*Brit. J. Pharmacol.* (1948), **3**, 91.

# A STUDY OF ANTHELMINTHIC POTENCY IN RELATION TO CHEMICAL CONSTITUTION

BY

ERNEST BALDWIN

*From the Biochemical Laboratory, Cambridge*

(Received July 7, 1947)

## INTRODUCTION

The results described in this paper are not by any means complete, nor are they in every respect unequivocal, but the author feels justified in publishing them as they stand if only because, in the present state of our knowledge of anthelminthics, almost any serious contribution to the field is likely to be of value to others engaged upon its many problems. The results of biological tests of about 200 synthetic compounds for anthelminthic properties are described, and the author wishes to express his thanks and profound indebtedness to Prof. E. Friedmann, to whom the broad plan of the work was due, and without whose expert chemical work the investigation could not have been undertaken. With a few exceptions, all the substances tested were prepared in this laboratory by Prof. Friedmann, latterly with the assistance of Mrs. B. Berrill.

The aim of the investigation was to discover as much as possible about the relationship between chemical constitution and anthelminthic potency and to find, if possible, new drugs that might be of value in the treatment of nematode infestations. It must be emphasized that, while the term "anthelminthic" as ordinarily employed refers indiscriminately to drugs acting upon parasitic worms of any kind, in the present context it relates specifically to compounds acting upon nematodes.

Recent work on the chemotherapy of microbial diseases has led to the discovery of the sulphonamides, penicillin and a number of other specific antibiotics such as atebrin and plasmoquine, and to the beginnings of a rational approach to chemotherapeutics. In the field of anthelminthics, however, although a considerable amount of work has been done, no comparable new drugs have been discovered, and advances can at present only be expected from the classical method of trial and error. While the comparatively recent introduction of hexylresorcinol in cases of nematode infestation undoubtedly represents a great advance in therapy, especially in ankylostomiasis, the drug most widely used in human ascariasis is still santonin, the active principle of *Artemisia maritima*, var. *anthelminthicum*, a remedy that has come down to us from antiquity.

## CHOICE OF A METHOD

A perusal of the literature indicates that the highest orders of anthelminthic activity may be expected among lactones, some of which have been reported (e.g., by Gluschke, 1932; Rosenmund and Schapiro, 1934) to be even more potent than santonin, itself a lactone. Our own results, however, indicate that the conclusions of earlier workers in the field must be accepted with considerable reserve, for, although we have tested more than 30 lactones of various types, not one has proved to have activity comparable with or even approaching that of santonin. That our results stand in marked contrast to those of other investigators we attribute to the use of different methods of *in vitro* testing. Relatively few tests appear to have been made on nematode-infested hosts, and many of the observations and conclusions reported in the literature have undoubtedly been prejudiced by the use of annelid material for the detection of anthelminthic potency *in vitro*. This method became current after its acceptance by Trendelenberg (1916), who, finding *Ascaris* a very refractory material, resorted to the earthworm as a convenient alternative. "Dass die Regenwürmer in derselben Weise wie die Spülwürmer auf Santonin mit Erregung reagieren würden schein höchst wahrscheinlich," he wrote. But Lamson and Ward (1936) condemned the use of earthworms as "irrational" and showed that "a comparative study of the lethality of 121 widely diversified chemical substances on both earthworms and pig *Ascaris* shows no correlation of action."

The extensive studies of Lamson *et al.* (1935, 1936), which led to the discovery of the valuable

anthelminthic properties of hexylresorcinol, were carried out on intact specimens of pig *Ascaris*, a notable advance in the technique of *in vitro* testing. But whole *Ascaris* is not a reliable test object. As long ago as 1885, von Schroeder studied the influence of various substances upon intact roundworms and was astonished by their resistance to drugs of many kinds. Most surprising of all was his observation that santonin, in spite of its established reputation as an anthelminthic, seemed to have little effect, even when applied in saturated solution over periods of many hours. Trendelenberg (1916) referred to a number of similar observations. Lamson and Brown (1936) likewise were unable to demonstrate that santonin has any significant effect upon intact *Ascaris*, and it was only when its action was tested upon isolated anterior fragments of the worm (Rebello and Rico, 1926; Baldwin, 1943a) that any effect of santonin was conclusively demonstrated *in vitro*. Baldwin came to the conclusion that, in fact, santonin acts by paralysing the central nervous system of the parasite without much affecting the peripheral neuro-muscular systems, so that the worms are, in effect, incoordinated by its action, though not "dead" or even moribund to casual observation.

Baldwin (1943a) used small, tied-off neuromuscular preparations of pig *Ascaris* with an intact cuticular layer. Unlike earthworm and leech preparations, these do not react to any of a large number of compounds known to be devoid of anthelminthic properties though possessing powerful physiological or pharmacological activity of other kinds, but respond nevertheless to most drugs of acknowledged anthelminthic potency. Earthworms and leeches are notoriously sensitive to a wide range of chemical compounds, many of which possess no known anthelminthic activity whatever, and we cannot believe that results obtained with these materials can necessarily be applied without reservation to nematodes.

As was pointed out in an earlier paper (Baldwin, 1943a), our normal test preparations of *Ascaris* fail to respond to acetylcholine, with or without eserine, even at concentrations as high as 1 : 5,000. Following up this interesting phenomenon we later devised an *Ascaris* preparation of which the muscle can be directly exposed to the action of any desired drug (Baldwin and Moyle, 1947) : in our normal test preparations the musculature is surrounded by an intact cuticular layer. With the new "exposed" preparations we found that the isolated muscle is stimulated by acetylcholine at concentrations of the order of $1 : 10^5$–$1 : 10^6$. The response is not demonstrably potentiated by eserine and is, apparently, a pure nicotine action (Baldwin and Moyle, 1948). Earthworm or leech muscle, by contrast, reacts both to the nicotine and muscarine effects of acetylcholine, as well as to adrenaline and a number of other compounds to which isolated *Ascaris* muscle shows no response whatever. To the evidence already adduced by Lamson and Ward (1936) we can thus add direct pharmacological evidence of the unsuitability of annelid material as a test object in anthelminthic studies.

The failure of our normal preparations to respond to acetylcholine shows, not that the muscle is insensitive to this compound, but rather that the cuticle is impermeable to it. There is other evidence to show that the cuticle of *Ascaris* displays highly selective permeability (Trim, 1944). It would appear, therefore, that a satisfactory anthelminthic must possess at least two attributes: it must (*a*) be capable of penetrating the nematode cuticle and (*b*), having so penetrated, have a deleterious action upon nematode tissues. Any sound *in vitro* method for the detection and measurement of anthelminthic activity should therefore be based upon material of nematode origin and with an intact cuticular layer. Earthworm and leech preparations of the kind usually employed possess nothing analogous to the cuticular barrier present in our *Ascaris* preparations, nor is there any guarantee, nor even any *a priori* probability, that their responses to a given drug can furnish any clue to the action of that drug upon the tissues of the nematode. Not only do they belong to an entirely different animal phylum, but the Nematoda as a whole display many unique morphological and physiological features (see, for example, Lapage, 1937).

The use of these *Ascaris* preparations has certain rather sharply defined limitations, however. First, some drugs that fail to gain access to the tissues by way of the cuticle might be able to penetrate by way of the mouth and the alimentary tract, which is occluded in our preparations. Further, our preparations cannot detect directly the anthelminthic potentialities of any compound which, like certain arsenicals (da Costa, 1931), relies upon the tissues or digestive secretions of the host for their evocation. Last, and most important, our preparations are unlikely to detect anthelminthic activity in compounds that act otherwise than upon the neuro-muscular apparatus, but the sharp positive correlation between acknowledged anthelminthic potency and positive *in vitro* responses observed in our earlier experiments (Baldwin,

1943a) would seem to prove that practically all the well-tried, acknowledged anthelminthics do, in fact, act upon the neuro-muscular apparatus. Nevertheless, two important drugs failed to evoke any response—viz., phenothiazine and gentian violet, both of which enjoy a high reputation in therapeutics. The mode of action of these compounds is still somewhat obscure. It is possible that they undergo conversion within the host into potent anthelminthic products, but it is possible also that they act otherwise than upon the neuromuscular mechanisms, possibly upon the reproductive organs. It accordingly follows that our present experiments do not cover the complete field of actual or potential anthelminthics, but in the absence of convenient routine methods for investigating substances that act through other channels the procedure used in the present study seems, in spite of the limitations already enumerated, to offer the most profitable line of immediate approach.

Essentially, therefore, our observations cover the activity only of compounds that act upon the neuromuscular apparatus and gain access thereto by way of the cuticle.

