SOME *IN VITRO* INHIBITORS OF CARBONIC ANHYDRASE

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Work on sulphonamides as *in vitro* inhibitors of carbonic anhydrase has been extended by the preparation and assay of novel types. Although diphenylsulphonamides, *p*-alkoxycarbonylbenzene sulphonamides and 5-alkoxycarbonylamino-1:3:4-thiadiazole-2-sulphonamides showed noteworthy activity *in vitro*, only the thiadiazole derivatives possessed diuretic activity in the rat.

IN 1940 Mann and Keilen[1] showed that certain aromatic sulphonamides could inhibit the enzyme "carbonic anhydrase" *in vitro*. This important discovery was confirmed shortly afterwards by Locke, Main and Mellor[2] and also by Höber[3] (who used an isolated frog kidney to determine enzyme inhibition) who likewise showed that whilst compounds possessing the free sulphonamide group were inhibitors of carbonic anhydrase, *N*-substituted sulphonamides were essentially inactive. Davenport[4] found that the five-membered heterocyclic ring structure thiophene-2-sulphonamide possessed up to forty times the activity of sulphanilamide. Krebs[5] carried out a systematic study of a series of aromatic sulphonamides and found that "Prontosil Red" (I) and "Prontosil Soluble" (II) were highly potent inhibitors of the enzyme. He also confirmed that substitution of the sulphonamide group, or its separation from the aromatic nucleus, as in ω-sulphonamidotoluene, were accompanied by virtual disappearance of activity.

Schwartz[6] pointed out in 1949 that as renal carbonic anhydrase catalysed the equilibrium reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

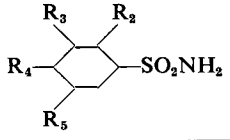
its inhibition would lead to a decrease in the rate of conversion of carbon dioxide into carbonic acid and consequently in the rate of production of hydrogen ions. The excretion of hydrogen ions was known to represent the normal mechanism for the conservation of sodium ions. Inhibition of renal carbonic anhydrase might consequently be expected to lead to increased excretion of sodium ions. Carbonic anhydrase inhibitors might consequently offer a new approach to the production of diuretics. In support thereof Schwartz reported on the use of sulphanilamide in the control of oedema associated with congestive heart failure. He also drew the attention of Roblin and his co-workers who were interested both in heterocyclic sulphonamides and in carbonic anhydrase inhibitors, to the implications of his work. In following up these developments, Roblin and others[7,8] found that, in general, increasing inhibitory activity was associated with increasing acidity of the sulphonamide, maximum activity being obtained by attachment of a sulphonamide group to a 5-membered heterocyclic ring (cf. Davenport)[4]. The 1:3:4-thiadiazoles proved particularly active, 5-acetamido-1:3:4-thiadiazole-2-sulphonamide being

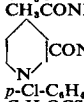
selected for further study. Its introduction into clinical practice was subsequently reported[9].

Initially we directed attention to simple derivatives of benzene sulphonamide, but failed to find therein compounds of significant activity.

TABLE I

MISCELLANEOUS DERIVATIVES OF BENZENE SULPHONAMIDE



R_2	R_3	R_4	R_5	Activity
—	—	—	—	3
—	—	CH_3CONH-	—	17
—	Cl-	CH_3CONH-	Cl-	<1
—	—		—	9
—	—	*p*-Cl-C_6H_4CONH-	—	50
—	—	C_6H_5OCSNH-	—	55
—	—	C_2H_5OCSNH-	—	35
—	CH_3-	CH_3-	—	3
CH_3-	—	CH_3-	—	<1
CH_3-	—	—	CH_3-	<1
Cl-	—	CH_3-	—	105
—	Br-	CH_3-	—	105
—	Cl-	-COOH	—	5
—	Br-	-COOH	—	10
—	CH_3O-	-COOH	—	<1
—	CH_3O-	*n*-C_4H_9OCO-	—	30
—	Cl-	Cl-	—	240
—	CH_3-	Cl-	—	55
—	-COOH	Cl-	—	20
—	—	Br-	—	90
—	—	I-	—	200

