STUDIES IN THE FIELD OF DIURETIC DRUGS

PART II. 5-CHLORO-2,4-DISULPHAMYLTOLUENE (DISULPHAMIDE)

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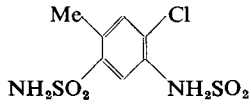
The preparation of 5-chloro-2,4-disulphamyltoluene, its properties and estimation in biological fluids are described.

IN Part I of this series[1] the preparation and carbonic anhydrase inhibiting activity (CAIA) of some new sulphamyl compounds were reported. Many of these, in particular the esters of *p*-sulphamylbenzoic acid, 4,4′-disulphamyldiphenyl, 4,4′-disulphamyldiphenyl sulphide and the 2-alkoxycarbonylamido-1,2,4-thiadiazoles[2] possessed outstanding activity in the CAIA assay employed. Nevertheless, with the exception of the thiadiazole derivatives, they were virtually inactive as diuretics in the saline-loaded rat. In extending this work we turned to the disulphamyl derivatives of benzene, a group of compounds independently studied in other laboratories[3,4].

Employing standard methods of synthesis, the 1,3-disulphamyl derivatives of benzene listed in Table VI were prepared and screened for carbonic anhydrase inhibiting activity. Study of their diuretic properties (oral administration to the saline-loaded rat) by Dr. A. David and his colleagues[5] led to the selection of 5-chloro-2,4-disulphamyltoluene (I) for further examination.



(I)

5-Chloro-2,4-disulphamyltoluene (disulphamide) (I) is a new compound prepared by chlorosulphonation of *m*-chlorotoluene under regulated conditions, followed by reaction of the resultant 5-chlorotoluene-2,4-disulphonchloride[6] with ammonia. It crystallises from alcohols, aqueous alcohols or glycols in colourless needles or prisms of m.p. 259·5° to 260·5° (corr.). Though possessing identical melting points, the two crystalline forms differ slightly in their infra-red spectra in Nujol suspension. Its pharmacological properties are described elsewhere[5].

EXPERIMENTAL

Solubility

The solubility of disulphamide was accurately determined by equilibrating a saturated solution of the compound in the presence of excess solid for 48 hours in a thermostat bath at 0° and 25°. In the case of the solubility determination at 0° the solution was equilibrated in a refrigerator

fitted with a recording thermometer. The saturated solutions were hand-centrifuged, samples were weighed into volumetric flasks, diluted, and the sulphamide determined by ultra-violet spectrophotometry. Replicate readings showed that the method was comparable in accuracy to the conventional macro-method whilst permitting the use of a sample of about 20 mg. for each determination. The solubilities at 0° and at 25° were found to be 0·02 g. and 0·06 g. per 100 g. of water, respectively.

Disulphamide is soluble to about 2 per cent in boiling water and is readily soluble in aqueous alkali hydroxides or carbonate.

TABLE I

SOLUBILITY OF 5-CHLORO-2,4-DISULPHAMYLTOLUENE IN AQUEOUS ETHANOL AT 25°

Ethanol (per cent by volume)	Solubility (g. solute per 100 g. of solvent)
100	1·89
90	2·23
80	2·41
70	2·08
60	1·44
50	1·17
40	0·63
20	0·15

The solubility in water-ethanol at 25° reached a maximum at about 80 per cent (v/v) of ethanol (Table I).

The solubility of the compound in other organic solvents at 25° (g. solute per 100 g. solvent) was as follows: hexane (0·0004); chloroform (0·001) and isopropanol (0·35).

Ultra-violet Absorption Spectra

Ultra-violet absorption spectra were determined using a Beckman recording spectrophotometer, Model DK-2. The results obtained in

TABLE II

ULTRA-VIOLET ABSORPTION SPECTRA OF 5-CHLORO-2,4-DISULPHAMYLTOLUENE IN VARIOUS SOLVENTS

Solvent	λ max (mμ)	ε max
Isopropanol	285	920
	276	1,035
	235	12,550
Ethanol	285	805
	276	897
	235	11,930
	210	46,000
50 per cent ethanol	285	957
	276	1,050
	235	11,790

isopropanol, ethanol and 50 per cent (v/v) aqueous ethanol are summarised in Table II. Acidification of a 1 : 1 aqueous-ethanolic solution to 0·05N with hydrochloric acid had no effect upon the spectral absorption. Addition of potassium hydroxide (0·05N) completely eliminated all spectral detail and the curve rose steadily from *ca.* 295 mμ to a maximum

at 221 mμ (εmax = 17,800). Thereafter there was a shallow minimum at 220 mμ when the curve rose again to an arbitrarily chosen lower wavelength limit of 218 mμ. These observations indicate a simple acid-base reaction between relatively stable species, the spectra in acid and alkali being mutually interconvertible by excess reagent on equilibration.

