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Biological Activities of Drugs. VI.[1] Structure-Activity Relationship of Sulfonamide Carbonic Anhydrase Inhibitors. (1)

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A study was made of correlation of carbonic anhydrase inhibitory activity with the chemical structures of 19 derivatives of benzenesulfonamide and 3 heterocyclic sulfonamides in reference to the physicochemical properties of the compounds.

A linear correlation was observed for Hammett's σ factor against pK_a, chemical shift of the sulfamoyl protons, and valence-force constant of the S=O bond.

Inhibitory activity increased with a decrease of pK_a, and with an increase of Hammett's σ factor, chemical shift of the sulfamoyl protons, and the S=O valence-force constant of the sulfamoyl group.

A close correlation was also found between the inhibitory activity and water solubility of the unionized molecules of benzenesulfonamides except for *p*-CH_3NH and *o*-NO_2 derivatives.

The physicochemical properties of drugs seem to have been increasingly taken up for the study of pharmacodynamic activity of drugs. They seem to refer to the physiological activities of variously substituted compounds in clarifying the action of compounds *in vitro*. They have been often studied in view of the pharmacodynamic activities of drugs, *e.g.*, bacteriostatic activity of sulfonamides[3-10] and carbothionamides[5,11,14] anticholinesterase activity of N-alkyl-substituted amides[13,14], and anesthetic activity of local anesthetics.[15-20]

Hansch, Fujita, *et al.* have introduced a method in expressing the physiological activity of a series of substituted compounds using substituent constant.[21-26]

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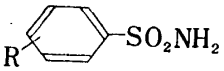
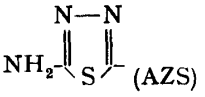
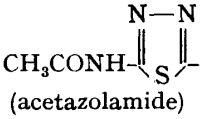
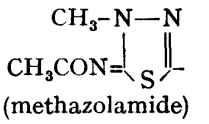
Most studies of carbonic anhydrase inhibitors are concerned with dissociation constant,[27-29] partition coefficient,[29] and binding ability with proteins and red blood cells.[28]

Presently, with series of derivatives of benzenesulfonamide and heterocyclic sulfonamides, a possible correlationship was studied between the physicochemical properties and inhibitory activity of the derivatives against carbonic anhydrase. The inhibitory activity was discussed on the basis of pK_a, chemical shift of the sulfamoyl proton, valence-force constant of S=O bond, and Hammett's σ factor of the substituted groups.

Table I. Physicochemical Properties of Sulfonamide Derivatives

No.	R	$\sum\sigma$ (or σ^-)	pK_a	Chemical shift of sulfamoyl protons (ppm)	SO_2 frequency (cm^{-1}) ν_{as}	ν_s	S=O valence-force constant $f_r \times 10^{-5}$ dyn/cm	Solubility in water at 15° (mmole/liter)
								
1	*p*-CH_3NH	−0.840	11.00	5.78	1304	1147	9.44	5.0
2	*p*-NH_2	−0.660	10.48	5.85	1312	1150	9.53	26.6
3	*p*-CH_3O	−0.268	10.17	6.01	1309	1161	9.59	15.6
4	*p*-CH_3	−0.170	10.11	6.06	1328	1155	9.69	11.8
5	*m*-CH_3	−0.069	10.06	6.07	1331	1155	9.71	17.5
6	H	0.000	9.95	6.12	1334	1161	9.77	16.0
7	*p*-Cl	0.227	9.88	6.26	1331	1155	9.71	6.9
8	*p*-Br	0.232	9.87	6.25	1332	1152	9.70	4.2
9	*m*-Cl	0.373	9.80	6.30	1332	1169	9.82	3.5
10	*p*-CH_3CO	0.502 (0.874)	9.66	6.34	1346	1163	9.89	2.3
11	*p*-CN	0.660 (1.000)	9.26	6.42	1344	1168	9.92	6.1
12	*m*-NO_2	0.710	9.42	6.51	1338	1190	10.04	2.2
13	*p*-NO_2	0.778 (1.270)	9.04	6.48	1353	1167	9.98	3.0
14	3,4-di-Cl	0.600	9.60	6.37	1311	1167	9.85	3.5
15	3-NO_2-4-Cl	0.937	9.34	6.50	1332	1174	9.91	0.95
16	3-CF_3-4-NO_2	1.208 (1.700)	9.09	6.62	1336	1167	9.88	0.65
17	*o*-CH_3	—	9.93	6.30	1337	1155	9.55	18.6
18	*o*-Cl	—	9.58	6.39	1337	1162	9.77	2.6
19	*o*-NO_2	—	8.67	6.59	1340	1166	10.04	1.6
	$R\text{-}SO_2NH_2$							
20	 (AZS)	—	7.8[a]	—	1342	1176	9.96	26.3
21	 (acetazolamide)	—	7.4[a]	—	1367	1179	10.17	2.7
22	 (methazolamide)	—	7.2[a]	7.05	1361	1178	10.13	2.0

a) from Maren[28]

