RESEARCH PAPERS

NUCLEAR DERIVATIVES OF 4:4′-DIAMINODIPHENYL SULPHONE

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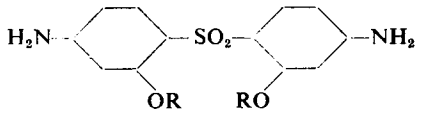
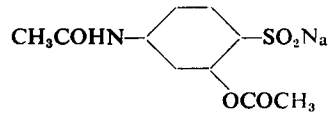
ATTEMPTS to lower the toxicity of 4:4′-diaminodiphenyl sulphone have resulted in numerous modifications of its chemical structure. Few, however, of the important group of *N*-substituted derivatives are more active against *Mycobacterium tuberculosis* than the parent compound and there is some evidence that they are converted in the body into 4:4′-diaminodiphenyl sulphone[1]. Nuclear substituents, too, contribute little to the level of *in vitro* activity[2,3] and in many cases marked reductions have been reported. Two compounds, of interest because of their structural relationship to *p*-aminosalicylic acid, 4:4′-diamino-2-hydroxydiphenyl sulphone[2,4] and 4:4′-diamino-3-methoxydiphenyl sulphone[3], possessed respectively one quarter and twice the *in vitro* activity of the parent compound, but *in vivo* studies and toxicities of these compounds have not been recorded.

The established activity of *p*-aminosalicylic acid against *M. tuberculosis* and its low toxicity, coupled with the known biological oxidation of sulphanilamide to 4-amino-2-hydroxy-benzenesulphonamide which occurs in certain animals[5], prompted a further investigation of ortho hydroxylated substituents in the molecule of 4:4′-diaminodiphenyl sulphone. The synthesis of 4:4′-diamino-2:2′-diethoxydiphenyl sulphone (I, $R = OC_2H_5$) and 4:4′-diamino-2:2′-dihydroxydiphenyl sulphone (I, R = H) is now reported. Since the conclusion of this work the preparation of the latter compound has been described by Amstutz[6].



I.

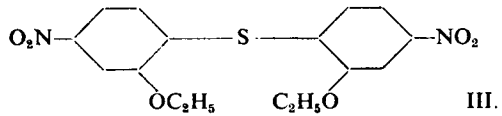


II.

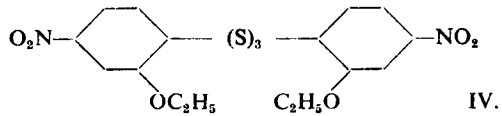
Attempts to condense sodium 4-acetylamino-2-acetoxy-benzenesulphinate (II) with 2-chloro-5-nitrophenol, according to the method described by Ferry, Buck and Baltzly[7] for the preparation of 4:4′-diaminodiphenyl sulphone, were unsuccessful. Under similar conditions *p*-bromonitrobenzene also failed to condense with II. Burton and Hu[8] have reported the failure of comparable reactions between various unspecified aryl iodides and sodium *p*-cyanobenzenesulphinate and, more recently, Peak and Williams[9] described unsuccessful attempts to obtain sulphones by the condensation of 2:4:6-trichlorobenzenesulphinate with diethylaminoethyl chloride.

The required compounds were obtained by the repeated oxidation of

2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide (III) with an excess of potassium permanganate to yield the corresponding sulphone, followed by combined reduction and de-ethylation with hydriodic acid. The latter reagent, with suitable adjustment of the reaction conditions, gave either one or the other of the required products, 4:4′-diamino-2:2′-diethoxydiphenyl sulphone or 4:4′-diamino-2:2′-dihydroxydiphenyl sulphone.



III.



IV.

Condensation of 4-chloro-3-ethoxynitrobenzene with sodium sulphide by the method of Hodgson and Dodson[10] failed to yield the monosulphide III, and 2-chloro-5-nitrophenol was isolated in 73 per cent. yield, together with a small quantity of a second product, molecular formula $C_{16}H_{16}O_6N_2S_3$, which has been tentatively assigned the structure IV. The nature of the sulphide link has not yet been established. 4-Chloro-3-ethoxy-nitrobenzene readily reacted with sodium hydrosulphide[11], though direct condensation yielded a mixture of the trisulphide $C_{16}H_{16}O_6N_2S_3$, described above, and 2:2′-diethoxy-4:4′-dinitrodiphenyl disulphide. A modified procedure, in which sodium 2-ethoxy-4-nitrothiophenoxide was first formed by the action of sodium hydrosulphide on 4-chloro-3-ethoxynitrobenzene and then condensed *in situ* with the latter substance, gave III in 30 per cent. yield (crude). Purification of the product obtained by this method was troublesome and wasteful, involving multiple chromatographic adsorptions and recrystallisations.

