indoloquinone (**2**) and 180 mg (0.78 mmole) of diphenylcarbamoyl chloride in 4 ml of pyridine was heated on the steam bath for about 16 hr. H_2O was added, and the mixture was extracted with CH_2Cl_2; the combined extracts were washed with saline, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a magnesia–silica gel absorbent. The material eluted by ether was recrystallized from ether–petroleum ether to give 57 mg of crystals, mp 159–161°. *Anal.* ($C_{27}H_{26}N_2O_5$) N. Elution of the column with $CHCl_3$ gave 65 mg of **2**.

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Adamantane Derivatives of *p*-Aminobenzamide and Sulfanilamide[1]

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A new antagonist of *p*-aminobenzoic acid was found to differ from sulfonamides in its mode of action. Inhibition of the growth of sensitive microorganisms by N^1-adamantyl-*p*-aminobenzamide was prevented competitively by *p*-aminobenzoic acid. When combined with sulfanilamide, synergistic inhibition occurred and this compound inhibited the growth of a strain of *Escherichia coli* resistant to sulfanilamide. Adamantyl derivatives of various drugs and antimetabolites have been synthesized in other laboratories.[2-5] Some of these compounds had remarkable pharmacological activity. In the course of the synthesis of folate antagonists, N^1-adamantyl-*p*-aminobenzamide (**3a**) and N^1-adamantylsulfanilamide (**6**) were synthesized. For comparison, the N^1-cyclohexyl-*p*-aminobenzamide (**3b**)[6] and N^1-phenyl-*p*-aminobenzamide (**3c**)[7] were also prepared. The synthesis of these compounds and preliminary evaluation of their biological activity are reported in this paper.

The general route of the synthesis of *p*-aminobenzamide derivatives is presented in Chart I. The synthetic sequence for the preparation of N^1-adamantylsulfanilamide is shown in Chart II.

The biological activity of **3a** was tested in three organisms: *Streptococcus faecalis*, *Escherichia coli* K12, and *Saccharomyces carlsbergensis*. The details concerning these tests will be published elsewhere. The concentration of **3a** required for 50% inhibition of growth of *E. coli* was 2×10^{-4} *M* and of *S. carlsbergensis*, 3×10^{-5} *M*. The concentration of sulfanilamide required for 50% inhibition of growth was

CHART I

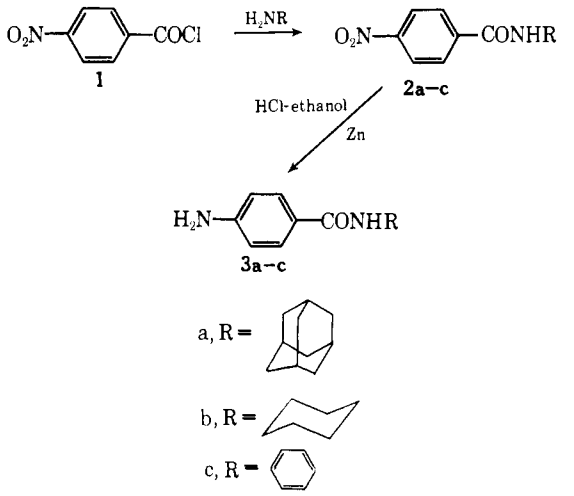
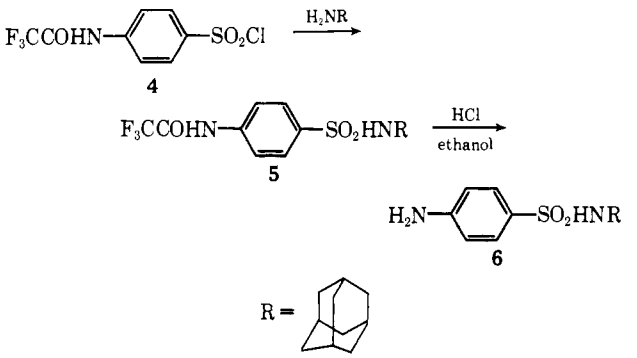


CHART II



4×10^{-4} and 5×10^{-4} *M*, respectively. *S. faecalis* was insensitive to **3a**. *E. coli* was not inhibited by **3b**, **3c**, and **6**. The growth inhibition caused by **3a** was reversed competitively by *p*-aminobenzoic acid and noncompetitively by dihydrofolic acid and folinic acid. The growth inhibition caused by sulfanilamide was reversed only by *p*-aminobenzoic acid. There was a strongly synergistic inhibition of the growth of *E. coli* when sulfanilamide and **3a** were used in combination. A strain of *E. coli* made resistant to sulfanilamide was as sensitive to **3a** as was the parent strain. It is evident from these results that **3a**, like sulfanilamide, is an antagonist of *p*-aminobenzoic acid. The metabolic pathway for the utilization of *p*-aminobenzoic acid in *E. coli*[8] may be represented by the following steps: 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine + *p*-aminobenzoic acid → dihydropteroic acid → dihydrofolic acid → tetrahydrofolic acid → N^5-formyltetrahydrofolic acid.