27) W.H. Miller, A.M. Dessert and R.H. Robin, *J. Am. Chem. Soc.*, **72**, 4893 (1950).
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Results

Physicochemical Properties

Physicochemical properties of 22 derivatives of sulfonamide were presented in Table I.

pK_a of the benzenesulfonamides was plotted against their substituent constants, *i.e.*, Hammett's σ factors (Fig. 1). It was found that σ^- factors for *p*-NO_2 and *p*-CN were more appropriate for the present comparison than σ factors.

A linear correlation was also observed between σ factors and NMR chemical shifts of protons of the sulfamoyl group (Fig. 2). The chemical shifts showed a linear correlationship with dissociation constants (pK_a) (Fig. 3), except for *o*-NO_2, *p*-CN, *p*-NO_2, *p*-CH_3NH and methazolamide.

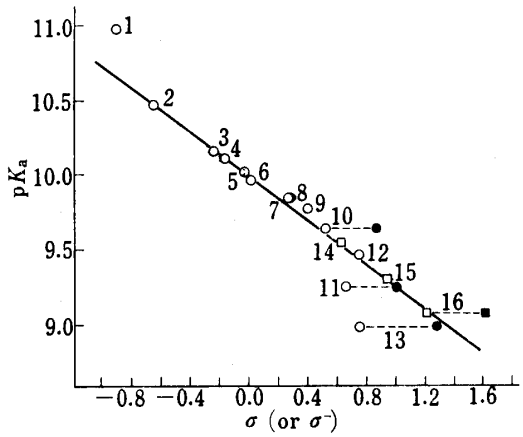


Fig. 1. Relationship between Hammett's σ (or σ^-) Factor and pK_a

Substituent numbers correspond to those in Table I.
open symbols: σ close symbols: σ^-
circles: *m*- and *p*-substituted benzenesulfonamides
squares: disubstituted benzenesulfonamides

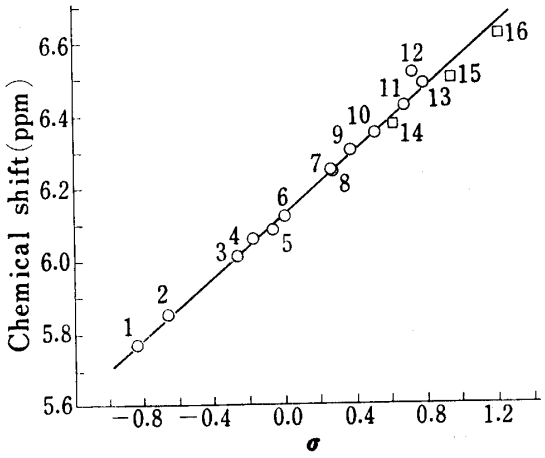


Fig. 2. Relationship between Hammett's σ Factor and Chemical Shift of Sulfamoyl Protons

Substituent numbers correspond to those in Table I.
○: *p*- and *m*-substituted benzenesulfonamides
□: disubstituted benzenesulfonamides

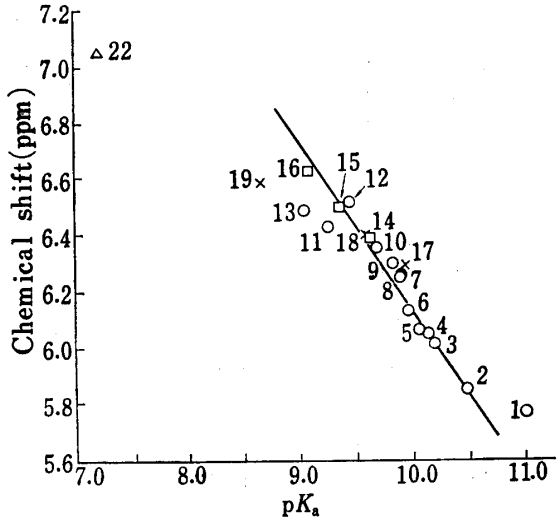


Fig. 3. Relationship between pK_a and Chemical Shift of Sulfamoyl Protons

Substituent numbers correspond to those in Table I.
○: *p*- and *m*-substituted benzenesulfonamides
×: *o*-substituted benzenesulfonamides
□: disubstituted benzenesulfonamides
△: heterocyclic sulfonamide