Determination of 5-Chloro-2,4-Disulphamyltoluene in Biological Fluids

Determination of disulphamide was carried out by measurement of the carbonic anhydrase inhibiting activity, using the procedure described

TABLE III

5-Chloro-2,4-disulphamyltoluene found in urine of four groups of 10 rats dosed with 300 mg./kg. (total dose = 3·2 g.) of the diuretic

Urine		5-Chloro-2,4-disulphamyl toluene found (mg.)	
Time (hours)	Volume (ml.)	By enzyme inhibition	By isolation
0–2	200	64	76
2–4	130	203	138
4–6	62	54	92
6–24	100	199	—

previously[1]. This method is known to have an error of ±50 per cent. A similar assay has previously been described for the assay of acetazolamide[7].

Preparation of Biological Samples for Assay

Urine samples. These were acidified with hydrochloric acid and extracted three times with ethyl acetate. The ethyl acetate solutions were then extracted three times with 5 per cent sodium carbonate solution, and residual ethyl acetate was removed from the aqueous solution by aeration. The solutions were made up to 200 ml. with 5 per cent sodium carbonate solution, and a minimum dilution of this solution of 1 in 100 with 50 per cent ethanol was taken for assay.

Blood samples. These were separated into cells and serum, each being made up to a 1 in 10 dilution with normal saline, and further 1 in 5 dilutions were made with distilled water. These solutions were heated for 5 minutes in a boiling water bath to destroy endogenous enzyme activity. Not more than 0·8 ml. of these solutions could be used in the assay procedure, since other constituents caused interference if larger quantities were taken. Recovery of material added to normal specimens was within experimental error.

Added to urine: 7·6 mg./l. Recovered: 9·9 mg./l. Added to 1 in 50 cell dilution: 0·24 mg./l. Recovered: 0·38 mg./l. Added to 1 in 50 serum dilution: 0·24 mg./l. Recovered: 0·34 mg./l.

Standards for the assay of disulphamide in biological specimens were prepared by addition of the compound to normal biological specimens. Both sets were then prepared for assay as described above.

Determination of the nature of the inhibitory activity in urine from dosed animals. Forty rats were dosed orally with 300 mg./kg. of a drug suspension, and their urine was subjected both to the assay procedure and to direct isolation of the drug. The drug was isolated by the following procedure. The urine was acidified, extracted three times with ethyl acetate, and the bulked ethyl acetate extract was extracted three times with sodium carbonate solution. Acidification of the concentrated carbonate solution led to the formation of crystals which were filtered off on a sintered crucible, dried, and weighed. Identity was established by determination of mixed melting point with an authentic sample. Results obtained are

TABLE IV

5-CHLORO-2,4-DISULPHAMYLTOLUENE FOUND IN URINE OF 8 RATS DOSED WITH 10 MG./KG. OF THE DIURETIC

5-hour volume of urine (ml.)	Total dose administered (mg.)	Recovery	
		mg.	per cent of dose
16	22·75	5·04	22·2
19	22·25	5·76	25·3
25	25·44	5·85	23·0
23·6	23·10	7·43	32·2
24·2	22·75	11·01	48·4

listed in Table III. Agreement was sufficiently close, within the limits of experimental error, to warrant the conclusion that the measured carbonic anhydrase inhibitory activity was due to the presence of unchanged 5-chloro-2,4-disulphamyltoluene.

Recovery of dose from urine. In the above high-dose experiment approximately 15 per cent of the dose was accounted for as being excreted unchanged in the urine. In a second experiment five groups of 8 rats were given an oral dose of 10 mg./kg., and 20 to 50 per cent of the dose was excreted in 5 hours. Results are listed in Table IV.

TABLE V

5-CHLORO-2,4-DISULPHAMYLTOLUENE IN SERUM AND RED CELLS OF RABBITS RECEIVING 10 MG./KG. OF THE DIURETIC BY MOUTH

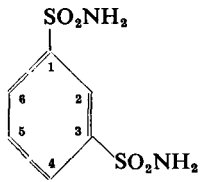
Hours	5-Chloro-2,4-disulphamyltoluene in serum (μg./ml.)		5-Chloro-2,4-disulphamyltoluene in red cells (μg./ml.)	
	Rabbit A	Rabbit B	Rabbit A	Rabbit B
1	<0·5	2·3	<0·5	3·6
2	0·9	1·7	1·3	1·4
4	<0·5	<0·5	2·1	0·6
6	<0·5	0·7	1·7	<0·5
24	<0·5	<0·5	<0·5	<0·5

Distribution of drug in blood. Two rabbits were given an oral dose of 10 mg./kg., and blood samples were taken periodically and assayed in serum and red cells. Results obtained are given in Table V.

