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EFFECT OF RING-SUBSTITUENTS ON THE ACTIVITY OF DIPHENOXYALKANES AGAINST *SCHISTOSOMA MANSONI*

BY

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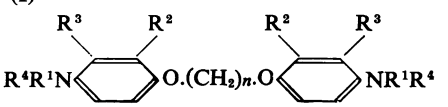
The discovery by Raison and Standen (1955) of the schistosomicidal activity of symmetrical diaminodiphenoxyalkanes led us to examine similar compounds in which the benzene rings have been further substituted as depicted in the general formula (I)



(I)

where alternatively R^2 or $R^3 = CH_3$, OCH_3, Cl, OH, NH_2 ; *n*, R^1 and R^4 have also been subjected to considerable variation. A few compounds with two methyl substituents in each ring and some analogous naphthalene compounds have also been studied.

The literature does not offer clear guidance on what might be expected from such modifications. According to Northey (1948) uniformly low activities have been encountered in derivatives of the sulphonamides in which the benzene nucleus has been substituted, and various compounds with alkyl, halogen, hydroxyl and nitro- substituents have been reported as inactive ; but Quan, Daniels, and Meyer (1954) have found that three derivatives of sulphathiazole of this type with methyl or chloro-substituents are at least as active against bacteria as sulphathiazole itself, though they are somewhat more toxic. The introduction of alkyl groups into an N^4-heterocyclic nucleus of such sulphanilamide derivatives as sulphadiazine modifies, but never destroys, the activity. Recently the effect of substitution on the antitubercular activity of *iso*nicotinic hydrazide has been investigated in detail (Isler, Gutman, Straub, Fust, Bohni, and Studer, 1955) ; all compounds with functional substituents are practically inactive, and alkyl substituents markedly reduce activity except with 2-methyl*iso*nicotinic hydrazide, which has similar activity to the unsubstituted compound. With the diamidines, a series formally analogous to ours, introduction of a hydroxyl group into stilbamidine has afforded hydroxystilbamidine, a versatile drug, free of the toxicity that has precluded the therapeutic use of stilbamidine itself ; with other drugs of this series, substituents have modified, without usually destroying, activity (Wien, 1946). The chemical effect of a substituent may be to block the metabolic course followed by the unsubstituted compound, or itself to serve as a point of metabolic attack—as has been postulated by Walker (1947) for the methyl group of inactive quinaldine analogues of certain quinoline antimalarial drugs —or to activate or deactivate positions of the benzene ring with consequential effects on metabolism. It is probably for the last-named reason that the 6-methoxyl group of the 8-aminoquinoline antimalarial drugs is essential to activity, facilitating their conversion into 5:6-quinones (Drake and Pratt, 1951 ; Taylor, Josephson, Greenberg, and Coatney, 1952).

In our series, introduction of any of the groups named above has always resulted in a big fall of activity, more often than not in complete loss, at our chosen maximum dose of 200 mg./kg. The effect on toxicity has not been studied in detail, but there were clear indications that several of the substituted compounds, particularly those of greater chain-length, are more acutely toxic than the unsubstituted analogues.

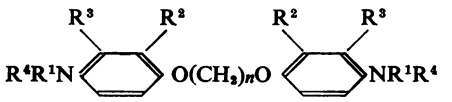
MATERIALS AND METHODS

The compounds described in this paper were given orally to mice infected with an Egyptian strain of *Schistosoma mansoni*. Treatment was started 63 days after the animals had been exposed to infection, and dosage was carried out twice daily for 5 consecutive days. Autopsy was made 7 days after completion of treatment and the result assessed as described by Raison and Standen (1955).