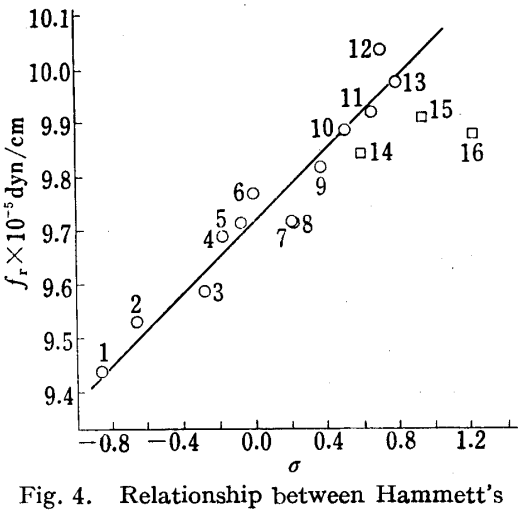


Fig. 4. Relationship between Hammett's σ Factor and S=O Valence-Force Constant

Substituent numbers correspond to those in Table I.
○: *p*- and *m*-substituted benzenesulfonamides
□: disubstituted benzenesulfonamides

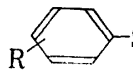
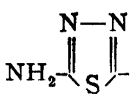
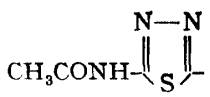
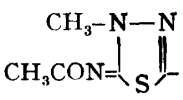
Hammett's σ factors were significantly correlated with valence-force constants (f_r) of S=O bond in the sulfamoyl group except for disubstituted compounds (Fig. 4).

The water solubility of unionized benzenesulfonamides increased with a decrease of Hammett's σ factors and valence-force constants of S=O bond except for p-CH_3NH derivative (Table I).

Inhibitory Activity for Carbonic Anhydrase

The inhibition constant (K_I in equation 3) of sulfonamide derivatives for carbonic anhydrase was studied at 0.2° and 15° (Table II). A linear correlationship of the inhibition constant between 0.2° and 15° was observed. In the table, a compound having a small K_I represents a strong inhibitory activity.

Table II. Inhibitory Activity of Sulfonamide Derivatives for Carbonic Anhydrase

No.	R	$K_I \times 10^7$M 0.2°	15°
			
1	p-CH_3NH	150	110
2	p-NH_2	230	250
3	p-CH_3O	45	50
4	p-CH_3	38	32
5	m-CH_3	50	60
6	H	61	75
7	p-Cl	19	11
8	p-Br	12	11
9	m-Cl	23	12
10	p-CH_3CO	11	13
11	p-CN	11	6.5
12	m-NO_2	13	7.6
13	p-NO_2	9.0	5.5
14	3,4-di-Cl	4.0	3.0
15	3-NO_2-4-Cl	1.7	2.5
16	3-CF_3-4-NO_2	1.4	2.2
17	o-CH_3	160	120
18	o-Cl	30	24
19	o-NO_2	85	35
	$R\text{-}SO_2NH_2$		
20		2.5	5.4
21		0.66	1.2
22		0.58	1.0

The inhibition constants were revealed to have a significant correlationship with the substituent constants (Fig. 5A and B).

The inhibition constants were found to decrease with a decrease of pK_a except for o-NO_2 (Fig. 6A and B).

