Brit. J. Pharmacol., 1946, **1**, 153.

THE PHARMACOLOGY OF BASIC ESTERS OF THIAZOLE CARBOXYLIC ACIDS

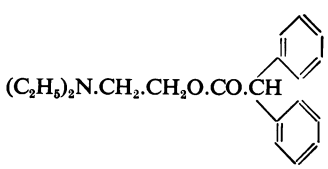
BY

M. R. A. CHANCE, P. DIRNHUBER AND F. A. ROBINSON

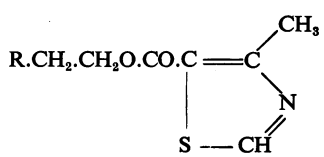
From Glaxo Laboratories, Ltd., Greenford, Middlesex

(Received March 2, 1946)

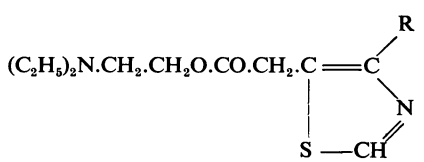
During an investigation into the pharmacological properties of thiazole compounds, several basic esters of 4-methyl-thiazole-5-carboxylic acid (II) and the diethylaminoethyl esters of 4-methyl-thiazole-5-acetic acid (III,R = CH_3), thiazole-5-acetic acid (III,R = H) and 2-amino-4-methyl-thiazole-5-carboxylic acid (IV) were prepared (Jones, Strachan and Robinson, 1946); these have now been examined pharmacologically. The close chemical relationship between these compounds and Trasentin (I) suggested that they might possess spasmolytic properties, and this has been confirmed *in vitro*. The appearance, moreover, of convulsions produced by the injection of non-toxic doses of these compounds into rabbits, mice, and guinea-pigs revealed stimulant properties on the C.N.S., which have been studied. Finally, the similarity in structure of diethylaminoethyl-2-amino-4-methylthiazole-5-carboxylate (IV) and procaine prompted us to investigate the local anaesthetic properties of this compound.



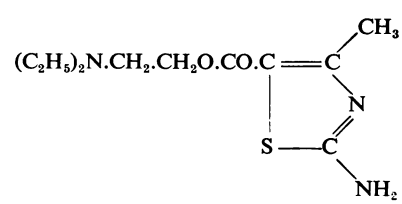
I



II



III



IV

NOTE.—Trasentin-6H is hexahydro-trasentin, i.e., one phenyl group of (I) replaced by cyclohexyl

EXPERIMENTAL

I. *Spasmolytic Properties*

1. *Action on isolated organs.*—The normal rhythm of the isolated ileum of the rabbit in Ringer's solution* was suppressed after a few minutes by a concentration of 1.25 mg./ml. diethylaminoethyl 4-methyl-thiazole-5-carboxylate (II,R = $(C_2H_5)_2N$) and was reduced, but not inhibited, by one-fifth of this concentration. In different experiments the tone fell, rose, or remained the same. Recovery took place within a few minutes after removal of the drug. Trasentin-6H, in a concentration of 2.5 μg./ml., produced no effect, but 12.5 μg./ml., i.e., 1/100 the concentration of the thiazole compound, produced a lowering of the tone with suppression of the rhythm.

The diethylaminoethyl ester, unlike Trasentin, which lowers muscle tone, raised the tone of the isolated uterus of the rabbit and guinea-pig in Ringer's solution. The effect was similar to that of posterior pituitary extract, except that the amplitude of the spontaneous contractions following this rise in tone was increased by the diethylaminoethyl ester, but remained the same with posterior pituitary extract. The increase of tone produced and maintained by 2 mg. of the ester was approximately the same as that produced by 0.05 I.U. posterior pituitary extract or by 60μg. atropine sulphate on guinea-pig uterus.

A concentration of 1.25 mg./ml. of the diethylaminoethyl ester contracted the bladder muscle of the guinea-pig.

