more than one motor end-plate, vary between 20 and 150μ; there is not a clearly identifiable relationship between the sizes and the number of motor end-plates in each fibre.

(4) The distance between one motor end-plate and the next within a single muscle fibre ranges from 20–30μ to more than 1,000μ (in some cases).

(5) About 5 per cent of the motor end-plates appear to have a small accessory plate (Fig. 1, *B*): a similar finding has been reported for the lateral rectus muscle of the orbit and interpreted as the ending of a sympathetic or parasympathetic nerve fibre[2].

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PHARMACOLOGY

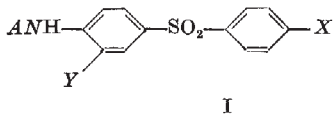
Repository Antimalarial Drugs: *N,N′*-Diacetyl-4,4′-diaminodiphenylsulphone and Related 4-Acylaminodiphenylsulphones

A sustained effort to develop antimalarial substances with prolonged action was initiated in these laboratories more than seven years ago. Recently, we reported the synthesis of *cyclo*guanil pamoate ('Camolar^R', *CI*-501) (refs. 1 and 2), a pamoic acid salt of 4,6-diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (DHT), and various other dihydrotriazine[1,3] and pyrimethamine[3,4] salts that exhibit remarkable repository antimalarial properties[2,4,5]. A single intramuscular dose of *cyclo*guanil pamoate has an unusual capacity to protect man for many months against challenges with susceptible strains of *Plasmodium vivax* and *P. falciparum*[6]. *Cyclo*guanil pamoate has so far not shown a liability to induce rapid resistance during numerous observations on *P. cynomolgi* in monkeys[2,5], although parasites known to be resistant to proguanil, DHT, or pyrimethamine are less susceptible to it[7,8]. A systematic search has been continued for a well-tolerated repository preparation that would destroy both normal and DHT-resistant parasites and simultaneously block the emergence of resistance *denovo*. Efforts were directed toward the synthesis of long-acting antimalarials possessing a different mode of action from DHT or pyrimethamine, both of which apparently inhibit the conversion of folic acid to folinic acid[9].

Although it has been known for several decades that various sulphones and sulphonamides exhibit antimalarial activity[9–11], none has been used extensively in the treatment of human malarias. This stemmed from the knowledge that other more active drugs were available, from apprehension about the toxicity of certain compounds, and from recognition that frequent dosing is necessary. The sulphones and sulphonamides reportedly act at a different site than DHT or pyrimethamine, presumably by preventing the incorporation of *p*-aminobenzoic acid into folic acid[9,11–14]. The synthesis of long-acting sulphone and sulphonamide derivatives was undertaken to provide antimalarial substances that, in combination with *cyclo*guanil pamoate or related compounds[1,3], might enable a sequential block in the metabolic synthesis of nucleotides and afford broader repository action against drug-resistant lines than either drug alone.

Plasmodia can acquire resistance to the sulphones and sulphonamides as well as to DHT and pyrimethamine[8,9,13,14]. However, in the course of this work it became apparent that certain lines of *P. berghei*[8], *P. cynomolgi*[13] and *P. gallinaceum*[14], made resistant to 4,4′-diaminodiphenylsulphone (DDS) or to DHT hydrochloride or pyrimethamine, were still susceptible to the heterologous drug, with only a low order of cross-resistance. Further, a 1 : 1 mixture of DHT hydrochloride and DDS proved highly effective against the parent, DHT-resistant, and DDS-resistant lines of *P. berghei*, and the rate of emergence of resistance in the parent strain was significantly less with the mixture than with either drug alone[8]. These observations reinforced interest in a combination of the two drugs in repository form.