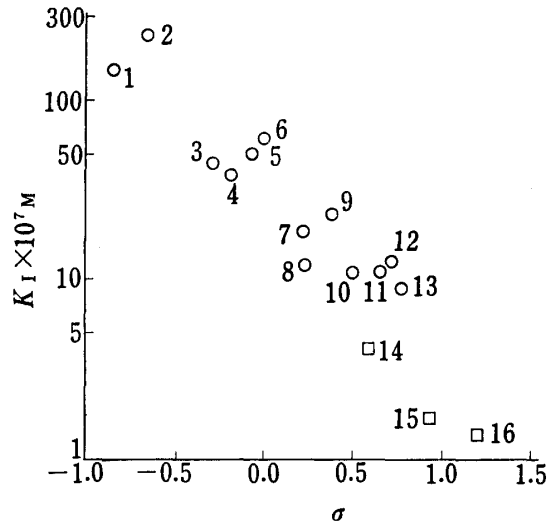


Fig. 5A. Relationship between Hammett's σ Factor and Carbonic Anhydrase Inhibitory Activity at 0.2°

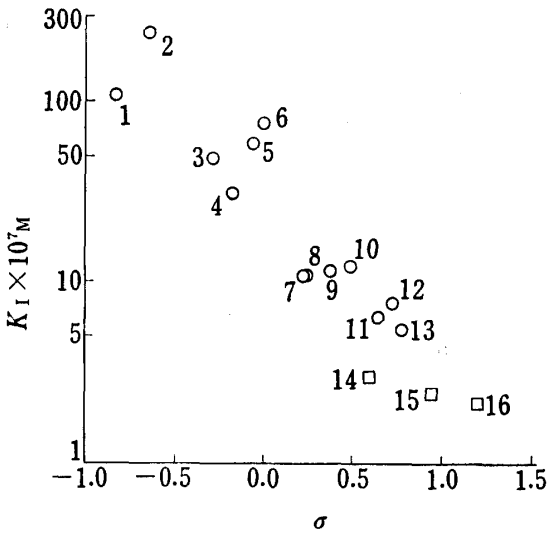


Fig. 5B. Relationship between Hammett's σ Factor and Carbonic Anhydrase Inhibitory Activity at 15°

Substituent numbers correspond to those in Table I.
○: *p*- and *m*-substituted benzenesulfonamides □: disubstituted benzenesulfonamides

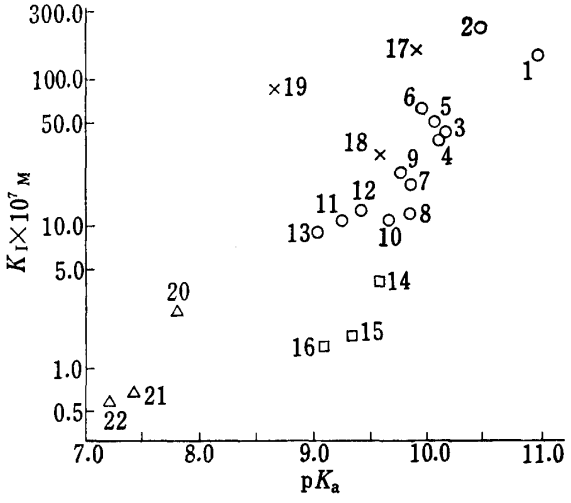


Fig. 6A. Relationship between pK_a and Carbonic Anhydrase Inhibitory Activity at 0.2°

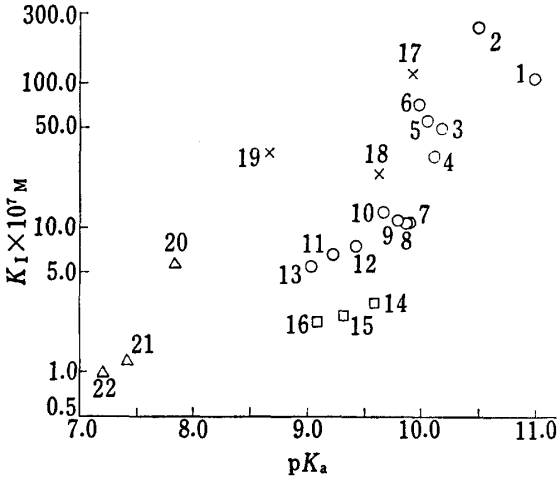


Fig. 6B. Relationship between pK_a and Carbonic Anhydrase Inhibitory Activity at 15°

Substituent numbers correspond to those in Table I.
○: *p*- and *m*-substituted benzenesulfonamides ×: *o*-substituted benzenesulfonamides
□: disubstituted benzenesulfonamides △: heterocyclic sulfonamides

The inhibition constants showed a linear correlationship with chemical shift of sulfamoyl protons except for *o*-substituted compounds (Fig. 7A and B).

