. Solutions of the compounds were prepared and injected intraperitoneally into the tumor mice. The animals were sacrificed at fixed time intervals after injection to obtain biopsy specimens for boron analysis. The boron tissue concentrations are recorded in Table II.

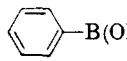
Results and Discussion

The aqueous/benzene and aqueous/chloroform partition coefficients of several mono- and disubstituted aromatic boronic acids are recorded in Table I. The group includes 3-amino-4-methylbenzeneboronic acid, *m*-aminobenzeneboronic acid, 3,5-diaminobenzeneboronic acid and 3,5-diamino-2-methylbenzeneboronic acid. The last three compounds have high values for their aqueous/lipid solvent coefficients in contrast with the low values for benzeneboronic acid and *p*-chlorobenzeneboronic acid. The coefficients for these amines are comparable to those observed with *p*-carboxybenzeneboronic acid. On this basis, were low lipid solubility the sole re-

(9) The authors are greatly indebted to Dr. D. M. Perese of the Department of Neurosurgery at the Roswell Park Memorial Institute in Buffalo, N. Y., for supplying us with the original subcutaneously-grown ependymoma.

TABLE II

TUMOR/BRAIN BORON LOCALIZATION FACTORS[a]

	Dose[b]	Time of sacrifice[d]	Tumor	Brain[c]	Localization factor[e]
3-NO_2-4-NH_2	35	15	7.0	12.8	0.5
	35	30	8.5	15.0	0.6
	70	30	12.0	18.7	0.6
3-NO_2-5-NH_2	35	15	9.0	4.9	1.8
	35	30	16.7	7.9	2.1
	70	30	34.0	10.6	3.2
2-NO_2-4-NH_2	35	15	24.4	8.8	2.8
	70	15	26.9	11.8	2.3
	140	30	48.8	22.0	2.4
3,5-$(NH_2)_2$	35	15	13.4	1.8	7.5
	35	30	21.7	2.9	7.5
	140	30	38.4	5.3	7.5
2-CH_3-3,5-$(NH_2)_2$	35	15	18.8	3.9	4.8
	35	30	17.8	3.7	4.8
	70	30	30.6	4.0	7.7

[a] The tumor and brain concentrations are tissue averages of one or two mice. [b] Dose in μg. of boron/g. of mouse. [c] Concentrations are in μg. of boron per g. of tissue. [d] Time is in min. [e] Localization factor is the tumor/brain boron ratio.

quirement for a compound in order to achieve a high tumor-to-brain ratio, it may be anticipated that all these compounds would be restricted in their penetration of normal brain but not brain tumor.

As shown in Table II and in previous work[6] this is not completely the case. Those compounds containing a single amino function, such as *m*-aminobenzeneboronic acid, penetrate normal brain as readily as tumor at the times in which they were examined.[10] Also these compounds were more toxic to the central nervous system in comparable doses, than those substances which showed a high tumor/brain ratio.

Introduction of a second amino function, however, markedly increased the tumor/brain boron ratio relative to the monoamines and appreciably lowered the toxicity of these compounds. Thus, it would appear that the transport of the diamines into the central nervous system may be related to their lipid solubility but the entrance of compounds such as *m*-aminobenzeneboronic acid may involve a mechanism which is independent of this property.

(10) The tumor/brain boron ratios for monoamines, 3-amino-4-methylbenzeneboronic acid and *m*-aminobenzeneboronic acid have been reported in Paper II and ranged from 0.9–1.2.

Some workers[11,12] have suggested that the affinity of a compound for certain cellular components may be a contributing factor in its concentration and penetration of the central nervous system. It is conceivable that certain functional groups such as amino and hydroxyl moieties can interact with cellular binding sites, become fixed in the cell and, as a result, no longer be freely diffusible. Since these sites may have specific requirements for adsorption, the arrangement of the substituent groups may be important.

To contribute information about the effect of the group position on an aromatic nucleus and its penetration of the central nervous system, three nitroaminobenzeneboronic acids were partitioned between an aqueous and a lipid solvent and were then examined in C3H mice bearing gliomas. These isomers are the 4-amino-2-nitro-, the 5-amino-3-nitro- and 4-amino-3-nitrobenzeneboronic acids. Of these compounds the *o*-nitroaniline derivative has distribution coefficients of less than 30 while the others have values in excess of 67. This higher lipid solubility of 4-amino-3-nitrobenzeneboronic acid may account for the fact that it penetrates brain readily and gives tumor/brain boron ratios of approximately 0.5, while the two other isomers have localization factors ranging from 1.8 to 3.2 as shown in Table II. The physical and chemical properties of compounds with certain groups in an *ortho* position are known to be different from other position isomers, especially with regard to their greater solubility in non-aqueous solvents. The theory that this difference is due to intramolecular hydrogen bonding with the formation of cyclic structures is supported by spectral evidence.[13] It would appear that such compounds, exemplified by 4-amino-3-nitrobenzeneboronic acid, do show an increased permeability of the brain relative to the other isomers. This may be due to its higher lipid solubility but it is also conceivable that the vicinal position of these groups may result in a greater binding potential.

In conclusion, it can be stated that lipid solubility is undoubtedly an important factor in the penetration of the brain by a drug. However, there must be other mechanisms involved to account for the

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(12) S. E. Mayer, R. P. Maickel and B. B. Brodie. *J. Pharmacol. Exptl. Therap.*, **127**, 205 (1959).

(13) L. G. Bellamy. "The Infra-red Spectra of Complex Molecules," John Wiley and Sons, New York. N. Y., 1954.

ready permeation of the central nervous system by certain compounds with low lipid solubility. It would seem important to determine in which cellular fraction, if any, the drug is fixed and the degree of stability of any drug-cellular substituent bonds.

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Aspects of the Metabolism of Isoniazid and Acetylisoniazid in the Human and the Dog[1,2]

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After administration of 1-acetyl-2-isonicotinylhydrazine, a known metabolite of isonicotinylhydrazine, an adult male excreted 1,2-diacetylhydrazine. In contrast, the dog, following administration of 1-acetyl-2-isonicotinylhydrazine, excreted acetylhydrazine. In view of the foregoing and previous data indicating the inability of the dog to form 1,2-diacetylhydrazine from hydrazine or acetylhydrazine, it is concluded that the metabolism of isoniazid in man and other species such as the rabbit involves the route: isonicotinylhydrazine → 1-acetyl-2-isonicotinylhydrazine → acetylhydrazine → 1,2-diacetylhydrazine.

The metabolism of isonicotinylhydrazine (isoniazid) to yield 1-acetyl-2-isonicotinylhydrazine (acetylisoniazid) has been recorded for

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