Sulfonamides inhibit the first step in the metabolic sequence by competing with *p*-aminobenzoic acid. Further, there is strong evidence that sulfanilic acid is coupled with the pteridine to form an analog of dihydropteroate.[9] This compound seems to inhibit the synthesis of dihydropteroate irreversibly. The growth inhibition caused by **3a** and by sulfanilamide is competitively reversed by *p*-aminobenzoate. This indicates that both compounds interfere with the utilization

(1) This investigation was supported in part by a grant (CA-02906) from the National Cancer Institute of the U. S. Public Health Service.

(2) K. Gerzon, E. V. Krumkalus, R. L. Brindle, F. J. Marshall, and M. A. Root, *J. Med. Chem.*, **6**, 760 (1963).

(3) R. T. Rapala, R. J. Kraay, and K. Gerzon, *ibid.*, **8**, 580 (1965).

(4) K. Gerzon and D. Kau, *ibid.*, **10**, 189 (1967).

(5) J. R. Geigy, Belgian Patent 629,371 (1963); *Chem. Abstr.*, **60**, 9284*b* (1964).

(6) Kuhlman, French Patent 820,736 (Nov. 17, 1937); *Chem. Abstr.*, **32**, 3628 (1938).

(7) G. Lockemann, *Ber.*, **75**, 1911 (1942).

(8) G. M. Brown, R. A. Weisman, and D. A. Molnar, *J. Biol. Chem.*, **236**, 2534 (1961).

(9) G. M. Brown, *ibid.*, **237**, 536 (1962).

of *p*-aminobenzoic acid for the synthesis of dihydropteroate. Dihydrofolate and folinic acid reverse the inhibition caused by **3a** but not by sulfanilamide, indicating that sulfanilamide or its metabolites exert additional inhibition at a metabolic point beyond the reduction of dihydrofolate to tetrahydrofolate. This does not appear to be the case with **3a**. Whether **3a** is actually coupled with the pteridine in a manner similar to the sulfonamides is under investigation.

The biological activity of the adamantyl derivative of *p*-aminobenzamide as compared to the inactivity of the cyclohexyl and phenyl derivatives raises the question whether the latter residues prevent the binding of *p*-aminobenzamide moiety to the active site of dihydropteroate synthetase, whereas the adamantyl group enhances it. Alternatively, differences in uptake of the compounds into the cell might account for the difference in their activity. The observation that joining of the adamantyl group to the active sulfanilamide produces an inactive compound does not aid in making a choice between these alternatives. Studies with isolated enzymes designated to pinpoint accurately the mode of action of **3a** in comparison with sulfanamides are now being conducted in our laboratory.

Experimental Section[10]

N¹-Adamantyl-*p*-aminobenzamide (3a).—Adamantylamine hydrochloride (5 g) was dissolved in 55 ml of H_2O. A solution of 1.4 g of NaOH in 55 ml of H_2O was added. The precipitated free base of adamantylamine was extracted with 125 ml of Et_2O. Et_2O was dried for at least 1 hr over KOH and then 4.95 g of *p*-nitrobenzoyl chloride (**1**) was added. The precipitate was filtered off and kept on the filter until all Et_2O was removed. This product was stirred for 15 min with H_2O, filtered, washed with H_2O, and dried at room temperature under vacuum (**2a**); yield 6 g (79%), mp 172–175°. N¹-Adamantyl-*p*-nitrobenzamide (**2a**) (6 g) was dissolved in a mixture of 196 ml of EtOH and 92 ml of 2 *N* HCl. This solution was stirred for 30 min with 6.5 g of Zn dust. The Zn was filtered off and the solution was poured into 1300 ml of H_2O which was then adjusted to pH 4. After standing overnight at 5°, the white crystals were collected by filtration, washed with H_2O, and dried at room temperature under vacuum; yield 3.6 g. The crude material was dissolved in 590 ml of 1 *N* HCl, the solution was clarified with Darco G60 and the pH was adjusted to 10; yield 2.8 g (52%), mp 169–171°. *Anal.* ($C_{17}H_{22}N_2O$) C, H, N.