Compound III was more readily obtained in 21 per cent. over-all yield by an alternative route, the condensation of 2-acetylamino-4-chloro-5-ethoxynitrobenzene with sodium sulphide by the method of Hodgson and Dodson[10]; the product, a mixture of 5:5′-diacetylamino-2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide and 5-acetylamino-5′-amino-2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide was de-acetylated and de-aminated by the method of van Erp[12]. Condensation of sodium 5-acetylamino-2-ethoxy-4-nitrothiophenoxide with 2-acetylamino-4-chloro-5-ethoxynitrobenzene using the procedure described above, gave the same two monosulphides, although the higher yield anticipated by this method was not obtained.

The preliminary pharmacological tests which are recorded below were kindly carried out by Professor G. A. H. Buttle of this School.

In vitro tuberculostatic activities of 4:4′-diamino-2:2′-diethoxydiphenyl sulphone (I, R = C_2H_5) and of 4:4′-diamino-2:2′-dihydroxydiphenyl sulphone (I, R = H, as dihydrochloride) were examined in Dubos medium using *M. tuberculosis* (H37Rv); sodium 4-aminosalicylate and 4:4′-diaminodiphenyl sulphone were used for comparison. The results are recorded in Table I.

A limited investigation has also been made of the *in vivo* activity of 4:4'-diamino-2:2'-dihydroxydiphenyl sulphone in mice infected intravenously with 0·01 mg. (dry bacterial substance) of *Mycobacterium murium* (NCTC 5676), with results which are summarised in Table II.

TABLE I

	Inhibitory Dilution
Substance I ($R=C_2H_5$)	1 : 16,000
Substance I ($R=H$)	1 : 256,000
4 : 4'-Diaminodiphenyl sulphone	1 : 128,000
Sodium 4-aminosalicylate	1 : 64,000

TABLE II

Substance	Number of Mice	Daily Dose mg.	Mortality after 3 weeks
4 : 4'-Diaminodiphenyl sulphone	6	1	3/6
Sodium 4-aminosalicylate...	6	1	3/6
4 : 4'-Diamino-2 : 2'-dihydroxydiphenyl sulphone	6	1	0/6*
Nil (controls)	6	0	4/6

* Post mortem examination of the survivors (killed after 3 weeks) revealed that four of the six mice were normal throughout.

Examination of the oral toxicity in mice of 4:4'-diamino-2:2'-dihydroxydiphenyl sulphone has shown it to be non-toxic at doses of 500 mg./kg., a dose level at which the parent compound, 4:4'-diaminodiphenyl sulphone is known to exhibit toxic reactions.

This work is part of a thesis presented to the University of London in May, 1950, for the degree of Ph.D.

EXPERIMENTAL

All m.pts. are uncorrected.

The following were prepared by the method of van Erp[12]:—

2-Acetylamino-4-chloro-5-ethoxynitrobenzene
4-Chloro-3-ethoxynitrobenzene
2-Chloro-5-nitrophenol.

4-Acetylamino-2-acetoxybenzenesulphinic Acid (II). 4-Acetylamino-2-acetoxybenzenesulphonyl chloride (16g., Thorpe and Williams[13]) was stirred with a solution of sodium sulphite ($Na_2SO_3,7H_2O$, 28·6 g.) in water (60 ml.). The solution was made just alkaline and maintained so for 2 hours at room temperature. The brown sludge which formed was removed by filtration and the filtrate mechanically stirred and acidified by the slow addition of 60 per cent. sulphuric acid. The crude product (7·34 g.), m.pt. 143°C. (decomp.) was precipitated as a buff crystalline solid, which on recrystallisation from ethyl alcohol (charcoal) gave white prisms, m.pt. 145°C. (decomp.) of *4-acetylamino-2-acetoxybenzene-*

sulphinic acid. Found: C. 45·5; H, 4·3; N, 5·7; S, 13·0 per cent. Eq.wt. 259. $C_{10}H_{11}O_5NS$ requires C. 46·7; H, 4·3; N, 5·5; S, 12·5 per cent. Eq.wt. 257.