The constants were also found to decrease with an increase of S=O valence–force constant except for *o*-NO_2 (Fig. 8A and B).

Water solubility of unionized molecules of benzenesulfonamides was found to have a great influence on the inhibition constants except for *p*-CH_3NH and *o*-NO_2 derivatives (Fig. 9A and B).

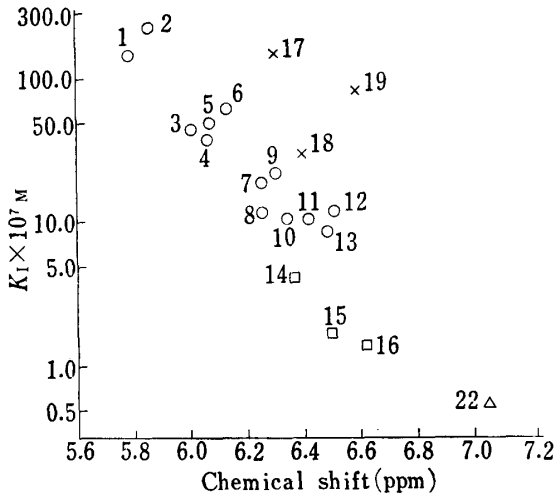


Fig. 7A. Relationship between Chemical Shift of Sulfamoyl Protons and Carbonic Anhydrase Inhibitory Activity at 0.2°

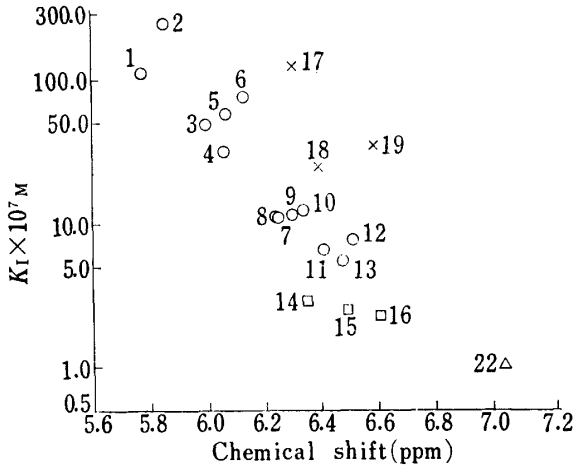


Fig. 7B. Relationship between Chemical Shift of Sulfamoyl Protons and Carbonic Anhydrase Inhibitory Activity at 15°

Substituent numbers correspond to those in Table I.
○: *p*- and *m*-substituted benzenesulfonamides × : *o*-substituted benzenesulfonamides
□: disubstituted benzenesulfonamides △: heterocyclic sulfonamides

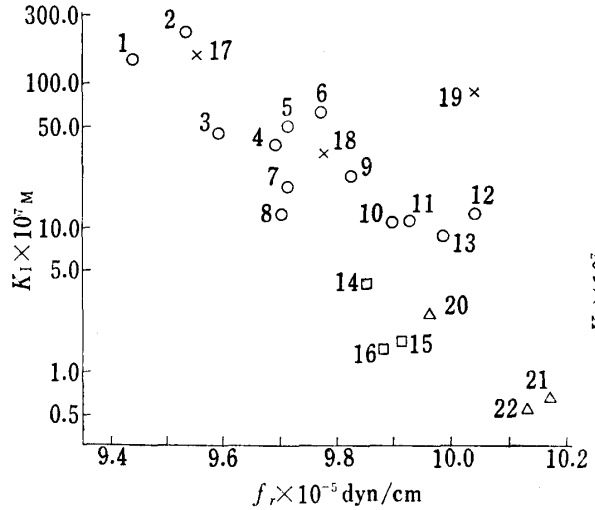


Fig. 8A. Relationship between S=O Valence-Force Constant and Carbonic Anhydrase Inhibitory Activity at 0.2°

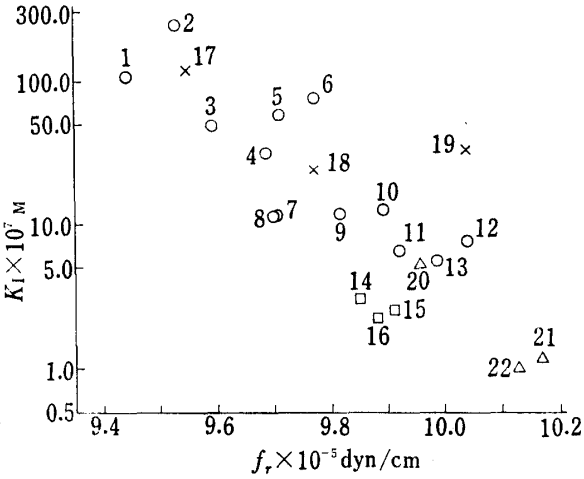


Fig. 8B. Relationship between S=O Valence-Force Constant and Carbonic Anhydrase Inhibitory Activity at 15°

