Brit. J. Pharmacol. (1952), 7, 219.

THE INFLUENCE OF GROUPS IN THE MOLECULE OF 2 : 3-DIHYDRO-3-KETOBENZO-1 : 4-THIAZINE ON ITS EFFECT ON LIVER FLUKE (*FASCIOLA HEPATICA*) *IN VITRO*

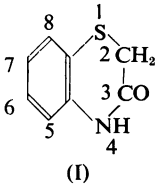
BY

ALEXANDER MACKIE AND JOHN RAEBURN

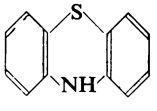
From the Department of Chemistry, Heriot-Watt College, Edinburgh

(Received January 17, 1952)

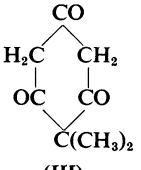
The parent compound chosen for the investigation described in this paper was 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine (I), as it contains in its molecule certain features present in substances which possess anthelmintic properties, e.g. phenothiazine (II). It also contains a $-CH_2CO-$ unit, which is a characteristic feature in filicic acid (III), the most important of the organic acids present in the anthelmintic *Filix mas.*



(I)



(II)



(III)

On account of these similarities, it was reasonable to assume that 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine might show some anthelmintic activity, and consequently this compound and a number of its derivatives with substituents in the 6- and 6 : 7-positions were tested against liver fluke (*Fasciola hepatica*) *in vitro*, so that the influence of the various groups on anthelmintic activity could be compared.

METHODS

Preparative.—2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine and derivatives were prepared by methods described elsewhere (Mackie and Raeburn, 1952). Unfortunately it was not possible to prepare the 6-bromo-derivative analytically pure, so that a complete comparison of the influence of the four halogens could not be made.

Biological testing.—The method of *in vitro* testing adopted was Baldwin's kymographic technique (1943) as modified by Chance and Mansour (1949) for liver fluke.

The maximum concentration used was 1 : 1,000, either as solution, emulsion, or suspension, and the tests were carried out at lower concentrations till no effect on the helminth was observed. The minimum effective concentration was noted for each compound, so that it was possible to arrange most of the substituent groups in order according to their effect on potency. There were, however, a few compounds whose minimum effective concentration could not be determined owing to their insolubility, for even at 1 : 4,000 heavy suspensions were obtained.

At least four determinations were carried out on every compound at each concentration, unless the compound had no effect, when two tests were considered sufficient. Moreover, two or sometimes three determinations were carried out at the same concentration for a given compound with different batches of parasites. Except in a very few determinations, the kymographic records for a given compound at a particular concentration were remarkably similar in form.

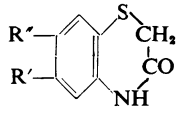
Results and Discussion

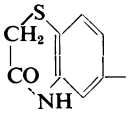
2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine and its derivatives, which have been fully tested *in vitro*, have shown a paralysant effect on liver fluke. The most potent was the 6-chloro-derivative, which produced a paralysant effect at 1 : 8,000, the minimum effective concentration. At a concentration of 1 : 1,000 the 6-amino- and 6-mercapto-compounds had so powerful an effect that the addition of amphetamine produced only small irregular movements after 10 min. At 1 : 2,000, the 6-amino-derivative produced a paralysant effect for about 20 min., which was then followed by small convulsive movements.

The 6-arsonic and -stibonic acids, the 6-chloromercuri-derivative, and bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl) were very insoluble and the minimum

TABLE I

The minimum effective concentration is the lowest at which a paralysant effect was observed.
Derivatives of 2: 3-dihydro-3-ketobenzo-1 : 4-thiazine