## Procedure

Different anthelminthics undoubtedly act in different ways. Lamson and Ward (1932) have divided anthelminthics into five groups according to whether they (i) cause temporary narcosis or paralysis followed by recovery, (ii) narcosis or paralysis followed by death, (iii) injury to the cuticle, (iv) digestion of the parasites, or (v) unexplained death. All the drugs in general use have one property in common when applied to our test preparations: all lead to paralysis of the anterior region of *Ascaris*. This paralysis is sometimes followed by other phenomena: for example by contracture with phenolic drugs such as hexylresorcinol, thymol, and β-naphthol; occasionally the sequelae are strongly characteristic of individual drugs. We have not been able to rule out the possibility that the paralysis observed in our tests might sometimes be followed by spontaneous recovery, but no such recovery has been observed in the course of the work. Since, therefore, our experiments were mainly of an exploratory nature, we decided to use the common feature of paralysis as our criterion. This is certainly convenient and for the most part probably reliable; its use is, we felt, preferable to setting up different criteria for individual drugs or groups of drugs, none of which might be quite comparable with another in any case.

All the biological tests reported here were carried out on " anterior preparations " by the method already described (Baldwin, 1943a). The drugs were prepared for testing by the methods described in the same paper, and each compound was tested at several different concentrations, each test usually lasting for 30 min. This enabled us to obtain approximate quantitative data descriptive of the potencies of the compounds by awarding " marks " according to the following scale:

| | '*Mark*' |
|---|---|
| Producing paralysis in < 10 min. | ++++ |
| ,, ,, ,, 10 min. | +++ |
| ,, ,, ,, 10–20 min. | ++ |
| ,, ,, ,, 20–30 min. | + |
| ,, ,, ,, > 30 min. | (+) |
| Very weak or doubtful | ± |
| Inactive | − |

In order to obtain more precise data it would have been necessary to work statistically, and the time taken to do so would more than have outweighed the advantages gained by using this relatively rapid *in vitro* technique. The more active and therefore more interesting compounds were tested several times and the results averaged, but two or three tests were carried out with every compound. As a precaution against unwilful selection of evidence, each substance was allotted a code number and tested before its identity was disclosed.

As standards of reference we carried out numerous experiments with a number of well-known drugs, the results of which are set forth in Table I in terms of the concentration of each required to produce complete paralysis of the anterior region in 20–30 min.

TABLE I

ACTIVITIES OF SOME STANDARD COMPOUNDS

| Compound | Concentration producing paralysis in 20–30 min. | Nature of preparation |
|---|---|---|
| Santonin | 1 : 100,000 | Solution |
| Hexylresorcinol | 1 : 10,000 | ,, |
| *p*-Benzylphenyl carbamate | 1 : 5,000 | ,, |
| Thymol | 1 : 5,000 | ,, |
| β-Naphthol | 1 : 5,000 | ,, |
| Oil of *Chenopodium* | 1 : 5,000 | Emulsion |
| Carbon tetrachloride | 1 : 2,000 | ,, |
| Tetrachloroethylene | 1 : 2,000 | ,, |
| Chlorbutol | 1 : 1,000 | Solution |

## The Action of Santonin

Santonin has been used in the treatment of certain nematode infestations since the dawn of history and its efficacy in practice has never been seriously questioned. But to this day we do not

know what group or radical is responsible for its outstanding activity. Trendelenberg (1916) discovered that previously denervated fragments of earthworm muscle remain practically motionless in Ringer's solution but that, on the addition of santonin, their tone rises sharply, leading to the onset of powerful rhythmic contractions which persist as long as santonin is present in the medium. The same effect, which is freely reversible, was also evoked by *desmotropo*santonin, by santonin oxime and by tetrahydrosantonin, all of which contain unmodified the lactone ring present in santonin itself. Santoninic acid, in which the lactone ring is opened, was quite inert, even in relatively concentrated solutions, and Trendelenberg therefore concluded that santonin owes its stimulant action to the presence of its lactone ring. In support of this conclusion he pointed out that other lactones—e.g., pilocarpine and coumarine, similarly lose their characteristic effects if the lactone ring is opened.

Oswald (1924) pointed out that the physiological activity of many substances is destroyed by the introduction of a carboxyl group into the molecule and suggested that the inactivity of santoninic acid might be due to the presence of its free carboxyl radical rather than to the absence of the lactone ring. In support of Trendelenberg's view, Josephson (1931) found that santoninic amide, like the acid, is inert. On the other hand, Oshika (1921) found that the ethyl esters of santoninic and santonic acids were both active towards earthworm muscle, the corresponding free acids being inert. It would therefore appear that, at least as far as earthworm muscle is concerned, the activity of santonin cannot be due solely to the lactone ring.

Caius and Mhaskar (1923), working on patients infested with *Ascaris*, administered a number of santonin derivatives and determined the percentages of cases cured by one test treatment. Some of their results are recorded in Table II. The behaviour of santoninic acid might, of course, be due to the ease with which, under acid conditions such as prevail in the stomach, the free acid reverts to the lactone.

But lactonization is not possible in either santonic (unless perhaps after previous reduction) or in santonous acid, both of which were active. Caius and Mhaskar came therefore to the conclusion that the active centre of the santonin molecule is the ketonic group of the unsaturated ring. This is present in santonin, santoninic acid and santonic acid. Santonous acid is usually figured in its enolic form, but that it can undergo ketonization is evident since it forms an oxime with hydroxylamine and a hydrazone with phenylhydrazine. *Desmotropo*santonin, which forms neither an oxime nor a hydrazone, does not ketonize and is inactive; santonone, also inert, likewise possesses no ketonic grouping.

Two further possibilities come to mind. Anthelminthic activity might be attributable to the presence of an unsaturated ring. Lamson *et al.* (1935), for example, found that 4–phenylphenol was very much more active than 4–*cyclo*hexylphenol; Lautenschläger (1921) and Rosenmund and Schapiro (1934) similarly found great increases in physiological activity among lactones when phenyl groups were introduced, and we too have found that the introduction of a phenyl radical leads to great increases in anthelminthic activity in several groups of substances, notably among lactones, thiazoles, and pyridines. But tetrahydrosantonin (*272*), although fully saturated, proved in our tests to be as active as santonin itself.

A further possibility, that the angular methyl group present in santonin and all its active derivatives may be involved, seems worth investigating in view of the importance of groups of this kind in determining the action of sex hormones. The masculinizing hormones (androsterone, testosterone) possess two angular methyl radicals and the oestrogenic hormones (oestrone, oestradiol) one only, while progesterone, which suppresses some of the characteristic features of feminine

TABLE II

(Results of Caius and Mhaskar, 1923)

| Compound | % patients cured by one test treatment |
|---|---|
| Santonin | 80 |
| Santoninic acid | 73 |
| Santonic acid | 84 |
| Santonous acid | 67 |
| *Desmotropo*santonin | 0 |
| Santonone | 0 |

TABLE III

| Active as santonin | Inactive |
|---|---|
| Santonin (*31*) | *d-Desmotropo*santonin (*223*) |
| *β-Santonin (*32*) | *l-Desmotropo*santonin (*224*) |
| Santoninic acid (*225*) | *l-Desmotropo*-β-santonin) (*263*) |
| Tetrahydrosantonin (*272*) | *d*-Santonous acid (*261*) |
| Santonin oxime (*239*) | *l*-Santonous acid (*241*) |
| | Santonic acid (*226*) |
| | Ethyl santonate (*256*) |
| | Allantolactone (*34*) |
| | ψ-Santonin (*33*) |

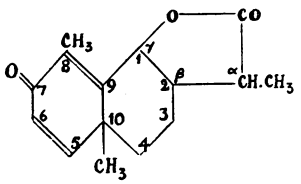
* Isomeric with santonin (Clemo, 1934); activity *ca.* 20% that of santonin (Baldwin, 1943b).

sexuality while emphasizing others, resembles the androgens in containing two such groups.

We have not attempted an exhaustive survey of the structure-activity relationships of the santonin group, but the results listed in Table III, which summarize the results of our experiments on these substances, are of some interest. In all the active compounds with the exception of santoninic acid, which probably owes its activity to the ease with which it reverts to santonin in aqueous solution, we find three structural features in common. These are:

(*a*) an intact γ–lactone ring,
(*b*) a double bond at position 7, and
(*c*) an angular methyl group at position 10.

The numbering, which is arbitrary, refers to the following structure:




One or more of these characters is absent from all the inert derivatives. The inactivity of santonic acid is evidently not due simply to the fact that it contains a free carboxyl radical, for its ethyl ester also is inert; probably, therefore, the inactivity of the acids cannot be attributed solely to their free carboxyl groups.

Although there is insufficient evidence to prove that the methyl group at position 10 is essential, it seems very probable indeed that the other two features must both be present if the substance concerned is to possess anthelminthic action. We may therefore suspect that santonin owes its efficiency to the simultaneous presence of *both* these features rather than to the presence of either alone. One of the difficulties that stand in the way of the further pursuit of the santonin problem is the considerable doubt that still attaches to the structure of some members of the group. We may tentatively suggest however that both the lactonic and the ketonic groups contribute to the total anthelminthic potency of the santonin molecule and of its active derivatives. As we shall show in later sections of this paper, both these groupings possess potentialities for anthelminthic activity, and the outstanding potency of santonin itself may perhaps be due to the unique manner in which these two active centres are linked together. Finally, though at present there is insufficient evidence to show that the angular methyl group plays any part in determining the activity of members of this group of compounds, this is a possibility that cannot at present be eliminated and would probably repay further investigation.