Substituent numbers correspond to those in Table I.
○: *p*- and *m*-substituted bfenzenesulfonamides × : *o*-substituted benzenesulfonamides
□: disubstituted benzenesulfonamides △: heterocyclic sulfonamide

Discussion

Physicochemical Properties

Hammett's σ factors, a measure of the effect of substituents on displacement of electronic charges in a molecule, were found to have a linear correlationship with pK_a, chemical shift of sulfamoyl protons and valence-force constant of S=O bond.

It is interesting that σ^- factors for *p*-NO_2 and *p*-CN are more appropriate than σ factors when compared with pK_a. Here it must be noticed that pK_a was measured in water, unlike other two constants. The σ^- factors were obtained following reactions of phenols and anilines.

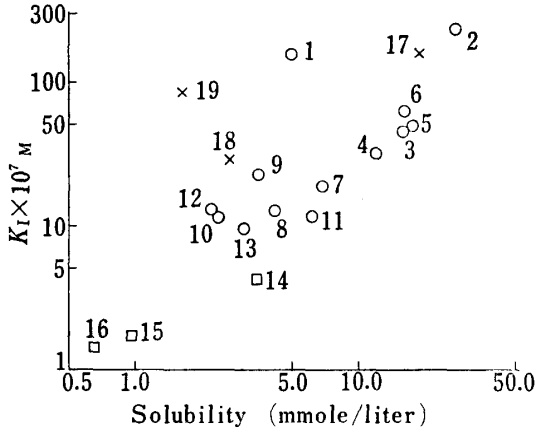


Fig. 9A. Relationship between Solubility in Water and Carbonic Anhydrase Inhibitory Activity at 0.2°

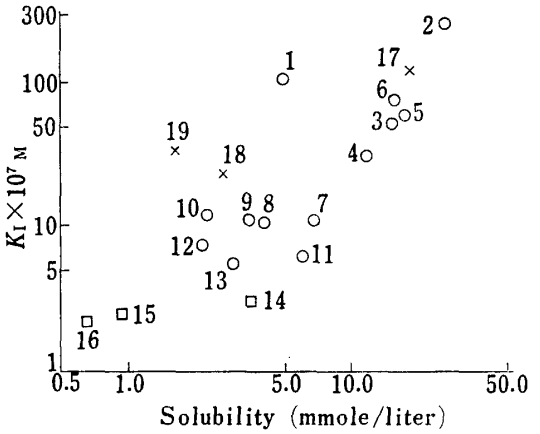


Fig. 9B. Relationship between Solubility in Water and Carbonic Anhydrase Inhibitory Activity at 15°

Substituent numbers correspond to those in Table I.
○: *p*- and *m*-substituted benzenesulfonamides ×: *o*-substituted benzenesulfonamides;
□: disubstituted benzenesulfonamides

For the σ^- factors, a correction of resonance arising from *p*-position of electronattracting substituents was assumed. The deviation of *p*-CH_3NH derivative from the linearity may be also ascribed to the effect of resonance arising from *p*-position of the electron-releasing group. Thus, it may be considered that the contribution of resonance of *p*-CH_3NH, *p*-NO_2 and *p*-CN to the sulfamoyl group was important for the dissociation of protons in aqueous solution.

In studying pK_a in terms of chemical shift (Fig. 3), the deviation of *p*-CH_3NH, *p*-CN, *p*-NO_2, *o*-NO_2 and methazolamide from linearity may be also ascribed to the difference of solvents, *i.e.*, water for dissociation constant and dioxane for chemical shifts.

In a comparison of Hammett's σ factors with S=O valence-force constants (Fig. 4), the disubstituted benzenesulfonamides were found to deviate from a linearity. It may be considered that the S=O valence-force constants were calculated from the results of the infrared absorption spectrum of crystalline compounds. Thus, a resonance between benzene ring and sulfamoyl group of the solid compounds was inhibited by the buttressing effect between the neighboring substituents.