2. *Action on isolated organs treated with acetylcholine (Ach).*—Preliminary tests on the Ach spasm of the isolated rabbit ileum in Ringer's solution showed that the esters listed in Table I had a weak spasmolytic action. Their activities were compared by measuring the degree of relaxation induced in a strip of ileum which was responding to a concentration of 0.4 p.p.m. Ach with constant submaximal contractions. The degree of

TABLE I

In vitro ACTIVITY AGAINST ACETYLCHOLINE (4×10^{-7}) IN RABBIT ILEUM

Compound. (10^{-4})	Per cent Relaxation of Contracted Muscle
Piperidinoethyl 4-methylthiazole-5-carboxylate	58
Diethylaminoethyl „ „	53
Dimethylaminoethyl „ „	50
β-Diethylaminopropyl „ „	48
Morpholinoethyl „ „	33
Diethylaminoethyl 2-methylthiazole-4-acetate	22

relaxation, expressed as the percentage reduction of this contraction, was measured for a standard dose of thiazole compound (see Table I). The piperidinoethyl ester of 4-methyl-thiazole-5-carboxylic acid (II,R = $C_5H_{10}N$) produced the maximum relaxation, followed by the dimethylaminoethyl ester (II,R = $(CH_3)_2N$) and, because the former was also the most active musculotropic compound, its spasmolytic activity was assayed against Trasentin by a method which involved "bracketing" doses of test and standard spasmolytic, as in the method of Dale for the assay of posterior pituitary extracts. It was found impossible to treat a single piece of gut with a sufficient number of doses of spasmolytic to make a randomized order feasible; this was due to the effects of Trasentin persisting for long periods and interfering with subsequent doses. Tested by this method the piperidinoethyl ester had approximately 1/1,000 of the activity of Trasentin.

*The bath containing the isolated tissue had a capacity of approximately 40 ml., and this value was used in calculating the concentrations recorded in the text.

A concentration of 0.5 mg./ml. of the diethylaminoethyl ester completely suppressed the contractions produced by a concentration of 0.1 p.p.m. Ach, whilst 1.25 mg./ml. was required to antagonize the effect of 0.4 p.p.m. Ach. Under the same conditions, 5 μg./ml. Trasentin was sufficient to suppress the contractions produced by 0.4 p.p.m. Ach, so that Trasentin was apparently 250 times as active. The recovery of the normal rhythm after treatment with Trasentin was proportional to the degree of relaxation induced, whereas the recovery of rhythm by the ileum relaxed by the thiazole compound was not dependent on the degree of relaxation and was very irregular.

B. B. Dikshit (1938) has reported that isolated rabbit ileum kept in Ringer's solution for 98 hours at 1° C. is unable to synthesize Ach, and he considered this synthesis to be mainly a function of the nerve plexus in the intestinal wall. This may provide a method of inhibiting Auerbach's plexus and so producing a denervated preparation on which the action of plain muscle stimulators and spasmolytic substances can be tested free from interference by the nerve plexus. These preparations are not as sensitive to Ach as is the normal isolated gut, but good contractions can be obtained with 10 μg. Ach, which is the quantity used throughout this work to produce contractions of the isolated gut. Observations on four strips of ileum from two rabbits showed that the response to 0.1 p.p.m. Ach was completely inhibited by a concentration of 1 mg./ml. of the diethylaminoethyl ester, and that the contracted ileum was relaxed to twice its original length by 0.2 mg./ml.

A concentration of 0.2 mg./ml. of the dimethylaminoethyl ester also reduced the contraction to 0.1 p.p.m. Ach, but to a smaller extent than an equal concentration of the diethyl compound. In two further tests a concentration of 1 mg./ml. completely inhibited the action of 0.1 p.p.m. Ach on a strip of ileum from another rabbit. These results suggest that the spasmolytic action is exerted directly on the muscle and is not mediated through Auerbach's plexus.

3. *Action on isolated organs treated with histamine or barium chloride.*—The effects of each compound on the spasm produced in the isolated guinea-pig ileum suspended in Tyrode by a concentration of 2 p.p.m. histamine hydrochloride was tested at four different levels and the percentage relaxation plotted against dosage. The resulting graphs proved to be straight lines. The relative activities of the compounds, as set out in Table II, were calculated from the concentrations required to produce half-relaxation. The dimethylaminoethyl ester was the most active member of the series and had one-quarter the activity of Trasentin-6H.