During this investigation, several hundred sulphones and sulphonamides were supplied to Dr. P. E. Thompson *et al.* of these laboratories for antimalarial evaluation. Among them, various 4-acylaminodiphenylsulphones (I)



I

where X =
(*a*) —NO_2
(*b*) —NH_2
(*c*) —$NHCOCH_3$

exhibited promising repository activity against *P. berghei* infections in the mouse. In formula I, *A* represents an acyl group containing from 2 to 12 carbon atoms inclusive; *X* is an amino, acetamido, or nitro group; and *Y* is hydrogen or methyl. As in previous work[2], drugs were given subcutaneously to mice as either aqueous or lipid suspensions, and repository activity was assessed on the basis of the period of protection against patent infections afforded by a single dose prior to challenge with blood stages of *P. berghei*. Results indicated that a 400-mg/kg dose of representative acylaminodiphenylsulphones (I*a–c*) prevented or greatly suppressed patent infections for periods ranging from 4 to 14 weeks. All preparations were well-tolerated locally and systemically by gross examination.

The 4-acylamino-4′-nitrodiphenylsulphones (I*a*) were prepared by acylation of a 4-amino-4′-nitrodiphenylsulphone with the appropriate acid chloride in an acetone–pyridine mixture. Catalytic hydrogenation of the nitro compounds (I*a*) in dimethylformamide over Raney nickel gave the corresponding *N*-acyl-4,4′-diaminodiphenylsulphones (I*b*), which were afterwards converted to the unsymmetrical *N,N′*-diacyl-4,4′-diaminodiphenylsulphones (I*c*) utilizing the appropriate acid chloride or anhydride[15]. Symmetrical *N,N′*-diacyl-4,4′-diaminodiphenylsulphones were prepared by acylation of DDS with an excess of the acid chloride or anhydride.

N,N′-Diacetyl-4,4′-diaminodiphenylsulphone (DADDS) was selected for expanded studies. The compound is a colourless crystalline solid, m.p. 289°–292° C. A representative sample exhibited the following absorption spectrum: λ_{max} in MeOH (ε) 256 (25,500), 284 (36,200) mμ. The solubility of DADDS in water or *p*H 7 0·1 M phosphate buffer is 0·003 mg/ml. (0·0003 per cent), in 40 per cent benzyl benzoate–60 per cent castor oil 0·026 mg/ml. (0·0026 per cent). Suspensions of DADDS in the benzyl benzoate–castor oil vehicle can be sterilized in an autoclave without detectable change.

Against trophozoite-induced *P. berghei* infections in the mouse, a single 100–400-mg/kg dose of DADDS almost invariably prevented or strongly suppressed patent infections from up to 6–14 weeks. In rhesus monkeys, a 50-mg/kg dose of DADDS prevented patent *P. cynomolgi* infections for 63–268 (average 158) days and greatly suppressed the parasitaemia for many weeks longer. Therapeutic tests against established patent infections in monkeys showed that DADDS suppressed the parasitaemia slowly. A comparison of DADDS, *cyclo*guanil pamoate, and a 1 : 1 mixture against lines of *P. berghei*

highly resistant to either DDS or to DHT demonstrated that the mixture had broader repository action against the drug-resistant lines than either drug alone. These results[7] encourage further evaluation of the DADDS-*cyclo*guanil pamoate mixture in connexion with the prevention and eradication of malaria.

The prolonged antimalarial effects and low toxicity of DADDS concur with chemical evidence of slow removal from the injection site and very low sulphone blood levels[7]. It is logical to assume that the antimalarial action of DADDS is due to the liberation of *N*-acetyl-4,4′-diaminodiphenylsulphone (MADDS), DDS or close relatives thereof inasmuch as DADDS has diminished activity against DDS-resistant *P. berghei*[7]. Presumably deacetylases present in host tissues are involved in these transformations.

Repository activity is abolished or drastically reduced when DADDS is modified by: (1) replacement of the sulphone moiety by a sulphinyl, thio, oxalyl or 2,2,2-trichloroethylidene linkage; (2) introduction of a chlorine atom adjacent to one acetamide function; (3) introduction of hydroxyl groups adjacent to both acetamide functions; (4) alkylation of one acetamide group; (5) replacement of both acetamide groups with amide functions containing more than two carbon atoms. A group of *N*,*N*″-alkylenedicarbonyl*bis*[*N*′-acetyl-4,4′-diaminodiphenylsulphones] was likewise devoid of appreciable repository action. Among congeners of thiazolsulphone, sulphadiazine and sulphamethoxypyridazine, acyl derivatives such as N^4,$N^{2\prime}$-diacetyl-4-aminophenyl-2′-aminothiazolyl-5′-sulphone, N^1,N^4-diacetyl-N^1-2-pyrimidinylsulphanilamide, and N^1,N^4-diacetyl-N^1-(6-methoxy-3-pyridazinyl)sulphanilamide lacked substantial repository action. Solubility studies with representative 4-acylaminodiphenylsulphones indicate that the length of repository action of a given amide cannot be reliably predicted on the basis of aqueous solubility.