Seydel, *et al.*[5] found that the water solubility of derivatives of sulfapyridine was increased by substituents which increased the polarity of S=O bond. In the present study, a derivative of benzenesulfonamide which had a large electron density at the sulfamoyl group showed a high water solubility except for *p*-CH_3NH derivative.

Inhibitory Activity for Carbonic Anhydrase

Miller[22] found that the inhibitory activity of heterocyclic sulfonamides for carbonic anhydrase was increased with a decrease of pK_a of the compounds.

A similar result was obtained in the present study for benzenesulfonamides and heterocyclic sulfonamides.

It was found that a compound which had a large Hammett's σ factor as well as a large force constant of S=O bond and a large chemical shift showed a strong inhibitory activity for carbonic anhydrase. The weak activity of the *o*-NO_2 derivative, irrespective of a low pK_a, a large S=O valence-force constant and a large chemical shift, was considered to be a steric inhibition of the interaction of the compound with active sites of the enzyme.

It was also interesting that the water solubility of the benzenesulfonamides had a good correlationship with the inhibitory activity. This correlationship strongly supports Freguson's principle.[30] The water solubility of the compounds was subjected to the electron

30) J. Ferguson, *Proc. Roy. Soc.* (London), Ser. **B127**, 387 (1939).

density of the sulfamoyl group which had an important influence on the solvation of water molecules to benzenesulfonamide molecules.

Thus, it may be concluded that the electronic properties of sulfonamide derivatives, such as Hammett's σ factor, pK_a, chemical shift of the sulfamoyl protons, and S=O valence–force constant, are influential for the carbonic anhydrase inhibitory activity of the compounds. The water solubility of the compounds is also influential for the activity of the derivatives.

Experimental

Materials——Benzenesulfonamide derivatives were prepared following known procedures and purified by recrystallization. Their melting points were as follows: *p*-CH_3NH, 166°; *p*-NH_2, 163°; *p*-CH_3O, 113°; *p*-CH_3, 138°; *m*-CH_3, 108°; *o*-CH_3, 153°; benzenesulfonamide, 153°; *p*-Cl, 144°; *m*-Cl, 148°; *o*-Cl, 188°; *p*-Br, 166°; *p*-CH_3CO, 176°; *p*-CN, 168°; *p*-NO_2, 179°; *m*-NO_2, 166°; *o*-NO_2, 191°; 3,4-di-Cl, 135°; 3-NO_2-4-Cl, 174°; 3-CF_3-4-NO_2, 184°.

Acetazolamide and methazolamide were supplied from the Lederle Co., Ltd. 2-Amino-1,3,4-thiadiazole-5-sulfonamide (mp 206°) was prepared from acetazolamide on hydrolysis.

Carbonic anhydrase was supplied from the Sigma Chemical Co.

Dissociation Constant——Dissociation constant (pK_a) was measured spectrophotometrically at 20±2° using a Shimadzu spectrometer, model QR-50, pH being measured by a Yanagimoto pH–meter, model 42–A.

NMR Spectrum——Chemical shift of protons in the sulfamoyl group was measured with a Hitachi NMR spectrometer of 60 Mc at 20°. Materials were dissolved in dioxane at a concentration of 0.3 mole/liter. Tetramethyl silane used as an internal standard.

Infrared Spectrum——A compressed disc method of KBr was employed using a Hitachi IR spectrometer.

Valence-force Constant of S=O Bond——Valence–force constant of S=O bond (f_r) was calculated as follows[31,32]:

$$f_r = \frac{\pi^2 C^2 (\nu_s + \nu_{as})^2}{1/M_s + 1/M_o} \text{ dyn/cm} \qquad (1)$$

where, ν_s and ν_{as} are frequencies of symmetric stretching and anti–symmetric stretching of S=O bond; M_s, atomic weight of sulfur; M_0, atomic weight of oxygen; C, velocity of light.

Solubility to Water——Ten ml of aqueous 0.005N HCl was pipetted into an L-shaped tube which had a glass stopper. A sufficient amount of a compound was suspended in HCl solution. The suspension was kept at 15° for 48 hr under shaking. After an equilibrium was established, the suspension was filtered off through filter paper. Concentration of the compound in the filtrate was measured spectrophotometrically. In case of *p*-aminobenzenesulfonamide, *p*-methylaminobenzenesulfonamide and 2-amino-1,3,4-thiadiazole-5-sulfonamide, solubility was measured in a 0.001N phosphate buffer of pH 5.5 to avoid the ionization of the amino group.