TABLE II

In vitro ACTIVITY AGAINST HISTAMINE HYDROCHLORIDE (2×10^{-6}) IN GUINEA-PIG ILEUM

Compound	Concentration Producing Half Relaxation
Dimethylaminoethyl 4-methylthiazole-5-carboxylate	$3{\cdot}8 \times 10^{-5}$
Diethylaminoethyl ,, ,, ...	$6{\cdot}0 \times 10^{-5}$
Piperidinoethyl ,, ,, ...	$7{\cdot}2 \times 10^{-5}$
β-Diethylaminopropyl ,, ,, ...	$1{\cdot}7 \times 10^{-4}$
γ-Diethylaminopropyl ,, ,, ...	$2{\cdot}1 \times 10^{-4}$
Morpholinoethyl ,, ,, ...	$2{\cdot}7 \times 10^{-4}$
Diethylaminoethyl 2-amino-4-methylthiazole-5-carboxylate	$2{\cdot}2 \times 10^{-4}$
Diethylaminoethyl 2-methylthiazole-4-acetate	$1{\cdot}5 \times 10^{-3}$
Diethylaminoethyl thiazole-4-acetate	$1{\cdot}8 \times 10^{-3}$
Trasentin-6H	$1{\cdot}0 \times 10^{-5}$

The effects of the compounds on the barium chloride contractions of the isolated guinea-pig ileum resembled those produced on the rabbit ileum, except that the compounds

permanently disturbed the normal rhythmical contractions. The barium chloride contractions of the isolated rabbit's ileum were also inhibited.

4. *"In vivo" experiments.*—Attempts to obtain in the living animal the reactions shown by the isolated tissues have failed in every instance except for the heart rate. The movements of a loop of ileum in a rabbit were recorded by a kymograph needle connected to it by a thread. 10 mg. of the diethylaminoethyl ester failed to diminish the violent movements induced by 25 mg. $BaCl_2$ injected intravenously. Similar results were obtained in the guinea-pig after intracardial injections. A dose of 2.5 mg. intravenously produced a lowering of the heart rate in three rats, as recorded by the electrocardiograph, but this is probably a result of vagal activity following C.N.S. stimulation (see below), for this dose is close to the convulsive level by the intravenous route.

II. Central Nervous Stimulation

The convulsions produced by the dimethyl- and diethyl-aminoethyl esters of 4-methyl-thiazole-5-carboxylic acid were compared with those produced in rabbits by intravenous injection of leptazol and Trasentin-6H in quantities shown in Table III. The doses quoted in the Table were determined by injecting six rabbits with graded doses starting at the LD 50 and diminishing in size.

TABLE III

MINIMAL CONVULSIVE DOSES BY INTRAVENOUS INJECTIONS TO RABBITS

Substance	*Dose* (mg./kg.)
Diethylaminoethyl 4-methyl-thiazole-5-carboxylate	87.5
Dimethylaminoethyl 4-methyl-thiazole-5-carboxylate	62.5
Leptazol	10.0
Trasentin-6H	15.0

At the onset of a convulsion produced by either thiazole compound, the rabbit sits back on its haunches with its forelegs extended. This takes place within a minute. A clonic phase ensues with violent running motions of the fore and hind legs, and passes into a tonic phase with opisthotonus. At this point the animal loses its balance and becomes unconscious. The tonic phase lasts no longer than one minute and is followed immediately by a return of consciousness and clonic movements which pass off leaving the animal exhausted. During the first clonic phase and part of the tonic phase the respiration is completely inhibited, but as the latter passes off the rate of respiration increases and apparently also the amplitude. Rabbits convulsed with the dimethyl compound always recovered their normal sitting position within ten minutes, usually soon after they recovered consciousness. Convulsions produced by the diethyl compound, on the other hand, were followed by a long period of exhaustion and the animal rarely recovered its balance in less than fifteen minutes; sometimes it took as long as half an hour. These convulsions are identical with those produced by leptazol, except that it is not possible with the thiazole compounds to induce such violent clonic movements involving the whole of the trunk and limbs, and the convulsions do not last as long.

Observations were also made to discover whether any difference existed between the convulsions produced in intact frogs and in frogs in which the higher nervous centres were separated from the spinal cord. It was observed that with intact frogs the thiazole compounds and leptazol produced convulsions similar to those obtained with strychnine, whereas in pithed frogs the effect was less marked and lasted for a much shorter period than with strychnine. The results suggest

that the higher centres as well as the spinal cord are involved in the stimulation produced by the thiazole compounds and by leptazol.

