The Metabolism of N-Alkyl-4-bromobenzenesulfonamides in the Mouse. Correlation with Anticonvulsant Activity

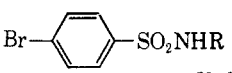
DAVID L. SMITH, HUGH H. KEASLING, AND ARLINGTON A. FORIST

Biochemical Research Division, The Upjohn Company, Kalamazoo, Michigan

Received February 12, 1965

The anticonvulsant potency of several N-alkyl-4-bromobenzenesulfonamides in mice was found to be directly related to their extent of metabolism to 4-bromobenzenesulfonamide. The extent of *in vivo* dealkylation of the monoalkylated derivatives varied inversely with the bulk of the alkyl substituent. After oral administration, plasma concentrations of the N-alkylsulfonamides and their dealkylated metabolites were determined at the microgram level by quantitative thin layer chromatography. Pretreatment of the mice with the metabolic inhibitor, β-diethylaminoethyl diphenylpropylacetate hydrochloride, decreased the rate of dealkylation slightly but did not significantly affect anticonvulsant activity. Pretreatment of the mice with CCl_4, however, considerably decreased both the extent of dealkylation and the anticonvulsant potencies of the N-alkylsulfonamides. Since N,N-dimethyl-4-bromobenzenesulfonamide was metabolized to N-methyl-4-bromobenzenesulfonamide and 4-bromobenzenesulfonamide, a free hydrogen atom on the sulfonamide nitrogen is not a prerequisite for *in vivo* dealkylation.

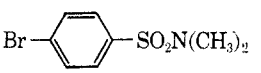
Biological data from these laboratories on a series of benzenesulfonamides and related compounds[1] have shown the 4-bromobenzenesulfonamides to be especially active anticonvulsant agents as revealed by antagonism of maximal electroshock seizures and thiosemicarbazide and strychnine lethality in mice. 4-Bromobenzenesulfonamide (I) and N-ethyl-4-bromobenzenesulfonamide (II), which were tested most extensively, were found to have about equal potency after oral administration (Table I). The anticonvulsant activity of I was thought to result from carbonic anhydrase inhibitory activity, which is commonly shown by nonsubstituted sulfonamides.[2-4] The high potency of II and the other substituted members of the series was unexpected, however, since it is generally accepted that substitution on the sulfonamide nitrogen destroys



I, R = H
II, R = C_2H_5
III, R = CH_3
IV, R = $CH_2CH{=}CH_2$
V, R = $CH(CH_3)_2$
VI, R = $(CH_2)_2CH_3$
VII, R = $(CH_2)_3CH_3$
VIII, R = OH

carbonic anhydrase inhibitory activity.[2,4,5] *In vitro* carbonic anhydrase inhibitory activity of I as well as lack of such activity by II has been established in these laboratories,[6] using the method of Maren.[7]

The data of Maren[5] on 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (acetazolamide) and its N^5-isopropyl derivative show the latter to be metabolized *in vivo* to a carbonic anhydrase inhibitor, thus explaining its activity. These data indicated to us that N-alkyl compounds of the present series (II–VII) might be dealkylated to I, which could be responsible for the observed anticonvulsant activity. However, since Maren[5] only presumed the carbonic anhydrase inhibitor to be acetazolamide (the product was not isolated and identified), we could not assume *a priori* that the activity of the N-substituted bromobenzenesulfonamides was due to metabolic dealkylation. Moreover, the similarity in potency of I and III in mice was not in accord with Maren's observation that only about 4% of the N^5-methyl derivative of acetazolamide was converted to a carbonic anhydrase inhibitor in this species. The present situation was complicated also by the fact that the dimethyl derivative (IX) possessed considerable activity. If the activity of IX were due to its conversion to I, this would con-



IX

tradict the suggestion of Wiseman, *et al.*,[8] that a free hydrogen on the sulfonamide nitrogen is necessary for *in vivo* dealkylation of sulfonamides.

In the present study, plasma and brain of mice were examined for both the N-alkyl-4-bromobenzenesulfonamides and possible metabolites by thin layer chromatography, followed by ultraviolet measurement. This procedure allowed quantitation at the microgram level of both drug and metabolites and their correlation with pharmacological response.