Compound	R′	R″	Nature of preparation	Minimum effective concentration
2: 3-Dihydro-3-ketobenzo-1 : 4-thiazine	H	H	Emulsion	1 : 2,000
6-Amino- ,, ,, ...	NH_2	H	,,	1 : 1,000
6-Amino- ,, ,, HCl	NH_2,HCl	H	Solution	1 : 3,000
6-Acetoamino- ,, ,, ...	$CH_3CO.NH$	H	Suspension	1 : 1,000
6-Fluoro- ,, ,, ...	F	H	,,	1 : 2,000
6-Chloro- ,, ,, ...	Cl	H	Emulsion	1 : 8,000
6-Iodo- ,, ,, ...	I	H	Suspension	1 : 5,000
6-Thiocyano- ,, ,, ...	CNS	H	,,	1 : 1,000
6-Triazo- ,, ,, ...	N_3	H	Emulsion	1 : 6,000
6-Nitro- ,, ,, ...	NO_2	H	,,	1 : 4,000
6-Nitroso- ,, ,, ...	NO	H	,,	1 : 6,000
6-Mercapto- ,, ,, ...	SH	H	,,	1 : 1,000
6-Arsonic Acid ,, ,, ...	H_2AsO_3	H	Suspension	Not obtained
6-Stibonic Acid ,, ,, ...	H_2SbO_3	H	,,	,, ,,
6-Chloromercuri- ,, ,, ...	HgCl	H	,,	,, ,,
6: 7-Dimethoxy- ,, ,, ...	CH_3O	CH_3O	Emulsion	1 : 4,000
6: 7-Dihydroxy- ,, ,, ...	OH	OH	,,	1 : 1,000
Bis-(2: 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl)		H	Suspension	Not obtained

effective concentration was not obtained. At a concentration of 1 : 4,000, the 6-chloromercuri-compound had a powerful depressant action but was not completely paralysant, the 6-arsonic acid showed a slight depressant effect, but the 6-stibonic acid and bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl) had no effect.

The results are summarized in Table I.

A typical kymographic record is shown in Fig. 1. This illustrates the effect of the compound on the helminth. The normal rhythmic movement of the worm in Ringer's solution at 37–38° C. is shown from the beginning of the experiment to the first long stroke on the signal line marked in minutes, when an emulsion of the compound under test replaced the original Ringer's solution. Amphetamine sulphate (1 : 5,000) in Ringer's solution, as employed by Chance and Mansour (1949), replaced the compound after approximately 45 to 50 min., as indicated by the second long stroke on the signal line. Restoration of the movement of the parasite on addition of amphetamine sulphate indicated that the compound was paralysant.

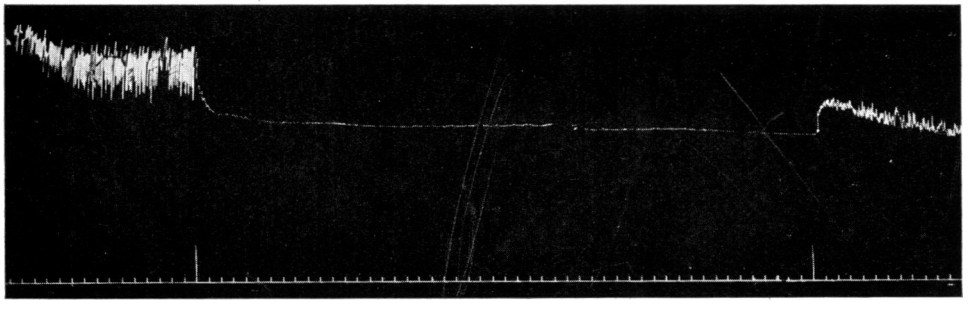


FIG. 1.—Paralysant effect of 6-chloro-2: 3-dihydro-3-ketobenzo-1 : 4-thiazine (1 : 8,000), followed by response to amphetamine sulphate (1 : 5,000).

In the following remarks it should be pointed out that comparisons between solutions, emulsions, and suspensions are not strictly accurate, but may be considered to be approximate (cf. Baldwin, 1943).

The introduction of the amino-group lowers the anthelmintic potency of 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine by one half, but the amino-hydrochloride is three times more effective than the free base ; this difference may, however, be due to free acid in solution. Acetylation does not appreciably alter the potency of the base. This is perhaps surprising, as acetylation might be expected to effect some change.