Sodium salt. Found: Na,8·4 per cent. $C_{10}H_{10}O_5NSNa$ requires Na.8·2 per cent.

Condensation of 4-chloro-3-ethoxynitrobenzene with sodium sulphide. Sodium sulphide ($Na_2S,9H_2O$, 1·29 g.) dissolved in water (3 ml.) and ethyl alcohol (97 per cent. 2 ml.) was slowly added to a mechanically stirred solution of 4-chloro-3-ethoxynitrobenzene (2 g.) in alcohol (10 ml.). The solution, which immediately became deep red, commenced to deposit a yellow solid after being refluxed for 10 minutes. Refluxing was continued for another 2 hours. The crystalline precipitate (0·2 g.) was recrystallised from glacial acetic acid forming yellow platelets, m.pt. 209°C. Found: C, 45·3; H, 3·8; N, 6·6; S, 22·8 per cent. Mol.wt. (Rast) 492. $C_{16}H_{16}O_6N_2S_3$ requires C, 44·9; H, 3·8; N, 6·5; S, 22·5 per cent. Mol. wt. 428.

The filtrate was evaporated to dryness, the product dissolved in benzene and then chromatographed on alumina. The first, yellow benzene eluate gave, on concentration and crystallisation, yellow rosettes of 2-chloro-5-nitrophenol (0·95 g.) m.pt. and mixed m.pt. 121° to 122°C. Found: C, 41·7; H 1·8; N, 7·9; Cl, 19·7 per cent. Calc. for $C_6H_4O_2NCl$ C, 41·5; H, 2·3; N, 8·1; Cl, 20·4 per cent.

Condensation of 2-acetylamino-4-chloro-5-ethoxynitrobenzene with sodium sulphide. Sodium sulphide ($Na_2S,9H_2O$, 2·4 g.), dissolved in water (2·5 ml.) was slowly added to a stirred solution of 2-acetylamino-4-chloro-5-ethoxynitrobenzene in alcohol (97 per cent.) (20·5 ml.). After refluxing for one hour and cooling, the red solid (1·57 g.), m.pt. 210° to 218°C., which deposited, was separated into two components (A and B) by boiling with a limited volume (60 ml.) of 80 per cent. acetic acid.

A. On cooling, the acetic acid solution yielded red crystals (0·525 g.) m.pt. 180° to 195°C., which, after recrystallisation from 80 per cent. acetic acid formed orange crystals of *5-acetylamino-5'-amino-2:2'-diethoxy-4:4'-dinitrodiphenyl sulphide,* m.pt. 209°C. Found: C, 49·0; H, 4·7; N, 12·8 per cent. $C_{18}H_{20}O_7N_4S$ requires C, 49·5; H, 4·6; N, 12·8 per cent.

B. The insoluble fraction was recrystallised from a large volume of 80 per cent. acetic acid yielding yellowish-orange needles of *5 : 5'-diacetylamino-2 : 2'-diethoxy-4 : 4'-dinitrodiphenyl sulphide,* m.pt. 240° to 242·5°C. Found: C, 49·4; H, 4·2; N, 11·5; S, 6·5 per cent. $C_{20}H_{22}O8N_4S$ requires C, 50·2; H, 4·6; N, 11·7; S, 6·7 per cent.

5:5'-Diamino-2:2'-diethoxy-4:4'-dinitrodiphenyl Sulphide.

Method A. 2-Acetylamino-4-chloro-5-ethoxynitrobenzene (8·1 g.) was suspended in ethyl alcohol (97 per cent., 160 ml.) and refluxed for 8 hours with a solution of sodium sulphide ($Na_2S,9H_2O$, 3·8 g.) in water (8 ml.). The crude mixture of monosulphides, which separated after removal of the alcohol, was heated with 0·66 N alcoholic potassium hydroxide for

1 hour under reflux. On distillation of the alcohol, a solid product was obtained which formed red needles of *5:5'-diamino-2:2'-diethoxy-4:4'-dinitrodiphenyl sulphide* (2·98 g.), m.pt. 196° to 198°C., from 60 per cent. acetic acid. Found: C, 48·9; H, 4·7; N, 13·90; S, 8·0 per cent. $C_{16}H_{18}O_6N_2S$ requires C, 48·6; H, 4·6; N, 14·2; S, 8·1 per cent.

