TABLE I
CYTOTOXICITY OF CARDIAC PRINCIPLES

Compd.	Structure	Disk-plate assay,[a] Bu./mg.	Tube-dilution assay,[b] LD_{50}, γ/ml.
Strophanthidin	I, $R_1 = R_4 = R_5 = R_6 = H$; $R_2 = OH$; $R_3 = CHO$	140	0.11
Convallatoxin	I, R_1 = L-rhamnose; $R_2 = OH$; $R_3 = CHO$; $R_4 = R_5 = R_6 = H$	8000	0.002
K-Strophanthin-β	I, R_1 = glucose–cymarose; $R_2 = OH$; $R_3 = CHO$; $R_4 = R_5 = R_6 = H$	1300	0.023
Ouabain	I, R_1 = L-rhamnose; $R_2 = R_4 = R_5 = OH$; $R_3 = CH_2OH$; $R_6 = H$	1300	0.024
Digitoxigenin	I, $R_1 = R_2 = R_4 = R_5 = R_6 = H$; $R_3 = CH_3$	400	0.078
Digitoxigenin 3-acetate	I, $R_1 = CH_3CO$; $R_2 = R_4 = R_5 = R_6 = H$; $R_3 = CH_3$	130	0.11
Gitoxigenin	I, $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = CH_3$; $R_6 = OH$	40	0.58
Oleandrigenin	I, $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = CH_3$; $R_6 = CH_3CO_2$	460	0.059
Oleandrin	I, R_1 = L-oleandrose; $R_2 = R_4 = R_5 = H$; $R_3 = CH_3$; $R_6 = CH_3CO_2$	900	0.013
Telocinobufagin	II, $R_1 = H$; $R_2 = CH_3$	800	0.02
Hellebrin	II, R_1 = glucose–L-rhamnose; $R_2 = CHO$	8000	0.006
Cinobufagin	III, R = H	800	0.028
Cinobufotalin	III, R = OH	800	0.063

[a] See ref. 4. [b] See ref. 3.

the solvent system: water–ethanol–1-butanol–Skellysolve B (10:2:8:5); the volume of each phase was 50 ml. After distribution, the contents of tubes 5–18, containing most of the activity, were combined and evaporated *in vacuo*. The residue (13.3 g.) was dissolved in 20 ml. of ethylene dichloride–methanol (1:1) and applied to a column of 350 g. of silicic acid. The column was eluted in 25-ml. fractions with 1250 ml. each of ethylene dichloride–methanol (9:1) and ethylene dichloride–methanol (3:1). Fractions 41–89, which contained most of the activity were combined and evaporated to dryness *in vacuo*. The residue (3.9×10^6 Bu.) was subjected to a 600-transfer countercurrent distribution between the two phases of the solvent system: chloroform–acetone–water (1:2:2); the volume of each phase was 10 ml. After distribution the contents of tubes 411–460, which contained the activity, were combined and evaporated *in vacuo* almost to dryness. On cooling, the concentrate deposited crystalline material which, on crystallization from acetone–water, yielded 452 mg. of convallatoxin, m.p. 228–231°, identical with an authentic sample of convallatoxin (melting point, mixture melting point, ultraviolet, infrared, and n.m.r. spectra).

Anal. Calcd. for $C_{29}H_{42}O_{10}$: C, 63.25; H, 7.69. Found: C, 62.95; H, 7.94.

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The Relationship between Structure and Anticonvulsant Activity in a Series of Benzenesulfonamides

H. H. KEASLING, E. L. SCHUMANN, AND W. VELDKAMP

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan

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During the course of routine testing, it was noted that 4-bromobenzenesulfonamide exerted a potent anticonvulsant effect as measured by protection against maximal electroshock (ES) seizures, thiosemicarbazide (TSC) lethality, or strychnine (Strych.) lethality. The availability of many closely related compounds suggested a unique opportunity to explore the relationship between chemical structure and activity. The present report details the synthesis of several new sulfonamides and the pharmacological study of the entire series.