J. W. Schultz, L. M. Tainter, and J. M. Dill (1939) describe a method of distinguishing between cortical and sub-cortical stimulation by measuring the total activity exhibited by rats during a period of seven hours. The rat, which has received a subcutaneous dose of the test substance, is suspended in a cage by a spring which oscillates as the animal moves about the cage. By recording the number and extent of the oscillations, an arbitrary measure of the amount of the activity exhibited in unit time can be recorded. The authors distinguish by this method between leptazol and picrotoxin, on the one hand, and nikethamide and caffeine on the other, the last two substances producing a marked increase in activity which, after nikethamide injections, extends over a period of six hours and, after injection of caffeine, over a period of three hours. Leptazol and picrotoxin, on the other hand, only produce a brief period of activity, extending to not more than one hour, and this may be partly due to convulsions. We have made similar observations with the thiazole compounds and amphetamine. The thiazole compounds did not appear to increase the total activity, but, indeed, appeared to depress it soon after the injection. It may, therefore, be concluded from the results reported in these three sections that central nervous stimulation by these compounds is primarily a medullary stimulation with involvement of the spinal cord.

Analeptic Activity

The results described in the previous three sections suggested that the thiazole compounds might antagonize the action of anaesthetics on the central nervous system. Tests were therefore made to ascertain their effect on: (*a*) the duration of anaesthesia, and (*b*) the toxicity of anaesthetics. Two different types of anaesthetics were chosen for the main investigations, namely, amytal sodium as a representative of the barbiturates, and paraldehyde which belongs to a different group of compounds, but produces anaesthesia of approximately the same duration.

(a) *Reduction of Anaesthetic Time*

From the dosage-mortality curve of amytal it was ascertained that no significant mortality would be expected in groups of animals receiving a dose equal to 60 per cent of the LD 50; as this dose produced a satisfactory period of anaesthesia, it was selected to produce the standard degree of anaesthesia. The same proportion of the LD 50 of paraldehyde was also found to be satisfactory.

Two analeptic drugs differing in their action on the nervous system were chosen for comparison with the two thiazole compounds, namely, picrotoxin and β-phenyl-*iso*propylamine sulphate (amphetamine). Picrotoxin is used as an antidote in barbiturate poisoning because it is safe in doses far exceeding those lethal to unanaesthetized animals, although its action is relatively short and somewhat irregular. Amphetamine, on the other hand, although as toxic to unanaesthetized as to anaesthetized animals, has a more prolonged action than picrotoxin (compared by the percentage recovery of mice), and is more effective at dose levels proportionately further removed from the LD 50 for unanaesthetized animals. It was, however, found impossible to obtain even an approximate value for the toxicity of amphetamine, and a search of the literature showed that other workers had experienced the same difficulty; as much as a tenfold difference is reported in the figures quoted by different authors using the same route for mice. It was therefore considered necessary to make an exhaustive study of the matter, the results of which finally enabled

us (Chance, 1946), after the conditions of the test had been sufficiently defined and controlled, to determine the toxicity of this substance with the same degree of accuracy as is usually encountered in biological assays. Meanwhile, a comparison was made of the two substances already mentioned with picrotoxin. Groups of sixteen mice received the standard dose of 120 mg./kg. by intraperitoneal injection followed immediately by a subcutaneous injection of the analeptic.

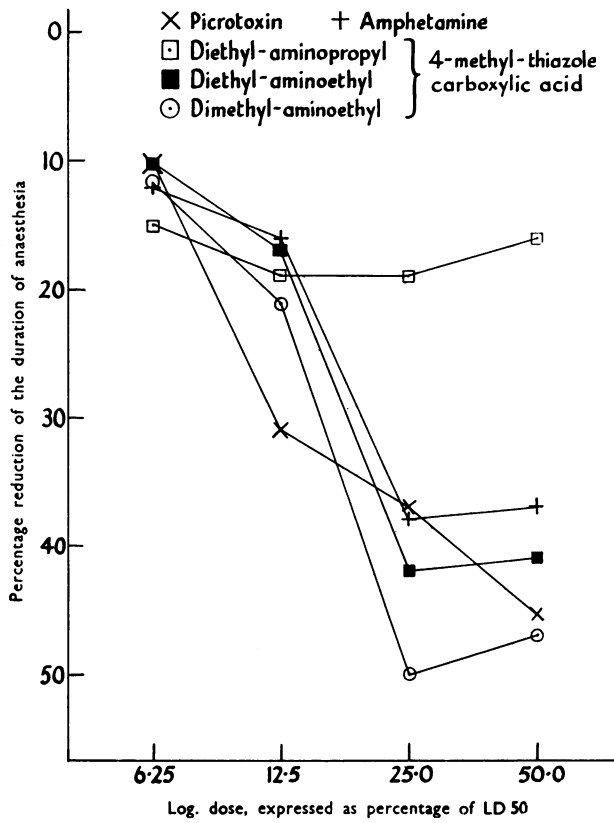


FIG. 1.—Shortened duration of amytal anaesthesia.

