# A Heterocyclic-Substituted Sulfone with Activity against *Mycobacterium tuberculosis*. (18948)

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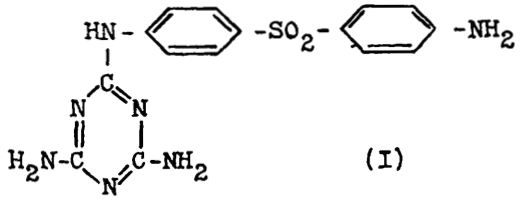
N-substituted derivatives of 4,4'-diaminodiphenyl sulfone are thought to owe their *in vivo* antibacterial activity to the catabolic removal of the substituent groups, since the N-substituted compounds generally have low *in vitro* activities and lead to the presence of diazotizable amine in the blood after administration to animals. High potency is believed to be encountered only when both amino groups of the parent sulfone can be readily liberated. It has been speculated that in the case of N-heterocyclic derivatives catabolic cleavage cannot occur and that such compounds are inactive *per se*(1).

An N-heterocyclic-substituted sulfone (I)



(I)

has now been synthesized, which in contrast to previously reported compounds of its type, is highly active *in vitro* against *Mycobacterium tuberculosis* and *Escherichia coli*.

*Synthesis.* 4,4'-Diaminodiphenyl sulfone (24.8 g) and an equimolar amount of 2-chloro-4,6-diamino-s-triazine (14.5 g) are permitted to react over a period of 10 minutes in 1 liter boiling 0.35 N hydrochloric acid, according to the method described by Banks and co-workers(2). On cooling to 13°C, a white precipitate of the very sparingly soluble dihydrochloride of bis[p-(2,4-diamino-s-triazinyl-6)-aminophenyl] sulfone is obtained and removed by filtration. The clear filtrate on keeping at 5°C for 72 hours deposits the dihydrochloride of the desired product, p-(2,4-diamino-s-triazinyl-6)-aminophenyl p'-aminophenyl sulfone (I), which is purified by recrystallization from dilute hydrochloric acid. The yield is 13.4 g (31%).

*Analysis:*

| | |
|---|---|
| Calculated for $C_{15}H_{15}O_2N_7S$, 2 HCl: | N 22.8 |
| Found | N 22.8 |

This hydrochloride is converted to the corresponding free base with sodium carbonate in 88% yield. The base is a white powder which is only slightly soluble in water, sparingly soluble in dilute hydrochloric acid and soluble in propylene glycol. The product may be diazotized and coupled with phenols. When heated rapidly, the base melts at 240-245°C.

*Analysis:*

| | |
|---|---|
| Calculated for $C_{15}H_{15}O_2N_7S$: | N 27.4, S 9.0 |
| Found | N 27.3, S 8.8 |

*Microbiological tests.* The H37Ra strain of *Mycobacterium tuberculosis* and the B strain of *Escherichia coli* were used. The test media for the two organisms were slight modifications of those described by Dubos and Davis(3) and Wood(4) respectively.

TABLE I. Effect of p-(2,4-diamino-s-triazinyl-6)-aminophenyl p'-Aminophenyl Sulfone (DTS) and 4,4'-Diaminodiphenyl Sulfone (DADS) on Growth* of *Mycobacterium tuberculosis* and *Escherichia coli*.

| Molarity of compound in test medium | *Mycobacterium tuberculosis* | | *Escherichia coli* | |
|---|---|---|---|---|
| | DTS | DADS | DTS | DADS |
| $5 \times 10^{-4}$ | 0 | 0 | 0 | 0 |
| $1.2 \times 10^{-4}$ | 0 | 1 | 0 | 0 |
| $3.1 \times 10^{-5}$ | 2 | 2 | 1 | 0 |
| $7.8 \times 10^{-6}$ | 3 | 3 | 2 | 2 |
| 0 (controls) | 4 | | 3 | |

* Amount of growth: 0, none; 1, slight; 2, moderate; 3, heavy; 4, very heavy. Incubation period: 2 weeks for *M. tuberculosis*, 2 days for *E. coli*, both at 37°C.

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2. Banks, C. K., Gruhzit, O. M., Tillitson, E. W., and Controulis, J., *J. Am. Chem. Soc.*, 1944, v66, 1771.

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As shown in Table I, the *in vitro* activity of p-(2,4-diamino-s-triazinyl-6)-aminophenyl p'-aminophenyl sulfone is of the same order as that of 4,4'-diaminodiphenyl sulfone. Since p-aminobenzoic acid ($5 \times 10^{-5}$ molar) was found to reverse completely the antibacterial action of the heterocyclic sulfone ($5 \times 10^{-4}$ molar), the latter presumably acts through a mechanism similar to that reported for 4,4'-diaminodiphenyl sulfone(4).

*Summary.* p-(2,4-Diamino-s-triazinyl-6)-aminophenyl p'-aminophenyl sulfone has been synthesized and shown to inhibit the growth of *M. tuberculosis* and *E. coli in vitro* at a concentration of $1.2 \times 10^{-4}$ moles per liter, the inhibition being reversed by p-aminobenzoic acid.

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## Production of Acrodynia in Mice Fed Diets Low in Pyridoxine.* (18949)

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During the course of an investigation on the influence of vitamins of the B complex on the induction of tumors in mice, a fortuitous observation of an acrodynia was made. This condition was cured by the injection of pyridoxine(1). However the diet fed to these mice was low in several of the B vitamins.

The present report describes further studies on the production of acrodynia in mice. The results are of interest because it has been reported that the pyridoxine deficient mouse, in contrast to the rat, does not develop acrodynia(2-5) except with the aid of desoxypyridoxine(6).

*Methods and results.* Since the original observation of acrodynia was made on female albino mice that were 2-3 months of age at the start of the experiment(1), similar mice were used in the present study. Weanling male mice, capable of rapid growth on a complete diet, were also used. These mice were obtained from a commercial source,† and upon arrival were immediately placed in screen bottom cages and were given water and the experimental diets *ad libitum*.

The following 4 diets were used: *Diet A*, a semi-purified diet plus pyridoxine: Glucose monohydrate (Cerelose), 78; ordinary commercial casein, 15; corn oil (Mazola), 2; liver concentrate (Wilson Laboratories 1-20 liver powder), 1; salt mixture (modified Phillips-Hart), 4. This was supplemented with the following vitamins per kg of diet: pyridoxine-HCl, 30 mg; thiamine-HCl, 1.55 mg; biotin, 0.1 mg; inositol, 20 mg; para aminobenzoic acid, 20 mg; choline-Cl, 20 mg; and 6 drops of halibut liver oil premixed with the corn oil. *Diet B*, a semi-purified diet without added pyridoxine: This diet was the same as diet A except that no pyridoxine was added. *Diet C*, a purified diet with low pyridoxine: This diet differed from diet A as follows. The crude casein was replaced by purified casein (Smaco) and the liver concentrate was replaced by an equivalent weight of glucose. The following vitamins were added per kg: pyridoxine-HCl,

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† Arthur Sutter, Springfield, Mo. The mice were described as "Rockland Strain."