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THE ACTION OF SUBSTANCES ANALOGOUS TO DIAMINODIPHENOXYALKANES AGAINST *SCHISTOSOMA MANSONI*

BY

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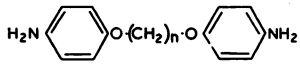
The effects on activity against *Schistosoma mansoni* of further modifications of the diaminodiphenoxyalkanes are reported. Replacement of the amino-group by any of a large number of other groups destroys activity. Activity is retained in the presence of certain substituted (hydroxyalkyl, carboxyalkyl) amino-groups and in some of their aldehyde-bisulphite derivatives. Many variations of the central chain lead to compounds of reduced activity, the outstanding exceptions being a number of but-2-ene derivatives, which retain full activity.

Previous papers from these laboratories (Raison and Standen, 1955; Caldwell and Standen, 1956; Standen and Walls, 1956) have reported the schistosomicidal activities of diaminodiphenoxyalkanes (I) and related compounds. Two further variations of this structure are now considered,



(I)

namely that of the terminal amino-group, apart from mono- and di-alkyl substitution (Raison and Standen, 1955), and that of the aliphatic chain linking the two benzene rings.

MATERIALS AND METHODS

As in the previous papers in this series, the compounds described were tested orally against adult *Schistosoma mansoni* in mice. Treatment commenced 63 days after infection of the mice and was continued twice daily for 5 consecutive days. The animals were killed 7 days after completion of treatment and were examined to determine the drug effects against the parasites. Assessment of results was made according to the methods described by Raison and Standen (1955).

RESULTS

The results of routine screening of those types of compound which showed activity are given in Tables I to IV. The "unit dose" referred to is that given orally twice daily for five days.

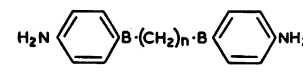
Variation of the Amino-group

Inactive Compounds.—When the amino-groups of (I) were replaced by one of the following groups, inactivity or only slight activity at toxic dose levels resulted: aminomethyl, guanidino, biguanido, amidino, ureido, thioureido, guanidinomethyl, benzylamino, aminoalkylamino, hydrazino, hydroxy, cyanomethylamino, carboxy, azidocarbonyl (.CON_3), nitrosomethylamino, toluene-*p*-sulphonamido, acetamido, ethoxycarbonamido, formyl, succinylamino, and sulphamino. Replacement of the *p*-aminophenyl group by 4-pyridyl or 6- or 8-quinolyl also led to loss of activity.

Active Compounds.—Comparatively few variations led to retention of high activity and the following types are almost the only examples: (i) hydroxyalkylamino (Table I, A), (ii) carboxyalkylamino (Table I, B), (iii) aldehyde-bisulphite derivatives of primary and secondary amines (Table I, C).

Variation of the Aliphatic Chain

Inactive Compounds. — Substances of the general type in which the ether oxygen atoms were omitted, namely diaminodiphenylalkanes (II; B=CH_2, n=7, 8), and those in which the oxygen atoms were replaced by carbonyl (II;



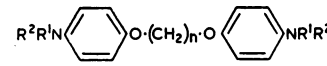
(II)

B=CO, n=7, 8), by ester groups derived either from *p*-aminophenol (II; B=O.CO, n=5) or *p*-aminobenzoic acid (II; B=CO.O, n=5), by

TABLE I

ACTIVITIES OF HYDROXYALKYLAMINES, CARBOXYALKYLAMINES, AND ALDEHYDE-BISULPHITE DERIVATIVES

For explanation of "unit dose" see Results. The doses in section C are those equivalent to 50 mg. of the parent amine