Clearly, the santonin problem is still a long way from its solution and much more work is needed before any final conclusions can be drawn.

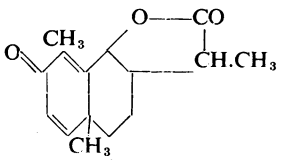
Taking santonin as our starting point it seemed desirable to discover whether its powerful activity is shared by other unsaturated ketones on the one hand, or by lactones on the other. Our experiments on these groups of compounds are presented in the next two sections; the remaining sections are devoted to certain other groups—viz., phenols, thiazoles, pyridines, and miscellaneous substances.

## Results and Discussion

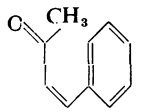
In the Tables (IV–XXIII) recording the experimental results the name or formula of each compound is followed by the "mark" awarded, the concentration tested and the code number, thus: $C_6H_5CO.CH_3$ ++ 1:1,000 *105*.

### 1. *Aliphatic-aromatic ketones*

Benzylidene acetone can be derived (on paper) by partial "dissection" of santonin:



*santonin*



*benzylidene acetone*

This compound and a group of related ketones (Table IV, A) proved to possess appreciable anthelminthic potency and attempts were made to increase this by chemical manipulation of the molecules. Activity here seems to be mainly associated with the ketonic group, the presence of one or more unsaturated linkages in the side-chain increasing the activity somewhat. Replacement of the phenyl by a furfuryl radical (*86*) reduced activity considerably.

Notable increases in activity were obtained by the introduction of alkyloxy radicals into position 4 of benzylidene acetone but not of acetophenone (Table IV, B). This suggests that the unsaturated side-chain of benzylidene acetone carries greater anthelminthic potentialities than the saturated side-chain of acetophenone. Among homologous alkyloxy derivatives maximal activity was found in the 4-ethoxy compound, a fall in potency occurring when the length of this radical was further increased. The influence of the position

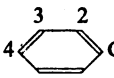
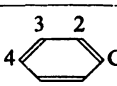
TABLE IV

| | | | | |
|---|---|---|---|---|
| A | $C_6H_5CO.CH_3$ | ++ | 1 : 1000 | *105* |
| | $C_6H_5CH_2CO.CH_3$ | + | 1 : 1000 | *80* |
| | $C_6H_5CH:CH.CO.CH_3$ | ++ | 1 : 1000 | *92* |
| | $C_6H_5CH:CH.CH:CH.CO.CH_3$ | ++ | 1 : 1000 | *120* |
| | $C_4H_3O.CH:CH.CO.CH_3$ | (+) | 1 : 1000 | *86* |
| B | $C_6H_5CO.CH_3$ | ++ | 1 : 1000 | *105* |
| | $4\text{-}CH_3O.C_6H_4CO.CH_3$ | ++ | 1 : 1000 | *95* |
| | $4\text{-}C_2H_5O.C_6H_4CO.CH_3$ | ++ | 1 : 1000 | *93* |
| | $C_6H_5CH:CH.CO.CH_3$ | ++ | 1 : 1000 | *92* |
| | $4\text{-}CH_3O.C_6H_4CH:CH.CO.CH_3$ | ++ | 1 : 2000 | *113* |
| | $4\text{-}CH_3CH_2O.C_6H_4CH:CH.CO.CH_3$ | + | 1 : 5000 | *81* |
| | $4\text{-}CH_3CH_2CH_2O.C_6H_4CH:CH.CO.CH_3$ | (+) | 1 : 5000 | *82* |
| | $4\text{-}(CH_3)_2CHO.C_6H_4CH:CH.CO.CH_3$ | ± | 1 : 2000 | *89* |
| | $4\text{-}CH_2:CH.CH_2O.C_6H_4CH:CH.CO.CH_3$ | + | 1 : 2000 | *84* |
| | $4\text{-}CH_3CH_2CH_2CH_2O.C_6H_4CH:CH.CO.CH_3$ | + | 1 : 2000 | *83* |
| C | $4\text{-}CH_3O.C_6H_4CH:CH.CO.CH_3$ | ++ | 1 : 2000 | *113* |
| | $3\text{-}CH_3O.C_6H_4CH:CH.CO.CH_3$ | + | 1 : 2000 | *100* |
| | $2\text{-}CH_3O.C_6H_4CH:CH.CO.CH_3$ | (+) | 1 : 2000 | *98* |
| | $4\text{-}C_2H_5O.C_6H_4CH:CH.CO.CH_3$ | + | 1 : 5000 | *81* |
| | $3\text{-}C_2H_5O.C_6H_4CH:CH.CO.CH_3$ | + | 1 : 2000 | *99* |
| | $2\text{-}C_2H_5O.C_6H_4CH:CH.CO.CH_3$ | (+) | 1 : 2000 | *97* |

of the alkyloxy group was next determined, maximal and minimal activities being found for positions 4 and 2 respectively (Table IV, C). The effects of pairs of alkyloxy radicals are not additive in the acetophenone series, and in the benzylidene series are actually antagonistic (Table V, A). The introduction of allyl groups served only to diminish the existing activity (Table V, B).

The hitherto unsubstituted methyl group of the ketonic side-chain was now modified by the introduction of further $CH_3$ or $C_2H_5$ radicals. This

TABLE V

| A. Acetophenone derivatives | | | |
|---|---|---|---|
| |  | | |
| 4-ethoxy | + | 1 : 2000 | *93* |
| 2 : 4-diethoxy | + | 1 : 2000 | *218* |
| Benzylidene acetone derivatives | | | |
| |  | | |
| 4-methoxy- | ++ | 1 : 2000 | *113* |
| 4-ethoxy- | + | 1 : 5000 | *81* |
| 3 : 4-dimethoxy- | − | 1 : 2000 | *91* |
| 3 : 4-methylenedioxy- | (+) | 1 : 2000 | *85* |
| 3-methoxy-4-ethoxy | ± | 1 : 2000 | *88* |
| 2-methoxy-4-ethoxy | (+) | 1 : 2000 | *104* |

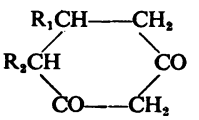
| B. Benzylidene acetone derivatives | | | | Same compound without allyl | | |
|---|---|---|---|---|---|---|
| 2-methoxy-4-allyl- | ± | 1 : 2000 | *109* | (+) | 1 : 2000 | *98* |
| 2-ethoxy-4-allyl | − | 1 : 2000 | *110* | (+) | 1 : 2000 | *97* |
| 4-ethoxy-3-allyl | ± | 1 : 2000 | *106* | + | 1 : 5000 | *81* |
| 2-methoxy-4-ethoxy-3-allyl | − | 1 : 2000 | *108* | (+) | 1 : 2000 | *104* |
| 3-methoxy-4-ethoxy-5-allyl | − | 1 : 2000 | *107* | ± | 1 : 2000 | *88* |

TABLE VI

| | | | |
|---|---|---|---|
| $4\text{-}CH_3O.C_6H_4CH_2CH_2.CO.CH_3$ | (+) | 1 : 2000 | *121* |
| $4\text{-}CH_3O.C_6H_4CH_2CH_2.CO.CH_2CH_3$ | + | 1 : 2000 | *122* |
| $4\text{-}CH_3O.C_6H_4CH_2CH_2.CO.CH_2CH_2CH_3$ | (+) | 1 : 2000 | *123* |
| $4\text{-}CH_3O.C_6H_4CH:CH.CO.CH_3$ | ++ | 1 : 2000 | *113* |
| $4\text{-}CH_3O.C_6H_4CH:CH.CO.CH_2CH_3$ | ++ | 1 : 2000 | *115* |
| $4\text{-}CH_3O.C_6H_4CH:CH.CO.CH_2CH_2CH_3$ | (+) | 1 : 2000 | *117* |
| $4\text{-}C_2H_5O.C_6H_4CH:CH.CO.CH_3$ | + | 1 : 5000 | *81* |
| $4\text{-}C_2H_5O.C_6H_4CH:CH.CO.CH_2CH_3$ | + | 1 : 5000 | *112* |
| $4\text{-}C_2H_5O.C_6H_4CH:CH.CO.CH_2CH_2CH_3$ | insoluble | | *111* |
| $4\text{-}Cl.C_6H_4CH:CH.CO.CH_3$ | +++ | 1 : 2000 | *101* |
| $4\text{-}Cl.C_6H_4CH:CH.CO.CH_2CH_3$ | insoluble | | *102* |
| $C_4H_3O.CH:CH.CO.CH_3$ | (+) | 1 : 1000 | *86* |
| $C_4H_3O.CH:CH.CO.CH_2CH_3$ | ++ | 1 : 2000 | *114* |

TABLE VII

A. Cyclohexadiones



| $R_1$ | $R_2$ | | | |
|---|---|---|---|---|
| $C_6H_5$ | H | – | 1 : 1000 | *118* |
| $C_6H_5$ | CN | – | 1 : 1000 | *124* |
| $C_6H_5$ | $CO_2H$ | – | 1 : 1000 | *103* |
| $4\text{-}CH_3O.C_6H_4$ | H | – | 1 : 1000 | *126* |

B. α-Ketonic acids

| | | | |
|---|---|---|---|
| $C_6H_5CH:CH.CO.COOH$ | – | 1 : 1000 | *96* |
| $4\text{-}CH_3O.C_6H_4CH:CH.CO.COOH$ | – | 1 : 1000 | *125* |
| $C_6H_5CH:CH.CH:CH.CO.COOH$ | – | 1 : 1000 | *116* |
| $C_4H_3OCH:CH.CO.COOH$ | – | 1 : 1000 | *87* |

was done with derivatives of anisylacetone, 4–methoxybenzylidene acetone, 4–ethoxybenzylidene acetone, 4–chlorobenzylidene acetone, and furfurylidene acetone. The results are shown in Table VI. There is a slight but probably significant increase of activity with the addition of one $-CH_2-$ unit, followed by a decline when a second such group is introduced.

