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Review Article

Release of 5-hydroxytryptamine from blood platelets*

M. K. PAASONEN, M.D.

THE results of Rand & Reid (1951) originally pointed to the high content of 5-hydroxytryptamine (5-HT) in blood platelets. Later Shore, Pletscher, Tomich, Kuntzman & Brodie (1956) demonstrated that reserpine caused a depletion of 5-HT from the platelets. The interest in the relation between blood platelets and 5-HT arises mainly from these two observations. The pharmacological, physiological and biochemical questions concerning 5-HT have been reviewed, for example, by Lewis (1958), Page (1958) and Erspamer (1961). A review by Maupin (1960) was particularly concerned with the role of 5-HT in platelets. The purpose of the present text is to report some of the results obtained while studying this amine in platelets and in particular to discuss its release from platelets.

Although platelets have no nucleus they have typical subcellular structures (cf. Telkkä, Nyholm & Paasonen, 1964) and an active metabolism. Glycolysis is the main source of energy (Waller, Löhr, Grignani & Gross, 1959) and at least 30 enzymes are known to be present in platelets (Zucker & Borrelli, 1958). From the pharmacological point of view it is important that platelets contain, or are able to absorb, adrenaline and noradrenaline *in vitro* (Born, Hornykiewicz & Stafford, 1958). The platelets of some species, especially the rabbit, contain large amounts of histamine (Code, 1952). The high content of adenosine triphosphate (ATP) in the platelets (Born, 1956) has also attracted attention. Although there are great differences in the content of biogenic amines in the platelets, the content of 5-HT in platelets is generally relatively high in all mammalian species. Some of the approximate figures, compiled from the literature and our own results, are presented in Table 1.

The platelets of the rabbit, human carcinoid tumour, the posterior salivary glands of *Eledone moschata* and of *Octopus vulgaris*, and the sting fluid of *Urtica dioica* all contain about the same amount of 5-HT/g. Baker, Blaschko & Born (1959) isolated granules containing 5-HT and ATP from human platelets while Wurzel, Marcus & Zweifach (1965) isolated them from rabbit platelets. It is conceivable that most of the 5-HT in the platelets is bound to the particles since it would otherwise be metabolised by the monoamine oxidase which they also contain (see p. 692). Only traces of 5-HT are present in the platelet-free plasma, and it seems that most of the amine measured outside the platelets may arise from platelet damage.

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* Based on a Special University of London lecture given at Charing Cross Hospital Medical School, London, May 20th, 1964.

TABLE 1. AMOUNTS OF 5-HT IN PLATELETS OF SOME MAMMALIAN SPECIES

Species	µg/10^9 platelets	Whole blood µg/ml	µg/ml platelet substance
Human	0·57	0·16	49
Rabbit	10·0	3·8	1100
Pig	2·2	0·4	
Dog	1·7	0·2	
Cat	0·9	3·8	
Guinea-pig	0·3	0·2	
Rat	0·3	0·4	

Uptake of 5-HT by platelets

Platelets and bone marrow are unable to decarboxylate 5-hydroxytryptophan (5-HTP) to 5-HT *in vitro* (Clark, Weissbach & Udenfriend, 1954; Gaddum & Giarman, 1956) and so 5-HT must originate elsewhere. Portal blood contains more 5-HT than arterial blood (Erspamer & Testini, 1959). It seems probable that the origin of platelet 5-HT is in the enterochromaffin cells of the intestinal mucosa, a view supported by a number of findings. Kärki, Paasonen & Peltola (1960) found that increasing the intraluminal intestinal pressure in rabbits increases the plasma 5-HT 2- to 4-fold in the venous blood coming from this portion of the intestine. The role of other tissues, however, cannot be entirely excluded. Thus the kidney decarboxylates 5-HTP and excretes 5-HT in the urine (Sandler & Spector, 1961) and the amount of 5-HT in blood from the kidneys is higher than that in the arterial blood (Uuspää & Airaksinen, 1963).