Results and Discussion

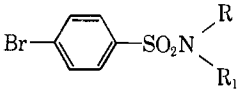
In preliminary experiments, analysis of mice plasma and brain collected 2 hr. after administration of II revealed only I. The identity of metabolically produced I was established by infrared and ultraviolet spectroscopy following thin layer chromatography. Since most of the pharmacological data for II had also been obtained 2 hr. after dosing, these preliminary results were consistent with the observed similarity in potency of I and II. They did not prove, however, that II was devoid of activity.

The concentration of I in the brain 2 hr. after oral administration of II is essentially identical with that attained 2 hr. after the same dose of I (Table II). These concentrations are also very similar to the corresponding plasma concentrations at 2 hr., indicating that these compounds distribute themselves equally

(1) H. H. Keasling, E. L. Schumann, and W. Veldkamp, *J. Med. Chem.*, **8**, 548 (1965).
(2) H. A. Krebs, *Biochem. J.*, **43**, 525 (1948).
(3) W. H. Miller, A. M. Dessert, and R. O. Roblin, Jr., *J. Am. Chem. Soc.*, **72**, 4893 (1950).
(4) T. Mann and D. Keilin, *Nature*, **146**, 164 (1940).
(5) T. H. Maren, *J. Pharmacol. Expl. Therap.*, **117**, 385 (1956).
(6) H. H. Keasling and L. J. Peterson, unpublished results.
(7) T. H. Maren, *J. Pharmacol. Expl. Therap.*, **130**, 26 (1960).
(8) E. H. Wiseman, E. C. Schreiber, and R. Pinson, Jr., *Biochem. Pharmacol.*, **11**, 88 (1962).

TABLE I

ANTICONVULSANT POTENCIES, R_f VALUES, ULTRAVIOLET MAXIMA, AND ULTRAVIOLET ABSORPTIVITIES OF SULFONAMIDES WITH THE GENERAL STRUCTURE



Compd.	R	R_1	Anticonvulsant activity ED_{50} and 95% C.I.[a] at 30 min., mg./kg. (electroshock)	Relative potency I = 1	R_f[b]	λ_{max}, mμ	Absorptivity
I	H	H	36[c] (33–40)	1.0	0.32	235	72.0
II	CH_2CH_3	H	45 (30–67)	0.80	0.86	235.5	54.4
III	CH_3	H	56 (42–75)	0.64	0.56	235	65.3
IV	$CH_2CH{=}CH_2$	H	56 (42–75)	0.64	0.74	235.5	53.4
V	$CH(CH_3)_2$	H	50 (37–68)	0.72	0.67	236	53.1
VI	$(CH_2)_3CH_3$	H	77 (47–106)	0.47	0.76	235	49.9
VII	$(CH_2)_4CH_3$	H	>200	<0.18	0.68	235	54.4
VIII	OH	H	45 (34–59)	0.80	0.21	235	55.5
IX	CH_3	CH_3	89 (67–118)	0.40	0.78	235	59.3

[a] 95% confidence interval computed by method of Spearman and Karber. [b] Thin layer chromatography on silica gel G; developing solvent: $CHCl_3$–HCOOH (19:1). [c] Weighed mean of several ED_{50} determinations.

TABLE II

CONCENTRATIONS OF 4-BROMOBENZENESULFONAMIDE (I) AND N-ETHYL-4-BROMOBENZENESULFONAMIDE (II) IN MICE AFTER ORAL ADMINISTRATION OF 400 MG./KG.[a]

	Plasma concn., γ/ml.			
	After oral administration of II			After oral administration of I[b]
Time, min.	of II	of I	Total (I + II)	of I
15	56.3 (6a)	73.8 (6a)	130.1 (6a)	135.0 (6a)
	50.7 (6e)	67.2 (6e)	117.9 (6e)	
Mean	53.5	70.5	124.0	
30	38.0 (6a)	116.1 (6a)	154.1 (6a)	167.5 (6a)
	42.1 (6e)	101.7 (6e)	143.8 (6e)	
	19.3 (6g)	71.4 (6g)	90.7 (6g)	106.0 (6g)
Mean	33.1	96.4	129.5	136.8
45	14.3 (6b)	84.6 (6b)	98.9 (6b)	113.0 (6b)
60	11.0 (6b)	89.4 (6b)	100.4 (6b)	117.0 (6b)
	16.9 (6f)	99.2 (6f)	99.2 (6f)	110.0 (6f)
Mean	14.0	94.3	108.3	113.5
75	<1.0 (6c)	108.3 (6c)	108.3 (6c)	123.3 (6c)
90	<1.0 (6c)	112.3 (6c)	112.3 (6c)	127.9 (6c)
105	<1.0 (6c)	119.3 (6c)	119.3 (6c)	136.1 (6c)
120	<1.0 (15d)	134.4 (15d)	134.4 (15d)	146.6 (15d)
	Brain concn., γ/ml.			
120	<1.0 (15d)	139.0 (15d)	139.0 (15d)	147.2 (15d)