Examination of the results (Table I) revealed that, in general, inhibitory activity is increased by electronegative *p*-substituents and in particular by halogens. More potent sulphonamides were found among dicyclic aromatic structures (Table II). Naphthalene 1- and 2-sulphonamides

TABLE II

COMPOUNDS CONTAINING 2 ARYL NUCLEI

	Activity
Naphthalene-1-sulphonamide	17
Naphthalene-2-sulphonamide	14
Diphenyl-4-sulphonamide	360
Diphenyl-4:4′-disulphonamide	690
2-Aminodiphenyl-4-sulphonamide	80
2-Acetamidodiphenyl-4-sulphonamide	40
2:2′-Diaminodiphenyl-4:4′-disulphonamide	135
Diphenylether-4-sulphonamide	50
Diphenylether-4:4′-disulphonamide	140
Diphenylsulphide-4-sulphonamide	80
Diphenylsulphide-4:4′-disulphonamide	515
Phenoxthin-3-sulphonamide	470
Phenoxthin-3:7-disulphonamide	245
N^4-*p*-Bromobenzenesulphonsulphanilamide	4
N^4-*p*-Tolylsulphonsulphanilamide	13

were of little interest (cf.[5]). Diphenyl derivatives, in contrast, were uniformly active. Thus diphenyl-4-sulphonamide was 360 times more potent than sulphanilamide itself, the activity being increased to nearly

700 times that of sulphanilamide by substituting a second sulphonamide group into the *p'*-position. Amino groups diminished potency, a result similar to that observed by Krebs[5] in the case of benzene sulphonamide. Phenoxthin-3 : 7-disulphonamide, in contrast, was less potent than the 3-monosulphonamide.

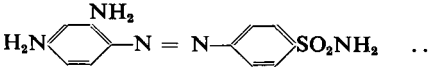
The high activity of Prontosil (I) led us to study the stilbene and anil derivative shown in Table III. The observed activities clearly demon-

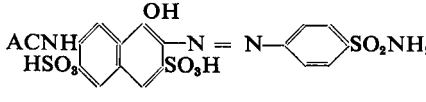
TABLE III

TWO RINGS LINKED BY UNSATURATED BOND(S)

	Activity
Prontosil	185
Stilbene-4-sulphonamide	155
Benzylidene sulphanilamide (III; R = H)	30
p-Chlorobenzylidene sulphanilamide (III; R = *p*-Cl)	55
2 : 4-Dichlorobenzylidene sulphanilamide	330
2-Hydroxybenzylidene sulphanilamide	35
3-Methoxy-4-hydroxy-5-iodobenzylidene sulphanilamide	120
p-Sulphamylbenzylidene *p*-chloroaniline	85
p-Sulphamylbenzylidene *p*-toluidine	230
p-Sulphamylbenzylidene aniline (IV; R = NH_2)	40

strate the superiority of the azo-linked structure (I) over the related anil types (III) and (IV). It is tempting to correlate this result with the


(I)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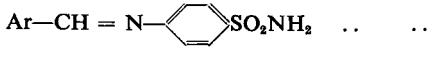
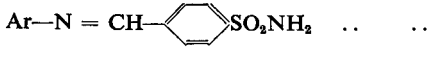

(III)


(IV)

observation that replacement of the =CH–CH= bridge in thiophene-2-sulphonamide by the diaza-group =N–N= is accompanied by impressive gain in inhibitory action (cf.[4,7,8]). Some derivatives of *p*-sulphamylphenyl urea and *p*-sulphamylphenylthiourea were also prepared, but none of these showed appreciable *in vitro* activity (Table IV) apart from products of unknown structure obtained by oxidation of *p*-sulphamylphenylthiourea with iodine or hydrogen peroxide.

Examination of *p*-carboxybenzenesulphonamide confirmed the earlier findings of Krebs[5] that this compound has relatively little activity. Its esters, in contrast, proved to be remarkably potent inhibitors of carbonic anhydrase *in vitro*. Butyl to dodecyl esters were particularly noteworthy, showing from 1000 to 2000 times the activity of sulphanilamide (Table V). Further increase in molecular weight was accompanied by rapid decrease in activity, possibly owing to the low solubility of the compounds in

aqueous solvents. Some substituted 2-amino-1:3:4-thiadiazoles[10] were also examined (Table VI) and proved moderately active.