It is well established that platelets take up 5-HT from the surrounding fluid *in vitro* and *in vivo* (Humphrey & Toh, 1954; Hardisty & Stacey, 1955; Weissbach, Bogdanski & Udenfriend, 1958; Born & Gillson, 1959) and the concentration of amine in the platelets may reach a value several hundred times that of the surrounding fluid. Absorption continues for 1 to 2 hr. The capacity of uptake is not the same in all instances and it does not occur in the cold (Stacey, 1958). The rate of uptake increases with increasing concentrations of 5-HT up to a concentration of about 0·5 µg/ml (Born & Gillson, 1959), this uptake being facilitated by 2,4-dinitrophenol and potassium and decreased by high concentration of glycolytic inhibitors like cyanide and iodoacetate. These workers also found that the platelet 5-HT can be exchanged completely if the amine is also present outside. This process of uptake is most probably an *active transport* as has been much emphasised by Brodie and his colleagues (Hughes & Brodie, 1959).

At 5-HT concentrations of 50 µg/ml or higher, and especially at higher pH values, another mechanism of uptake exists. This involves a nearly linear dependence of 5-HT accumulation on the amine concentration (Weissbach, Redfield & Titus, 1960). It is unlikely that endogenous 5-HT is absorbed *in vivo* by this mechanism, which is mainly due to *diffusion*.

Reserpine was found by Brodie, Shore & Pletscher (1956), Brodie, Tomich, Kuntzman & Shore (1956) and Born, Ingram & Stacey (1956) to prevent the uptake of 5-HT by platelets *in vitro* and *in vivo*. As was

shown by Stacey (1961), there are a number of other agents which also inhibit this process, although less effectively than reserpine. The release and uptake are closely related phenomena and some questions of the uptake will be dealt with later (for further discussion about uptake, see Born & Gillson, 1959; Stacey, 1961).

The work of Born & others (1956) indicates that the property of platelets to take up 5-HT against a concentration gradient is related to their ATP content. Platelets are rich in this nucleotide and human platelets contain about 22 $\mu g/10^9$ platelets. These authors also found that the amount of ATP in platelets is related to the amount of 5-HT and in *in vitro* experiments the ratio of ATP molecules to 5-HT molecules becomes one. ATP would provide the energy for the uptake process—a situation somewhat comparable to that in the adrenal medulla. According to Carlsson, Hillarp & Waldeck (1963) even the cells of the adrenal medulla take up more 5-HT than catecholamines if equal amounts of these amines are present in the perfusion fluid.

In vivo the platelets hold their 5-HT for a long time. After an intravenous infusion of 20 mg/kg of 5-HT into dogs, elevated values were found for at least 4 days by Weissbach & others (1958). This means that many, if not all, of the platelets retain the amine content, or some portion of it, for as long as they exist. Of course, some of the platelet 5-HT could originate in stores in other tissues after the infusion.

Release of 5-HT from platelets

5-HT is known to be released from platelets in a number of different ways *in vitro*, *in vivo* or both. These include: 1, mechanical trauma; 2, factors present normally or in pathological conditions in tissue fluids; 3, drugs or other foreign agents.

MECHANICAL TRAUMA

As discussed by Stacey (1958), mechanical trauma of any kind, such as contact with unsiliconed glass, frothing of plasma or freezing and thawing, will break up the platelets and release 5-HT as well as other constituents. Ultrasonic waves also cause platelets to lose their vasoactive material (Cole, Livingston, Loughry & Holden, 1953), including 5-HT (Buckingham & Maynert, 1964). For these reasons platelet damage must be considered as a factor in some observed complications in extracorporeal circulation of blood, such as the heart-lung apparatus (Sarajas, Kristoffersson & Frick, 1959).

The best way to minimise platelet damage during the handling of samples *in vitro* is to use plastic vessels and pipettes. Inadequate siliconising may cause much release of 5-HT especially during the incubation of samples. In this connection, it is surprising that platelets retain their 5-HT in plasma made hypotonic by adding water (Ahtee & Paasonen, unpublished). The liberation of the amine begins at a concentration of about 0·5% and is parallel to the haemolysis of red cells. Addition of sodium chloride to give a final salinity equivalent to 1·3% causes no release of 5-HT within a one hr period of incubation.