[a] The numbers in parentheses refer to the number of mice used for the determination; the letter designates a series of parallel experiments. [b] As expected, II was not detected after administration of I.

between plasma and brain tissue. Like the plasma data, the brain data also indicated quantitative conversion of II to I. Since the peak anticonvulsant activity of II occurs at about 2 hr. after either i.p. or *p.o.* administration, and since no II can be detected in the plasma or brain 2 hr. after administration, the activity observed at this time interval seems to be associated exclusively with I produced *in vivo*, especially since it is known that the latter itself has anticonvulsant activity.

In an attempt to determine if II had intrinsic anticonvulsant activity, its rate of conversion to I was followed and compared with the time-course of anticonvulsant activity. The plasma concentrations of

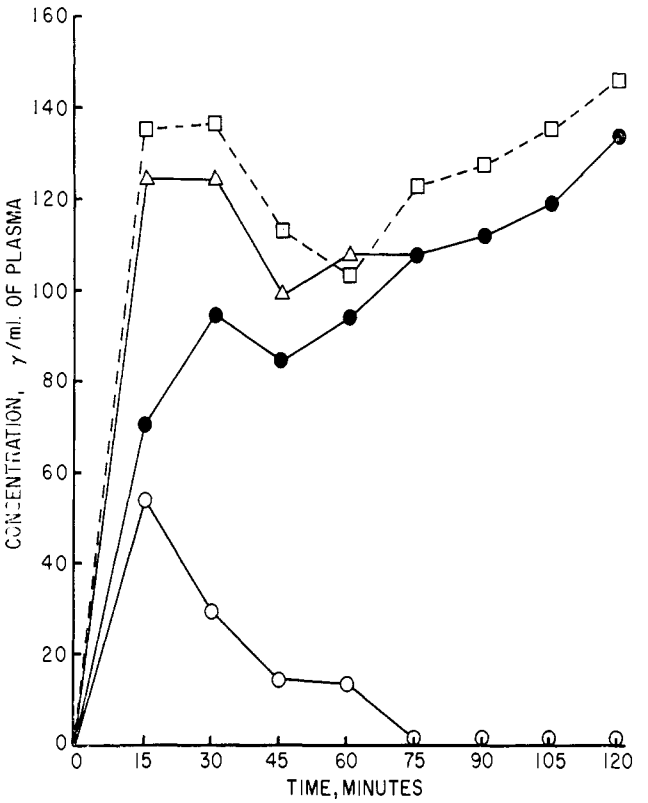


Figure 1.—Plasma concentration of N-ethyl-4-bromobenzenesulfonamide (-⊖—⊖-), 4-bromobenzenesulfonamide (-●—●-), and total sulfonamide (-△—△-) after oral administration of 400 mg./kg. of N-ethyl-4-bromobenzenesulfonamide. Plasma concentrations of 4-bromobenzenesulfonamide (-□---□-) after oral administration of 400 mg./kg. of 4-bromobenzenesulfonamide.