Biological study of the above compounds by Dr. A. David and Mr. K. P. Fellowes, B.Sc. (Biological Laboratories, Godalming, Surrey) failed to

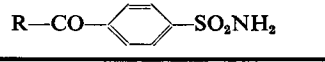
TABLE IV

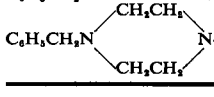
p-SULPHAMYLPHENYLUREA AND RELATED COMPOUNDS

	Activity
1-*p*-Sulphamylphenylurea	7
1:3-Bis-(*p*-sulphamylphenyl)-urea	210
1-*p*-Sulphamylphenylthiourea	4
1-Phenyl-3-(*p*-sulphamylphenyl)-thiourea	25
2-*p*-Sulphamylphenylaminothiazole	30
1:3-Bis-(*p*-sulphamylphenyl)-formamidine	135
p-Sulphamylphenyldiguanidine hydrochloride	3
2-Amino-4-(*p*-sulphamylphenylamino)-*sym*-triazine	18
5-Sulphamyl-1:3:4-triazolo-1:2:1′:2′-quinoline	20
Unidentified oxidation products of *p*-sulphamylphenylthiourea:	
With iodine (i)	315
(ii)	335
With H_2O_2	220

TABLE V

ESTERS AND AMIDES OF *p*-SULPHAMYLBENZOIC ACID



R	Activity (Sulphanilamide = 1)
HO-	4
CH_3O-	100
C_2H_5O-	260
C_3H_7O-	505
C_4H_9O-	1140
$C_5H_{11}O$-	930
$C_6H_{13}O$-	2290
$C_7H_{15}O$-	1430
$C_8H_{17}O$-	2080
$C_9H_{19}O$-	2280
$C_{10}H_{21}O$-	1220
$C_{11}H_{23}O$-	2250
$C_{12}H_{25}O$-	1040
$C_{16}H_{33}O$-	1
$C_{18}H_{37}O$-	3
iso-C_3H_7O-	115
sec-C_4H_9O-	155
iso-C_4H_9O-	530
CH_2:$CH.CH_2O$-	900
$C_6H_5CH_2O$-	2400
$C_6H_5.C_2H_4O$-	945
cyclo-$C_6H_{11}O$-	505
HOC_2H_4O-	135
NH_2-	40
NH_2NH-	15
$NH_2CSNHNH$-	18
$(HOC_2H_4)_2N$-	1
$C_6H_5CH_2NH$-	215
	3

reveal correlation between *in vitro* and *in vivo* activity. With the exception of the thiadiazole derivatives (Table VI), the more potent inhibitors of the enzyme *in vitro* were uniformly inactive *in vivo* in inducing diuresis in the rat on oral administration.

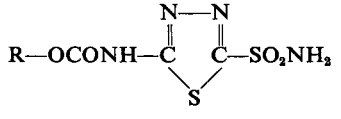
EXPERIMENTAL

N^4-(p-*Bromobenzenesulphonyl*)-*sulphanilamide.* *p*-Bromobenzenesulphonyl chloride (2·6 g.) was added with stirring at room temperature to a solution of sulphanilamide (1·7 g.) dissolved in the minimum volume of pyridine. The orange solution was allowed to stand at room temperature for 24 hours and was then diluted with water. The product was crystallised from aqueous ethanol in small needles of m.p. 210° to 212°. Found: C, 36·7; H, 2·8; N, 7·5; S, 16·1. $C_{12}H_{11}O_4N_2BrS_2$ requires C, 36·8; H, 2·8; N, 7·2; S, 16·4 per cent.

N^4-(p-*Toluenesulphonyl*)-*sulphanilamide* crystallised from acetic acid in shining needles of m.p. 188° to 189°. Found: C, 47·9; H, 4·6; N, 8·4; S, 19·3. $C_{13}H_{14}O_4N_2S_2$ requires C, 47·8; H, 4·3; N, 8·6; S, 19·6 per cent.