FACTORS PRESENT IN TISSUE FLUIDS

Clotting of blood. Several authors (Zucker & Borrelli, 1955a,b; Humphrey & Jaques, 1955; Grette, 1959) have shown that during clotting about one half of the 5-HT from platelets is released into the serum. Clotting of plasma by recalcification releases all 5-HT from previously washed platelets (Zucker & Borrelli, 1955b). The reason for the incomplete release or recovery or both, of 5-HT from serum is not clear. Shaking of blood during clotting decreases the amount of the amine in serum (Sharman & Sullivan, 1956).

The release itself is known to be due to the action of *thrombin* (Zucker & Borrelli, 1955a,b; Grette, 1962). It does not arise from the mechanical effects of clot formation, as was demonstrated by the use of a fibrinogenic blood containing no fibrinogen to form fibrin and thus to clot (Hardisty & Pinniger, 1956). The release of 5-HT from platelets by a sufficient amount of thrombin occurs in a few seconds (Zucker & Borrelli, 1956b). Binding of the intracellular calcium by ethylenediaminetetra-acetate (EDTA) decreases the release of 5-HT, and the subsequent addition of calcium liberates the rest of the amine (Markwardt & Barthel, 1964). During the release by thrombin the platelets remain microscopically intact in calcium-free medium (Zucker & Borrelli, 1955b) and viscous metamorphosis occurs only if calcium is present (Markwardt & Barthel, 1964). Grette (1962) has shown that as well as releasing 5-HT, thrombin also releases adenine nucleotides, inorganic phosphate, free amino-acids, but only a small amount of cellular protein.

The release by thrombin is dose dependent from about 0·01 to 1·0 or 10·0 u/ml (Gaintner, Jackson & Maynert, 1962). This means that thrombin may release 5-HT at a concentration of about 10^{-10} M. It is possible that thrombin is the only coagulation factor necessary for the 5-HT release during coagulation.

Trypsin. Trypsin and thrombin react in a similar manner with fibrinogen, and fibrinogen is present on the surface of platelets. Gaintner & others (1962) demonstrated that trypsin at a concentration of 0·1 μg/ml or more releases 5-HT from platelets *in vitro* and that the time-response curves are identical in both cases. Heparin, however, blocks the action of thrombin but not the action of trypsin. Trypsin also releases potassium from platelets but this release is less complete than that of 5-HT (Buckingham & Maynert, 1964). In spite of the presence of trypsin and thrombin in the platelet suspension, the concentration of 5-HT in the platelets begins to rise after the fast initial depletion (Gaintner & others, 1962; Buckingham & Maynert, 1964).

Bacterial endotoxins. Administration of bacterial endotoxin to the rabbit increases 5-HT in the plasma (Davis, Meeker & Bailey, 1961). Incubation of platelet-rich plasma (Des Prez, Horowitz & Hook, 1961) or whole blood of the rabbit (Davis, Meeker & McQuarrie, 1960) with endotoxin also causes an increase of 5-HT in the plasma. This release also concerns histamine. Davis, Bailey & Hanson (1963) demonstrated that warfarin or heparin prevented the release *in vivo*, but these agents did not prevent the transient thrombocytopenia caused by

endotoxin. The release of amines by bacterial endotoxin is almost instantaneous both *in vivo* and *in vitro* and there is a possibility that thrombin is involved in the mechanism of action. Des Prez (1964), however, has pointed to certain differences between the action of thrombin and endotoxin.

Antigen-antibody reaction. Humphrey & Jaques (1955) showed that the addition of purified antigen and antibody to platelet-rich plasma of normal rabbits released 5-HT and histamine. The release also occurs *in vivo* and a maximal depletion is reached within 2 min (Waalkes & Coburn, 1959). Calcium is necessary for the release of 5-HT induced by antigen. Heparin does not inhibit the release but it is prevented by tosylarginine and *p*-tosylarginine methyl ester (TAME), which are substrates for thrombin and thromboplastin. The release of amines from blood cells by antigen has been shown to be associated with the activation of proteolytic enzymes of plasma (Ungar, Yamura, Isola & Kobrin, 1961). The results of Shore & Alpers (1963a) suggested that a direct platelet damaging factor was associated with the early stages of blood coagulation and that antigen may activate this mechanism. It is also possible that the same heat-stable platelet-damaging factor in plasma and serum of the rabbit is responsible both for the *spontaneous* release of 5-HT and histamine as well as for the antigen-induced amine release (Shore & Alpers, 1963a).