5-Chlorotoluene-2,4-disulphonchloride. m-Chlorotoluene (126·5 g.) was added dropwise with stirring to chlorosulphonic acid (350 g.), the rate of addition being controlled to keep the temperature of the reaction below

TABLE VI

DISULPHONAMIDES



Substituent at position			CA* activity (w/w) acetazolamide = 1	m.p. °C.	Formula	Found					Required				
4	5	6				C	H	N	S	Cl	C	H	N	S	Cl
—	—	—	0·01	233–235											
Me	—	—	0·30	189–190	$C_7H_{10}O_4N_2S_2$	34·0	3·8	10·8	25·6	—	33·6	4·0	11·2	25·6	—
Et	—	—	0·04	185–187	$C_8H_{12}O_4N_2S_2$	36·6	4·3	10·6	24·0	—	36·4	4·6	10·6	24·3	—
Me_2CH	—	—	0·04	213–214	$C_9H_{14}O_4N_2S_2$	39·2	5·2	9·7	—	—	38·8	5·1	10·1	—	—
F	—	—	0·10	189–191	$C_6H_7O_4N_2S_2F$	28·6	2·4	11·0	—	—	28·3	2·8	11·0	—	—
Cl	—	—	0·09	212–214	—	—	—	—	—	—	—	—	—	—	—
Br	—	—	0·06	243–245	—	—	—	—	—	—	—	—	—	—	—
Me	Me	—	0·25	237–239	—	—	—	—	—	—	—	—	—	—	—
Me	—	Me	0·47	257–259	—	—	—	—	—	—	—	—	—	—	—
Me	—	F	0·28	216–218	$C_7H_9O_4N_2S_2F$	31·6	3·3	10·5	23·4	—	31·4	3·4	10·5	23·9	—
Me	—	Cl	0·40	259·5–260·5 corr.	$C_7H_9O_4N_2S_2Cl$	29·9	2·9	9·9	—	—	29·5	3·2	9·8	22·5	12·5
Me	Cl	—	2·00	237–239	$C_7H_9O_4N_2S_2Cl$	29·9	3·2	10·1	22·3	12·6	29·5	3·2	9·8	22·5	12·5
Cl	Me	—	0·67	239–240	$C_7H_9O_4N_2S_2Cl$	30·1	3·2	9·8	22·7	12·5	29·5	3·2	9·8	22·5	12·5
Me	—	Br	0·43	264–265	$C_7H_9O_4N_2S_2Br$	25·8	2·8	8·2	—	—	25·5	2·7	8·5	—	—
Et	—	Cl	0·56	194–195	$C_8H_{11}O_4N_2S_2Cl$	32·1	3·4	9·4	21·5	11·9	32·2	3·7	9·4	21·5	11·9
n-Pr	—	Cl	0·55	179–180	$C_9H_{13}O_4N_2S_2Cl$	34·3	3·8	8·9	20·9	11·7	34·6	4·2	9·0	20·5	11·4
Cl	Cl	—	2·00	234–236	$C_6H_6O_4N_2S_2Cl_2$	—	—	—	—	—	—	—	—	—	—
Cl	—	Cl	0·66	265	$C_6H_6O_4N_2S_2Cl_2$	23·4	2·3	9·6	20·7	23·0	23·6	2·0	9·2	21·0	23·3
Br	—	Br	0·40	271–273	$C_6H_6O_4N_2S_2Br_2$	18·7	1·8	7·2	15·8	44·4†	18·3	1·5	7·1	16·2	40·5†
Cl	—	F	0·40	234–235	$C_6H_6O_4N_2S_2ClF$	25·0	2·2	9·7	—	11·8	25·0	2·1	9·7	—	12·3
Cl	—	Br	0·32	290	$C_6H_6O_4N_2S_2ClBr$	20·9	1·9	—	—	—	20·7	1·7	—	—	—
$-+$ ONH_4	Cl	—	0·0002	252 decomp.	$C_6H_{10}O_5N_2S_2Cl$	23·9	3·2	13·5	—	11·6	23·7	3·2	13·8	—	11·7
$-+$ ONH_4	SO_2NH_2	—	<0·0002	266 decomp.	$C_6H_{12}O_7N_4S_3$	21·2	3·1	16·1	27·7	—	20·7	3·5	16·1	27·6	—
Cl	SO_2NH_2	—	0·17	344 decomp.	$C_6H_5O_6N_3S_3Cl$	20·9	2·6	11·6	27·8	10·0	20·6	2·8	12·0	27·5	10·1

* Carbonic Anhydrase.

† Bromine.