Compound No.	n	R^1	R^2	Unit Dose (mg./kg.)	Worms Killed %
A. Hydroxyalkylamines					
221C54	3	$CH_2.CH_2.OH$	H	200 50	5 0
122C54	4	$CH_2.CH_2.OH$	H	100 50	90 57
346C53	5	$CH_2.CH_2.OH$	H	100 50	100 36
347C53	6	$CH_2.CH_2.OH$	H	100 50	96 63
261C53	7	$CH_2.CH_2.OH$	H	50 25 15	100 57 14
262C53	8	$CH_2.CH_2OH$	H	50 25 15	86 47 16
394C53	9	$CH_2.CH_2.OH$	H	100 50	100 70
161C54	10	$CH_2.CH_2.OH$	H	200	0
281C54	4	$CH_2.CH_2.CH_2.OH$	H	200 50	12 0
295C54	5	$CH_2.CH_2.CH_2.OH$	H	200 50	84 0
165C54	6	$CH_2.CH_2.CH_2.OH$	H	200 50	100 43
184C54	7	$CH_2.CH_2.CH_2.OH$	H	50 25	98 0
166C54	8	$CH_2.CH_2.CH_2.OH$	H	200 50	100 68
616C54	9	$CH_2.CH_2.CH_2.OH$	H	50	83
217C54	6	$CH_2.CH(OH).CH_3$	H	50	53
218C54	7	$CH_2.CH(OH).CH_3$	H	50 25 12·5	100 90 21
219C54	8	$CH_2.CH(OH).CH_3$	H	50 25 12·5	98 93 4
483C55	4	$CH_2.CH_2.OH$	CH_3	200 50	19 0
381C53	5	$CH_2.CH_2.OH$	CH_3	100 50	87 11
270C54	6	$CH_2.CH_2.OH$	CH_3	200 50	85 0
271C54	7	$CH_2.CH_2.OH$	CH_3	50 25	88 0
272C54	8	$CH_2.CH_2.OH$	CH_3	200 50	100 35
484C55	9	$CH_2.CH_2.OH$	CH_3	200 50	99 74
265C54	2	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200	0
197C54	3	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200	0
105C54	4	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50	93 0
125C53	5	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50	100 0

TABLE I—*continued*

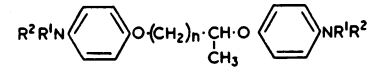
Compound No.	*n*	R^1	R^2	Unit Dose (mg./kg.)	Worms Killed %
197C53	6	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50 25	100 80 0
252C53	7	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50 25	100 90 0
253C53	8	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	50 25	100 12
330C53	9	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50	100 34
2C54	10	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50	90 0
106C54	11	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200	3
284C54	4	$CH_2.CH(OH).CH_3$	$CH_2.CH(OH).CH_3$	200 50	62 1
287C54	5	$CH_2.CH(OH).CH_3$	$CH_2.CH(OH).CH_3$	200 50	100 0
187C54	6	$CH_2.CH(OH).CH_3$	$CH_2.CH(OH).CH_3$	200 50 25	100 36 0
241C54	7	$CH_2.CH(OH).CH_3$	$CH_2.CH(OH).CH_3$	200 50	93 0
220C54	8	$CH_2.CH(OH).CH_3$	$CH_2.CH(OH).CH_3$	200 50	100 0
454C54	9	$CH_2.CH(OH).CH_3$	$CH_2.CH(OH).CH_3$	200 50	47 0
547C54	8	$CH_2.CH_2.CH_2.OH$	$CH_2.CH_2.CH_2.OH$	200	100
198C54	7	$CH_2.CH(OH).CH_2.OH$	H	50 25 12·5	99 86 1
185C54	7	$CH_2.CH(OH).CH_2.OH$	$CH_2.CH(OH).CH_2.OH$	200	0
B. Carboxyalkylamines					
63C55	4	$CH_2.CO_2H$	H	50	0
13C53	5	$CH_2.CO_2H$	H	50	2
543C54	6	$CH_2.CO_2H$	H	50	96
22C54	7	$CH_2.CO_2H$	H	50 25	96 30
544C54	8	$CH_2.CO_2H$	H	50	90
131C55	9	$CH_2.CO_2H$	H	50	94
167C54	7	$CH_2.CH_2.CO_2H$	H	50 25	92 6
143C55	7	$CH(CH_3).CO_2H$	H	50 25	100 24
358C55	7	$CH_2.CH_2.CH_2.CO_2H$	H	50	98
240C55	5	$CH_2.CO_2H$	$CH_2.CO_2H$	200	0
566C54	7	$CH_2.CO_2H$	$CH_2.CO_2H$	50	87
C. Aldehyde-bisulphite derivatives					
66C55	6	$CH_2.SO_3Na$	H	70	14
398C53	7	$CH_2.SO_3Na$	H	75	89
67C55	5	$CH_2.SO_3Na$	CH_3	75	85
111C55	5	$CH_2.SO_3Na$	$CH_2.CH_2.OH$	85	44
167C55	6	$CH(CH_3).SO_3Na$	H	75	27
236C55	7	$CH(C_6H_5).SO_3Na + NaHSO_3$	H	90	70
168C55	7	$CH(SO_3Na).CH_2—CH(C_6H_5).SO_3Na$	H	125	95
234C55	7	$CH(SO_3Na).(CH.OH)_4.CH_2.OH$	H	110	93

sulphonyl (II; B=SO_2, n=2–6), or by sulphonamido (II; B=SO_2NH, n=4, 5, 8, 10; B=$NH.SO_2$, n=4), were all inactive at the maximum unit dose of 200 mg./kg. Replacement of the oxygen atoms by imino-groups (II; B=NH, n=2–6) gave toxic products which were inactive at the maximum tolerated unit dose, which usually did not exceed 20 mg./kg. Insertion of a *p*-phenylene group into the carbon chain destroyed the activity.