Glycogen. Rocha e Silva (1950) showed that intravenous administration of glycogen causes a rapid clumping and disappearance of platelets and leucocytes from the circulation in rabbits and dogs. This action is similar to that of antigen-antibody reaction and, accordingly, Waalkes & Coburn (1959) found that glycogen released 5-HT and histamine from platelets of rabbit both *in vivo* and *in vitro*. In both instances the lung content of the amines rose many times above the control values, obviously as a result of the occlusion of platelet-leucocyte clumps or emboli in the lung (Waalkes & Coburn, 1959).

Tissue extracts. According to Toh (1956, 1957) alkaline tissue extracts from some mammalian tissues, particularly from the kidney, stomach mucosa and submucosa, accelerate the release of 5-HT and histamine from platelets *in vitro*. When given intraperitoneally for three days, the extracts depleted the 5-HT content of the spleen of rats (Toh, 1957). The release of amines from platelets is inhibited by EDTA, oxalate or citrate, but this inhibition is not due to the binding of calcium since it also happens in calcium-free salt solution (Toh, 1956). This releasing action of tissue extracts has been confirmed by using another model system, in the shape of the neoplastic mast cells of mouse, as a source of 5-HT and histamine (Giarman, Potter & Day, 1960). The release is time and dose dependent and the active principle is stable to heat, acid or alkali and cannot be separated from protein. Although the extracts caused some damage to the cells, these authors believe that lysis of the cells is not in itself sufficient to explain the activity observed. The release of 5-HT by tissue extracts points to the possibility of a selective local release in tissues *in vivo*.

Fatty acids. Several long chain saturated fatty acids will, at 37° and in the presence of plasma and calcium, release both 5-HT and histamine

from platelets of rabbit *in vitro* (Shore & Alpers, 1963b). The most active are stearic, arachidic and behenic acids, which, in concentrations of 1 to 25 μg/ml, release most of the amines within 30 min. The platelet damage occurs in the presence of heparin used to block the action of thrombin. It is of interest that these saturated long chain fatty acids greatly accelerate thrombus formation in an *in vitro* thrombus-producing system (Connor, 1962). It is suggested that in both instances the mechanism involves the activation of the Hageman factor.

DRUGS

Reserpine and related agents. As for other tissues, reserpine releases 5-HT from platelets *in vivo* (Shore & others, 1956) and *in vitro* (Carlsson, Shore & Brodie, 1957). This action is common to those rauwolfia alkaloids that release 5-HT from the brain (Brodie, 1958). There is a dose-response relationship when the reserpine concentration ranges from about 10^{-7} to 10^{-6} M but it is difficult to effect the release *in vitro* of more than about half of the platelet 5-HT by reserpine-like drugs. After reserpine administration, rabbit platelets *in vivo* lose most, if not all, of their 5-HT within a day. It is possible that this would happen also *in vitro* if such extended incubations were possible. In any case the rate of release *in vitro* becomes very much slower after about 50% of 5-HT is liberated.

Reserpine prevents the uptake of catecholamines (Born & others, 1958; Hughes & Brodie, 1959), and it is therefore likely that these amines, although less concentrated in platelets, are also released by reserpine. Since one molecule of reserpine releases several hundred 5-HT molecules and since the rate or degree of depletion is not related to the 5-HT content of platelets, the mechanism of reserpine action cannot be one of simple displacement. Experiments on lysed platelets have failed to show any binding of 5-HT with platelet components (Hughes, Shore & Brodie, 1958; Sano, Kakimoto & Taniguchi, 1958). Electron microscopy reveals no obvious change in the structure of platelets (Telkkä & others, 1964). Impairment of the active transfer mechanism has been emphasised by Brodie and his colleagues and this would mean that reserpine acts by blocking this carrier mechanism at the platelet membrane (Hughes & Brodie, 1959). As a consequence, the amine could diffuse out passively. Although the best explanation available, it is not compatible with the findings that reserpine-induced release is prevented by lowering the temperature. Bartholini, Da Prada & Pletscher (1965) have recently demonstrated that reserpine prevents spontaneous 5-HT release in a glucose-free potassium phosphate medium probably through an action on the membrane of platelets, but accelerates the release when glucose is supplied. The utilisation of glucose in the cold is inhibited and, as a consequence, this membrane-stabilising action of reserpine might be dominant at low temperatures. Active transport as a mechanism in the outward flux of amines from nerve granules, has been suggested by von Euler, Stjärne & Lishajko (1964). If active outward transport occurs in platelets it could explain the failure of reserpine to act in the cold.