RESULTS

Methyl Substituents.—Whereas the primary amines (I ; $R^1 = R^4 = H$) of the unsubstituted

TABLE I



Compound Number	n	R¹	R⁴	R²	R³	Unit Dose (mg./kg.)	Worms Killed %
A. Methyl Substituents: Primary							
170C53	3	H	H	H	CH_3	200	0
171C53	4	H	H	H	CH_3	200	Toxic
186C53	5	H	H	H	CH_3	200	64
						50	0
187C53	6	H	H	H	CH_3	200	67
						50	0
	7–10	H	H	H	CH_3	200	0
142C53	3	H	H	CH_3	H	200	60
						50	0
117C53	4	H	H	CH_3	H	200	79
						50	1
143C53	5	H	H	CH_3	H	200	2
144C53	6	H	H	CH_3	H	200	0
	7–10	H	H	CH_3	H	50	Toxic
B. Methyl Substituents: Secondary							
28C53	3	CH_3	H	CH_3	H	200	67
						50	0
29C53	4	CH_3	H	CH_3	H	200	68
						50	0
30C53	5	CH_3	H	CH_3	H	200	94
						50	8
31C53	6	CH_3	H	CH_3	H	200	94
						50	4
141C53	7	CH_3	H	CH_3	H	200	99
						50	28
75C53	8	CH_3	H	CH_3	H	200	78
						50	4
	9–10	CH_3	H	CH_3	H	200	Toxic
69C53	3	C_2H_5	H	CH_3	H	200	100
						50	0
70C53	4	C_2H_5	H	CH_3	H	200	99
						50	35
						25	8
101C53	5	C_2H_5	H	CH_3	H	200	68
						50	0
77C53	6	C_2H_5	H	CH_3	H	200	100
						50	3
78C53	8	C_2H_5	H	CH_3	H	200	96
						50	0
79C53	10	C_2H_5	H	CH_3	H	200	Toxic
C. Methyl Substituents: Tertiary							
	3, 5–8	CH_3	CH_3	H	CH_3	200	0
225C53	3	CH_3	CH_3	CH_3	H	200	45
103C53	4	CH_3	CH_3	CH_3	H	200	70
						50	0
290C53	5	CH_3	CH_3	CH_3	H	200	52
116C53	6	CH_3	CH_3	CH_3	H	200	95
494C55	7	CH_3	CH_3	CH_3	H	200	100
						50	3
169C53	8	CH_3	CH_3	CH_3	H	200	73
						50	0
	9–10	CH_3	CH_3	CH_3	H	200	Toxic
D. Methoxyl Substituents: Primary							
	3, 4, 6	H	H	H	OCH_3	200	0
	3, 4	H	H	OCH_3	H	200	0
72C53	5	H	H	OCH_3	H	200	12
73C53	6	H	H	OCH_3	H	200	5
102C53	7	H	H	OCH_3	H	200	94
						50	5
97C53	8	H	H	OCH_3	H	200	92
						50	6
223C53	9	H	H	OCH_3	H	200	80
						50	0
	10, 11	H	H	OCH_3	H	200	0

TABLE I—*continued*

Compound Number	n	R¹	R⁴	R²	R³	Unit Dose (mg./kg.)	Worms Killed %
E. Methoxyl Substituents: Secondary							
169C55	3	C_2H_5	H	OCH_3	H	200	0
170C55	4	C_2H_5	H	OCH_3	H	200	6
171C55	5	C_2H_5	H	OCH_3	H	200	24
172C55	6	C_2H_5	H	OCH_3	H	200	67
						50	0
596C55	7	C_2H_5	H	OCH_3	H	200	75
						50	0
173C55	8	C_2H_5	H	OCH_3	H	200	98
						50	5
635C55	9	C_2H_5	H	OCH_3	H	200	100
						50	34
F. Methoxyl Substituents: Tertiary							
	3–6	CH_3	CH_3	OCH_3	H	200	0
319C53	7	CH_3	CH_3	OCH_3	H	200	100
257C53	8	CH_3	CH_3	OCH_3	H	200	52
						50	0
60C55	9	CH_3	CH_3	OCH_3	H	200	93
						50	0
224C53	10	CH_3	CH_3	OCH_3	H	200	8
G. Other Substituents: Primary							
367C53	6	H	H	H	Cl	200	0
	3–7	H	H	Cl	H	200	0
370C53	8	H	H	Cl	H	200	24
	9–10	H	H	Cl	H	200	0
	6, 7	H	H	H	OH	200	0
	5, 7	H	H	OH	H	200	0