TABLE II

ACTIVITIES OF BRANCHED-CHAIN COMPOUNDS HAVING THE FORMULA GIVEN BELOW COMPARED WITH THOSE FOR RELATED STRAIGHT-CHAIN COMPOUNDS

The values given in the last column are those for the compounds with the same total number of carbon atoms in the chain and are taken from Raison and Standen (1955) and from Table I, A.



Compound No.	n	R^1	R^2	Unit Dose (mg./kg.)	Worms Killed %	
					Branched	Straight
115C54	2	H	H	200 50	74 0	99 66
47C53	3	H	H	100 50	96 52	100 78
89C55	3	H	CH_3	200 50	80 —	99 99
64C55	3	H	$CH_2.CH_2.OH$	200 50	97 0	100 36
65C55	3	CH_3	CH_3	200 50	98 1	100 96
91C55	3	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200	39	100

Active Compounds.—The variations of (I) which preserved some degree of activity were: (1) replacement of ether oxygen by sulphur, (2) substitution by alkyl in the carbon chain (Table II), (3) replacement of a methylene group by another atom or group, (4) the introduction of unsaturated linkages into the carbon chain (Tables III and IV).

DISCUSSION

Variation of the Amino Group

The influence of the changes in structure indicated in Table I (A, B, and C) on schistosomicidal activity is best assessed by comparison with the corresponding parent compounds, results for which have already been published by Raison and Standen (1955). Considering first the *hydroxyalkylamines* (Table I, A), the simplest examples are the secondary hydroxyethylamino-compounds; this series shows the same general picture as the ethylamino-compounds, with a well-marked peak of activity at n=7, but the level of activity is lower. The γ-hydroxypropylamino-series shows a peak at n=7, whereas the propylamino-series had a similar level of activity but with two peaks at n=4 and 7. Only three examples are given of secondary β-hydroxypropylamino-compounds, but their activities are much higher than the corresponding propylamines for the same values of n; 218C54 and 219C54 have very high activities.

In the tertiary hydroxyethylmethylamino-compounds there are indications of an activity alternating with n, but the level of activity is lower than in the related ethylmethylamines. Bis(hydroxyethyl)-amino-compounds show high activity over the range n=4 to 10 and at the lower dose of 50 mg./kg. a single peak at n=7 to 8; the related diethylamines showed an additional peak at n=4 and were in general somewhat more active. The favourable influence of β-hydroxylation of the propyl group is shown again in the tertiary compounds, which show high activity at 200 mg./kg. over the range n=4 to 9, a much higher level of activity than the di-*n*-propylamines. The activity almost disappears at 50 mg./kg., however, making the series less active than their lower homologues, the bis(hydroxyethyl)amines.

Among the *carboxyalkylamines* (Table I, B), the carboxymethylamino-compounds have been most extensively studied. High activity is found in the range n=6 to 9, but in general the compounds are not so active as the parent methylamines. The corresponding esters are much less active. α- and β-Carboxyethyl- and γ-carboxypropyl-amino-compounds have been studied with the single chain length of 7 and high activity was found. It is interesting to compare the activity of the latter compound (358C55) with the related, but inactive, succinylamino-derivative.

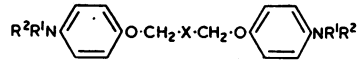
Aldehyde-bisulphite derivatives of a few representative types are shown in Table I, C, in which the doses indicated are equivalent to a dose of 50 mg./kg. of the parent amine. It seems likely that the activity of these derivatives is caused by their hydrolysis, on oral administration, to the original amine.

As far as the amino-group is concerned, therefore, it appears from the results now presented, together with those of Raison and Standen (1955), that very little modification is possible if activity is to be retained. Only alkyl, and such substituted alkyl groups as those described above, are permissible substituents in the primary amines (I). It appears to be essential to retain a group, attached directly to the benzene ring, having the order of basicity of an aromatic amino-group and

TABLE III

COMPARISON OF ACTIVITIES OF RELATED COMPOUNDS HAVING UNSATURATED AND SATURATED CHAINS

The values given in the last column are those for the corresponding saturated compounds of the same chain length and are taken from Raison and Standen (1955) and from Table I, A.



