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IN VITRO TESTS OF CHEMICAL COMPOUNDS ON *ASCARIS LUMBRICOIDES* AND *FASCIOLA HEPATICA*

BY

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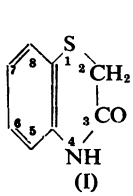
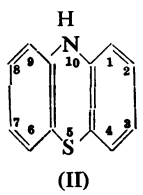
Baldwin (1948) tested over 200 chemical compounds *in vitro* against *Ascaris lumbricoides* ("roundworm") and found that anthelmintic potency was influenced by the presence of certain groups and by the arrangement of groups within the molecule. Mackie and Raeburn (1952a) found that 2:3-dihydro-3-ketobenzo-1:4-thiazine (I) and a number of its derivatives produced a paralysant effect on *Fasciola hepatica* (liver fluke) *in vitro*, and were able to arrange substituent groups in order of potency. Azo-dyestuffs from 6-amino-2:3-dihydro-3-ketobenzo-1:4-thiazine were either inactive or only depressant when tested *in vitro* against liver fluke and the anterior preparations of roundworm (Mackie and Cutler, 1952).

The present paper describes an extension of this work, to include the *in vitro* testing against roundworm and liver fluke, not only of additional derivatives of (I), but also of derivatives of phenothiazine (II), rhodanine (III), and a number of miscellaneous compounds.



(I)



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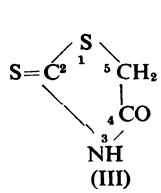


(III)

Derivatives of phenothiazine were tested because it has been so successful as an anthelmintic in veterinary practice, despite certain disadvantages. Compound (III), which contains the -S-CH_2-CO-NH- group (cf. I), might be expected to show some activity, and the testing of its derivatives seemed desirable. The miscellaneous compounds tested included those which were promising against the free-living stages of sclerostomes (Parnell and Mackie, 1952).

From a study of these various compounds, some observations on *in vitro* anthelmintic effect and chemical constitution have been made.

METHODS

Preparative.—Many of the compounds were prepared by known methods, but the 2:3-dihydro-3-ketobenzo-1:4-thiazine derivatives and most of the phenothiazine and rhodanine derivatives were either new compounds or prepared by improved methods (Mackie and Raeburn, 1952b; Mackie and Cutler, 1953, 1954; Mackie and Misra, 1954).

Biological Testing.—The compounds were tested *in vitro*, employing Baldwin's kymographic technique for roundworms (1943) and Chance and Mansour's modification of this method for liver flukes (1949). Anterior preparations of roundworms were used, since they contain the so-called "nerve-ring" (cf. Baldwin, 1943). Certain details of procedure have already been recorded (Mackie and Raeburn, 1952a).

RESULTS

2:3-Dihydro-3-ketobenzo-1:4-thiazine Derivatives.—Mackie and Raeburn (1952b) stated that this compound, and the derivatives described, had practically no effect on intermediate preparations of the roundworm. Nineteen of the 37 compounds of this type, described in the paper cited, and by Mackie and Cutler (1952, 1953), however, showed depressant effects on anterior preparations. Table I summarizes the effects of the new compounds

TABLE I

IN VITRO EFFECT OF 2:3-DIHYDRO-3-KETOBENZO-1:4-THIAZINE DERIVATIVES ON *ASCARIS LUMBRICOIDES* AND ON *FASCIOLA HEPATICA*

(P = paralysant)

Substituent	Effect On	
	Ascaris	*Fasciola*
6-Chloroacetamido-	–	P (1:3,000)
6-Benzoylamido-	–	+
6-Toluene-*p*-sulphonamido-	–	+
6-*p*-Acetamidobenzenesulphonamido-	–	+
6:7-Diethoxy-	–	P (1:1,000)
6-Bromo-	–	P (1:20,000)
		+(1:100,000)
6-Methyl-	++	P (1:5,000)
6-*Tert.*-butyl	–	+
Benzo-1:4-thiazine	P (1:2,000)	P (1:1,000)

active against roundworm or liver fluke, or both (for previous results see Mackie and Raeburn, 1952a; Mackie and Cutler, 1952). In this and other tables, all concentrations are 1:1,000, except for those compounds with paralysant (P) and lethal (L) effects, where the figures in parentheses are minimum effective concentrations. Other effects are indicated as follows: strongly depressant + +; depressant +; little or no effect −.

The derivatives already investigated by Mackie and Raeburn (1952a) had the following effects against *Ascaris*:

Strongly Depressant: 6-acetamido-; 6-chloro-; 6-iodo-; 6-triazo-; 6-nitroso-.

Depressant: Unsubstituted; 6-amino-; 6-nitro-; 6:7-dimethoxy-.