Since cyclization of the side-chain of physiologically active substances sometimes leads to important increases in their potency, and we had so far failed to obtain activities greater than about + at 1 : 5,000, a series of cyclic diketones was tested: these may, for our purposes, be regarded as derived from cinnamylidene acetone (*120*). These diones proved to be completely inert and so too did a series of α-keto-acids corresponding to some of the parent ketones (Table VII).

The first halogenated products tested seemed to offer a prospect of greater activities, but no further increases could be obtained by the further introduction of alkyloxy radicals (Table VIII). The influence of phenolic (OH) groupings was next studied. Numerous phenols are known to possess important anthelminthic properties and some of these, notably thymol, β–naphthol, and hexylresorcinol have found extensive employment in clinical medicine and veterinary science. The first attempts in this direction were somewhat discouraging, for the introduction of (OH) at position 4 in acetophenone, and at position 2 in benzylidene acetone, completely destroyed such activity as was formerly present (Table IX). Further work, however, brought to light some interesting phenomena. Whereas the introduction of (OH) at position 4 in acetophenone resulted in inactivation, substitution

TABLE VIII

| | | | |
|---|---|---|---|
| $C_6H_5CO.CH_3$ | ++ | 1 : 1000 | *105* |
| $C_6H_5CO.CH_2Br$ | +++ | 1 : 2000 | *171* |
| | + | 1 : 5000 | |
| $4\text{-}CH_3O.C_6H_4CO.CH_3$ | ++ | 1 : 1000 | *95* |
| $4\text{-}CH_3O.C_6H_4CO.CH_2Cl$ | ++ | 1 : 1000 | *181* |
| $C_6H_5CH:CH.CO.CH_3$ | ++ | 1 : 1000 | *92* |
| $4\text{-}Cl.C_6H_4CH:CH.CO.CH_3$ | +++ | 1 : 2000 | *101* |

TABLE IX

| | | | |
|---|---|---|---|
| $C_6H_5CO.CH_3$ | ++ | 1 : 1000 | *105* |
| $4\text{-}HO.C_6H_4CO.CH_3$ | – | 1 : 1000 | *94* |
| $2\text{-}HO.C_6H_4CO.CH_3$ | ++ | 1 : 2000 | *213* |
| $2:4\text{-}(HO)_2C_6H_3CO.CH_3$ | – | 1 : 2000 | *127* |
| $2\text{-}C_2H_5O.C_6H_4CO.CH_3$ | +++ | 1 : 1000 | *228* |
| | (+) | 1 : 2000 | |
| $4\text{-}C_2H_5O.C_6H_4CO.CH_3$ | ++ | 1 : 1000 | *93* |
| $2:4\text{-}(C_2H_5O)_2.C_6H_3CO.CH_3$ | + | 1 : 2000 | *218* |
| $2\text{-}C_2H_5O,\ 4\text{-}HO.C_6H_3CO.CH_3$ | – | *ca* 1 : 2000 | *217* |
| $2\text{-}HO,\ 4\text{-}C_2H_5O.C_6H_3CO.CH_3$ | +++ | 1 : 1000 | *215* |
| | (+) | 1 : 2000 | |
| $C_6H_5CH:CH.CO.CH_3$ | ++ | 1 : 1000 | *92* |
| $2\text{-}HO.C_6H_4CH:CH.CO.CH_3$ | – | 1 : 1000 | *119* |
| $2\text{-}CH_3O.C_6H_4CH:CH.CO.CH_3$ | (+) | 1 : 2000 | *98* |
| $2\text{-}C_2H_5O.C_6H_4CH:CH.CO.CH_3$ | + | 1 : 2000 | *97* |

at position 2 approximately doubled the activity, while simultaneous hydroxylation at positions 4 and 2 yielded an inert product. But the introduction of (OH) at position 2 in 4–ethoxyacetophenone had little influence, nor was there much change when, instead of (OH), a second ethoxy radical was placed in position 2. Further evidence of the inactivating influence of the 4–hydroxy group was obtained with 2–ethoxy–4–hydroxyacetophenone (*217*). Thus a phenolic (OH) abolishes the activity of acetophenone when placed in the 4–position but tends to increase it when placed in position 2, *unless* the latent potentialities of the substance have already been evoked by the substitution of an alkyloxy radical in position 4, when the effects of the two substituents are not additive. Essentially the same phenomena were observed in a series of hydroxylated derivatives of halogenated acetophenones (Table X). Benzylidene acetone, unlike acetophenone, is inactivated by hydroxylation at position 2.

Thus no combination of the potentiating radicals used in these experiments raised the activity of the compounds beyond a value of about + at 1 : 5,000. When, by the introduction of one potentiating grouping a relatively high order of potency had been developed, the addition of a

TABLE X

| | | | |
|---|---|---|---|
| $C_6H_5CO.CH_3$ | ++ | 1 : 1000 | *105* |
| $C_6H_5CO.CH_2Br$ | +++ | 1 : 2000 | *171* |
| | + | 1 : 5000 | |
| $4\text{-}HO.C_6H_4CO.CH_2Cl$ | – | 1 : 1000 | *183* |
| $2\text{-}HO.C_6H_4CO.CH_2Cl$ | + | 1 : 5000 | *200* |
| $2:4\text{-}(HO)_2C_6H_3CO.CH_2Cl$ | + | 1 : 1000 | *170* |
| $3:4\text{-}(HO)_2C_6H_3CO.CH_2Cl$ | – | 1 : 1000 | *168* |
| $4\text{-}CH_3O.C_6H_4CO.CH_2Cl$ | ++ | 1 : 1000 | *181* |
| | + | 1 : 2000 | |
| $2\text{-}HO,\ 4\text{-}C_2H_5O.C_6H_3CO.CH_2Cl$ | + | 1 : 2000 | *203* |

TABLE XI

| | | | | | |
|---|---|---|---|---|---|
| 2 : 4-$(HO)_2C_6H_3CO.CH_3$ | | | − | 1 : 2000 | *127* |
| 2 : 4-$(HO)_2C_6H_3CO.CH_2CH_3$ | + | 1 : 2000 | ++ | 1 : 1000 | *229* |
| 2 : 4-$(HO)_2C_6H_3CO.CH_2CH_2CH_3$ | + | 1 : 2000 | +++ | 1 : 1000 | *230* |
| 2 : 4-$(HO)_2C_6H_3CO.CH_2CH_2CH_2CH_3$ | +++ | 1 : 2000 | (+) | 1 : 5000 | *237* |
| 2 : 4-$(HO)_2C_6H_3CO.CH_2CH_2CH_2CH_2CH_3$ | (+) | 1 : 10,000 | ++ | 1 : 5000 | *240* |
| 2 : 4-$(HO)_2C_6H_3CO.CH_2CH_2CH_2CH_2CH_2CH_3$ | − | 1 : 10,000 | + | 1 : 5000 | *242* |
| 2 : 4-$(HO)_2C_6H_3CH_2CH_2CH_3$ | | | + | 1 : 2000 | *259* |
| 2 : 4-$(HO)_2C_6H_3CH_2CH_2CH_2CH_2CH_2CH_3$ | | | + | 1 : 10,000 | *5* |
| 2 : 4-$(HO)_2C_6H_3CH_2CH_2CH_2CH_2CH_2CH_2CH_3$ | | | ++ | 1 : 10,000 | *258* |

TABLE XII

| | | | |
|---|---|---|---|
| $C_6H_5CO.C_6H_5$ | (+) | 1 : 1000 | *220* |
| 4-$HO.C_6H_4CO.C_6H_5$ | ++ | 1 : 1000 | *132* |
| 4-$C_2H_5O.C_6H_4CO.C_6H_5$ | (+) | 1 : 2000 | *133* |
| 2-$HO.C_6H_4CO.C_6H_5$ | − | 1 : 1000 | *219* |
| 2-$C_2H_5O.C_6H_4CO.C_6H_5$ | − | *ca* 1 : 1000 | *227* |

second potentiating radical was liable to diminish rather than increase it.