series (Raison and Standen, 1955) give 100% activity at 200 mg./kg. for $n=3$ to 9 and at 25 mg./kg. for $n=6$ to 8, none of the corresponding compounds in which R^2 or $R^3=CH_3$ is 100% active at 200 mg./kg. for $n=3$ to 10, only a few showing any activity at all (Table I*A*).

The secondary amines of the ($R^2=CH_3$, $R=H$) series (Table I*B*) are markedly more active than the primary amines, a difference not shown in other series studied here, but their activity at 200 mg./kg. is hardly superior to that of the unsubstituted compounds at 25 mg./kg. although a certain parallelism can be observed (Fig. 1). Full comparison is not possible owing to the high toxicity of the compounds of greater chain-length. As with the unsubstituted series the methylamino- and ethylamino-compounds behave very similarly (Fig. 2).

The tertiary amines of the (I; $R^2=H$, $R^3=CH_3$) series (Table I*C*) are inactive for the examples tested, whereas those of the (I; $R^2=CH_3$, $R^3=H$) series show some activity for $n=3$ to 8, but greatly reduced compared with the unsubstituted series and without the marked alternation of properties characteristic of the latter.

Methoxyl Substituents.—The primary amines of the series (I; $R^2=H$, $R^3=OCH_3$) are inactive at the maximum dosage for the examples tested ($n=3$, 4, 6) (Table I*D*), but those of the series (I; $R^2=OCH_3$, $R^3=H$) show full activity at 200 mg./kg. for $n=7$ to 9 with almost complete loss of

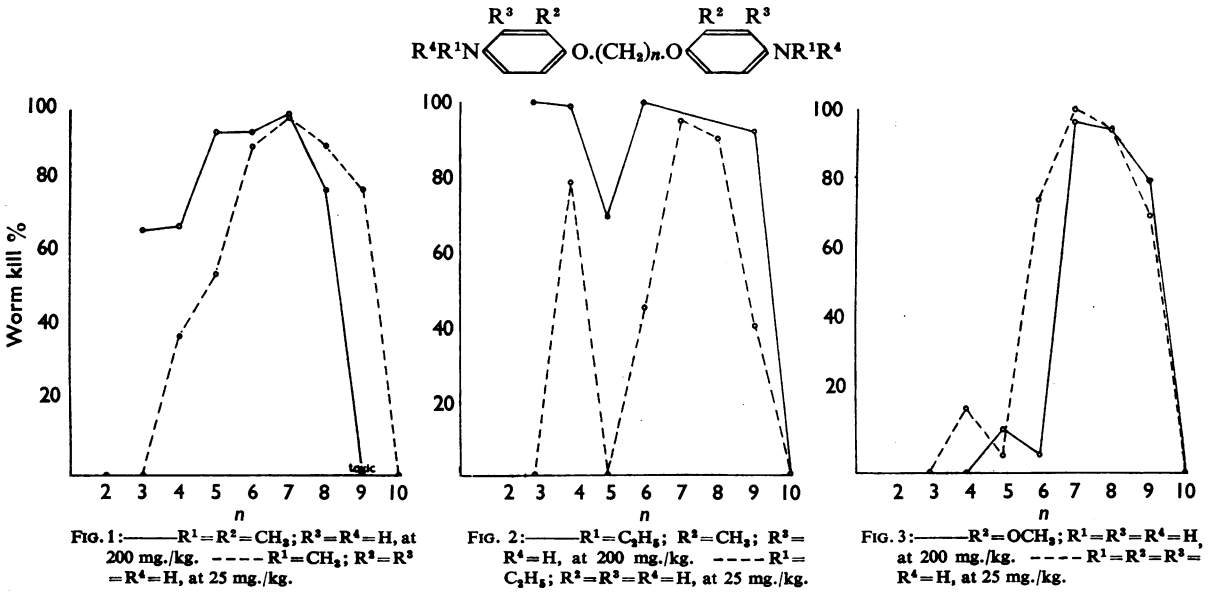


FIG. 1:——$R^1=R^2=CH_3$; $R^3=R^4=H$, at 200 mg./kg. - - - - $R^1=CH_3$; $R^2=R^3=R^4=H$, at 25 mg./kg.