I and II, which resulted from 400 mg./kg. of orally administered II, were determined at 15-min. intervals for 2 hr. (Table II; Figure 1); also, plasma concentrations of I were determined after oral administration of I itself (Table II; Figure 1). The plasma concentrations indicate rapid absorption of I and II. Peak plasma concentrations were achieved within 15–30 min. after oral administration. Metabolism of II to I was also very rapid; the half-life of conversion in mice was about 20 min. at a dose of 400 mg./kg. The

TABLE III

THE EFFECTS OF PRIOR ADMINISTRATION OF β-DIETHYLAMINOETHYL DIPHENYLPROPYLACETATE HYDROCHLORIDE (X) AND CCl_4

Treatment[a]	Concn. in plasma, γ/ml., at 60 min. of I	of II	of III	Total	ED_{50},[b] mg./kg. At 60 min.	At 15 min.
I	110.0 (6f)[c]	...	...	110.0 (6f)	40 (0.90)	80 (0.451)
X + I	98.8 (6f)	...	...	98.8 (6f)	56 (0.64)	81 (0.44)
% change	−10.2			−10.2		
II	99.2 (6f)	16.9 (6f)	...	116.1 (6f)	56 (0.64)	120 (0.30)
X + II	74.7 (6f)	47.8 (6f)	...	122.5 (6f)	50 (0.72)	100 (0.36)
% change	−24.7	+183		+5.5		
III	133.0 (6f)	...	20.2 (6f)	153.2 (6f)	56 (0.64)	...
X + III	83.1 (6f)	...	44.4 (6f)	127.5 (6f)	50 (0.72)	...
% change	−37.5		+120	−16.7		

	At 30 min. of I	of II	Total	At 30 min. Oral	I.p.
I	106.0 (6g)	...	106.0 (6g)	66 (0.55)	54 (0.67)
CCl_4 + I	66.7 (6g)	...	66.7 (6g)	108 (0.33)	57 (0.63)
% change	−37.1		−37.1		
II	71.4 (6g)	19.3 (6g)	90.7 (6g)	93 (0.39)	66 (0.55)
CCl_4 + II	4.2 (6g)	58.6 (6g)	62.8 (6g)	287 (0.13)	100 (0.36)
% change	−1600	+204	−30.8		

[a] The compounds were administered orally as follows: I, II, and III, 400 mg./kg.; X, 50 mg./kg. 30 min. prior to the administration of I, II, or III; CCl_4, 0.2 ml. 24 hr. prior to administration of I or II as a 40% solution in cottonseed oil. [b] Dose which protects 50% of mice against supramaximal electroshock. The numbers in parentheses following the ED_{50} values are the relative potencies (*cf.* Table I). [c] The numbers in parentheses following the concentrations refer to the number of mice used for the determination; the letter designates parallel experiments.

conversion of II to I was essentially complete at 75 min. No other metabolites were detected by thin layer chromatography of chloroform extracts of the plasma.

Neglecting possible differences in the gastrointestinal absorption of I and II, a comparison of the plasma concentrations, resulting from equal doses of I and II in parallel experiments, indicates that the conversion of II to I is essentially quantitative[9] (Table II). Although urine was not examined, it is very unlikely that II was excreted intact in significant amount.

The fact that only about 50%[10] of II at a dose of 400 mg./kg. was converted to I at 15 min. (Table II) led us to test for anticonvulsant activity at this time interval. These tests revealed II to be less active than I, which supported the concept that I was necessary for activity.

The Effect of Metabolic Inhibitors.—In a further attempt to show conclusively whether or not II has intrinsic anticonvulsant activity, inhibition of metabolism was attempted by pretreating the mice with β-diethylaminoethyl diphenylpropylacetate hydrochloride[11] (X), which has been reported to inhibit dealkylating enzymes *in vitro*, and which has been used extensively as a tool in drug metabolism studies *in vivo*.[12]

X, 50 mg./kg., a dose which has been recommended,[13] was administered orally 30 min. prior to 400 mg./kg. of each sulfonamide. Pretreatment with X in this manner was found to decrease the extent to which II and III were converted to I (Table III). The total drug in the plasma, however, remained essentially unchanged. Although 50 mg./kg. of X did decrease the rate of conversion of II to I, relatively large amounts of I were still found in the blood 60 min. after the administration of II. It was not surprising, therefore, that prior administration of X did not produce a significant difference in the anticonvulsant activity of II at 60 min.

Although only about 50% of II was converted to I at 15 min. even without prior administration of X, tests for anticonvulsant activity at this time interval suggested that the activity of II was potentiated by prior administration of X (Table III). Swinyard, *et al.*,[14] have reported that X itself exhibits anticonvulsant action, but is ineffective at a dose of 50 mg./kg. However, in combination with II, the action of X may be potentiated. Although Swinyard, *et al.*,[14] reported potentiation of the anticonvulsant action of several drugs by X, the reverse may have been the true situation; these workers recognized that prevention of demethylation of nitrogen and inhibition of aliphatic side-chain oxidation are not the only mechanisms by which X potentiates antiepileptic drugs.