Little or No Effect: 6-amino- hydrochloride; 6-fluoro-; 6-thiocyano-; 6-mercapto-; 6-arsonic acid; 6-stibonic acid; 6-chloromercuri-; 6:7-dihydroxybis-(2:3-dihydro-3-ketobenzo-1:4-thiazin-6-yl).

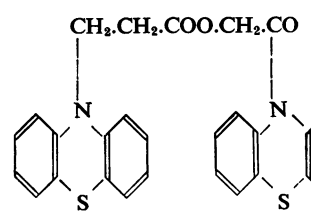
Phenothiazine Derivatives.—The effect of phenothiazone, thionol, and phenothiazine sulphoxide on the liver fluke and roundworm has already been described (Mackie and Raeburn, 1952c; Mackie, 1953). Since the 10-aminoacetylphenothiazines possess interesting pharmacological properties (Dahlbom and Ekstrand, 1951), some of these, and a number of other phenothiazine derivatives, were tested against roundworm and liver fluke *in vitro*. The results are recorded in Table II, except for the salts and esters of β-10-phenothiazinylpropionic acid and compounds weakly active or inactive.

The sodium, α-phenylethylammonium, S-benzyl-isothiuronium, and piperazinium salts of β-10-phenothiazinylpropionic acid were paralysant towards the liver fluke at 1:1,000, but only the sodium salt had any depressant action against the roundworm. Of the esters (*normal* from methyl to octyl; *iso*propyl; *iso-*, *sec.-*, and *tert.*-butyl; *p*-nitrobenzyl, and 10-phenothiazinylcarbonylmethyl; formula IV) only the *iso*butyl ester was active against both helminths: it was strongly depressant towards roundworm and depressant towards liver fluke. The latter helminth was strongly depressed by the *iso*propyl ester, but the *sec.*- and *tert.*-butyl esters produced stimulant effects.

TABLE II
IN VITRO EFFECT OF PHENOTHIAZINE DERIVATIVES ON *ASCARIS LUMBRICOIDES* AND *FASCIOLA HEPATICA*
(P = paralysant; L = lethal)

Compound	Effect On	
	Ascaris	*Fasciola*
Lauth's violet	−	+
Methylene blue	−	L (1:2,000) P (1:3,000)
3:7-Dinitro-10-acetylphenothiazine sulphoxide	+	−
10-Dimethylaminoacetylphenothiazine	+	−
10-Ethylaminoacetylphenothiazine	+	P (1:2,000)
10-Diethylaminoacetylphenothiazine	++	P (1:1,000)
β-10-Phenothiazinylpropionic acid	−	L (1:3,000)
γ-10-Phenothiazinyl-γ-ketobutyric acid	−	++
5′:6′-Dihydro-4′-ketopyridino-(3′:2′:1′-1:10a:10)phenothiazine semicarbazone	+	−

The following phenothiazine derivatives had little or no effect on either helminth: 1:3:7:9(?)-tetrachloro-; 10-methyl-; 3-formyl-10-methyl-; 10-acetyl-; 10-chloroacetyl-; 10-phenylacetyl-; 10-benzoyl-; 10-(2′:4′-dichlorobenzoyl)-; 10-(4′-nitrobenzoyl)-; 10-(3′:5′-dinitrobenzoyl)-; 10-anisoyl; 10-(toluene-*p*-sulphonyl)-; 10-(*p*-acetamidobenzenesulphonyl)-; 10-piperidylacetyl-; 10-morpholinylacetyl-phenothiazines; β-10-phenothiazinylpropionitrile; β-10-phenothiazinylpropion-*p*-toluidide; β-10-phenothiazinylpropion-*p*-bromoanilide; 5′:6′-dihydro-4′-ketopyridino-(3′:2′:1′-1:10a:10)phenothiazine (V), and its benzylidene derivative.



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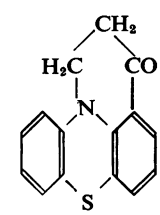

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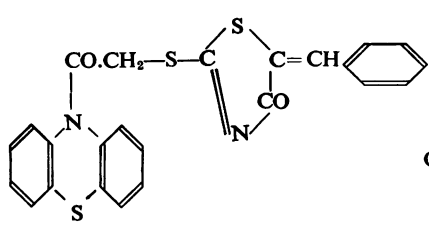

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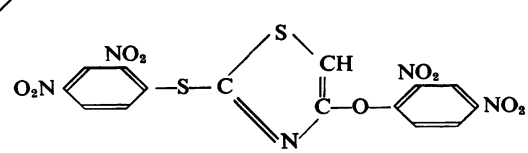

(VII)

Rhodanine Derivatives.—The results of the tests are summarized in Table III.