FIG. 2:——$R^1=C_2H_5$; $R^2=CH_3$; $R^3=R^4=H$, at 200 mg./kg. - - - - $R^1=C_2H_5$; $R^2=R^3=R^4=H$, at 25 mg./kg.

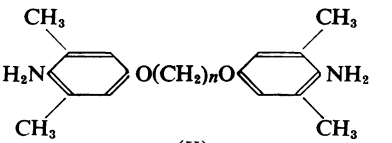
FIG. 3:——$R^2=OCH_3$; $R^1=R^3=R^4=H$, at 200 mg./kg. - - - - $R^1=R^2=R^3=R^4=H$, at 25 mg./kg.

FIGS. 1, 2, and 3.—The effect of further substitution (methyl in Figs. 1 and 2; methoxyl in Fig. 3) in the benzene rings on the activity of di-*p*-methylamino- (in Fig. 1); di-*p*-ethylamino- (in Fig. 2); and di-*p*-amino- (in Fig. 3) diphenoxyalkanes of chain-length $n=3$–10, compared with the unsubstituted compounds when tested against *S. mansoni* in mice.

activity at 50 mg./kg. There is once more similar activity at 200 mg./kg. to that of the unsubstituted compounds at 25 mg./kg. (Fig. 3). The secondary amines of this series (Table I*E*) do not show the marked superiority over the primary amines observed in the corresponding methyl substituted series. The tertiary amines (Table I*F*) resemble the primary amines with only the compounds $n=$ 7 to 9 showing marked activity at maximum dosage, and, like the primary amines, differ from the corresponding methyl series in that activity is only apparent at greater chain-length.

Other Substituents.—Primary amines, with various other substituents, were also tested (Table I*G*). In the (I; $R^2=Cl$, $R^3=H$) series, none of the compounds $n=3$ to 10 shows activity except $n=8$, which is slightly active. For (I; $R^2=H$, $R^3=Cl$) the only compound tested, $n=6$, is inactive, and for (I; $R^2=NH_2$, $R^3=H$) the compounds $n=4$ to 7 are all inactive.

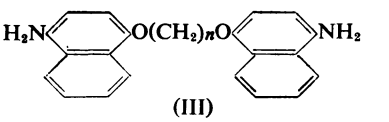
There is considerable evidence that an early step in the metabolism of aromatic amines is the substitution of a hydroxyl group into the para-position to the amino-group, or if this position is blocked, as in our compounds, substitution into the ortho-position. Consequently, compounds of the series (I; $R^2=H$, $R^3=OH$) might represent metabolites of the symmetrical diaminodiphenoxyalkanes, and it was therefore of interest to examine this type. The two primary amines, $n=6$ and 7, are inactive at 200 mg./kg. orally, and also at 50 mg./kg. by parenteral routes; the isomeric compounds (I; $R^2=OH$, $R^3=H$) are also inactive for $n=5$ and 7. These results suggested that the normal metabolic processes might lead to inactive compounds, and that blocking of the free ortho-positions might prevent this dystherapeutic effect and have a favourable influence on activity, but all the compounds ($n=3$ to 8) of the general formula (II)



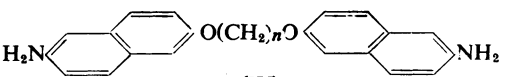
(II)

that were tested are devoid of activity at 200 mg./kg.