Method B. Sodium sulphide ($Na_2S,9H_20$, 8·0 g.) dissolved in water (65 ml.) was neutralised by the careful addition of sodium bicarbonate (2·75 g.), the solution being cooled below 20°C. Sodium carbonate was precipitated by the slow addition of ice-cold methyl alcohol (65 ml.) and the filtrate (mainly sodium hydrosulphide) was refluxed with 2-acetylamino-4-chloro-5-ethoxynitrobenzene (8·36 g.) for 10 minutes, under nitrogen, to give a clear red solution. Sodium carbonate (1·73 g.) and 2-acetylamino-4-chloro-5-ethoxynitrobenzene (8·36 g.) were added and the solution stirred and heated under reflux for 2·5 hours. The crude product was treated as described under A above. Yield 5·68 g.

2 : 2'-Diethoxy-4 : 4'-dinitrodiphenyl disulphide. A solution of sodium hydrosulphide (3·06 g. $Na_2S.9H_2O$), prepared as described above, was refluxed for 4 hours with 4-chloro-3-ethoxynitrobenzene (2 g.). The solution was cooled and poured onto ice (100 g.) when a yellowish-green solid (0·37 g.) was precipitated, which, after recrystallisation from glacial acetic acid formed yellow platelets, m.pt. 209°C., identical with the trisulphide $C_{16}H_{16}O_6N_2S$ isolated previously. The filtrate was extracted with ether, the ether solution dried (anhydrous sodium sulphate) and the solvent removed. The yellow solid product was recrystallised from ethyl alcohol-acetone (2:1) forming bright yellow microcrystalline platelets (0·52 g.) m.pt. 152°C. of *2 : 2'-diethoxy-4 : 4'-dinitrodiphenyl disulphide.* Found: C, 48·5; H, 4·4; N, 6·9; S, 15·7 per cent. Mol.wt. (Rast) 348. $C_{16}H_{16}O_6N_2S_2$ requires C, 48·5; H, 4·1; N, 7·1; S, 16·2 per cent. Mol.wt. 394.

2 : 2'-Diethoxy-4 : 4'-dinitrodiphenyl Sulphide.

Method A. Nitrosylsulphuric acid (13 g.) prepared by the method of Gattermann and Liebermann[14] was slowly heated to 50°C. with 5:5'-diamino-2:2'-diethoxy-4:4'-dinitrodiphenyl sulphide (2 g.) in a 500 ml. flask and maintained at that temperature until the solid had completely dissolved (*ca.* 1/2 to 1 hour). The flask was cooled by immersion in ice and ice-cold ethyl alcohol (100 ml.) slowly added. After the effervescence had ceased, the flask was heated at 100°C. for 1/2 hour. Acetaldehyde and ethyl alcohol were removed by steam distillation and the black resinous mass which solidified on cooling was chromatographically adsorbed on alumina from acetone. Evaporation of the solvent from the first, reddish-yellow, acetone eluate yielded a brown solid, which, after recrystallisation from acetic acid (60 per cent.) (charcoal), formed yellow platelets (0·9 g.) of *2 : 2'-diethoxy-4 : 4'-dinitrodiphenyl sulphide,* m.pt. 115°C.

Method B. A solution of sodium hydrosulphide (≡14·2 g. $Na_2S.9H_2O$) was refluxed with 4-chloro-3-ethoxynitrobenzene (11·85 g.) for 5 minutes under nitrogen. Sodium carbonate (4·94 g.) and 4-chloro-3-ethoxynitro-

benzene (11·85 g.) was added and the solution stirred and heated under reflux for 16 hours. Methyl alcohol and unchanged 4-chloro-3-ethoxynitrobenzene (9·56 g.) were removed by steam distillation and the solid product extracted with boiling acetone. Evaporation of the acetone solution yielded a yellow solid, which, on recrystallisation from 60 per cent. acetic acid, gave crude 2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide (7·28 g.) in yellow needles, m.pt. 108° to 115°C. A pure specimen for analysis was obtained by successive recrystallisation from 80 per cent. acetic acid (twice) and from benzene and light petroleum (b.pt. 60° to 80°C.) followed by chromatographic adsorption (twice) on alumina and elution in benzene, the first yellow eluate being collected. Evaporation of the benzene solution and recrystallisation of the product from 40 per cent. acetic acid yielded bright yellow needles of pure 2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide, m.pt. 115°C. Found: C, 52·6; H, 4·5; N, 7·7; S, 8·1 per cent. $C_{16}H_{16}O_6N_2S$ requires C, 52·8; H, 4·4; N, 7·7; S, 8·8 per cent.