Results and Discussion

The LD_{50} and anticonvulsant data obtained in these studies are detailed in Table I. Examination of the data on effects of 4-substitution (compounds **1**–**16**) reveals a wide variation between anticonvulsant activity and lethality. The most toxic compound (**13**) is devoid of anticonvulsant activity. The four most active compounds (**3**–**6**) are active in all three test procedures. The TSC and Strych. tests do not discriminate among these four agents; however, ES reveals the order $CF_3 > Br = Cl > I$. Further, it is clear that, in terms of ratio of activity to lethality, the 4-bromo compound is the most effective agent. Subsequent studies of the effect of variation in substitution on the sulfonamide N were carried out utilizing the 4-Br substitution.

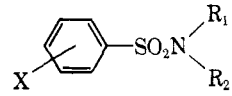
The study of N-substituted compounds (**17**–**34**) reveals that introduction of simple monoalkyl substituents does not abolish activity but, in general, increases toxicity. When the substituent is *n*-butyl or larger (**25**–**30**), activity is generally lost. With the exception of dimethyl (**31**), the disubstituted compounds (**32**–**34**) are inactive in the anticonvulsant tests. This pattern of activity suggested that the activity of these compounds might be the result of biotransformation. Studies designed to clarify this hypothesis are reported by Smith, *et al.*, in a subsequent manuscript.

Experimental

Methods.—All experiments were carried out in Carworth Farm male mice weighing 20–25 g. Toxicity was determined

TABLE I

STRUCTURE, LETHALITY, AND ANTICONVULSANT ACTIVITY OF SULFONAMIDES



No.	Method of prepn.	X	R_1	R_2	LD_{50}, mg./kg.	PD_{50}, mg./kg. E.S.	PD_{50}, mg./kg. TSC	PD_{50}, mg./kg. Strych
1	*a*	H	H	H	1000	89 (67–118)	—	—
2	*b*	4-F	H	H	562	113 (85–149)	—	—
3	*b*	4-Cl	H	H	233	45 (34–59)	100 (60–166)	225 (169–299)
4	*b*	4-Br	H	H	1000	36 (33–40)[c]	65 (55–77)[c]	167 (143–196)[c]
5	*b*	4-I	H	H	933	62 (50–77)[c]	79 (55–114)	146 (109–193)[c]
6	*d*	4-CF_3	H	H	533	23 (18–29)[c]	79 (54–118)	178 (116–274)
7	*a*	4-COOH	H	H	>1000	—	—	—
8	*a*	4-NH_2	H	H	>1000	—	—	—
9	*a*	4-OCH_3	H	H	>1000	142 (127–157)	—	—
10	*a*	4-CN	H	H	650	28 (18–43)	—	—
11	*a*	4-CH_3	H	H	>1000	142 (127–157)	—	—
12	*e*	4-$COCH_3$	H	H	>1000	—	—	—
13	*f*	4-CH_2CN	H	H	100	—	—	—
14	*g*	4-SO_2CH_3	H	H	767	—	—	—
15	*h*	4-$SOCH_3$	H	H	>1000	146 (100–192)	—	—
16	*i*	4-SCH_3	H	H	>1000	—	—	—
17	*f*	4-Br	H	OH	1000	45 (34–59)	159 (127–199)	—
18	*f*	4-Br	H	OCH_3	>1000	126 (101–158)	200 (148–271)	—
19	*f*	4-Br	CH_3	OH	562	—	—	—
20	*j*	4-Br	H	CH_3	562	56 (42–75)	89 (56–140)	126 (100–158)
21	*k*	4-Br	H	CH_2CH_3	562	45 (30–67)	114 (91–142)[c]	132 (119–147)[c]
22	*k*	4-Br	H	$CH_2CH_2CH_3$	562	77 (47–105)	178 (122–259)	159 (111–228)
23	*f*	4-Br	H	$CH_2CH{=}CH_2$	178	56 (42–75)	112 (82–155)	—
24	*k*	4-Br	H	$CH{<}^{CH_3}_{CH_3}$	562	50 (37–68)	112 (85–149)	—
25	*k*	4-Br	H	$CH_2CH_2CH_2CH_3$	562	—	—	—
26	*j*	4-Br	H	$CH_2C_6H_5$	>1000	—	—	200 (148–271)
27	*l*	4-Br	H	$CH_2CH_2C_6H_5$	>1000	—	159 (105–240)	—
28	*m*	4-Br	H		>1000	—	—	—
29	*f*	4-Br	H		237	—	—	—
30	*f*	4-Br	H		>1000	—	—	—
31	*j*	4-Br	CH_3	CH_3	1000	89 (67–118)	126 (101–158)	200 (148–271)
32	*j*	4-Br			>1000	—	—	—
33	*n*	4-Br			>1000	—	—	—
34	*f*	4-Br			562	—	—	—