Reserpine does not influence the amount of ATP in platelets. It is also without effect *in vitro* on the intra-platelet content of potassium and causes no change in the protein content or packed volume of platelets (Paasonen, 1964; Paasonen & Solatunturi, 1965). In man, about 1 mg/day is sufficient to maintain platelet 5-HT depletion (Sjoerdsma, Weissbach, Terry & Udenfriend, 1957).

Tetrabenazine is a benzoquinolizine derivative which releases 5-HT from platelets *in vitro* (Paasonen & Pletscher, 1959). In concentrations that *per se* cause some 5-HT depletion it causes inhibition of the reserpine-induced 5-HT release *in vivo* from brain (Quinn, Shore & Brodie, 1959) and from platelets *in vitro* (Paasonen, 1964). This is taken as an indication of the same point of action for tetrabenazine and reserpine. Chlorpromazine, on the other hand, potentiates the 5-HT releasing action of reserpine (Paasonen, 1964). Monoamine oxidase inhibitors also antagonise the 5-HT depletion from platelets (Paasonen & Pletscher, 1960; Paasonen, 1961b). Although reserpine liberates histamine from rabbit platelets *in vivo* (Waalkes, Coburn & Terry, 1959) it is not known why this does not occur *in vitro* (Burkhalter, Cohn & Shore, 1960).

Phenothiazines and allied drugs. It was reported by Marshall, Stirling, Tait & Todrick (1960) that, during imipramine treatment, the 5-HT content of platelets decreases in three weeks to about one-sixth of the original level. These authors also demonstrated that imipramine *in vitro* prevents the uptake of 5-HT by human platelets. Of a number of substances studied by Stacey (1961), imipramine causes 50% inhibition of the 5-HT uptake at a concentration of 5×10^{-7} M. The next most active agents, cocaine and chlorpromazine, are equipotent at concentrations of $2{\cdot}5 \times 10^{-5}$ M and $3{\cdot}5 \times 10^{-5}$ M respectively. *In vitro*, chlorpromazine, imipramine and related agents also cause 5-HT release from platelets of man and rabbit (Bartholini, Pletscher & Gey, 1961). The release by chlorpromazine is dose dependent (Fig. 1) and a high enough concentration causes a total 5-HT depletion. The release is fast with high concentrations and at 10^{-3} M, chlorpromazine releases more than 90% of the amine within 15 min. This would mean that the depletion cannot be due to a simple inhibition of the uptake mechanism. It has been suggested by Paasonen (1964) that chlorpromazine facilitates the permeation of 5-HT through the limiting membranes. Proof of a morphological change in the platelets is given by the finding that chlorpromazine, unlike reserpine, decreases the packed platelet volume (Paasonen, 1964). The size of the platelets is also reduced when the platelet-rich plasma is observed under the microscope. Therefore, the result cannot be due merely to centrifugation. Electron microscopical studies by Telkkä & others (1964) show that incubation of platelet-rich plasma with chlorpromazine damaged the limiting membranes, allowing part of the cytoplasm to leak out (Fig. 2).

Consistent with the structural change are the findings that, *in vitro*, other components—histamine, ATP and potassium—are liberated from platelets by chlorpromazine (Fig. 1) (McLean, Nicholson & Hertler, 1963; Paasonen 1964; Paasonen & Solatunturi, 1965b). The liberation

of histamine proceeds parallel to that of 5-HT but the potassium liberation does not (Fig. 1). Potassium has been demonstrated by Buckingham & Maynert (1964) to exist largely in the free state in platelets, unlike 5-HT, and the faster initial release of potassium may be because of this fact.