To obtain more definitive information regarding the intrinsic potency of the N-substituted sulfonamides, the effect of pretreatment with CCl_4 was investigated; the latter causes liver damage[15,16] and would be expected to inhibit dealkylation.

Oral administration to mice of 0.2 ml. of CCl_4 as a 40% solution in cottonseed oil 24 hr. prior to oral administration of 400 mg./kg. of II produced a significant change in the ratio of II to I at 30 min. after drug administration (Table III). The rate of de-

(9) It should be noted that 130 γ of II is equivalent to 112 γ of I by virtue of their molecular weight differences.

(10) Note, however, that the data of Table II were obtained from mice which had been given about four times the maximum dose used in testing for activity. It must be pointed out that the per cent conversion could be dose related.

(11) This compound, referred to in the literature as SKF-525A, was a gift from Smith Kline and French Laboratories, Philadelphia 1, Pa.

(12) B. B. Brodie, *J. Pharm. Pharmacol.*, **8**, 1 (1956).

(13) L. Cook, E. Mucha, and E. J. Fellows, *J. Pharmacol. Exptl. Therap.*, **112**, 382 (1954).

(14) E. A. Swinyard, J. A. Madsen, and L. S. Goodman, *ibid.*, **111**, 54 (1954).

(15) R. J. Williams, "Deconication Mechanisms," John Wiley and Sons, Inc., New York, N. Y., 1959, pp. 27, 91.

(16) G. G. Villela, *Biochem. Pharm.*, **13**, 665 (1964).

TABLE IV

PLASMA CONCENTRATIONS OF 4-BROMOBENZENESULFONAMIDE AND THE N-SUBSTITUTED SULFONAMIDE 30 MIN. AFTER ADMINISTRATION OF 100 MG./KG. OF THE LATTER TO MICE[a]

Administered compd.	R	R_1	Plasma concn., γ/ml. of I	Plasma concn., γ/ml. of administered compd.	Plasma concn., γ/ml. Total
I	H	H	130.7	130.7	130.7
VIII	OH	H	91.5	<1.0	91.5
III	CH_3	H	82.5	53.3	134.8
II	CH_2CH_3	H	57.0	47.3	104.3
IV	$CH_2CH{=}CH_2$	H	46.5	81.4	127.9
V	$CH(CH_3)_2$	H	38.6	26.1	64.7
IX	CH_3	CH_3	35.6	32.2	120.5[b]
VI	$CH_2CH_2CH_3$	H	27.1	21.6	48.7
VII	$CH_2CH_2CH_2CH_3$	H	19.8	36.1	55.9

[a] Plasma from six mice were used for each determination. All values in this table represent parallel runs (series h). [b] Includes 52.7 γ/ml. of III as a metabolite.

ethylation was markedly decreased, *e.g.*, whereas 79% of II was converted to I at 30 min. in normal mice, only 6.7% was converted in CCl_4-pretreated mice. The CCl_4 pretreatment also decreased by about 40% the total amount of drug in the plasma 30 min. after oral administration of either I or II, indicating that this agent causes a decrease in the absorption of these compounds.[17] Pharmacological data obtained after both intraperitoneal and oral administration of I and II in CCl_4-pretreated mice, when correlated with the plasma level data, strongly support the postulate that much, if not all, of the activity of II results from bioconversion to I (Table III). The pharmacological data also reflect the fact that these compounds are less efficiently absorbed after carbon tetrachloride pretreatment.

Since the effective brain tissue level of I is not known, it is impossible from the CCl_4 data alone to state with absolute certainty that II does not have anticonvulsant activity; however, it can be stated that I is intrinsically much more potent than II. The association of carbonic anhydrase inhibitory activity with nonsubstituted sulfonamides and the fact that I is very effective as a carbonic anhydrase inhibitor while II does not have this activity support the conclusion that the anticonvulsant action of II results solely from its *in vivo* conversion to I.