50°. After the addition was complete, pentachloroethane (250 ml.) was added to the stirred mixture which was then gradually heated to reflux temperature. The heating was continued until evolution of hydrogen chloride was complete (usually about 8 hours). The mixture was then heated on the steam bath at about 20 mm. pressure to remove all volatile material. The hot residue was extracted with a high boiling (100–120°) light petroleum solvent, two extractions usually being sufficient. The *product* separated from the light petroleum extract on cooling; it had m.p. 122–124°, and was sufficiently pure for the next stage of the process. A portion, crystallised from ligroin, had m.p. 125–126°. Found: S, 20·1; Cl, 32·8. Calc. for $C_7H_5O_4S_2Cl_3$: Cl, 32·9; S, 19·8 per cent.

5-*Chlorotoluene*-2,4-*disulphonamide*. The foregoing disulphonchloride (100 g.) was added in portions with stirring to liquid ammonia and when the addition was complete, excess of ammonia was allowed to evaporate at room-temperature. The semi-solid residue was dissolved in water (500 ml.) and the solution acidified with hydrochloric acid. The *product* which separated had m.p. 257–259°. The m.p. was 259·5–260·5° (corr.) after crystallisation from aqueous ethanol.

Fluorobenzene-2,4-*disulphonchloride*. A mixture of fluorobenzene-4-sulphonchloride (150 g.) and chlorosulphonic acid (300 ml.) was heated at 160–170° for 20 hours. The cooled mixture was poured on to ice and the *product* isolated with carbon tetrachloride and purified by distillation under reduced pressure. It had b.p. 132–139° at 0·5 mm., and m.p. 69–71° after crystallisation from light petroleum (b.p. 60–80°). Found: C, 24·9; H, 1·3; Cl, 24·5. $C_6H_3O_4S_2Cl_2F$ requires C, 24·6; H, 1·0; Cl, 24·2 per cent.

5-*Fluorotoluene*-2,4-*disulphonchloride*, prepared by chlorosulphonation of 3-fluorotoluene in pentachloroethane, had m.p. 99–100° after crystallisation from ligroin. Found: S, 21·3; Cl, 22·8. $C_7H_5O_4S_2Cl_2F$ requires S, 20·9; Cl, 23·1 per cent.

5-*Bromotoluene*-2,4-*disulphonchloride*, crystallised from ligroin in white needles, m.p. 121–122°. Found: C, 22·8, H, 1·4; S, 17·1; Br, 21·9; Cl, 19·4; $C_7H_5O_4S_2Cl_2Br$ requires C, 22·8; H, 1·4; S, 17·4; Br, 21·7; Cl, 19·3 per cent.

5-*Chlorofluorobenzene*-2,4-*disulphonchloride*, had m.p. 103–105° after crystallisation from ligroin. Found: C, 22·4; H, 0·9; Cl, 32·1. $C_6H_2O_4S_2Cl_3F$ requires C, 22·0; H, 0·6; Cl, 32·5 per cent.

5-*Bromochlorobenzene*-2,4-*disulphonchloride*, had m.p. 139–141° after crystallisation from ligroin. Found: C, 18·9; H, 0·9; S, 16·6. $C_6H_2O_4S_2Cl_3Br$ requires C, 18·6; H, 0·5; S, 16·5 per cent.

2,4-*Dibromobenzenesulphonchloride*, was obtained as a low melting solid of b.p. 128° at 0·2 mm. by chlorosulphonation of *m*-dibromobenzene (129 g.) with chlorosulphonic acid (200 g.) in carbon tetrachloride (125 g.) at reflux temperature for 16 hours. Found: C, 21·7; H. 1·0. $C_6H_3O_2SClBr_2$ requires C, 21·6; H, 0·9 per cent.

4,6-*Dibromobenzene*-1,3-*disulphonchloride*, was obtained by heating the foregoing compound (100 g.) with chlorosulphonic acid (150 ml.) at 150° for 20 hours. It had m.p. 159–162° after crystallisation from ligroin.

Found: C, 17·0; H, 0·7; S, 14·5. $C_6H_2O_4S_2Cl_2Br_2$ requires C, 16·6; H, 0·5; S, 14·8 per cent.

4-*Chloro*-3,5-*xylenol*-2,6-*disulphonamide*, prepared by chlorosulphonation of 4-chloro-3,5-xylenol at room temperature followed by reaction of the resultant disulphonchloride with liquid ammonia, had m.p. 242–244° after crystallisation from aqueous ethanol. Found: C, 31·0; H, 3·7; N, 9·2; Cl, 11·5. $C_8H_{11}O_5N_2S_2Cl$ requires C, 30·6; H, 3·5; N, 8·9; Cl, 11·3 per cent.

Other disulphonamides prepared by methods already illustrated are summarised in Table VI.

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