The following rhodanine derivatives had little or no effect on either helminth: cupric, silver, and mercuric

TABLE III
IN VITRO EFFECT OF RHODANINE DERIVATIVES ON *ASCARIS LUMBRICOIDES* AND *FASCIOLA HEPATICA*

Compound	Effect On	
	Ascaris	*Fasciola*
Rhodanine	+	P (1: 1,000)
3-Allylrhodanine	++	P (1: 1,000)
5-*iso*Nitroso-3-allylrhodanine	P (1: 1,000)	L (1: 2,000)
		P (1: 4,000)
Benzylidenerhodanine	−	L (1: 10,000)
Benzylidene-3-allylrhodanine	+	−
o-Chlorobenzylidenerhodanine	−	L (1: 2,000)
p-Chlorobenzylidenerhodanine	−	P (1: 1,000)
o-Nitrobenzylidenerhodanine	++	L (1: 2,000)
		P (1: 3,000)
Salicylidenerhodanine	+	L (1: 16,000)
p-Hydroxybenzylidenerhodanine	−	L (1: 1,000)
		P (1: 4,000)
Anisylidenerhodanine	−	++
Cinnamylidenerhodanine	−	++
o-Nitrocinnamylidenerhodanine	−	++
Furfurylidenerhodanine	−	L (1: 3,000)
		P (1: 4,000)
10-Methylphenothiazine-3-formylidenerhodanine	+	+
Quinrhodine	++	++

rhodanides; 3-allylrhodanyl-5-*p*-dimethylaminoanil; 4:5′ - dehydro - 4:5′ - bis - 3 - allylrhodanyl - 5 - *p* - dimethylaminoanil; 5:5′ - dehydro - 5:5′ - bis - 3 - allylrhodanine; benzylidenerhodanine 2-phenylhydrazone; piperonylidenerhodanine; *p*-dimethylaminobenzylidenerhodanine; 10-methylphenothiazine-3-formylidene-3-allylrhodanine; S-(10-phenothiazinylcarbonylmethyl)-5′-benzylidenerhodanine (VI); S-(10-phenothiazinylcarbonylmethyl) - 5′ - (*p* - chloro - benzylidene)rhodanine; 2:4-di-(2′:4′-dinitrophenyl)-rhodanine (VII).

Miscellaneous Compounds.—A number of miscellaneous compounds, such as aliphatic and aromatic halogen compounds, allyl compounds, mercury compounds, aromatic amines, phenols, pyridines, etc., were tested *in vitro* against both helminths. Some of the compounds had shown promise as sclerostome larvicides (Parnell and Mackie, 1952).

Of the halogenated compounds tested, carbon tetrabromide was the most promising, since it was six times more lethal (1:6,000 against liver fluke) than the tetrachloride. The tetrabromide was paralysant at 1:10,000, and had about the same potency against *Ascaris* (paralysant at 1:2,000) as the tetrachloride. Of the α-, β-, γ-, and δ-isomers of benzene hexachloride only the δ- was active (paralysed liver fluke at 1:12,000).

Some allyl compounds were effective, especially the iodide (roundworm paralysed at 1:5,000; liver fluke killed at 1:5,000) and the *iso*thiocyanate (liver fluke killed at 1:2,000, paralysed at 1:8,000).

Mercuric chloride and two of its organic derivatives were lethal towards liver fluke (mercuric chloride and ethylmercuric chloride at 1:20,000; ethoxyethylmercuric chloride at 1:16,000), but only ethylmercuric chloride was paralysant towards roundworm (1:2,000).

Diphenylamine was lethal towards liver fluke at 1:20,000 and paralysant towards roundworm at 1:1,000. Its derivatives, and other amino-compounds tested, had little or no effect.

Ortho- and *p*-nitrophenols were active, especially the *p*-isomer, towards liver fluke (lethal at 1:4,000; paralysant at 1:12,000). The *o*-isomer was more potent towards roundworm (paralysant at 1:3,000).

No lethal effects were observed on liver fluke with pyridine, the picolines, 2:6-lutidine, quinoline and isoquinoline, and only α-picoline was paralysant, whereas all paralysed the anterior preparations of roundworm. Pyridine and α-picoline were the most effective against the latter preparation (cf. Baldwin, 1948).

*pseudo*Thiohydantoin had little effect (cf. rhodanine).

Essential oil from *Artemisia maritima*, containing 65% β-thujone and 16% cineol-1:8, was lethal to liver flukes at 1:2,000 and paralysed at 1:3,000.

2 - Amino - 5 - nitrothiazole (Enheptin - T), a remedy for coccidiosis, was tested against both helminths, but had no effect.

Conessine dihydrochloride had no effect on either helminth. Janot and Cavier (1949) indicated that it might be of value in the treatment of helminthiasis, although they found that it did not kill the anterior preparation of roundworm *in vitro*.