[a] Commercially available (Eastman Kodak Co.). [b] W. Lenz, *Ber.*, **12**, 580 (1879). [c] Weighted mean of multiple determinations. [d] H. L. Yale and F. Sowinski, *J. Org. Chem.*, **25**, 1824 (1960). [e] J. B. Wright, U. S. Patent 3,041,331 (1962). [f] See Experimental. [g] B. Blank, F. A. Farina, J. F. Kerwin, and H. Saunders, *J. Org. Chem.*, **26**, 1551 (1961). [h] F. J. Marshall, M. V. Sigal, Jr., H. R. Sullivan, C. Cesnik,and M. A. Root, *J. Med. Chem.*, **6**, 60 (1963). [i] H. Burton and P. F. Hu, *J. Chem. Soc.*, 604 (1948). [j] C. S. Marvel and F. E. Smith, *J. Am. Chem. Soc.*, **45**, 2697 (1923). [k] W. Ssolonina, *J. Russ. Phys. Chem. Soc.*, **31**, 643 (1899). [l] W. H. Carothers and G. A. Jones, *J. Am. Chem. Soc.*, **47**, 3054 (1925). [m] T. A. Mastrukova, Yu. N. Sheinker, I. K. Kuznetsova, E. M. Peresleni, T. B. Sakharova, and M. I.Kabachnik, *Tetrahedron*, **19**, 357 (1963). [n] J. S. Shupe, *J. Assoc. Offic. Agr. Chemists*, **25**, 227 (1942).

as previously described.[1] Groups of six mice were tested for anticonvulsant activity by intraperitoneal injection of compounds suspended in 0.25% aqueous methylcellulose in doses spaced at 0.3 log intervals from 200 mg./kg. Tonic extensor seizures were induced using a 60-cycle 25-amp. current for 0.2 sec., delivered through ear-clip electrodes (Hans Tech Apparatus). Protection against the tonic extensor component of the seizure was utilized as the end point. Thiosemicarbazide lethality was tested by injecting the test compound immediately prior to the injection of thiosemicarbazide (Eastman), 20 mg./kg. i.p. Protection against lethality 4 hr. after TSC was the end point. Protection against strychnine lethality was tested by injecting strychnine sulfate, 3 mg./kg. i.p., 30 min. after the test compound. Lethality was assessed 30 min. after strychnine administration. For each test the protective dose-50 of the compound was computed by the method of Spearman and Karber.[2]

4-Cyanomethylbenzenesulfonamide (XIII).—A mixture of 39.6 g. (0.3 mole) of *p*-aminophenylacetonitrile[3] and 102 ml. of concentrated HCl in 300 ml. of acetic acid was stirred and maintained at 0–5° during the dropwise addition of 22.8 g. (0.33 mole)

(1) G. A. Youngdale, *et al.*, *J. Med. Chem.*, **7**, 415 (1964).

(2) D. J. Finney, "Statistical Methodology in Biological Assay," Hafner Publishing Co., New York, N. Y., 1952, p. 524.