2 : 2′-Diethoxy-4 : 4′-dinitrodiphenyl Sulphone. Potassium permanganate (5·2 g.) in hot water (40 ml.) was added drop by drop to a stirred solution of 2:2′-diethoxy-4:4′-dinitrodiphenyl sulphide (8·18 g.) in boiling glacial acetic acid (120 ml.). Water (120 ml.) was added and the cooled mixture decolorised with sulphur dioxide. The pale yellow crystalline precipitate was redissolved in boiling glacial acetic acid (100 ml.) and reoxidised with potassium permanganate by the above procedure. Dilution and decolorisation of the cold solution yielded a white solid (4·06 g.), which, on recrystallisation from 60 per cent. acetic acid, formed shining white needles of *2 : 2′-diethoxy-4 : 4′-dinitrodiphenyl sulphone,* m.pt. 213°C. Found: C, 48·1; H, 4·2; N, 7·3; S, 8·3 per cent. $C_{16}H_{16}O_8N_2S$ requires C, 48·5; H, 4·0; N, 7·1; S, 8·1 per cent.

2 : 2′-Dihydroxy-4 : 4′-dinitrodiphenyl Sulphone. 2:2′-Diethoxy-4:4′-dinitrodiphenol sulphone (0·44 g.) was heated for 2 hours at 100°C. with concentrated sulphuric acid (1·2 g.). Upon cooling and pouring on to ice (20 g.), a white crystalline solid separated, which was recrystallised from 30 per cent. acetic acid (charcoal) to yield white needles of 2:2′-dihydroxy-4:4′-dinitrodiphenyl sulphone, m.pt. 233°C. Found: C, 42·0; H, 2·5; N, 8·2 per cent. $C_{12}H_8O_8N_2S$ requires C, 42·4; H. 2·4; N, 8·2 per cent.

4:4′-Diamino-2:2′-dihydroxydiphenyl Sulphone (I, R = H). 2:2′-Diethoxy-4:4′-dinitrodiphenyl sulphone (3·38 g.) was refluxed with constant-boiling hydriodic acid (100 ml.) at 140°C. for 24 hours, the solution being stirred continuously. The cooled solution was diluted with water (100 ml.), decolorised with sulphur dioxide and neutralised by the addition of sodium carbonate, when the product, a white solid (2·1 g.), was precipitated. The aqueous filtrate was extracted with ether, the ethereal solution washed with water, dried (anhydrous sodium sulphate) and evaporated to yield a further small quantity of product (0·1 g.). The combined products were crystallised from hot water forming small needles (1·7 g.), off-white in colour, of *4 : 4′-diamino-2 : 2′-dihydroxydiphenyl sulphone,* m.pt. 179° to 181°C. Found: C, 51·6; H, 4·4; N, 9·9; S, 11·1

per cent. $C_{12}H_{12}O_4N_2S$ requires C, 51·4; H, 4·3; N, 10·0; S, 11·4 per cent.

Hydrochloride ($C_{12}H_{12}O_4N_2S$),2HCl found white prisms, m.pt. 207°C., from alcohol-ether. Found: C, 40·5; H, 4·5; N, 8·2 per cent. Eq. wt. 355. $C_{12}H_{14}O_4Cl_2N_2S$ requires C, 40·8; H, 4·0; N, 7·9 per cent. Eq. wt. 353.

4 : 4'-Diamino-2 : 2'-diethoxydiphenyl Sulphone (I, R = OC_2H_5), 2:2'-Diethoxy-4:4'-dinitrodiphenyl sulphone (3·38 g.) was refluxed with constant boiling hydriodic acid (100 ml.) for 7 hours at 140°C., the solution being stirred continuously. The cooled solution was diluted with water (100 ml.) and decolourised with sulphur dioxide. The cream-coloured precipitate of *4 : 4'-diamino-2 : 2'diethoxydiphenyl sulphone* was crystallised from 30 per cent. acetic acid, in small white needles, m.pt. 269°C. Found: C, 57·1; H, 5·9; N, 8·5 per cent. $C_{16}H_{20}O_4N_2S$ requires C, 57·1; H, 5·9; N, 8·3 per cent.

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