A series of alkylated resorcinyl ketones was now examined. Resorcinyl methyl ketone itself was inert, but it was thought that the introduction of longer alkyl radicals into the side-chain might reveal some latent anthelminthic potency, much as the bacterial (Leonard, 1924) and the anthelminthic (Lamson, Brown, and Ward, 1935) potentialities of resorcinol are augmented. The results (Table XI) show that marked anthelminthic properties appear in resorcinyl ethyl ketone (*229*), increase in intensity as the alkyl chain is lengthened, and reach a maximum in the valeryl ketone (*240*). Further lengthening of the chain is attended by diminishing activity. These results run roughly parallel to those obtained by Lamson, Brown, and Ward (1935) for the alkyl resorcinols, but the ketones show appreciably less activity than the corresponding non-ketonic alkylresorcinols (Table XI).

Finally, a group of substances derived from benzophenone and containing two aromatic rings was investigated. The results (Table XII) contain little of interest beyond showing that the effects of hydroxylation at positions 2 and 4 are precisely opposite to those observed in the acetophenone series (Table IX). The effect of the ethoxy radical, as in benzylidene acetone, is greater in position 4 than in 2.

The conclusions reached regarding the behaviour of these ketones may be summarized as follows. (i) The ketonic group of aliphatic-aromatic ketones carries potentialities for anthelminthic activity which approach nearly to those of thymol and β–naphthol. (ii) This latent potency can be evoked by substitution of alkyloxy or phenolic radicals, in the benzene ring, or by halogenation. (iii) The influence of these potentiating radicals varies from one group of ketones to another and with the position of substitution. Finally (iv), the effects of these potentiating radicals are not additive and may, in fact, be antagonistic in certain compounds.

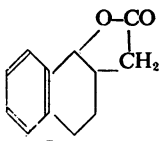
## 2. *Lactones*

Since Trendelenberg (1916) came to the conclusion that the anthelminthic properties of santonin are due to its lactone ring, many new lactones have been prepared and tested, for example by Lautenschläger (1921), von Oettingen (1929), Gluschke (1932), and Rosenmund and Schapiro (1934). Activity greater than that of santonin has been claimed for some of these products.

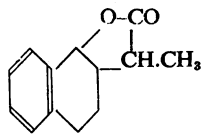
Lautenschläger (1921) tested a series of γ–lactones (γ–butyro–lactone, γ–valerolactone, paraconic acid lactone and a number of sugar lactones and betaines). The simpler compounds had little action upon earthworm muscle, intact earthworms or the cardiac muscle of the frog, but great increases in activity with respect to these materials were obtained by the introduction of phenyl radicals. Phenyl butyrolactone and phenyl paraconic acid lactone were about half as active as santonin upon earthworm preparations, while a third product, phthalide (α : β–benzbutyrolactone), was as active as santonin itself. A considerable number of related compounds also showed a high order of activity.

von Oettingen (1929) tested butyrolactone, valerolactone, valerolactone carboxylic acid, *iso*-caprolactone, α- and β-angelica lactones and the dilactone of acetone di-acetic acid, all of which had a more or less depressant action upon isolated earthworm muscle. This contrasts sharply with the powerful stimulant action of santonin upon the same tissue (Trendelenberg, 1916). von Oettingen found that activity was greatly increased by the introduction of methyl or carboxyl groups into the lactone ring or by the introduction of a double bond. At concentrations of 0.04 *M*, β-angelica lactone, valerolactone carboxylic acid and the dilactone were as active as santonin, though less so in more dilute solutions. von Oettingen and Garcia (1929) then showed that β-angelica lactone removed all the roundworms from 7 out of 10 infested cats—one of the few published experiments in which lactones other than santonin have been tested in infested hosts.

Gluschke (1932) prepared a number of lactones and claimed that certain lactones derived from α-tetralone, and nearly related to santonin itself, equalled or surpassed the latter in activity—viz., syntonins *a* and *b* (I and II). In these experiments again the test object consisted of earthworm muscle.




I

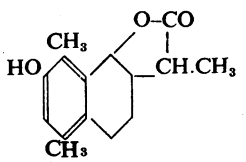



II

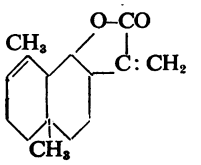
Rosenmund and Schapiro (1934), following up the work of Lautenschläger (1921), prepared a series of substituted γ-butyrolactones and tested their activity upon leech muscle and intact specimens of *Ascaris*. They state that the *o*-cresol ether and anisole derivatives of γ-butyrolactone were from 3 to 4 times more active than santonin and that there was, moreover, a close parallel between the responses of leech muscle and of the intact roundworms. Yet, as has been pointed out, santonin itself has little or no evident action upon intact *Ascaris* (von Schroeder, 1885 ; Lamson *et al.*, 1935, 1936). It is accordingly difficult to assess the validity of Rosenmund and Schapiro's observations and conclusions.

The variety of lactones which might have been made and tested is so large that we felt it necessary to restrict the scope of our work to some extent. We have however tested several groups of lactones in which a high order of activity was to be anticipated from the results of our predecessors in this field, together with a number of miscellaneous lactones representing a considerable variety of chemical types.

α-Angelica lactone (*231*) and its anisal derivative (*234*), the dilactone of acetone diacetic acid (*238*), and copper glycine (*236*), which has interesting structural resemblances to the dilactone, were tested at a concentration of 1:1,000. Only α-angelica lactone showed any activity (+ at 1 : 1,000) although, in view of the claims of von Oettingen (1929) and Rosenmund and Schapiro (1934), the anisal compound if no other might have been expected to show a very high order of activity indeed. More nearly related to santonin were the *d*- and *l*-*desmotropo*santonins (*223, 224* ; III) and *l*-*desmotropo*-β-santonin (*263*). These compounds are very insoluble and were tested in saturated solutions. Had they possessed activity in any way comparable with that of santonin it would, according to our estimates of their solubilities, have been detectable in our experiments, but uniformly negative results were obtained. Of




III




IV

particular interest in relation to Gluschke's (1932) claims was an observation that alantolactone (*34* ; IV), a substance even more closely allied to santonin than are the syntonins, was totally inert when tested at 1:2,000. According to von Oettingen (1929) the presence of a double bond augments the activity of the lactone ring so that a particularly high order of potency might have been expected here.

A series of derivatives of γ-butyrolactone was also examined and here it was found that the activity of γ-phenylbutyrolactone itself approaches that of santonin in fairly high concentrations. But the two substances are in no way comparable at lower concentrations, phenylbutyrolactone giving dubious or slight activity at 1:10,000 whereas santonin is still powerfully active at 1 : 100,000. Alkyloxylation removed the activity. Our results with this series of compounds (Table XIII) are entirely at variance with those of Rosenmund and Schapiro (1934).

In confirmation of an earlier observation (Baldwin, 1943a) coumarine (V) was found to be active and umbelliferone (7-hydroxycoumarine) inert. 3-Hydroxycoumarine and 7-ethoxycoumarine were also tested. Chromone (VI), 2-cou-

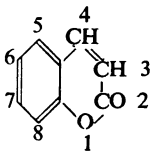
TABLE XIII

| | | | |
|---|---|---|---|
| Phenylbutyrolactone | +++ | 1 : 5000 | *260* |
| 4-Ethoxyphenylbutyrolactone | ± | 1 : 1000 | *262* |
| 2-Methyl-4-ethoxyphenylbutyrolactone | ± | 1 : 1000 | *266* |
| 3-Methyl-4-ethoxyphenylbutyrolactone | ± | saturated | *267* |
| 2-Ethoxy-5-methylphenylbutyrolactone | − | 1 : 1000 | *264/5* |
| 2-Naphthylbutyrolactone | − | saturated | *268* |

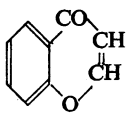
TABLE XIV

| | | | |
|---|---|---|---|
| Coumarine (V) | + | 1 : 1000 | *74* |
| 3-Hydroxycoumarine | − | 1 : 2000 | *211* |
| 7-Hydroxycoumarine | − | 1 : 1000 | *78* |
| 7-Ethoxycoumarine | (+) | 1 : 2000 | *210* |
| Chromone (VI) | ++ | 1 : 1000 | *245* |
| | (+) | 1 : 2000 | |
| Ethyl chromone-2-carboxylate | (+) | 1 : 1000 | *257* |
| 2-Coumaranone (VII) | ++ | 1 : 1000 | *233* |
| | ± | 1 : 2000 | |
| 3-Coumaranone (VIII) | +++ | 1 : 2000 | *186* |
| | + | 1 : 5000 | |
| 6-Hydroxy-3-coumaranone | − | 1 : 2000 | *191* |
| 6-Ethoxy-3-coumaranone | − | 1 : 2000 | *190* |

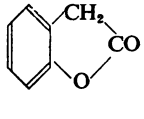
maranone (VII), and 3-coumaranone (VIII) were also examined, together with some of their derivatives, in view of their relation to phthalide (IX) which, according to Lautenschläger (1921), is as active as santonin. The results obtained with this group of compounds are listed in Table XIV. It



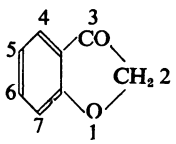
V



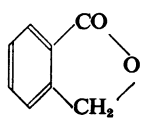
VI



VII



VIII



IX

is noticeable first of all that in none of these fused-ring compounds does there appear any activity approaching that of santonin, or even comparable with that of phenylbutyrolactone (Table XIII). This would appear to indicate that higher potencies are associated with separated than with fused rings, a phenomenon which, as we shall see, appears in other groups of compounds.