TABLE VI

2-ALKYLOXYCARBONYLAMINO-1 : 3 : 4-THIADIAZOLE-5-SULPHONAMIDES



R	Activity
CH_3-	230
C_2H_5-	105
C_3H_7- ..	280
C_4H_9- ..	195
C_5H_{11}- ..	410
C_6H_{13}- ..	330
$C_6H_5.CH_2$- ..	1020

2 : 2′-*Diaminodiphenyl*-4 : 4′-*disulphonamide.* 2 : 2′-Dinitrodiphenyl-4 : 4′-disulphonchloride prepared by the method of Feldmann[11] yielded the disulphonamide on treatment with liquid ammonia. The latter had m.p. 263° (softening 258°) after crystallisation from acetic acid. The disulphonamide (1 g.) was heated under reflux with 95 per cent ethanol (150 ml.), concentrated hydrochloric acid (1·5 ml.) and reduced iron powder (25 g.) for 8 hours. The suspension was filtered, the mother liquors neutralised and concentrated to dryness under reduced pressure. After addition of water to the residue the diamine (0·5 g.) was collected and crystallised from ethanol, m.p. 269°. Found: C, 42·2; H, 4·6; S, 18·5. $C_{12}H_{14}O_4N_4S_2$ requires C, 42·1; H, 4·1; S, 18·7 per cent.

Stilbene-4-*sulphonamide.* A mixture of sulphanilamide (8·6 g.) and sodium nitrite (3·8 g.) was dissolved in N sodium hydroxide solution (50 ml.), and this solution was added cautiously with stirring to ice cold sulphuric acid prepared from concentrated acid (9 ml.) and chopped ice (50 g.). After allowing to stand for 5 minutes a solution of cinnamic acid (7·4 g.) in acetone (270 ml.) was added, followed by a solution of cupric chloride dihydrate (2·1 g.) and sodium acetate (28·7 g.). The mixture was allowed to stand overnight, filtered and the product precipitated by dilution with water and crystallisation from ethanol. It had m.p. 249° to 250°[12]. Found: C, 64·4; H, 5·1; N, 5·4; S, 12·7. Calc. for $C_{14}H_{13}O_2NS$: C, 64·8; H, 5·0; N, 5·4; S, 12·4 per cent.

p-*Sulphamylbenzylidene* p-*toluidine.* A solution of benzaldehyde-4-sulphonamide was prepared by the method of Dakin[13]. Condensation with *p*-toluidine yielded the *product* which separated from ethanol in pale yellow needles, m.p. 218° to 219°. Found: N, 10·2. $C_{14}H_{14}O_2N_2S$ requires N, 10·2 per cent.

p-*Sulphamylbenzylidene* p-*chloroaniline* separated from aqueous ethanol in pale yellow needles, m.p. 194° to 196°. Found: C, 53·1; H, 3·7; N, 9·2. $C_{13}H_{11}O_2N_2ClS$ requires C, 53·0; H, 3·8; N, 9·5 per cent.

N^4-p-*Methylbenzylidene sulphanilamide.* A mixture of sulphanilamide (6·88 g.) and *p*-tolualdehyde (4·8 g.) in the minimum volume of ethanol was heated on the steam bath for 15 minutes. The *product* separated on cooling and was purified by crystallisation from a mixture of ethanol and acetone forming shining plates of m.p. 195°. Found: C, 61·5; H, 5·1; N, 10·3; S, 11·6. $C_{14}H_{14}O_2N_2S$ requires C, 61·3; H, 5·1; N, 10·2; S, 11·7 per cent.

N^4-p-*Chlorobenzylidene sulphanilamide*, after crystallisation from acetone, had m.p. 193° to 194°. Found: C, 52·9; H, 3·9; N, 9·5; Cl, 11·8. $C_{13}H_{11}O_2N_2ClS$ requires C, 53·0; H, 3·7; N, 9·5; Cl, 12·0 per cent.

N^4-(2′:4′-*Dichlorobenzylidene*)-*sulphanilamide*, after crystallisation from acetone had m.p. 205° to 206°. Found: C, 47·9; H, 2·9; N, 8·3; Cl, 21·4; S, 9·6. $C_{13}H_{10}O_2N_2Cl_2S$ requires C, 47·4; H, 3·0; N, 8·5; Cl, 21·5; S, 9·7 per cent.