N¹-Cyclohexyl-*p*-aminobenzamide (3b).—A mixture of *p*-nitrobenzoyl chloride (**1**) (1.8 g) and cyclohexylamine (1.7 g) was dissolved in 20 ml of absolute Et_2O. The precipitate was handled in exactly the same way as described for corresponding adamantylamine derivative; yield 1.84 g (76%), mp 200–203°. A sample of **2b** (1.8 g) was stirred with 60 ml of EtOH, 28 ml of 2 *N* HCl, and 2 g of Zn dust for 2 hr. After filtering, the solution was poured into 400 ml of H_2O and the pH of this mixture was adjusted to 4.0. The precipitate was then handled as described for **3a**; yield 550 mg (35%), mp 176–178°. *Anal.* ($C_{13}H_{18}N_2O$) C, H, N.

N¹-Adamantylsulfanilamide (6).—Trifluoroacetylsulfanilyl chloride (**4**) was prepared by a modification of the procedure by Schroeter.[11] The modification involved the use of trifluoroacetic anhydride (in place of Ac_2O) which was added in small portions (15 ml followed by two times 10 ml) to 10.65 g of solid sodium sulfanilate, yield 10.3 g, mp 144–149°. N-Trifluoroacetylsulfanily chloride (**4**) (5 g) and adamantylamine (from 5 g of adamantylamine hydrochloride) were refluxed for 60 min in 200 ml of EtOH. This solution was added to 2.5 l. of 1.0 *N* HCl. The precipitate (**5**) was filtered, washed with H_2O, and dried under vacuum at 60°; yield 3.7 g (47%), mp 220–225°. A sample of N¹-adamantyltrifluoroacetylsulfanilamide (**5**) (3.5 g) was refluxed for 2 hr with 350 ml of EtOH and 87.5 ml of 5 *N* HCl. The solution was evaporated to dryness under reduced pressure at 50° over KOH to trap HCl. The residue was taken up in 20 ml of H_2O, filtered, washed with H_2O, and dried under vacuum at room temperature over KOH; yield of the crude product was 2.31 g, mp 167–172°. For purification the material was dissolved in 40 ml of EtOH, the solution was clarified with Darco G60, and filtered. The filtrate was added to 400 ml of 0.05 *M* HCl. The initially formed fine precipitate was filtered off and discarded. After standing overnight in the refrigerator, the product crystallized. It was dried at 50° under vacuum; yield 0.4 g (15%), mp 179–180°.

Anal. ($C_{16}H_{22}N_2O_2S$) C, H, N, S.

(10) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The melting points reported are uncorrected. The melting points of *p*-aminobenzoic acid derivatives were taken in a Thiele tube; those of sulfanilic acid derivatives in Fisher-Johns melting point apparatus.

(11) G. Schroeter, *Ch. m. Ber.*, **39**, 1559 (1906).

Antimalarial Agents. III. Bis[*p*-(3-sydnonyl)phenyl] Sulfone

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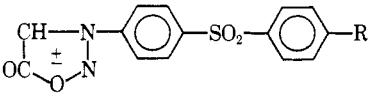
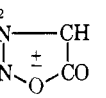
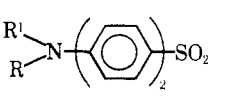
3-[*p*-(4-Aminophenylsulfonyl)phenyl]sydnone (Ia)[1] was found to be curative at the rate of 80 mg/kg of mouse infected with *Plasmodium berghei* and was devoid of any toxic effects at 640 mg/kg. Since Ia is the monosydnone derived from the antimalarial agent bis(*p*-aminophenyl) sulfone (DDS, IIa), it was of considerable importance to test the bissydnone of DDS. The synthesis and properties of this bissydnone, *i.e.*, bis[*p*-(3-sydnonyl)phenyl] sulfone (Ib), are reported here. Bis(*p*-aminophenyl) sulfone (IIa) was cyanomethylated on both nitrogens with paraformaldehyde and KCN in AcOH to give an excellent yield of bis{*p*-[*N*-(cyanomethyl)amino]phenyl} sulfone (IIb).



I

Ia, R = NH_2

b, R = 



II

IIa, R = R¹ = H

b, R = H; R¹ = CH_2CN

c, R = H; R¹ = CH_2COOH

d, R = NO; R¹ = CH_2COOH

e, R = NO; R¹ = $CH_2C(O)NHCH_2Ph$

f, R = H; R¹ = NH_2

The latter was hydrolyzed to bis{*p*-[*N*-(carboxymethyl)amino]phenyl} sulfone (IIc) by heating with aqueous KOH. It was nitrosated and the crude nitroso compound IId was treated with trifluoroacetic anhy-

(1) I. C. Popoff and G. H. Singhal, *J. Med. Chem.*, **11**, 631 (1968).