All four parent compounds (V–VIII) may be regarded as cyclized derivatives of *o*-phenols, and all possessed some activity. This seems to confirm the observation that hydroxylation of acetophenone in position 2 (Table IX) leads to marked increases of activity. The most active member of the group, 3-coumaranone (VIII), may be regarded as a cyclized form of 2-hydroxyacetophenone, with the activity of which (++ at 1:2,000) its own is comparable. 6-Hydroxy-3-coumaranone, which may be compared with 2:4-dihydroxyacetophenone (− at 1:2,000), was inert, but there was no return of activity when the hydroxyl group at position 6 was replaced by an ethoxy radical (cf. 2-hydroxy-4-ethoxyacetophenone, *215*). Hydroxylation of coumarine at position 7 similarly led to loss of activity, but in this case replacement of (OH) by an ethoxy radical was attended by the return of some degree of activity.

Three phenylated ketolactones derived from butyrolactone were also examined and found to be inert, while clavatin, which is believed to contain a lactone ring (Raistrick, 1943), and the azlactone of resorcinol aldehyde were also inert (Table XV).

Taken as a whole these results show that considerable anthelminthic activity is in some compounds associated with lactonic structure. But the appearance of such properties among lactones is very sporadic indeed, so much so that they may well be purely fortuitous and associated with other structural features. Certainly there is no evidence

TABLE XV

| | | | |
|---|---|---|---|
| Ethyl 2-phenyl-4: 5-diketotetrahydrofurane-3-carboxylate | — | 1: 1000 | *248* |
| 2-Phenyl-3-acetyl-4: 5-diketotetrahydrofurane | — | 1: 1000 | *255* |
| 2-Phenyl-3-cinnamyl-4: 5-diketotetrahydrofurane | — | saturated | *253* |
| Clavatin | — | 1: 5000 | *232* |
| Resorcinol aldehyde azlactone | insoluble | | *201* |

to support the notion of a specific relationship between anthelminthic potency and lactonic structure. One outstanding point is the greater activity of compounds with separated as opposed to fused rings.

3. *Phenols*

Many phenols are known to possess anthelminthic activity, and the work of Lamson *et al.* (1935, 1936) added many new ones to the list. Several phenols and phenolic derivatives have found wide clinical and veterinary employment, notably β-naphthol, thymol, and hexylresorcinol, all of which gave positive results in our tests. Our results with a number of phenolic derivatives of the aliphatic-aromatic ketones have already been described (Tables IX–XII).

Attempts were made to find new active derivatives of active phenols and a study was also made of several phenolic families that hitherto have not been systematically investigated. A number of phenolic acetates, chloroacetates, methylsulphonates, benzenesulphonates, *p*-toluenesulphonates, cinnamates, and carbamates have been prepared and tested, but proved for the most part to be inert or insoluble, apart from the carbamates (Tables XVI, XVII). The carbamates showed

TABLE XVI

| | | | |
|---|---|---|---|
| Phenyl chloracetate | + | 1: 2000 | *180* |
| Thymol | + | 1: 5000 | *7* |
| Thymyl chloracetate | — | 1: 1000 | *179* |
| Thymyl methylsulphonate | — | 1: 1000 | *243* |
| 1-Naphthol | + | 1: 5000 | *182* |
| 2-Naphthol | + | 1: 5000 | *6* |
| 2-Ethoxynaphthalene | ± | 1: 1000 | *204* |
| 2-Naphthyl acetate | — | 1: 2000 | *208* |
| 2-Naphthyl carbamate | + | 1: 5000 | *221* |
| 2-Naphthyl methylsulphonate | insoluble | | *247* |
| Ethyl salicylate | (+) | 1: 1000 | *206* |
| 2-Aminophenol | — | 1: 1000 | *165* |
| 2-Aminothiophenol | insoluble | | *166* |

activity of the same order as the parent phenols and, in view of their lesser toxicity, are likely to be of practical use.

Among the lactones previously examined, greater activity had been found among those containing independent than those containing fused ring systems. It seemed desirable, therefore, to discover whether the same rule might also apply among phenols. A number of phenylated phenols were accordingly examined with a view to comparing their activity with that of the naphthols. One member of this series, 4-benzylphenyl carbamate, is already in use on a fairly large scale under a variety of proprietary names. The results are listed in Table XVII where, as in Table XVI, the only useful derivatives were the carbamates. Several interesting relationships appeared, however, especially with reference to the position of the phenolic (OH) group.

2-Hydroxydiphenyl (*135*) was highly active (+ at 1: 10,000) and the 4-compound insoluble; these effects resemble those observed with acetophenone (Table IX) and contradict the results of Lamson *et al.* (1935), who found the 4-compound strongly and the 2-derivative only feebly active. In the diphenylmethane series these effects were reversed, 4-hydroxybenzylphenol (*128*) being about 3 times as active as the 2-compound (*130*); here our results are in agreement with those of Lamson *et al.* (1935). In strong contrast to our observations among the ketones we found that, among phenylphenols and benzylphenols alike, the replacement of (OH) by an ethoxy radical, whether in the 2- or the 4-position, always abolished activity.

Compared with 1-naphthol (+ at 1: 5,000), with its fused rings, 2-hydroxydiphenyl, with independent rings gave a higher order of activity (++ at 1: 5,000), thus falling into line with the results found among lactones. Further general confirmation is to be found in the fact that among the phenylphenols and benzylphenols alike, the active hydroxy derivatives showed potencies higher than that of 1- and 2-naphthols. The most potent substance discovered in this group was 2-hydroxydiphenyl carbamate (*136*), and it is noteworthy that this compound was found more active than the parent phenol (*135*). 4-Benzylphenyl carbamate, a drug that has done good service in practice, is less active than the phenol from

TABLE XVII

| | | | | |
|---|---|---|---|---|
| A | 2-HO.$C_6H_4C_6H_5$ | ++<br>+ | 1 : 5000<br>1 : 10,000 | *135* |
| | 2-$C_2H_5O.C_6H_4C_6H_5$ | – | 1 : 1000 | *206* |
| | 2-$CH_3CO.O.C_6H_4C_6H_5$ | – | 1 : 2000 | *207* |
| | 2-$H_2N.CO.O.C_6H_4C_6H_5$ | ++<br>(+) | 1 : 10,000<br>1 : 20,000 | *136* |
| | 2-$CH_3SO_2O.C_6H_4C_6H_5$ | – | *ca.* 1 : 2000 | *244* |
| | 2-$C_6H_5SO_2O.C_6H_4C_6H_5$ | insoluble | | *214* |
| | 2-(4$^1$-$CH_3.C_6H_4SO_2$)$O.C_6H_4C_6H_5$ | insoluble | | *212* |
| | 2-$C_6H_5CH$: $CH.CO.O.C_6H_4C_6H_5$ | insoluble | | *188* |
| | 4-HO.$C_6H_4C_6H_5$ | insoluble | | *134* |
| | 4-$C_2H_5O.C_6H_4C_6H_5$ | – | 1 : 2000 | *209* |
| | 4-$CH_3CO.O.C_6H_4C_6H_5$ | insoluble | | *205* |
| | 4-$H_2N.CO.O.C_6H_4C_6H_5$ | – | 1 : 5000 | *222* |
| B | 2-HO.$C_6H_4CH_2C_6H_5$ | + | 1 : 5000 | *130* |
| | 2-$C_2H_5O.C_6H_4CH_2C_6H_5$ | – | 1 : 2000 | *131* |
| | 4-HO.$C_6H_4CH_2C_6H_5$ | +++<br>+++ | 1 : 2000<br>1 : 5000 | *128* |
| | 4-$C_2H_5O.C_6H_4CH_2C_6H_5$ | – | 1 : 2000 | *129* |
| | 4-$H_2N.CO.O.C_6H_4CH_2C_6H_5$ | ++ | 1 : 5000 | *53* |
| | 4-$CH_3SO_2O.C_6H_4CH_2C_6H_5$ | – | 1 : 1000 | *246* |

which it is derived, and less active also than 2-hydroxydiphenyl carbamate.