N^4-(3′-*Iodo*-4′-*hydroxy*-5′-*methoxy*)*benzylidene sulphanilamide* crystallised from a mixture of ethanol and acetone, m.p. 208°. Found: C, 39·2; H, 2·9; N, 6·4; S, 7·4; I, 29·6. $C_{14}H_{13}O_4N_2IS$ requires C, 38·9; H, 3·0; N, 6·5; S, 7·4; I, 29·4 per cent.

1-(2′-*Quinolyl*)-*thiosemicarbazide.* To a solution of 2-hydrazinoquinoline hydrochloride (9·7 g.) in water (100 ml.) was added a solution of potassium thiocyanate (5·4 g.) dissolved in the minimum volume of water and the solution heated on the steam bath for 1 hour. The *product* which separated on cooling crystallised from ethanol in yellow needles m.p. 158°. Found: C, 55·1; H, 4·6; N, 25·4; S, 14·8. $C_{10}H_{10}N_4S$ requires C, 55·0; H, 4·6; N, 25·7; S, 14·7 per cent.

1:3:4-*Triazolo*-5-*mercapto*-1:2:1′:2′-*quinoline.* To a solution of 2-hydrazinoquinoline (1·59 g.) in ethanol (50 ml.) was added carbon disulphide (1·52 g.) followed by a solution of potassium hydroxide (0·56 g.) in water (5 ml.). The mixture was heated under reflux for 2 hours when evolution of hydrogen sulphide ceased. The solvent was largely distilled off, water added to dissolve the residual solid and the solution just acidified with hydrochloric acid. The *product* (1·8 g.) was collected, washed with water and crystallised from ethanol in needles, m.p. 258°. Found: C, 60·0; H, 3·5; N, 20·6; S, 15·6. Calc. for $C_{10}H_7N_3S$: C, 59·7; H, 3·5; N, 20·9; S, 15·9 per cent.

(Marchwald and Meyer[14], prepared this compound, m.p. 261°, by heating 4-phenyl-1-quinolyl(2)-thiosemicarbazide to 150°.)

1:3:4-*Triazolo*-5-*sulphamyl*-1:2:1′:2′-*quinoline.* The foregoing mercaptan (8 g.) was finely powdered, suspended in a mixture of glacial acetic acid (60 ml.) and water (30 ml.), cooled to 0° to 5° and treated with

a slow stream of chlorine gas for 3 hours. The product was collected, washed with cold water and added moist to concentrated ammonia solution (100 ml., d = 0·880). The sulphonamide crystallised and was purified by successive crystallisations from water, ethanol and glacial acetic acid, forming needles m.p. 255° to 256° (decomp.). Found: C, 48·4; H, 2·7; N, 22·3; S, 12·9. $C_{10}H_8O_2N_4S$ requires C, 48·4; H, 3·2; N, 22·6; S, 12·9 per cent.

n-*Propyl*-N-p-*sulphamyl phenyl thiocarbamate.* Powdered *p*-sulphamylphenyl *iso*-thiocyanate (4·3 g.) was suspended in *n*-propanol (100 ml.) and the mixture heated under reflux for 18 hours. The solid dissolved after several hours. Concentration to half-bulk followed by dilution with light petroleum (b.p. 60° to 80°) yielded the *product* which crystallised from a mixture of *n*-propanol and light petroleum (b.p. 60° to 80°) in white needles clusters, m.p. 165° to 167°. Found: C, 44·0; H, 5·3; N, 10·3. $C_{10}H_{14}O_3N_2S_2$ requires C, 43·8; H, 5·2; N, 10·2 per cent.

Ethyl-N-p-*sulphamylphenyl thiocarbamate* crystallised from aqueous ethanol in needles, m.p. 183° to 184° (decomp.). Found: C, 40·7; H, 4·7. $C_9H_{12}O_3N_2S_2$ requires C, 41·5; H, 4·7 per cent.

n-*Butyl*-N-p-*sulphamylphenyl thiocarbamate* crystallised from *n*-butanol in small prisms, m.p. 153° to 154°. Found: N, 9·7. $C_{11}H_{16}O_3N_2S_2$ requires N, 9·7 per cent.