(3) R. Pschoor, C. Sydal, and W. Stöher, *Chem. Ber.*, **35**, 4400 (1902).

of $NaNO_2$ in 36 ml. of water. To the resulting solution was then added a solution of 80 g. of SO_2 in 240 ml. of acetic acid to which had been added a solution of 12 g. of cuprous chloride dihydrate in 21 ml. of water. The mixture was stirred for 1 hr., then poured into an excess of ice water. The precipitated yellow solid was separated, washed with water, then added to 210 ml. of concentrated NH_4OH. This mixture was stirred for 1 hr. and allowed to stand overnight. The resulting solid was recrystallized from ethanol to give fine, yellow needles, m.p. 191–192°.

Anal. Calcd. for $C_8H_8N_2O_3S$: C, 48.97; H, 4.11; N, 14.28; S, 16.34. Found: C, 49.13; H, 4.69; N, 13.71; S, 16.24.

N-Hydroxy-4-bromobenzenesulfonamide (XVII).—A cold solution of 11.5 g. (0.51 g.-atom) of sodium in 150 ml. of absolute ethanol was added, at such a rate that no boiling occurred, to a stirred, hot solution of 32.5 g. (0.47 mole) of hydroxylamine hydrochloride in 12 ml. of water. After the addition, NaCl was removed by filtration and washed with 150 ml. of absolute ethanol. The stirred filtrate and washings were then treated, portionwise, with 36.2 g. (0.14 mole) of 4-bromobenzenesulfonyl chloride, stirred for 1 hr., then evaporated under reduced pressure. The residue was extracted with ether and the ether solution was evaporated. Recrystallization from water gave 15 g. (42%) of white prisms, m.p. 146–147°.

Anal. Calcd. for $C_6H_6BrNO_3S$: C, 28.58; H, 2.40; Br, 31.70; N, 5.56; S, 12.72. Found: C, 28.34; H, 2.39; Br, 31.72; N, 5.38; S, 12.84.

N-Methoxy-4-bromobenzenesulfonamide (XVIII).—A solution of 8.8 g. (0.22 mole) of NaOH in 100 ml. of water and 150 ml. of ethanol was treated with 16.6 g. (0.2 mole) of methoxyamine hydrochloride and 25.6 g. (0.1 mole) of 4-bromobenzenesulfonyl chloride, then stirred and heated under reflux for 2 hr. The cooled mixture was poured into 2.5 l. of ice water and the precipitated solid was recrystallized from aqueous ethanol to give 18.5 g. (70%) of white needles, m.p. 98–100°.

Anal. Calcd. for $C_7H_8BrNO_3S$: C, 31.39; H, 3.03; Br, 30.03; N, 5.26; S, 12.05. Found: C, 31.53; H, 3.12; Br, 29.90; N, 5.12; S, 12.31.

N-Methyl-N-hydroxy-4-bromobenzenesulfonamide (XIX).—This substance was made by the same method used for **18** above, using N-methylhydroxylamine hydrochloride. Recrystallization from aqueous ethanol gave 11.0 g. (41%) of white needles, m.p. 122–124°.

Anal. Calcd. for $C_7H_8BrNO_3S$: C, 31.59; H, 3.03; Br, 30.03; N, 5.26; S, 12.05. Found: C, 31.62; H, 3.14; Br, 29.98; N, 5.39; S, 12.23.

N-Allyl-4-bromobenzenesulfonamide (XXIII).—A mixture of 11.4 g. (0.2 mole) of allylamine and 25.6 g. (0.1 mole) of the 4-bromobenzenesulfonyl chloride in 350 ml. of benzene was stirred for 1 hr., then evaporated under reduced pressure. The dark residue was triturated with water and recrystallization from aqueous ethanol, using Darco G-60, to give 16.8 g. (61%) of white needles, m.p. 64–65°.

Anal. Calcd. for $C_9H_{10}BrNO_2S$: C, 39.14; H, 3.65; Br, 28.94; N, 5.07; S, 11.61. Found: C, 39.25; H, 3.82; Br, 28.63; N, 5.06; S, 11.69.