Compound No.	X	R^1	R^2	Unit Dose (mg./kg.)	Worms Killed %	
					Unsaturated	Saturated
A. Compounds with triple bonds						
410C53	C⋮C	H	H	200	0	99
412C53	C⋮C	H	CH_3	200	10	100
533C54	C⋮C	H	$CH_2.CH_2.OH$	200	3	100
467C54	C⋮C	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50	0 0	93 0
479C54	C⋮C	CH_3	$CH_2.CH_2.OH$	200	1	19
432C55	$CH_2.C$⋮C	H	H	200 50	88 0	100 78
430C56	$(CH_2)_2.C$⋮C	H	H	200 50	98 4	100 99
271C55	$CH_2.C$⋮$C.CH_2$	H	H	200 50	63 46	100 99
272C55	$CH_2.C$⋮$C.CH_2$	H	CH_3	200	37	100
370C56	$(CH_2)_4.C$⋮C	H	H	200 50	98 44	100 100
273C55	$(CH_2)_2C$⋮C—C⋮$C.(CH_2)_2$	H	H	200 50	39 11	0 —
296C55	$(CH_2)_2C$⋮C—C⋮$C.(CH_2)_2$	H	CH_3	200	0	39
297C55	$(CH_2)_2C$⋮C—C⋮$C.(CH_2)_2$	CH_3	CH_3	200	0	0
B. Compounds with double bonds						
411C53	CH:CH	H	H	200 50	98 18	99 66
8C54	CH:CH	H	CH_3	50 25	100 39	98 36
466C54	CH:CH	H	C_2H_5	50 25	100 24	100 78
311C54	CH:CH	H	n-C_3H_7	50 25	91 2	79 0
465C54	CH:CH	H	$CH_2.CH_2.OH$	200 50	44 0	100 0
480C54	CH:CH	H	$CH_2.CH(CH_3).OH$	200 50	88 8	— —
129C54	CH:CH	CH_3	CH_3	100 50 25	85 57 42	85 60 32
83C55	CH:CH	C_2H_5	C_2H_5	50 25	42 0	35 0
130C54	CH:CH	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50	7 0	93 0
478C54	CH:CH	CH_3	$CH_2.CH_2.OH$	200 50	25 2	19 0
131C54	$CH_2.CH$:$CH.CH_2$	H	H	200 50	100 46	100 99
192C54	$CH_2.CH$:$CH.CH_2$	H	CH_3	50 25	100 26	100 90
428C54	$CH_2.CH$:$CH.CH_2$	H	n-C_3H_7	200 50	5 0	66 16

[*continued overleaf*

TABLE III—*continued*

Compound No.	X	R^1	R^2	Unit Dose (mg./kg.)	Worms Killed %	
					Unsaturated	Saturated
468C54	$CH_2.CH:CH.CH_2$	H	$CH_2.CH_2.OH$	200 50	99 0	100 63
236C54	$CH_2.CH:CH.CH_2$	CH_3	CH_3	50 25	80 9	57 0
237C54	$CH_2.CH:CH.CH_2$	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50	92 0	100 80
10C54	$(CH_2)_2.CH:CH.CH_2$	H	H	200 50	100 13	100 100
193C54	$(CH_2)_2.CH:CH.CH_2$	H	CH_3	50 25	94 5	100 94
255C54	$(CH_2)_2.CH:CH.CH_2$	CH_3	CH_3	200 50	100 72	100 100
61C55	$(CH_2)_2.CH:CH.CH_2$	$CH_2.CH_2.OH$	$CH_2.CH_2.OH$	200 50	71 2	100 90

TABLE IV

ACTIVITIES OF BUTENE DERIVATIVES CARRYING ONE AMINO GROUP AND ONE URETHANE OR AMIDE GROUP

Compound No.	R	R^1	R^2	Unit Dose (mg./kg.)	Worms Killed %
427C54	H	H	OC_2H_5	200 50	98 64
425C54	H	CH_3	OC_2H_5	50 25	100 67
254C54	CH_3	H	OC_2H_5	200 50	97 58
224C54	CH_3	CH_3	OCH_3	25 12·5	100 85
7C54	CH_3	CH_3	OC_2H_5	25 12·5	95 25
636C55	CH_3	CH_3	$OC_3H_7(n)$	50	98
637C55	CH_3	CH_3	$OC_3H_7(i)$	50	99
541C54	CH_3	CH_3	$OC_4H_9(n)$	50 25	100 39
174C55	CH_3	C_2H_5	OCH_3	50 25	62 17
542C55	CH_3	C_2H_5	OC_2H_5	200 50 25	100 74 0
426C54	CH_3	H	CH_3	50 25	78 41
534C54	CH_3	CH_3	CH_3	25	93

not carrying too large a substituent. The introduction of a more basic group destroys the activity.