Oxidation of p-*sulphamylphenyl thiourea.* To a solution of *p*-sulphamylphenylthiourea (9·2 g.) in water (500 ml.) containing concentrated hydrochloric acid (2 ml.) was added a solution of iodine in potassium iodide until the iodine colour just disappeared. After cooling, the solid was collected, dissolved in diluted sodium hydroxide solution and filtered to remove sulphur. The process was repeated. Acidification of the filtrate yielded the *product* (7·5 g.) which separated from water in small needles of m.p. 241° (decomp.). Found: C, 34·2; H, 4·0; N, 16·2; S, 22·1 per cent. A second experiment using hydrogen peroxide (13·8 g. of 30 per cent solution) yielded a product which separated from water in feathery needles of m.p. 220° (decomp.) (the m.p. was depressed on admixture with the above compound). Found: C, 32·1; H, 4·2; N, 16·9; S, 19·4 per cent.

Esters of p-*sulphamyl benzoic acid.* Esters of *p*-sulphamyl benzoic acid were prepared by three methods: (*a*) Direct esterification of *p*-sulphamyl benzoic acid by the Fischer-Speier method. (*b*) Trans-esterification from methyl *p*-sulphamyl benzoate. (*c*) A solution of the acid and alcohol in pyridine was treated with benzene sulphonyl chloride[15].

An example of each method of preparation is given and the properties of the various esters are summarised in Table VII.

Method (*a*) *Methyl* p-*sulphamyl benzoate.* A suspension of *p*-sulphamyl benzoic acid (56 g.) in methanol (500 ml.) containing hydrochloric acid gas (5 g.) was heated under reflux for 2 hours. All solid dissolved after *ca.* 30 minutes. The product separated on cooling and crystallised from methanol in prismatic needles of m.p. 185°.

Method (*b*) n-*Octyl* p-*sulphamyl benzoate.* Methyl *p*-sulphamyl benzoate (5 g.) was dissolved in *n*-octanol (50 ml.) containing hydrochloric acid gas (1 g.) and the solution heated under reflux for 5 hours.

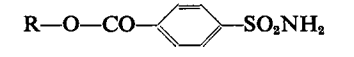
The product which separated on cooling crystallised from ethyl acetate in small shining plates of m.p. 106° to 107°.

Method (*c*) n-*Decyl* p-*sulphamyl benzoate*. A solution of *p*-sulphamyl benzoic acid (5 g.) in pyridine (150 ml.) was cooled in ice and treated with toluene *p*-sulphonyl chloride (9·5 g.). *n*-Decanol (4 g.) was then added with stirring. The mixture was allowed to warm up to room temperature over 1 hour and was then poured onto ice. The white solid was collected, washed with water and purified by crystallisation from a mixture of ethyl acetate and light petroleum (b.p. 60° to 80°) forming shining needles of m.p. 109° to 110°.

n-*Butyl*-2-*methoxy*-4-*sulphamyl benzoate* was prepared directly by esterification of 2-methoxy-4-sulphamyl benzoic acid. It crystallised from