Lowering the temperature antagonises the release of 5-HT caused by chlorpromazine but to a less extent than that caused by reserpine; also monoamine oxidase inhibition has hardly any effect (Paasonen, 1964). These findings are also compatible with the increased membrane permeability.

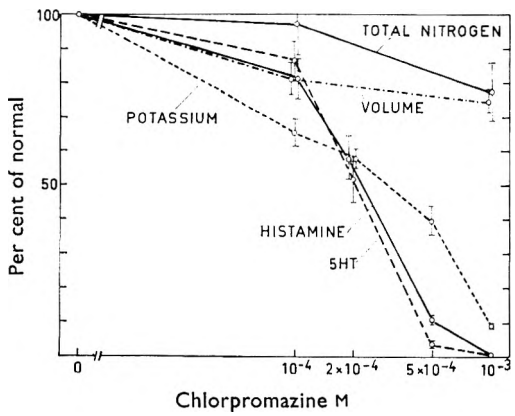

FIG. 1. The effect of chlorpromazine on the amount of various components and packed volume of platelets of rabbit. The platelet-rich plasma was incubated at 37° for 3 hr with the concentrations of chlorpromazine indicated (Paasonen & Solatunturi, 1965b). By permission of Editors of *Ann. Med. exp. Fenn.*

The following results of Ahtee & Paasonen (unpublished) point to a relationship between the 5-HT depletion and the content of the releasing agent in question inside, or in the membrane of, platelets. Chlorpromazine causes haemolysis *in vitro* and *in vivo* and red blood cells are able to concentrate this agent. Similarly the chlorpromazine content is considerably higher in the platelets than in the plasma. *N*-Hydroxyethylpromethazine (Aprobit), which is a quaternary phenothiazine compound,

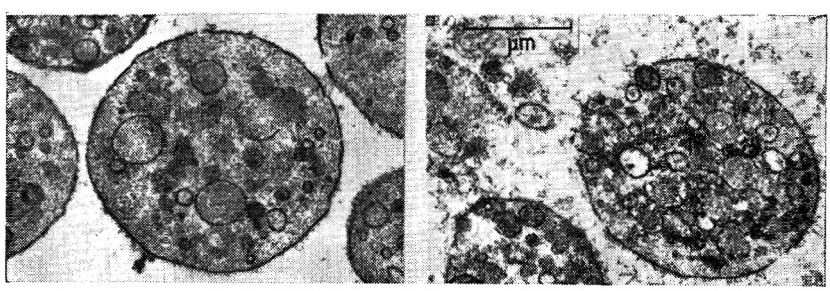

FIG. 2. Electronmicrograph of platelets of rabbit after 1 hr incubation at 37° with (on the right) and without (on the left) chlorpromazine (10^{-3} M). Chlorpromazine ruptures the cell membrane and part of the cytoplasmic material leaks out.

causes no haemolysis of red blood cells and no 5-HT release from platelets. Neither do red blood cells nor platelets absorb this substance as they absorb other phenothiazines.

In vivo, chlorpromazine does not lower the 5-HT content of platelets in acute experiments on rabbits or rats (Paasonen, unpublished). In chronic experiments on psychiatric patients it may cause some decrease in the platelet 5-HT but this effect is not consistent. The reason for this is probably the inadequate amount of chlorpromazine taken up by platelets *in vivo*. Preliminary experiments by Ahtee & Paasonen indicate that, in acute experiments, chlorpromazine decreases the content of 5-HT in the lungs of rabbits. It is known that the concentration of chlorpromazine is higher in this tissue than in others.

Nathan & Friedman (1962) have found that chlorpromazine increases the permeability of *Tetrahymena pyriformis*, a ciliate protozoan. On the other hand, phenothiazines also have a permeability decreasing action on membranes. Freeman & Spirtes (1963) have shown that chlorpromazine prevents the swelling of mitochondria and the haemolysis of red cells in hypotonic solutions. The membrane stabilising actions may well be operating when chlorpromazine acts in therapeutic concentrations *in vivo*. These actions may explain why, for example, chlorpromazine counteracts the reserpine-induced decrease of the monoamines in some experiments (Gey & Pletscher, 1961; Costa, Garattini & Valzelli, 1960).