The results obtained with the carbamates suggested an investigation of some unsaturated amides and substituted ureas, none of which however yielded results of any great interest or importance (Table XVIII). Tests on the tolyl compounds were limited by the very sparing solubilities of these substances.

The principal conclusion reached regarding the phenols may be summarized as follows. (i) The already abundant evidence for the anthelminthic potency of phenols and their carbamates has been generally confirmed. (ii) The position of the (OH) radical has different effects in different chemical groups. (iii) Phenols containing independent ring systems are more active than those in which the rings are condensed, and (iv) one compound with considerable promise has been discovered—viz., 2-hydroxydiphenyl carbamate.

4. *Thiazoles*

Recent work on the chemotherapy of bacterial diseases has emphasized the importance of certain

TABLE XVIII

| | | | |
|---|---|---|---|
| $C_6H_5CH$: $CH.CO.NH_2$ | – | 1 : 1000 | *189* |
| $C_6H_5CH$: $CH.CO.NH.CH_3$ | – | 1 : 2000 | *195* |
| $C_6H_5CH$: $CH.CO.N(CH_3)_2$ | – | 1 : 1000 | *196* |
| $C_6H_5CH$: $CH.CO.NH.CH_2CH_3$ | (+) | 1 : 1000 | *193* |
| $C_6H_5CH$: $CH.CO.N(CH_2CH_3)_2$ | (+) | 1 : 1000 | *194* |
| $C_6H_5CH$: $CH.CO.NH.CH_2CH_2CH_3$ | – | 1 : 2000 | *192* |
| $C_6H_5CH$: $CH.CO.NH.CH_2CH_2CH_2CH_3$ | (+) | 1 : 2000 | *187* |
| $C_6H_5NH.CO.NH_2$ | – | 1 : 1000 | *76/284* |
| $C_6H_5NH.CS.NH_2$ | – | 1 : 1000 | *285* |
| 2-$CH_3C_6H_4NH.CO.NH_2$ | – | 1 : 10,000 | *282* |
| 2-$CH_3C_6H_4NH.CS.NH_2$ | – | 1 : 10,000 | *280* |
| 3-$CH_3C_6H_4NH.CO.NH_2$ | – | 1 : 1000 | *283* |
| 4-$CH_3C_6H_4NH.CO.NH_2$ | – | 1 : 10,000 | *281* |
| 4-$CH_3C_6H_4NH.CS.NH_2$ | – | 1 : 10,000 | *279* |
| 2-Naphthyl urea | insoluble | | *286* |
| 2-Naphthylthiourea | insoluble | | *287* |

TABLE XIX

| | | | |
|---|---|---|---|
| 2-Aminothiazole | – | 1 : 1000 | *198* |
| 2-Amino-4-methylthiazole | – | 1 : 1000 | *175* |
| 2-Amino-4-phenylthiazole | ++ | 1 : 2000 | *172* |
| 2-Amino-4-(2′-hydroxyphenyl)-thiazole | – | 1 : 2000 | *199* |
| 2-Amino-4-(4′-hydroxyphenyl)-thiazole | – | 1 : 1000 | *185* |
| 2-Amino-4-(4′-methoxyphenyl)-thiazole | insoluble | | *184* |
| 2-Amino-4-(2′ : 4′-dihydroxyphenyl)-thiazole | – | 1 : 1000 | *167* |
| 2-Amino-4-(3′ : 4′-dihydroxyphenyl)-thiazole | – | 1 : 1000 | *169* |
| Benzthiazole | ++ | 1 : 2000 | *173* |
| 2-Phenylbenzthiazole | – | 1 : 2000 | *177* |
| 2-(4′-Aminobenzenesulphonamido)-thiazole (sulphathiazole) | – | 1 : 2000 | *52* |

ring compounds, notably thiazoles and pyridines, as potential antibiotics. It seemed worth while therefore to see whether derivatives of these compounds might hold out any promise of useful anthelminthic activity. Among the thiazoles, 2–aminothiazole provided a convenient starting point.

This compound was inactive, but appreciable activity appeared with the introduction of a phenyl radical to form 2–amino–4–phenylthiazole (Table XIX). Hydroxylation of the benzene ring, whether at position 2 or 4 only served to inactivate the products, while alkyloxylation rendered them insoluble. Sulphathiazole was quite inactive.

Further tests were made with two benzthiazoles, in which the component rings are fused. Contrary to our experience with lactones and phenols, ring fusion in the present series had little effect, while the introduction of a second phenyl radical at position 2 of the thiazole ring yielded an inert product. Little prospect of useful potency was found among these substances, therefore, and we went on to study a series of pyridine derivatives.

*5. Pyridines*

Pyridine itself already showed an activity of the same order as that of thymol and β–naphthol. A number of substituted products (Table XX) showed no greater activity, with the exception of 2–aminopyridine. A ketonic derivative, 1–methyl–2–pyridone, was inert. Nikethamide, a synthetic derivative of pyridine, was inactive, while arecoline, the active principle of the betel nut, proved to be relatively feeble. With the introduction of a second ring to form 4–benzylpyridine there was a sharp rise in activity ; sulphapyridine, however, was inert.

In order to explore further the effects of a second ring, a series of dipyridyls was examined, and here was found the highest order of activity encountered in the course of the work (Table XXI). 2–2′–Dipyridyl showed activity comparable with that of santonin and was far more active than any other of the four dipyridyls prepared. This high potency appears to be specifically associated with the 2–2′–linkage, for there was a profound fall of potency when this was shifted

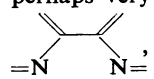
TABLE XX

| | | | |
|---|---|---|---|
| Pyridine | +++ | 1 : 2000 | *143* |
| | + | 1 : 5000 | |
| 2-Methylpyridine | +++ | 1 : 2000 | *144* |
| | (+) | 1 : 5000 | |
| 2-Chloropyridine | ++ | 1 : 1000 | *142* |
| | ± | 1 : 2000 | |
| 1-Methyl-2-pyridone | – | 1 : 1000 | *138* |
| 2-Aminopyridine | +++ | 1 : 2000 | *139* |
| | +++ | 1 : 5000 | |
| | – | 1 : 10,000 | |
| 4-Benzylpyridine | +++ | 1 : 5000 | *153* |
| | + | 1 : 10,000 | |
| 2-(4′-Aminobenzenesulphonamido)-pyridine (sulphapyridine) | – | 1 : 1000 | *59* |
| Arecoline (1-methyl-$\Delta^3$-tetrahydropyridine-3-carboxylic methyl ester) | ++ | 1 : 1000 | *58* |
| Nikethamide (pyridine-3-carboxylic diethylamide) | – | 1 : 1000 | *57* |

TABLE XXI

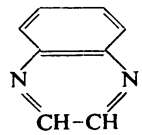
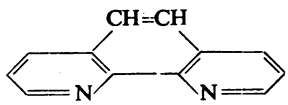
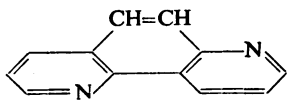
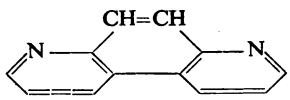
| | | | |
|---|---|---|---|
| 2-2′-Dipyridyl | +++<br>++ | 1 : 50,000<br>1 : 100,000 | *137* |
| 2-3′-Dipyridyl | + | 1 : 1000 | *158* |
| 3-4′-Dipyridyl | ± | 1 : 1000 | *152* |
| 4-4′-Dipyridyl | ± | 1 : 1000 | *147* |
| 2-Methyl-4-4′-dipyridyl | − | 1 : 1000 | *154* |
| 4-Pyridylpyridinium chloride | − | 1 : 1000 | *151* |
| 2-2′-2″-Tripyridyl | ++<br>± | 1 : 50,000<br>1 : 100,000 | *145* |
| 2-2′-2″-2‴-Tetrapyridyl | insoluble | | *155* |

to the 2–3′–position. 2–2′–2″–Tripyridyl was rather less active than the dipyridyl at the same concentrations, but the difference is probably attributable to its higher molecular weight. The corresponding tetrapyridyl was insoluble.

Following up the clue afforded by 2–2′–dipyridyl a number of other compounds were prepared containing pairs of nitrogen atoms linked to adjacent carbon atoms, but no activity was discovered here until we came to the phenanthrolines (Table XXII). Of these, 4 : 5–phenanthroline showed activity equal to that of 2–2′–dipyridyl, the other two being feebly active or inert. We are led, therefore, to the conclusion that a particularly high order of anthelminthic potency is associated, perhaps very specifically, with the bond system  , which is common to the two most active compounds.