TABLE VII

ESTERS OF *p*-SULPHAMYL BENZOIC ACID



R	Method	m.p. °C	Formula	Found				Required			
				C	H	N	S	C	H	N	S
Me	a	185	$C_8H_9O_4NS$	44·7	4·2	6·2		44·6	4·2	6·5	
n-Pr	a	109–110	$C_{10}H_{13}O_4NS$	49·6	5·4	5·9	13·6	49·4	5·4	5·8	13·2
iso-Pr.. ..	a	139–140	$C_{10}H_{13}O_4NS$	49·9	5·6	5·8	13·4	49·4	5·4	5·8	13·2
n-Bu ..	a	110	$C_{11}H_{15}O_4NS$	51·8	6·1	5·4	12·6	51·4	5·9	5·5	12·5
iso-Bu ..	a	136	$C_{11}H_{15}O_4NS$	51·8	6·0	5·3	12·2	51·4	5·9	5·5	12·5
n-Amyl ..	b	96	$C_{12}H_{17}O_4NS$	52·6	5·9	5·3	12·1	53·1	6·3	5·1	11·8
n-Hexyl ..	a	106–107	$C_{13}H_{19}O_4NS$	54·9	6·7	4·6	10·8	54·7	6·7	4·9	11·2
*cyclo*Hexyl ..	a	132–133	$C_{13}H_{17}O_4NS$	54·6	5·8	5·2	11·4	55·1	6·1	4·9	11·3
n-Heptyl ..	a	107–108	$C_{14}H_{21}O_4NS$	56·4	7·4	4·9	10·7	56·1	7·1	4·7	10·7
n-Octyl ..	b, c	107	$C_{15}H_{23}O_4NS$	57·6	7·3	4·5	10·3	57·5	7·4	4·5	10·2
n-Nonyl ..	b, c	107–108	$C_{16}H_{25}O_4NS$	58·6	7·5	3·9	9·4	58·7	7·7	4·3	9·8
n-Decyl ..	b, c	109	$C_{17}H_{27}O_4NS$	59·4	8·0	4·0	9·7	59·8	8·0	4·1	9·4
n-Undecyl ..	b, c	108–109	$C_{18}H_{29}O_4NS$	60·6	8·0	4·0	9·3	60·8	8·2	3·9	9·0
n-Dodecyl ..	b	107–108	$C_{19}H_{31}O_4NS$	61·3	8·2	4·3	9·2	61·8	8·5	3·8	8·7
n-Octadecyl	b	112–113	$C_{25}H_{43}O_4NS$	66·2	9·3	2·8	6·7	66·2	9·6	3·1	7·1
Allyl ..	a	108–109	$C_9H_9O_4NS$	49·4	4·7	5·8	13·4	49·8	4·6	5·8	13·3
Benzyl ..	a	162–163	$C_{14}H_{13}O_4NS$	57·9	4·5	4·8	11·4	57·7	4·5	4·8	11·0
β-Phenethyl ..	b	159	$C_{15}H_{15}O_4NS$	59·4	4·9	4·9	10·6	59·0	5·0	4·6	10·5
β-Hydroxy ethyl	b	126–128	$C_9H_{11}O_5NS$	44·1	4·5	5·4	12·7	44·1	4·5	5·7	13·1

water in silky needles of m.p. 98°. Found: C, 50·1; H, 5·8; N, 4·8; S, 11·3. $C_{12}H_{17}O_5NS$ requires C, 50·2; H, 6·0; N, 4·9; S, 11·2 per cent.

p-*Sulphamyl benzoic acid piperazine salt*, prepared to characterise the acid, crystallised from hot water in prisms, m.p. 280° (decomp.). Found: C, 44·5; H, 5·0; N, 11·2; S, 13·2. $C_{18}H_{24}O_8N_4S_2$ requires C, 44·3; H, 5·0; N, 11·5; S, 13·1 per cent.

p-*Sulphamyl benzamide* crystallised from water in needles, m.p. 242° to 244°. Found: N, 13·7. Calc. for $C_7H_8O_3N_2S$: N, 14·0 per cent.

N-(p-*Sulphamylbenzoyl*)di-β-*hydroxyethylamine* crystallised from a mixture of ethanol and ethyl acetate in needles, m.p. 155° to 156°. Found: C, 46·1; H, 5·2; N, 9·7. $C_{11}H_{16}O_5N_2S$ requires C, 45·8; H, 5·6; N, 9·7 per cent.

N-p-*Sulphamylbenzoyl benzylamine*. A mixture of methyl-4-sulphamyl benzoate (4 g.) and benzylamine (10 ml.) was heated on the steam bath for 8 hours. It was then cooled, stirred with dilute hydrochloric acid and the residual *solid* crystallised from ethanol in needles m.p. 186° to 188°.

The melt solidified rapidly and then remelted at 198° to 199°. Found: C, 57·5; H, 4·9; N, 9·7; S, 11·1. $C_{14}H_{14}O_3N_2S$ requires C, 57·9; H, 4·9; N, 9·7; S, 11·0 per cent.

p-*Sulphamylbenzhydrazide.* To a suspension of methyl *p*-sulphamyl benzoate (27 g.) in ethanol (50 ml.) was added hydrazine hydrate (24 g.). The solid dissolved rapidly and the solution was heated on the steam bath for 2 hours. The product separated on cooling and crystallised from ethanol in shining plates, m.p. 238° to 240° (decomp.). Found: N, 19·7. $C_7H_9O_3N_3S$ requires N, 19·5 per cent.