With the exception of fluorine, which does not affect the activity of the unsubstituted compound, the introduction of halogen atoms (Cl or I) increases its potency. The chloro-derivative is four times as effective as the unsubstituted compound, the order of potency of the halogens being $Cl > I > F$.

The triazo- and nitroso-groups also have a considerable influence on anthelmintic activity. It is particularly interesting to note that the latter group is 1.5 times as effective as the nitro-group.

The thiocyano- and mercapto-groups both halve the activity of the unsubstituted compound.

The anthelmintic potency of the unsubstituted compound is increased twofold by the introduction of two methoxy-groups in the 6 : 7-positions. The corresponding dihydroxy-derivative, however, is only half as effective as the unsubstituted compound ; this was unexpected, as many examples are known where the introduction of a hydroxy-group increases the activity. It is possible that the vicinal position of the hydroxy-groups may be a controlling factor.

The very insoluble derivatives, the 6-arsonic and 6-stibonic acids, the 6-chloromercuri-compound, and bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl) might have had a paralysant effect at concentrations higher than 1 : 4,000 if they had been more soluble.

From the results obtained, it will be seen that the order of potency of the radicals is as follows : Cl>N_3,NO >I >NO_2, 6 : 7-dimethoxy >NH_2,HCl > unsubstituted, F>NH_2, CH_3.CO.NH, CNS, SH, 6 : 7-dihydroxy.

The results of testing 2 : 3-dihydro-3-ketobenzo-1 : 4-thiazine and its derivatives suggest that the constitutional similarities of this compound to phenothiazine and to filicic acid, already noted, have some significance. Although Chance and Mansour (1949) found phenothiazine to be without effect on liver fluke *in vitro*, they did find that *Filix mas* was lethal at a concentration of 1 : 5,000.

Summary

1. 2 : 3-Dihydro-3-ketobenzo-1 : 4-thiazine and derivatives with substituents in the 6- and 6 : 7-positions were tested against liver fluke (*Fasciola hepatica*) *in vitro* and the effects recorded kymographically.

2. All the compounds, which could be tested over a wide range of concentrations, showed paralysant effects.

3. Minimum effective concentrations (MEC) were estimated and it was possible to arrange the substituent groups in the following order of potency (MEC in parentheses) : Cl(1 : 8,000)>N_3, NO(1 : 6,000) >I(1 : 5,000) >NO_2, 6 : 7-dimethoxy (1 : 4,000)>NH_2HCl(1 : 3,000)>unsubstituted compound, F(1 : 2,000)>NH_2, CH_3.CO.NH, CNS, SH, 6 : 7-dihydroxy (1 : 1,000).

4. The 6-amino- and 6-mercapto-derivatives were paralysant at a concentration of 1 : 1,000, and severely reduced the subsequent response to amphetamine sulphate.

5. Owing to the insolubility of the 6-arsonic acid, 6-stibonic acid, 6-chloromercuri-derivatives and bis-(2 : 3-dihydro-3-ketobenzo-1 : 4-thiazin-6-yl), no minimum effective concentration could be obtained, but a 1 : 4,000 suspension of the 6-chloromercuri-derivative produced almost complete paralysis ; the 6-arsonic acid was slightly depressant and the other two derivatives without effect at this concentration.

It is a pleasure to acknowledge our indebtedness to the Principal and Governors of the Heriot-Watt College for providing the laboratory facilities for this investigation and to the Agricultural Research Council for financial aid. Our thanks are also due to Professor Bell for his interest in this work, to Mr. A. W. Patterson for his helpful advice in the biological testing, and to Mr. Soulsby, of the Edinburgh City Abattoir, for the collection of the parasites.

References

Baldwin, E. (1943). *Parasitology*, **35**, 89.
Chance, M. R. A., and Mansour, T. E. (1949). *Brit. J. Pharmacol.*, **4**, 7
Mackie, A., and Raeburn, J. (1952). *J. chem. Soc.*, 787.