Both thiazole compounds, like picrotoxin, reduced the duration of anaesthesia produced by the standard dose of amytal (Fig. 1). The reductions in the duration of anaesthesia, brought about by equivalent proportions of the LD 50 of the two thiazole compounds and picrotoxin, were approximately equal; when the logarithm of the dose was plotted against the reduction in anaesthesic time the relationship was not linear for the thiazole compounds, though possibly so for picrotoxin.

The thiazole compounds were, however, less effective than picrotoxin against paraldehyde, and acted somewhat differently (Fig. 2). Over the same range of doses, expressed as proportions of the LD 50, the dimethyl compound was less

effective than the diethyl compound, which had an optimal activity at a level equal to 25 per cent of its LD 50. These compounds are clearly less active than picrotoxin, but their action is nevertheless pronounced, and against amytal their action is indistinguishable from that of picrotoxin when compared at the same proportions of their respective LD 50's.

After the investigation into the factors affecting the toxicity of amphetamine had been completed, two more compounds became available. These were the hydrochlorides of diethylaminopropyl 4-methyl-thiazole-5-carboxylate

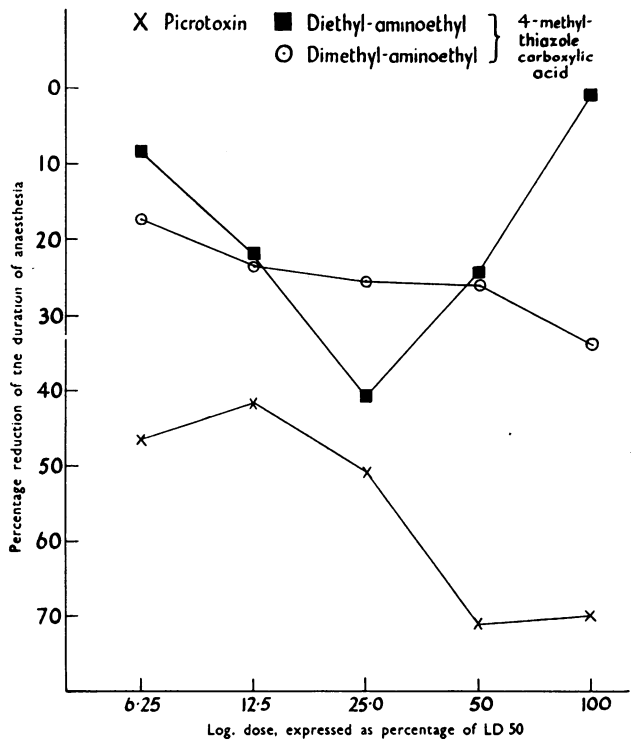


FIG. 2.—Shortened duration of paraldehyde anaesthesia.

(II,R = $(C_2H_5)_2N.CH_2$) and diethylaminoethyl 2-amino-4-methyl-thiazole-5-carboxylate (IV). The former produced convulsions on injection into mice, and was therefore tested for analeptic activity, together with the hydrochlorides of the diethyl- and dimethylaminoethyl esters of 4-thiazole-5-carboxylic acid. On this occasion amphetamine sulphate was also included in the comparison. The combined results are shown in Fig. 1.

(b) *Effect on the Toxicity of Amytal*

The LD 50 of amytal sodium on "Swiss" mice had been measured previously and found to be 200 mg. per kg. body-weight. When redetermined on a group of twenty mice it appeared to be slightly higher, and it is possible that the original estimate may have been

somewhat low. A dose of 200 mg./kg. was, however, chosen as the toxic dose in these experiments, and gave a mortality of 43 per cent.