1-(p-*Sulphamylbenzoyl*)-*thiosemicarbazide.* The foregoing hydrazide (2·15 g.) was dissolved by warming in water (50 ml.) containing concentrated hydrochloric acid (1 ml.), treated with potassium thiocyanate (1·2 g.) and the solution heated on the steam bath for 2½ hours with concentration to one-third volume. The *product* which separated on cooling crystallised from ethanol in shining plates, m.p. 233° (decomp.). Found: C, 35·4; H, 3·7; S, 22·6. $C_8H_{10}O_3N_4S_2$ requires C, 35·0; H, 3·7; S, 23·3 per cent.

Materials and Methods for Carbonic Anhydrase Assay

Carbonic Anhydrase. A freeze-dried preparation of crude "chloroform enzyme" was used, prepared by the method described by Roughton and Booth[16].

Assay procedure. The method is based on the colorimetric procedure of Roughton and Booth[16].

Three ml. of 0·05 M sodium veronal buffer of pH 8·2, 0·2 ml. of bromothymol blue (B.D.H. indicator solution), 1·8 ml. of water and 0·4 ml. of enzyme solution were pipetted into a 25 ml. weighing bottle, which was stoppered and equilibrated in ice for 20 minutes. Five ml. of ice-cold water saturated with carbon dioxide was then added from a chilled syringe, the nozzle of which was held below the liquid surface, and the mixture was rapidly mixed by rotation. The time taken for the indicator colour to match that of a standard of bromothymol blue made up in pH 6·3 phosphate buffer was timed with a stop-watch.

In the absence of enzyme, reaction times of 160–180 seconds were obtained. In the presence of enzyme (10 mg. in 100 ml. of water) reaction times were reduced to 40–50 seconds.

Assay of inhibitors. A solution of the inhibitor sample was made up in water, and 1·8 ml. of the solution was added to the weighing bottle in place of the 1·8 ml. of water. Where the sample was too insoluble in water the solution was made up in 50 per cent ethanol; 50 per cent ethanol was then also used for determination of the blank times.

About twelve readings were obtained in duplicate for serial dilutions of the inhibitor. The per cent inhibition was then plotted against $-\text{Log}$ (I) (where (I)=inhibitor concentration). A straight line was usually obtained over the concentration range giving 20–80 per cent inhibition, and the concentration causing 50 per cent inhibition could then be read off.

The molar concentration of substance causing 50 per cent inhibition was expressed relative to the concentration of sulphanilamide causing similar inhibition, this being given the arbitrary value of 1.

Results

In this system sulphanilamide was found to give 50 per cent inhibition at a concentration of $9{\cdot}5 \times 10^{-7}$M. Considerable variation was found in inhibitor activities from day to day, and all the figures quoted are subject to error of about $\pm$ 50 per cent. Similar variation was reported by Miller, Dessert and Roblin[8].

TABLE VIII

COMPARISON OF RESULTS WITH THOSE OBTAINED BY MILLER AND OTHERS[7,8] AND KREBS[5]

Inhibitor	Activity (Sulphanilamide = 1)		
	Observed	Miller and others	Krebs
Benzene sulphonamide	3	4	2
N^4-Acetylsulphanilamide	17	—	8
p-Sulphamylbenzoic acid	4	—	5
Naphthalene-1-sulphonamide	17	—	6
Naphthalene-2-sulphonamide	14	—	9
Prontosil	186	50	150
2-Acetamido-1 : 3 : 4-thiadiazole-5-sulphonamide (Diamox)	347	330	—
2-Amino-1 : 3 : 4-thiadiazole-5-sulphonamide	11	25	—

Results obtained appear to be roughly comparable with those quoted by previous workers. Table VIII gives comparative results obtained by Miller and others (*loc. cit.*), Krebs[5], and ourselves.

Activity values obtained for various types of sulphonamide are summarised in Tables I to VI.

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