It seems possible that, by suitable chemical manipulation, 2–2′–dipyridyl and 4 : 5–phenanthroline might be made the basis of new and valuable anthelminthics. Harwood (1934) has pointed out that the most useful anthelminthic drugs for the treatment of intestinal infestations have melting points below about 80°C. and are only sparingly soluble (1 : 1,000 or less) in water. Probably, therefore, these new substances themselves are too soluble to be of much value in the removal of intestinal nematodes but, if their toxicity is not excessive, they might conceivably prove useful in infestations of the blood and lymphatic systems without further modification. Conceivably, it might be possible to produce

TABLE XXII

| | | | |
|---|---|---|---|
| Ethylene diamine hydrate | − | 1 : 1000 | *163* |
| 1 : 2-Diaminobenzene (*o*-phenylene diamine) | − | 1 : 1000 | *165* |
| 2 : 5-Dimethylpyrazine | − | 1 : 1000 | *157* |
| 2 : 5-Disodium pyrazine dicarboxylate | − | 1 : 1000 | *150* |
| Benzpyrazine (quinoxaline)  | − | 1 : 1000 | *148* |
| 4 : 5-Phenanthroline  | ++ | 1 : 100,000 | *146* |
| 1 : 5-Phenanthroline  | + | 1 : 1000 | *156* |
| 1 : 8-Phenanthroline  | − | 1 : 1000 | *149* |

sparingly soluble derivatives that would be of value in intestinal infestations.

6. *Miscellaneous antibiotics*

In addition to the groups of substances already mentioned, a considerable number of miscellaneous antibiotics were tested in the course of the experiments. These included a number of well-known and important substances, but none proved to show any anthelminthic activity whatever (Table XXIII). These negative results help

TABLE XXIII

| | | | |
|---|---|---|---|
| Acetarsone | — | 1 : 500 | *54* |
| Alepol | — | 1 : 1000 | *159* |
| Atebrin | — | 1 : 1000 | *140* |
| Bayer 205 | — | 1 : 2000 | *71* |
| Clavatin | — | 1 : 5000 | *232* |
| 2 : 7-Diaminoacridine | — | 1 : 2000 | *67* |
| *Hydnocarpus* oil | — | 1 : 1000 | *161* |
| Neosalvarsan | — | 1 : 2000 | *55* |
| Penicillin | — | 480 O.U./ ml. | *66* |
| Sulphaguanidine | — | 1 : 1000 | *160* |
| Sulphamethazine | — | 1 : 1000 | *250* |
| Sulphanilamide | — | 1 : 100 | *51* |
| Sulphasuxidine | — | 1 : 1000 | *254* |
| Sulphathiazole | — | 1 : 2000 | *52* |
| Tartar emetic | — | 1 : 1000 | *164* |
| Tyrothricin | — | *ca.* 1 : 1000 | *270* |

to emphasize the necessity, already stressed in this paper, of choosing as test material a tissue preparation that properly represents the organism it is desired to attack. The biological activity of antibiotics seems in general to be specifically limited to particular organisms or groups of closely related organisms. Among parasitic helminths this same specificity is well known: santonin, which acts upon nematodes, is devoid of activity upon tape-worms, for example, while pelletierine acts upon tape- but not upon round-worms and so on. At the same time, however, agents are available that attack both types; these include numerous phenols, but these agents are bactericidal as well and are indeed, members of the category of "general protoplasmic poisons."

## Summary

1. This paper reports the results of tests carried out *in vitro* on over 200 chemical compounds for the detection of anthelminthic potency. The technique employed has certain limitations which are enumerated and discussed in the text.

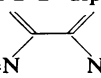
2. Significant activity is found among aliphatic-aromatic and aromatic-aromatic ketones, but in spite of numerous structural modifications and manipulations nothing approaching the activity of santonin has been discovered in this group.

3. Among lactones considerable activity was observed, but here again the activity of santonin far exceeds that of any other lactone tested in these experiments. These facts appear to support the suggestion that the efficacy of santonin is due in part to its ketonic and in part to its lactonic structure, but that its outstanding anthelminthic power is due to the simultaneous presence of both and to the unique manner in which they are combined together, rather than to either alone.

4. Among the lactones, phenols, and pyridines tested it was observed that anthelminthic activity increased with the addition of a second (usually a benzene) ring to the parent molecule, and that activity was greater when the two rings were independent than when they were fused. In the group of thiazoles, however, there was little to choose between the two types of structure.

5. Although numerous derived phenols were examined, none was found to compare with the carbamates; some of the latter were even more active than the parent phenols. The value of phenolic carbamates, already well known, has been confirmed, and an unusually high order of potency has been demonstrated in 2-hydroxydiphenyl carbamate. Several groups of derived phenolic ureas and amides were inert.

6. Among the thiazoles examined none showed much promise of useful potency, but among the pyridines an outstanding order of activity was revealed in 4-benzylpyridine and, more especially, in 2-2′-dipyridyl and in 4 : 5-phenanthroline. The



linkage in the last two compounds and in the corresponding tripyridyl possesses properties which, so far as the experiments have gone, seem to be unique and to offer considerable possibilities as a new starting point in the search for new and highly efficacious anthelminthics.

7. No activity was discovered among an assortment of microbial antibiotics. In particular, there is at present no reason to think that penicillin or the sulphonamides can yield new anthelminthic agents of any practical value.

8. The results reported here are at variance with those of earlier investigators in almost every respect, especially in the lactone field. This is attributed to the use by our predecessors of unsuitable methods of *in vitro* testing. The importance of using experimental material of nematode origin as the basis of methods of this kind is strongly emphasized and its necessity is, we believe, confirmed by the general outcome of this investigation.

9. The possibility that some of the substances tested may act otherwise than on the neuromuscular systems of the nematode has not been excluded.

The author wishes to record his deep and sincere thanks to Prof. E. Friedmann, whose chemical skill and co-operation made this work possible: his suggestions, criticisms and encouragement went far towards aiding the completion of the experiments described here. Thanks are due also to Miss L. A. Norris, Miss M. Cotton, and Miss V. Moyle, all of whom gave invaluable assistance at various stages of the investigation. To the Agricultural Research Council, who provided financial support for the work, and numerous friends who kindly presented samples of a variety of drugs, thanks are also offered. Finally, grateful recognition is made of the unfailing courtesy and kindness of the manager of the St. Edmundsbury Co-operative Bacon Factory, who arranged the regular supply of living nematode material at all times of the year, often under very difficult conditions.

## References

Baldwin, E. (1943*a*). *Parasitol.*, **35**, 89.
Baldwin, E. (1943*b*). *Pharm. J.* (July 17).
Baldwin, E., and Moyle, V. (1947). *J. exp. Biol.*, **23**, 277.
Baldwin, E., and Moyle, V. (1948). In preparation.
Caius, J. F., and Mhaskar, K. S. (1923). *Ind. J. med. Res.*, **11**, 377.
Clemo, G. R. (1934). *J. chem. Soc.*, 1343.
da Costa, G. F. G. (1931). *Arch. int. Pharmacodyn.*, **41**, 443.
Gluschke, A. (1932). *Arch. wiss. prakt. Tierheilk.*, **65**, 201.
Harwood, P. D. (1934). *Proc. Soc. exp. Biol.*, N.Y., **32**, 131.
Josephson, K. (1931). *Svensk. Farm. Tid.*, **35**, 69.
Lamson, P. D., and Brown, H. W. (1935). *J. Pharmacol.*, **53**, 227.
Lamson, P. D., and Brown, H. W. (1936). *Amer. J. Hyg.*, **23**, 85.
Lamson, P. D., and Ward, C. B. (1932). *J. Parasitol.*, **18**, 173.
Lamson, P. D., and Ward, C. B. (1936). *Science*, **84**, 293.
Lamson, P. D., Brown, H. W., and Ward, C. B. (1935). *J. Pharmacol.*, **53**, 198.
Lamson, P. D., Brown, H. W., Stoughton, R. W., Harwood, P. D., Baltzly, R., and Bass, A. (1935). *J. Pharmacol.*, **53**, 218, 234, 239.
Lamson, P. D., Stoughton, R. W., and Bass, A. D. (1936). *J. Pharmacol.*, **56**, 50, 60, 63.
Lapage, G. (1937). *Nematodes parasitic in animals*, London.
Lautenschläger, L. (1921). *Ber. dtsch. Pharm. Ges.*, **31**, 279.
Leonard, V. (1924). *J. Urol.*, **12**, 585.
Oettingen, W. F. von (1929). *J. Pharmacol.*, **36**, 335.
Oettingen, W. F. von, and Garcia, F. (1929). *J. Pharmacol.*, **36**, 355.
Oshika, H. (1921). *Acta Schol. med. Univ. imp. Kioto*, **4**, 421.
Oswald, A. (1924). *Chem. Const. u. Pharm. Wirking*, p. 266. Berlin: Springer.
Raistrick, H. (1943). *Lancet*, **245**, 635.
Rebello, S., and Rico, J. T. (1926). *C.R. Soc. Biol.*, Paris, **94**, 915.
Rosenmund, K. W., and Schapiro, D. (1934). *Arch. Pharm., Berl.*, **272**, 313.
Schroeder, W. von (1885). *Arch. exp. Path. Pharmak.*, **19**, 290.
Trendelenberg, P. (1916). *Arch. exp. Path. Pharmak.*, **79**, 190.
Trim, A. R. (1944). *Parasitol.*, **35**, 209.