The values of LD 50 for the diethyl and dimethyl compounds are 700 and 500 mg. per kg. respectively, for strychnine 1 mg. per kg., and for picrotoxin 5 mg. per kg. Each substance was tested by subcutaneous injection into a group of not more than ten mice immediately after intraperitoneal injection of the standard dose of amytal. Both the amytal and the various amounts of the test substances were administered in equal volumes of solution. The procedure was completed in a total of four minutes, so that each received the antidote at the onset of anaesthesia, which for amytal occurs two minutes after intraperitoneal injection. In each investigation carried out on one day the same number of animals was used for each dose of all four substances, and simultaneously the same number of animals received the standard dose of amytal alone. The whole investigation was then repeated until all doses of each substance had been tested on forty animals.

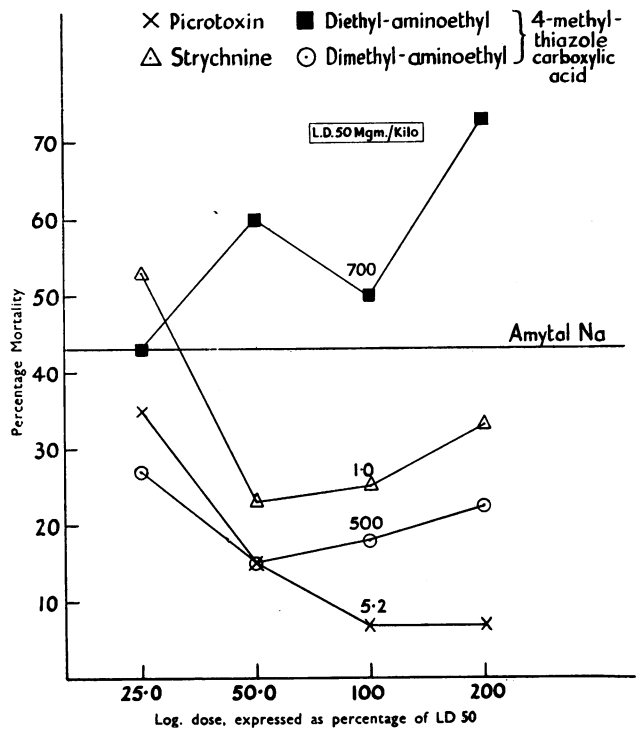


FIG. 3.—Effect of picrotoxin, strychnine and the dimethyl- and diethylaminoethyl esters of 4-methyl-thiazole carboxylic acid on the toxicity of amytal sodium (200 mg./kg.) in mice.

Fig. 3, in which the dose, expressed as the logarithm of the percentage of the LD 50, is plotted against the percentage mortality for each substance, shows that the mortality to the standard dose of amytal was 43 per cent—a slightly lower value than expected. Reductions in mortality are statistically significant when the mortality was 20 per cent or less. The three highest doses of picrotoxin produced a significant lowering of mortality from the standard dose of amytal, though the lowest did not. With strychnine, reductions of mortality due to the

LD 50 of amytal were produced at three dose levels (200, 100 and 50 per cent of the LD 50 of strychnine), but they were not statistically significant ; nor was a significant increase in mortality shown with the smallest dose of strychnine. The curve for the dimethyl compound shows that it behaves very like strychnine, but is, if anything, more active ; significantly lower mortalities were produced by 50 and 100 per cent LD 50 doses. The diethyl compound, on the other hand, increased the mortality due to amytal, significantly at the highest dose (200 per cent of its LD 50).

III. Local Anaesthetic Activity

Since it was thought possible that the amino substituted thiazole, diethylaminoethyl 2-amino-4-methyl-thiazole-carboxylate might possess local anaesthetic properties similar to those of procaine, it was tested for local anaesthetic activity by the method of Chance and Lobstein (1944). It was inactive at a concentration of 1 in 100, as was also diethylaminoethyl 4-methyl-thiazole-carboxylate.

