Brit. J. Pharmacol. (1955), **10**, 340.

CHEMICAL CONSTITUTION AND ANTHELMINTHIC ACTIVITY OF CYCLIC ANALOGUES OF PHENOTHIAZINE

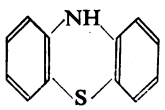
BY

W. P. ROGERS, J. CYMERMAN-CRAIG, AND G. P. WARWICK

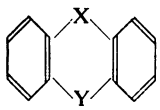
From the Department of Zoology, University of Adelaide, South Australia, and Department of Organic Chemistry, University of Sydney, N.S.W.

(RECEIVED APRIL 18, 1955)

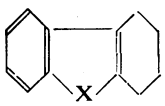
Thiodiphenylamine, or phenothiazine (I), is used extensively as an anthelminthic, particularly for the treatment of nematode infestations of the alimentary tract of sheep (Harwood, 1953). This drug is of particular interest because, compared to many other anthelminthics, it is relatively specific in its action, and because it forms a variety of oxidation products in treated animals (Lipson, 1940; De Eds and Thomas, 1942; Clare, 1947; Clare, Whitten, and Filmer, 1947; Harpur, Swales, and Denstedt, 1950). It is probable that phenothiazine itself, rather than its oxidation products, is the effective anthelminthic (Esserman, 1952). On the other hand, the oxidation products inhibit a number of mammalian enzyme systems (Collier, 1940; Collier and Allen, 1942a, 1942b; Collier and Allenby, 1952), and many of the toxic signs in treated animals are probably due to these compounds (Collier and Allen, 1942a; Clare *et al.*, 1947; Whitten, 1948). We decided to examine the



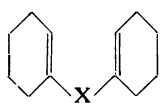
I



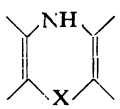
II



III



IV



V

anthelminthic activity of a number of cyclic analogues of phenothiazine, comprising the various possible combinations of X and Y in structure II (where X or Y or both may be carbon, nitrogen, oxygen, sulphur or selenium) and structure III (where X may be carbon, nitrogen, oxygen, or sulphur). Structures II and III represent tricyclic isosteres of phenothiazine, whereas structure IV—where X is nitrogen or sulphur—represents the corresponding open-chain analogues.

In this paper the anthelminthic activity of 25 analogues of phenothiazine of type II, III, and IV is reported.

MATERIALS AND METHODS

Preparation of Compounds.—All compounds were prepared by published methods. They were of analytical purity and had the melting points recorded in the literature.

Anthelminthic Activity.—Natural infestations of mixed populations of *Syphacia obvelata* and *Aspiculuris tetraptera* were used for assessing anthelminthic activity. There was no obvious difference in the reactions of these two parasites to phenothiazine, and, as a rule, no attempt was made to separate them when counting the parasites in the host or in the faeces.

The compounds were administered by a stomach tube to mice lightly anaesthetized with ether. Each mouse received the drug suspended in 0.4 ml. of 1/500 (v/v) "Wetsit," an anionic detergent. "Wetsit" alone showed no activity. The faeces of each mouse were collected separately on damp filter paper for 24 hr., after which the mice were killed. The parasites in the faeces of each mouse, and those in the colon, caecum, and rectum were counted. Activity was assessed as $\frac{\text{parasites in faeces}}{\text{total parasites found}} \times 100$.

When *post mortem* examination showed that the compound was retained in the stomach of the treated animal for long periods (as with phenoselenazine), tests were extended to cover 48 hr. In order to determine the action of phenothiazine at different time intervals, 7 mice were given 1 g./kg., the faeces collected 4, 19, 24, 27, and 44 hr. after the dose, and the activity at the different times assessed.

The mice were not fasted before or during the experiments. At least 5 mice were used in each test and the total number of parasites/mouse was usually well in excess of 80.

RESULTS

Phenothiazine.—The results of an experiment with phenothiazine are shown in Fig. 1. The linear regression is significant ($P<0.001$).

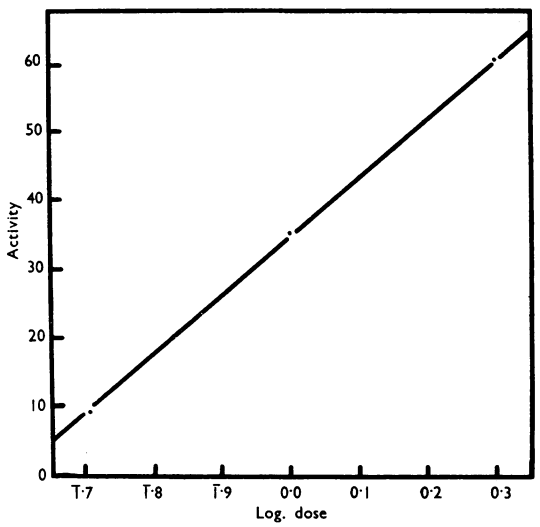


FIG. 1.—The relation between log. dose (g./kg.) of phenothiazine and activity. Activity was assessed as $\frac{\text{parasites in faeces}}{\text{total parasites}} \times 100$. The linear regression was significant ($P < 0.001$).