The Anticonvulsant Activity of N,N-Dimethyl-4-bromobenzenesulfonamide.—The observation by Wiseman, *et al.*,[8] that neither 2-methylbenzothiadiazine nor *o*-N-methylsulfamylaniline is demethylated *in vivo* suggested to them that a free hydrogen atom on the sulfonamide nitrogen is a prerequisite for metabolic dealkylation. Although *o*-N-methylsulfamylaniline, which does have a hydrogen atom on the sulfonamide nitrogen, did not demethylate, this was considered to be due to the contribution of a hydrogen-bonded form. This view was strengthened by the fact that replacement of the *o*-amino function by a methyl group, which has comparable bulk but cannot participate in such a system, resulted in a compound which was demethylated *in vivo*. If the hypothesis of Wiseman, *et al.*, is correct, neither N-methyl-4-bromobenzenesulfonamide (III) nor 4-bromobenzenesulfonamide (I) could be produced *in vivo* from N,N-dimethyl-4-bromobenzenesulfonamide (IX). Since the latter is active as an anticonvulsant (Table I), this would mean that metabolically produced I is not necessary for the activity of IX. With the developing solvent selected, the thin layer R_f values (Table I) of I, III, and IX are sufficiently different to allow separation and quantitation of these compounds in plasma.

Contrary to the hypothesis of Wiseman, *et al.*, demethylation of N,N-dimethyl-4-bromobenzenesulfonamide did occur; both the N-methyl and the nonsubstituted derivative were unequivocally identified and quantitated after the administration of 100 mg./kg. of IX by eluting the zones, followed by infrared and ultraviolet measurement.

The N-Substituted Analogs.—All of the other N-alkyl sulfonamide derivatives (Table IV) also are dealkylated to their parent compound, 4-bromobenzenesulfonamide. The latter also results from *in vivo* reduction of N-hydroxy-4-bromobenzenesulfonamide (VIII). The extent of *in vivo* dealkylation of the monoalkylated derivatives varies inversely with the length and complexity of the alkyl substituent. The concentration of I in the plasma at 30 min., resulting from equal doses (100 mg./kg.) of the sulfonamides, is in the following order of the substituent: H > OH > CH_3 > C_2H_5 > $CH_2CH{=}CH_2$ > $CH(CH_3)_2$ > $(CH_2)_2CH_3$ > $(CH_2)_3CH_3$. This order is in general agreement with the relative potency of these compounds as anticonvulsant agents (Table I). Statistical analysis of the data revealed that the ED_{50} values correlate better with the plasma concentrations of I than with the intact drug or total (drug plus metabolite) concentrations [correlation coefficients (P = 0.05) of −0.60 *vs.* −0.24 and −0.49, respectively]; *i.e.*, even if the N-alkyl compounds were *as active* as I, the ED_{50} values correlate best with the concentration of I. This further supports the conclusion that metabolically produced I is necessary for activity in this series. Given the consideration that I itself is the most active anticonvulsant agent in this series, inspection of all of the data shows that it is highly unlikely that any of the substituted compounds have significant intrinsic anticonvulsant activity.

Experimental

Materials.—The methods of synthesis of the sulfonamides have been described in a previous publication from these laboratories.[1]

Development of the Analytical Procedures.—Since the possibility existed from the outset that one or more of the N-substituted sulfonamides might be metabolically dealkylated to 4-

(17) The volume of distribution and/or rate of excretion, however, may also be affected.

bromobenzenesulfonamide, an analytical procedure was sought that would enable simultaneous determination of these compounds. The similarity of the sulfonamides investigated is such that separation prior to quantitation was required, *e.g.*, their ultraviolet maxima do not differ by more than 1 mμ. After the investigation of gas chromatography without success, separation was achieved by thin layer chromatography. The R_f values of the sulfonamides, which might conceivably be expected to occur together in the same sample, were sufficiently different using silica gel G as the support material and chloroform–formic acid (19:1) as the developing solvent (Table I). The zones were located by fluorescence quenching. After quantitative elution with a known volume of absolute ethanol, the concentrations were determined by ultraviolet analysis.

Determination of Sulfonamides in Plasma and Brain Tissue.—Six mice were usually used for each sulfonamide determination and were the same mice used in testing for anticonvulsant activity. The blood was heparinized, pooled, and centrifuged, and the plasma was decanted from the red cells and centrifuged a second time, if necessary.