Discussion

The piperidinoethyl, dimethylaminoethyl, diethylaminoethyl and β-diethylaminopropyl esters of 4-methyl-thiazole-5-carboxylic acid had approximately the same activity in inhibiting the contractions produced by acetylcholine in the isolated ileum (neurotropic action) but were only about 1/1,000 as active as Trasentin ; the morpholinoethyl ester of 4-methyl-thiazole-5-carboxylic acid and the diethylaminoethyl ester of 2-methyl-thiazole-4-acetic acid were even less active. The compounds did not produce mydriasis. Their effect on spasm induced by barium chloride or histamine (musculotropic action) was of the same order as that of Trasentin. In this respect the piperidinoethyl, dimethylaminoethyl and diethylaminoethyl esters were more active than the morpholinoethyl or the β- and γ-diethylaminopropyl esters of 4-methyl-thiazole-5-carboxylic acid ; the two isomeric propyl esters showed about the same activity. The diethylaminoethyl esters of 2-methyl-thiazole-4-acetic acid and thiazole-4-acetic acid had only a fraction of the activity of the corresponding ester of 4-methyl-thiazole-5-carboxylic acid, demonstrating the importance of direct attachment of the carboxylic group to the nucleus. The activity of the diethylaminoethyl ester of 2-amino-4-methyl-thiazole-5-carboxylic acid was 1/30 that of the corresponding ester of 4-methyl-thiazole-5-carboxylic acid, so that the introduction of an amino group materially reduced the musculotropic activity.

The diethylaminoethyl ester of 4-methyl-thiazole-5-carboxylic acid, although resembling Trasentin in its action on the isolated ileum, had a different effect on the isolated uterus of the rabbit or guinea-pig and, instead of relaxing the muscle, increased the tone in a manner similar to posterior pituitary extract or atropine. The substance was less potent, however, 2 mg. producing the same response as 0.05 I.U. posterior pituitary extract or 60 μg. atropine sulphate. This ester had no effect on the ileal contractions induced by barium chloride or histamine hydrochloride *in vivo*.

Some of the esters were found to be stimulants of the central nervous system, producing convulsions in mice and guinea-pigs. Some of them reduced the duration of narcosis induced by amytal and paraldehyde, though the effect was markedly different with the different compounds.

Trasentin and Trasentin-6H are convulsants and respiratory stimulants (Graham and Lazarus, 1940), whilst atropine also stimulates the medulla and higher cerebral centres, though at the usual clinical dosage its effects are manifest only as moderate respiratory stimulation and slight vagal excitation. The thiazole compounds are less potent stimulants than leptazol or Trasentin. They act on the medulla, and the effect resembles that of leptazol rather than that of strychnine. The diethylaminoethyl ester of 4-methyl-thiazole-5-carboxylic acid was slightly effective in antagonizing the effect of a lethal dose of amytal, but the dimethylaminoethyl ester was not. Neither compound was as effective as leptazol in inducing central nervous stimulation, the lethal dose being close to the convulsive dose.

SUMMARY

1. The basic esters of 4-methyl-thiazole-5-carboxylic acid and of thiazole-5-acetic acid possess spasmolytic and analeptic properties.

2. The spasmolytic activity of these compounds is exhibited only *in vitro*, and is most marked against histamine spasm. A comparison of the different substances revealed that the dimethylaminoethyl ester of 4-methyl-thiazole-5-carboxylic acid was the most active, having an activity against histamine spasm approximately one-quarter that of Trasentin-6H; the diethylamino- and piperidinoethyl esters were only a little less active than the dimethylaminoethyl ester.

3. The diethyl- and dimethylaminoethyl esters and the γ-diethylaminopropyl ester of 4-methyl-thiazole-5-carboxylic acid produced in guinea-pigs and rabbits convulsions similar to those caused by leptazol and compounds acting on the mid-brain, although there was evidence of spinal involvement. These esters have analeptic activity, demonstrated by their ability to reduce the duration of anaesthesia in mice. The diethyl- and dimethylaminoethyl esters of 4-methyl-thiazole-5-carboxylic acid are, however, inferior to picrotoxin in this respect; not only is the reduction of paraldehyde anaesthesia less marked than the reduction of amytal anaesthesia, but antidotal activity is exhibited by the dimethyl compound over a smaller dose range than that found for picrotoxin.

REFERENCES

Chance, M. R. A. (1946). *J. Pharmacol.* (In the press.)
Chance, M. R. A., and Lobstein, H. (1944). *J. Pharmacol.*, **82**, 203.
Dikshit, B. B. (1938). *Quart. J. Pharm.*, **28**, 243.
Graham, J. D. P., and Lazarus, S. (1940). *J. Pharmacol.*, **69**, 331.
Jones, E. R. H., Strachan, J., and Robinson, F. A. (1946). *J. chem. Soc.* (In the press.)
Schultz, J. W., Tainter, L. M., and Dill, J. M. (1939). *Proc. Soc. exp. Biol.*, **42**, 242.