Variation of the Aliphatic Chain

Replacement of the ether oxygen atoms by sulphur atoms led to substances either inactive or of much lower activity. Peaks of activity in the series of primary and secondary amines appeared at $n=4$ and $n=9$.

Alkyl (methyl) substitution in the carbon chain was studied in six compounds (Table II), but they proved to be, in general, much less active than corresponding compounds with an unbranched chain of the same number of carbon atoms.

Replacement of one or more methylene groups by an oxygen atom led to compounds the activity of which depended on the position of the substitution. The chain $.O.(CH_2)_2.O.(CH_2)_2.O.$ gave inactive compounds whether the terminal amino-group was primary, secondary or tertiary. With $.O.(CH_2)_3.O.(CH_2)_3.O.$, some activity was found at the highest dose, but with $.O.(CH_2)_4.O.(CH_2)_4.O.$ high activity, comparable with that of the parent diphenoxyalkane, was found. Furthermore, when two methylene groups were replaced by oxygen atoms, as with the chain $.O.(CH_2)_2.O.(CH_2)_2.O.(CH_2)_2.O.$, complete loss of activity resulted. The critical factor appears to be the length of carbon chain between the phenoxy-oxygen and the inserted oxygen atom and these findings may have significance in relation to the speculations of Caldwell and Standen (1956) as to the structural requirements for schistosomicidal activity in this group of compounds. When a methylene group in the chain was replaced by a symmetrically disposed sulphur atom or sulphone group, the compounds were only slightly active.

The fact that replacement of the ether oxygens by any atom or group other than sulphur results in complete loss of activity is consistent with the view that the biological activity of the diphenoxyalkanes is dependent on interaction at this site with a substrate, perhaps by hydrogen bonding, or

even after cleavage of the molecule. Substitution by the sulphur atom, which most resembles oxygen but which forms hydrogen bonds less readily, does not always result in complete loss of activity. Furthermore, the unfavourable effect of branching of the carbon chain at the position adjacent to an ether oxygen, like that of further substitution in the benzene ring (Standen and Walls, 1956), could be attributed to a steric hindrance to substrate-drug interaction.

Unsaturation in the Chain.—The compounds with a triple bond (Table III, A) are all less active than the corresponding saturated compounds, this effect being shown most clearly in the but-2-ynes, which are almost inactive. The distance of the triple bond from the oxygen atoms seems to be important; thus, the primary amines 432C55, 430C56, and 370C56 form a series of increasing activity in which one oxygen is progressively farther away from the triple bond. In the two examples studied (272C55 and 296C55), a methylamino-compound is less active than the corresponding primary amine, the reverse of what was usually found with the saturated compounds.

The comparative inactivity of the but-2-ynes and the low activity of some of the other acetylenes seem to be related to the fact that the triple bond is in the β-position to an oxygen atom. Under certain conditions such compounds are more readily split to yield *p*-aminophenol than are saturated compounds and this was thought to be a possible explanation of the low activity. It was surprising therefore to find that the *trans*-but-2-enes, which are more readily split than the but-2-ynes, are often no less active than the corresponding butanes (Table III, B). In fact, but-2-enes with one terminal basic and one terminal urethane group (Table IV) resemble the saturated compounds of this type (Caldwell and Standen, 1956) in their high level of activity; 224C54 is one of the most active compounds yet investigated. The activity is maintained at a high level for considerable variation of the urethane group, but compounds in which that group is derived from a secondary amine, for example 7C54, are more active than those in which it is derived from a primary amine, namely, 254C54. Replacement of urethane by acetamido (426C54, 534C54) also produced highly active compounds.

Compounds with *trans*-hex-3-ene and hept-3-ene (probably *trans*) chains proved to be usually somewhat less active than the corresponding saturated compounds (Table III, B).

The effect of unsaturation in the chain is interesting in that it does not always decrease activity. Unsaturation imposes a rigidity on the molecule, but in the but-2-ynes and but-2-enes, in which this factor would be expected to be most important, highly contrasting results have been observed. The ease of cleavage of a carbon chain, enhanced by unsaturation, has been discussed above and does not appear to provide an explanation of our results. It is always risky, though tempting, to seek analogies between the *in vivo* and *in vitro* behaviour of drugs. However, the approximate coincidence of the biological results with corresponding saturated and unsaturated compounds (excluding the but-2-ynes) leads one to speculate whether the latter may be hydrogenated *in vivo*. It may then be pointed out that, whereas the but-2-enes described here are smoothly hydrogenated in the presence of various catalysts to butanes, catalytic reduction of the but-2-ynes is much less satisfactory.

We wish to thank the many technical assistants who have contributed to the accomplishment of this work.

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