Without prior adjustment of pH, the plasma was extracted with four times its volume of chloroform; the $CHCl_3$ was evaporated to near dryness and then transferred quantitatively to the thin layer plate, together with standards, and developed. Plasma from nontreated mice was run in parallel with the drug-containing samples. The silica gel, corresponding to zones, was removed from the plate with a small spatula and eluted with 5 ml. of absolute ethanol, it being previously determined that the zones could be quantitatively eluted in this manner. The ethanol eluates were examined spectrophotometrically with a Cary spectrophotometer (the complete spectrum was recorded). Concentrations were calculated from the previously determined absorptivities (Table I). The concentrations of I, determined in this manner, agreed very closely with those based on carbonic anhydrase inhibition.[6] Infrared spectra of thin layer chromatographic eluates were also run in a few cases to confirm identities.

Brain tissues from groups of 15 mice were weighed and pooled and then added to *ca.* 30 ml. of water and homogenized with a Kontes glass Kel-F tissue grinder. The homogenates were extracted with two 50-ml. portions of $CHCl_3$. The total chloroform extract was washed first with 20 ml. of 0.2 *N* HCl followed with 20 ml. of ammonia buffer (pH 8.3). Approximately two-thirds (the exact volume was measured) of the 100-ml. extract, which was recovered free of the aqueous and emulsion phases, was evaporated to dryness, and then reconstituted to 10 ml. with $CHCl_3$. Measured volumes of this solution were quantitatively transferred to a fluorescent, thin layer plate along with standards carried through the same procedure as in the case of the plasma samples.

In experiments using CCl_4 and β-diethylaminoethyl diphenylpropylacetate hydrochloride, the mice were dosed in the same manner as below.

Anticonvulsant Activity.—In the electroshock procedure, groups of six Carworth Farms (CF) male mice (20–25 g.), treated with various amounts of the sulfonamides (0.3 log interval between doses) in test vehicle (0.25% aqueous methylcellulose), were shocked *via* ear clip electrodes with a 60 cycle current for 0.2 sec. at a current of intensity of 25 ma. Abolition of the tonic extension component of the seizure was utilized as the effect metameter and log dose as the dose metameter in computing ED_{50} values using the method of Spearman and Karber.[18]

The Effect of X on the Anticonvulsant Actions of I, II, and III (Table III).—Two groups of six CF male mice were dosed with I, II, and III at 100, 50, 25 and 12.5 mg./kg. *p.o.* (one group for each compound had received X, 50 mg./kg. *p.o.*, 30 min. before the sulfonamide; the other group had similarly received diluent, 0.2 ml.). Sixty minutes later the mice were subjected to supramaximal electroshock and ED_{50} values were determined. In experiments with X at 15 min. after sulfonamide administration, two groups of 10 mice were treated *p.o.* with I or II in doses of 200, 140, 100, 71, or 50 mg./kg. (One group of the mice in each case was pretreated (30 min. previously) with X, 50 mg./kg. *p.o.* Fifteen minutes after I or II, the mice were subjected to electroshock and ED_{50} values were determined.

The Effect of CCl_4 Pretreatment on the Anticonvulsant Action of I and II Given Orally.—Two groups of 10 CF male mice were treated with I or II at 280, 200, 140, 100, 71, 50, and 36 mg./kg. *p.o.*; one group at each dose level had been pretreated 24 hr. before with 0.2 ml. of CCl_4 (40% solution in cottonseed oil). (One group of CCl_4-pretreated mice received 400 mg./kg. *p.o.* of II.) Thirty minutes after sulfonamide treatment, mice were subjected to supramaximal electroshock and the ED_{50} values were determined.

The Effect of CCl_4 Pretreatment on the Anticonvulsant Action of I and II Given Intraperitoneally.—Two groups of 10 CF male mice were dosed with I or II at 140, 100, 71, 50, 36, or 25 mg./kg. i.p. (one group for each sulfonamide had received 0.2 ml. of 40% CCl_4 in cottonseed oil *p.o.* 24 hr. previously). Thirty minutes after sulfonamide the mice received electroshock and ED_{50} values were determined.

(18) D. J. Finney, "Statistical Methods in Biological Assay," Hafner Publishing Co., New York, N. Y., 1952, p. 524 *et seq.*