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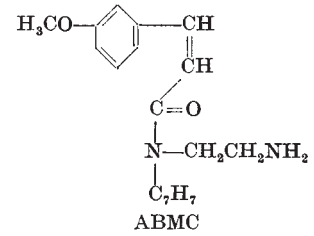
An Easily Obtainable Antiserotonin which has Little Effect on the Brain

Although several highly potent antimetabolites of serotonin, for example, hydrazindole, BAS and others, have been known for several years[1,2], they have been used very little, either in pharmacological research or in clinical medicine. This has been primarily because the compounds are difficult to synthesize. About the only antiserotonins now in use are lysergic acid diethylamide (LSD) and the related 1-methyl butanolamide (UML). These latter, although they have severe disadvantages (they affect the mind) when compared with the synthetic antiserotonins mentioned here, are often used in preference to them because they are commercially available. Even LSD and UML, however, are expensive.

Recently, Dombro and Woolley[3] have described a substituted cinnamamide which was highly potent as a specific antagonist of serotonin, and which was very easily prepared. This compound, namely, *N*-dimethylaminoethyl-*N*-benzyl-*m*-methoxycinnamamide, because it was a tertiary amine, was able to penetrate into the central nervous system and to exert pharmacological effects there. It also acted on peripheral nerves, as shown by its effects as a local anaesthetic.

Although antimetabolites of serotonin which act on the nervous system are desirable for some uses (cf. ref. 2), there are other instances in which a drug which is confined to the peripheral smooth musculature is required. For example, in the treatment of hypertension[4] or of carcinoid, an agent confined to the peripheral organs seems to be necessary. We have therefore attempted to alter the chemical structure of the previously mentioned cinnamamide in such a way as to retain its high potency and ease of synthesis, but to render it poorly capable of actions on the central nervous system. These goals were accomplished by synthesis of the corresponding primary amine, namely, *N*-aminoethyl-*N*-benzyl-*m*-methoxycinnamamide or ABMC. The reason why such a manoeuvre would be expected to succeed has been discussed by Dombro and Woolley[2,3] and is that primary aliphatic amines, in contrast to tertiary amines, do not readily pass into the brain or even into peripheral nerves.



ABMC

The new compound, ABMC, was synthesized easily in the following way. Commercially available *m*-methoxycinnamic acid was converted to its *N*-benzylamide by way of the acid chloride[3]. The sodium salt of this amide was prepared, either in dimethylformamide (DMF) or in dimethylsulphoxide (DMSO) solution, by addition of two equivalents of sodium hydride. Aminoethylchloride hydrochloride (1·1 equivalents) was then added slowly, dissolved either in DMF or in DMSO. When DMF was used as solvent the reaction mixture was heated to 120° for 20 h, but when DMSO was used the reaction was allowed to proceed at room temperature. The reaction mixture was diluted with ethyl acetate, and the basic compounds were extracted into aqueous hydrochloric acid. The free base was then liberated with alkali and extracted into ethyl acetate. The solvent was removed from the extract, and the desired compound was obtained as a crystalline bioxalate by addition of oxalic acid to an acetone solution. For the biological work, the oxalate was converted to the hydrochloride. Both the bioxalate and the hydrochloride crystallized from ethanol–ether or from acetone. The free base was amorphous. The bioxalate melted at 188°–190°, and contained: C, 63·05; H, 6·04; and N, 7·04 (theory: C, 62·99; H, 6·04; N, 7·00). The hydrochloride melted at 191°–193°.

Several other ways of synthesizing the new compound were explored, but all of them failed. Thus, when one amino group of ethylenediamine was protected by formyla-