N-(3-Pyridyl)-4-bromobenzenesulfonamide (XXIX).—A mixture of 25.5 g. (0.1 mole) of 4-bromobenzenesulfonyl chloride, 18.8 g. (0.2 mole) of 3-aminopyridine, and 350 ml. of water was brought to a boil, then allowed to cool and stand overnight. The precipitated solid was recrystallized from aqueous acetone, using Darco G-60, to give 14 g. (45%) of white crystals, m.p. 187–188°.

Anal. Calcd. for $C_{11}H_9BrN_2O_2S$: C, 42.18; H, 2.90; Br, 25.52; N, 8.95; S, 10.24. Found: C, 42.27; H, 2.66; Br, 25.66; N, 8.47; S, 10.28.

N-(4-Pyridyl)-4-bromobenzenesulfonamide (XXX).—This substance was prepared in the same manner as **29**, using 4-aminopyridine. Recrystallization of the precipitated solid from aqueous dimethylformamide gave 13 g. (42%) of white plates, m.p. 325–326° dec.

Anal. Calcd. for $C_{11}H_9BrN_2O_2S$: C, 42.18; H, 2.90; Br, 25.52; N, 8.95; S, 10.24. Found: C, 42.36; H, 2.76; Br, 25.41; N, 8.71; S, 10.16.

N-Methyl-N¹-(4-bromobenzenesulfonyl)piperazine (XXXIV).—A mixture of 22 g. (0.22 mole) of N-methylpiperazine, 350 ml. of benzene, and 25.6 g. (0.1 mole) of 4-bromobenzenesulfonyl chloride was stirred for 4 hr., diluted with 500 ml. of benzene, washed with 120 ml. of 10% aqueous NaOH, dried ($MgSO_4$), and evaporated. Recrystallization of the residue from absolute ethanol gave 23.8 g. (75%) of white needles, m.p. 153–154°.

Anal. Calcd. for $C_{11}H_{15}BrN_2O_2S$: C, 41.38; H, 4.74; Br, 25.03; N, 8.78; S, 10.05. Found: C, 41.62; H, 4.64; Br, 25.05; N, 8.80; S, 10.08.

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New Compounds

Some 2,3,6-Trisubstituted Quinazolones

K. KISHOR, R. C. ARORA, AND SURENDRA S. PARMAR[1]

Department of Pharmacology and Therapeutics, K. G. Medical College, Lucknow University, Lucknow, India

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In a series of ring-substituted benzylamines synthesized with regard to all possible electron distribution in the benzene ring by induction and resonance, Zeller[2] reported that *m*-iodobenzylamine was found to be 2–3 times better as a substrate for beef liver monoamine oxidase than benzylamine. We have now synthesized some iodo-substituted quinazolones from aliphatic and as well as aromatic amines in continuation of our work on the synthesis of 2,3-disubstituted quinazolones.[3] Such quinazolones have been reported to possess hypnotic[4] and anticonvulsant[5] properties. In the present study, 2,3-disubstituted 6-iodoquinazolones were synthesized following the method of Bogert, *et al.*[6]

Experimental[7]

Quinazolones. General Procedure.—Iodoacetanthranil (m.p. 150–154°) was synthesized by refluxing 5 g. of 5-iodoanthranilic acid[8] with 50 ml. of acetic anhydride for 1 hr. After distilling the excess acetic anhydride, 6-iodoacetanthranil separated out as a solid mass in 60–65% yield (*Anal.* Calcd. for $C_9H_6INO_2$: C, 37.6; H, 2.09; N, 4.8. Found: C, 37.1; H, 2.0; N, 4.48.) and was used without further purification. Molar proportions of 6-iodoacetanthranil and the appropriate amines were mixed together for the preparation of quinazolones as reported earlier.[3] The 2,3-disubstituted 6-iodoquinazolones, summarized in Table I, were characterized by their sharp melting points and by analyses.

(1) The authors wish to express their thanks to Professor T. R. Govindachari, Director, Ciba Research Laboratories, Bombay, India, for the microanalyses of the compounds and to the State Medical Research Council (U. P.) for a research grant and providing a research fellowship to R. C. Arora.

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