Inhibitory Activity for Carbonic Anhydrase——A colorimetric method was employed for the estimation of the carbonic anhydrase inhibitory activity following Philpot[33] and Miyake[34] with some modifications. An apparatus for the measurement is presented in Fig. 10. Aqueous solution of 0.0026M $NaHCO_3$ and 2.5 mg/ml bromothymol blue, an indicator, was taken into a test tube through a 50 ml burette. The test tube was kept in a 50% aqueous propylene glycol bath at 0.2° or 15°; CO_2 was bubbled into the test tube for 10 min to assure saturation. A bubbling rate was regulated by changing the depth of a leaking orifice of CO_2 in a pressure regulator (a 200 ml measuring cylinder).

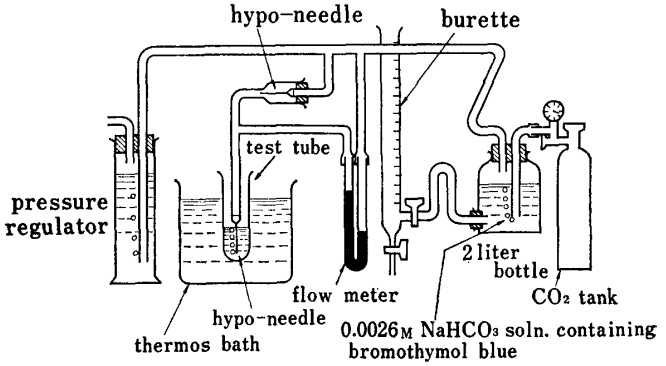


Fig. 10. Apparatus for Measuring the Carbonic Anhydrase Activity

31) D. Barnard, J.M. Fabian, and H.P. Koch, *J. Chem. Soc.*, **1949**, 2442.
32) S.F. Mason, *J. Chem. Soc.*, **1958**, 3619.
33) E.J. Philpot and J.L. Philpot, *Biochem. J.*, **30**, 2191 (1936).
34) T. Miyake and G. Pincus, *Endocrinology*, **63**, 816 (1958).

As a blank test, 1 ml of distilled water was added to the reaction tube under a continuous bubbling of CO_2. Two min later, 1 ml of buffer solution (0.206M $NaHCO_3$–0.3M Na_2CO_3, pH 10.0) was injected with a 1 ml syringe. The time was measured for the color to change from blue to yellowish green during the bubbling. The end point was judged by matching the color with a standard solution consisting of phosphate buffer at pH 7.0 and the same indicator. Time (t_0) required for the color change proved to 71 sec at 0.2° and 31 sec at 15° on an average.

For the measurement of carbonic anhydrase inhibitory activity, the concentration of the enzyme was adjusted as follows: Two parts of an aqueous stock solution of the enzyme which was kept at 0° was diluted with 5 parts of water. Following saturation with CO_2, 1 ml of the diluted enzyme solution was put into a test tube which contained 7 ml of the $NaHCO_3$–bromothymol blue solution. Subsequent procedures for the measurement of the color change were done as in the blank test. The concentration of the enzyme in the stock solution was adjusted so as to cause the color change at 20 sec at 0.2° or 8.5 sec at 15°. The time was designated as t_e.

The inhibitory activity of a compound was measured using 1 ml of a mixture of 2 parts of the adjusted stock solution of the enzyme and 5 parts of the compound solution with various concentrations instead of the adjusted enzyme solution alone for the measurement of t_e.

Per cent inhibition (i) was calculated as follows:

$$i = \frac{t_o - t}{t_o - t_e} \tag{2}$$

where t is the time to change the color in the presence of the enzyme and the compound.

Inhibition constant was obtained from i,[35,36]

$$\frac{I_o}{i} = K_I \frac{1}{1-i} + iE \tag{3}$$

where K_I is inhibition constant; i, per cent inhibition; I_0, concentration of the inhibitor; E, concentration of the carbonic anhydrase.

35) L.H. Easson and E. Stedman, *Proc. Roy. Soc.* (London), Ser. **B127**, 142 (1936).
36) T.H. Maren, *J. Pharmacol. Exptl. Therap.*, **139**, 140 (1963).