Fig. 2 shows that the maximum effect of a dose of 1 g./kg. of phenothiazine was attained in 24 hr. The graph shows the results from 6 of the

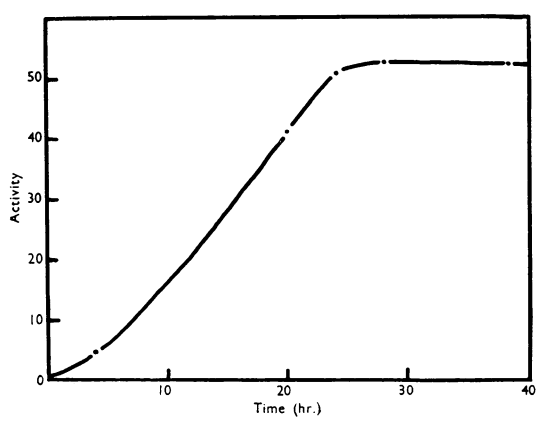


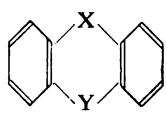
FIG. 2.—The relation between time and anthelminthic activity of phenothiazine (1·0 g./kg.). Activity as in Fig. 1. The standard deviations as percentages of the means were: 48 (0–4 hr.), 75 (0–19 hr.), 59 (0–24 hr.), and 55 (0–38 hr.).

7 mice treated. The remaining mouse became moribund after treatment and did not eat; worms continued to appear in the faeces for 44 hr. This result was quite different from all the others, and it was considered that the discrepancy was due to the moribund condition of the animal. From this and other experiments, it appeared that results obtained with animals which were markedly affected by the treatment were misleading, and they were therefore neglected.

Cyclic Analogues of Phenothiazine.—The results obtained with the active members of the series are summarized in Table I. Statistical analysis showed

TABLE I

ACTIVE TRICYCLIC PHENOTHIAZINE ANALOGUES OF STRUCTURE II



Compound	X	Y	Activity at 1 g./kg.	Activity at 2 g. kg.
Phenothiazine	NH	S	38	80
Phenoxazine	NH	O	—	80
Phenothiazine S-oxide	NH	SO	16	—
Acridine	N	CH	12	—
Thianthren	S	S	10	—
Dibenzdioxin	O	O	—	20

that there was no significant difference between the activities of phenothiazine and phenoxazine, and that these substances were significantly more active than the other compounds tested.

No activity was observed with phenoselenazine (II; X=NH, Y=Se), phenothiazine S-dioxide (X=NH, Y=SO_2), dihydroacridine (X=NH, Y=CH), acridone (X=NH, Y=CO), phenazine (X=N, Y=N), phenoxthiin (X=O, Y=S), anthracene (X=CH, Y=CH), xanthone (X=CO, Y=O), or thioxanthone (X=CO, Y=S).

No activity was observed with any of the compounds with structure III that were tested: carbazole (X=NH), dibenzfuran (X=O), dibenzthiophen (X=S), dibenzthiophen S-oxide (X=SO), or dioxide (X=SO_2), fluorenone (X=CO), fluorene (X=CH_2) or phenanthrene (X=CH:CH).

Two compounds of the structure IV were examined. Diphenyl sulphide (X=S) was inactive, and diphenylamine (X=NH) showed only slight activity at a dose of 2 g./kg.

DISCUSSION

The number of tests on each compound was sufficient only to indicate their approximate relative activities. However, for this part of the investigation it was required only to separate compounds of high activity from those of low

activity, and the minimum number of tests for this purpose was carried out. It appears possible that the optimal requirements for anthelminthic activity may be the presence of the central ring-system (V) containing an -NH group and a hetero-atom X (such as oxygen or sulphur) possessing one or more "lone pairs" of electrons. Further work on this topic is in progress.

SUMMARY

1. An examination of the anthelminthic activity of 23 tricyclic and 2 acyclic analogues of phenothiazine showed that only phenothiazine and phenoxazine possessed appreciable activity when tested against mixed infestations of *Syphacia obvelata* and *Aspiculuris tetraptera*.

2. The significance of the results is discussed.

The authors are indebted to Mr. R. Simmonds for technical assistance and to Mr. V. Bofinger, of C.S.I.R.O. Division of Mathematical Statistics, for advice. One of us (G. P. W.) wishes to thank the Division of Animal Health and Production for the award of an Ian McMaster Scholarship. A grant from the Agricultural Research Council for technical assistance is also acknowledged.

REFERENCES

Clare, N. T. (1947). *Aust. vet. J.*, **23**, 340.
—— Whitten, L. K., and Filmer, D. B. (1947). Ibid., **23**, 344.
Collier, H. B. (1940). *Canad. J. Res.*, **18B**, 345.
—— and Allen, D. E. (1942a). Ibid., **20B**, 189.
—— —— (1942b). Ibid., **20B**, 284.
—— and Allenby, G. M. (1952). *Canad. J. med. Sci.*, **30**, 443.
De Eds, F., and Thomas, J. O. (1942). *J. Parasit.*, **28**, 363.
Esserman, H. B. (1952). *Aust. J. sci. Res.*, *B***5**, 485.
Harpur, R. P., Swales, W. E., and Denstedt, O. F. (1950). *Canad. J. Res.*, **28D**, 134.
Harwood, P. D. (1953). *Exp. Parasitol.*, **2**, 428.
Lipson, M. (1940). *Aust. J. exp. Biol. med. Sci.*, **18**, 269.
Whitten, L. K. (1948). *Aust. vet. J.*, **23**, 336.