Pumpkin seed extract had little or no effect against liver fluke or roundworm *in vitro*, although the seeds have been used against tapeworm.

Sodium azide was very effective against both roundworm (paralysant at 1:10,000) and liver fluke (paralysant at 1:4,000).

DISCUSSION

2:3-Dihydro-3-ketobenzo-1:4-thiazine Derivatives.—Increase in the length of the side-chain in position 6 generally decreased the anthelmintic potency towards *Fasciola hepatica* (cf. Mackie and Raeburn, 1952a; Mackie and Cutler, 1952). The chloroacetamido-derivative was exceptional in this respect.

The order of potency of the radicals was Br> Cl>N_3, NO>I, CH_3>NO_2, 6:7-dimethoxy>

NH_2.HCl, $ClCH_2CONH$>unsubstituted, F>NH_2, CH_3CONH, CNS, SH, 6:7-dihydroxy, 6:7-diethoxy. The order of potency of the halogens was different from that obtained with *A. lumbricoides*. This was not surprising, since the two helminths belong to different phyla.

The result with benzo-1:4-thiazine was interesting, since liver fluke is generally more sensitive than roundworm: nevertheless the absence of the -CH_2-CO- group increased the *in vitro* activity against the latter, but decreased the potency against the former.

Phenothiazine Derivatives.—The introduction of methyl groups into Lauth's violet conferred lethal properties towards liver fluke (cf. methylene blue, which has been used as an anthelmintic). The presence of a second phenothiazine residue in (IV) does not produce any marked effect.

It was surprising that the presence of the -CH_2-CO- group in (V) and its benzylidene derivative had little or no effect on either helminth (cf. Baldwin, 1948).

Rhodanine Derivatives.—The position of the substituent groups in the benzene nucleus, and also any substitution in the 2 and 3 positions in the rhodanine nucleus, had a marked effect on the potency of the corresponding benzylidene-rhodanines.

The presence of a phenothiazine residue in the molecule usually produced little or no effect, and replacement of the -CH_2-CO- group in rhodanine by a quinoline residue (quinrhodine) destroyed the paralysant effect on liver fluke.

Rhodanine had only half the potency of 2:3-dihydro-3-ketobenzo-1:4-thiazine towards liver fluke (Mackie and Raeburn, 1952a), but produced the same effect on roundworm.

Miscellaneous Compounds.—In vivo experiments with carbon tetrabromide would be desirable, not only to ascertain its anthelmintic activity, but also to study its effect on the liver, which is damaged by carbon tetrachloride. The tetrabromide had high larvicidal potency (Parnell and Mackie, 1952).

Rico (1927) found that allyl *iso*thiocyanate paralysed *A. lumbricoides in vitro*, but did not give a minimum effective concentration. It has been suggested as an anthelmintic against lungworm (Mathey, 1945). The allyl compounds were very effective larvicides, especially the iodide and *iso*thiocyanate (Parnell and Mackie, 1952). The three mercury compounds tested had similar larvicidal properties, especially ethylmercuric chloride, which merits further investigation.

Although diphenylamine only paralysed roundworm at 1:1,000, Guthrie (1940) had found it to be effective against ascarids in dogs.

The greater potency of pyridine and its derivatives, and of quinoline and of isoquinoline, on roundworm than on liver fluke is exceptional. It may be that these compounds can penetrate the cuticle comparatively easily.

Summary

1. Derivatives of 2:3-dihydro-3-ketobenzo-1:4-thiazine, phenothiazine and rhodanine, and some miscellaneous compounds, have been tested *in vitro* against *Fasciola hepatica* and the anterior preparation of *Ascaris lumbricoides*.

2. 2:3-Dihydro-3-ketobenzo-1:4-thiazine derivatives showed only depressant effects, when active, towards *A. lumbricoides*, but paralysant effects were observed with some derivatives, particularly the 6-bromo-compound, on liver fluke. Increase in the length of the side-chain usually decreased the anthelmintic potency towards liver fluke.

3. Some of the aminoacetylphenothiazines were active against liver fluke, and β-10-phenothiazinylpropionic acid was lethal.

4. 5-*iso*Nitroso-3-allylrhodanine was the only rhodanine derivative which paralysed *Ascaris*, but some, especially the benzylidene compounds, were lethal to the liver fluke.

5. Amongst the miscellaneous compounds the following were very active: allyl iodide and sodium azide against *Ascaris*; carbon tetrabromide; benzene hexachloride; allyl iodide and *iso*thiocyanate; mercuric chloride; ethylmercuric chloride; ethoxyethylmercuric chloride; diphenylamine, and *p*-nitrophenol against liver fluke.

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