The naphthalene compounds (III; $n=5$, 7) and



(III)

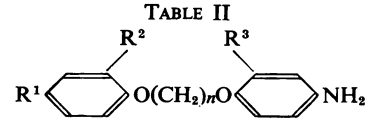


(IV)

(IV ; $n=2$ to 4), of which the former may be regarded as substituted diaminodiphenoxyalkanes, are all inactive at 200 mg./kg.

Table II gives details of the effect of substituting a methyl group into one ring only of (I). For a chain-length of four carbon atoms and with *p*-amino-groups in both rings there is a marked fall in activity as each ring is substituted in turn with a methyl group (384C50, 496C55, 117C53). For a chain-length of seven carbon atoms introduction of a methyl group into one ring reduces activity, but the compound with methyl groups in both rings is highly toxic (153C51, 120C54, 107C53). With a methyl group in one ring and a nitro-group in place of the amino-group in the other the results are anomalous, for whereas the 7-chain compounds have the expected order of activity (254C53> 48C54), with the 4-chain the methyl compound (495C55) is more active than the unsubstituted compound (325C54), or indeed than the unsubstituted diamine (384C50).

TABLE II



Compound Number	*n*	R^1	R^2	R^3	Unit Dose (mg./kg.)	Worms Killed %
325C54	4	NO_2	H	H	200	100
					50	76
495C55	4	NO_2	H	CH_3	200	100
					50	98
384C50	4	NH_2	H	H	200	99
					50	66
					25	17
496C55	4	NH_2	H	CH_3	200	100
					50	8
117C53	4	NH_2	CH_3	CH_3	200	79
					50	1
254C53	7	NO_2	H	H	200	97
					50	21
48C54	7	NO_2	H	CH_3	200	100
					50	0
153C51	7	NH_2	H	H	50	100
					25	96
					15	42
120C54	7	NH_2	H	CH_3	200	100
					50	29
107C53	7	NH_2	CH_3	CH_3	50	Toxic

Discussion

The striking dystherapeutic effect of further substitution of the aromatic rings of the diaminodiphenoxyalkanes is apparent, particularly when the substituent is *ortho* to the amino-group. This class shows complete loss of activity for all but two examples (186C53 and 187C53) (Table I*A*), which show slight activity at maximum dosage, and this suggests that some essential form of combination between the amino-group and substrate is prevented or hindered by the adjacent substituent and, in view of the varied polar nature of the groups studied, this effect may well be steric. Very great loss of activity also occurs when the substituent is in the ortho-position to the ether linkage, and although now the loss is not usually complete it is tempting to ascribe a similar cause also to this dystherapeutic effect. The implication is that at some stage cleavage of the drug occurs with liberation of a phenolic group, which in turn combines with the substrate, such combination being hindered by ortho-substituents. The parallelism in activity observed between the more active of the substituted and the corresponding unsubstituted compounds (Figs. 1–3), and the usually additive effect of substitution of each ring in turn (Table II) would not be incompatible with such a notion, but no evidence has yet accrued that any possible cleavage products of the active compounds have schistosomicidal activity.

Summary

1. Substitution of CH_3, OCH_3, OH, Cl, or NH_2 into the aromatic rings of the symmetrical diaminodiphenoxyalkanes invariably results in a great reduction in activity against *Schistosoma mansoni*, loss of activity usually being complete when a substituent ortho to the amino-group is present in both rings.

2. The most active of the compounds in which both rings are substituted are the primary amines with methoxyl ortho to the ether linkage, and secondary amines with methyl similarly placed, but even these are only as active at 200 mg./kg. as are the unsubstituted compounds at 25 mg./kg.

3. When one ring only is substituted with methyl the activity is intermediate between that of the unsubstituted and the disubstituted compounds, except for a nitro-amine substituted with methyl in the basic ring, which has high activity.

We are indebted to our colleagues Drs. Caldwell, Gorvin, and Raison for some of the compounds referred to, and for valuable technical assistance to Mr. R. Nightingale with the chemical work and to Mrs. Rosemary Richards with the biological work.

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