Guanethidine and prenylamine. Guanethidine lowers the content of catecholamines in the heart and spleen (Sheppard & Zimmerman, 1959.) Unlike reserpine, guanethidine does not reduce the catecholamine content of brain and adrenals (Cass, Kuntzman & Brodie, 1960). Guanethidine lowers the platelet 5-HT *in vitro* but again a concentration of 10^{-3} M or more is needed in platelet-rich plasma (Paasonen, unpublished).

Prenylamine (*N*-3′-phenylpropyl-2′-)1-diphenylpropyl-3-amine is a vasodilating agent which reduces 5-HT and noradrenaline levels both centrally and peripherally (Schöne & Lindner, 1960). Carlsson & others (1963) found it to be a very active inhibitor of the catecholamine uptake by granules of the adrenal medulla. Prenylamine depletes 5-HT from platelets too, and its activity *in vitro* is of the same order of magnitude as that of reserpine (Paasonen, unpublished). The following results (Paasonen, unpublished) indicate a certain difference in the action of guanethidine and prenylamine on the one hand and reserpine on the other. Addition of tetrabenazine to platelet-rich plasma does not clearly inhibit the action of guanethidine and prenylamine in the same way as it inhibits the action of reserpine. Monoamine oxidase inhibitors inhibit the effect of reserpine but less than that of these two agents. The action of guanethidine and prenylamine in these three tests lies somewhere between the action of reserpine and that of chlorpromazine. The failure of guanethidine and prenylamine to change the packed platelet volume indicates that they do not cause structural changes *in vitro* like chlorpromazine.

Sympathomimetic amines and related agents. Stacey (1961) showed that amphetamine, tyramine, cocaine and various other agents prevent the uptake of 5-HT by human platelets *in vitro*. As 5-HT releasers these

compounds seem to be less effective. Buckingham & Maynert (1964) found that 1 mg/ml of amphetamine releases 79% of 5-HT from human platelets in 60 min. The same concentration of adrenaline releases 24% and tryptamine 87%. A concentration of about 10^{-3} M of tyramine, cocaine and α-methyldopa is needed to lower the platelet 5-HT *in vitro* (McLean & others, 1963; Bartholini & others, 1961). According to Bartholini & Pletscher (1964) the *p*-chloro derivative of *N*-methylamphetamine (Ro 4–6861) also liberates 5-HT from platelets *in vitro*.

Nicotine. According to Schievelbein & Werle (1962) and Schievelbein (1963), nicotine causes depletion of 5-HT from rabbit platelets *in vitro* at concentrations higher than 4×10^{-4} M. Much more of the alkaloid seems to be necessary for a pronounced release and the concentrations employed by the authors usually seem to be ten times higher than the figure mentioned above. The depletion is not influenced by changes of temperature between 20 and 37°, but the amount of 5-HT released is proportional to the nicotine concentration used. Histamine and catecholamines are also released by nicotine (Schievelbein & Zitzelsberger, 1964). Monoamine oxidase inhibitors inhibit the release and they also antagonise the effect of nicotine in depressing the uptake of 5-HT. The authors conclude that the site of action of nicotine is in the activation of monoamine oxidase in the platelet.

Other agents. Various haemolytic agents, like desoxycholic acid, digitonin, lysolecithin or bee venom, cause 5-HT liberation from platelets (Habermann & Springer, 1958). Some of the agents mentioned above, for example phenothiazines, also induce haemolysis, and the 5-HT release and haemolysis have similarities (Ahtee & Paasonen, 1965). Although the monoamine oxidase inhibitors pheniprazine and iproniazid antagonise the reserpine-induced 5-HT liberation from platelets *in vitro*, they themselves cause 5-HT release at a concentration of about 10^{-3} M or higher.

High concentrations of ouabain, sodium fluoride, iodoacetic acid or *p*-chloromercuribenzoic acid release 5-HT as well as potassium and amino-acids from platelets *in vitro* (Buckingham & Maynert, 1964).

Daily doses of cortisone cause some depletion of 5-HT as well as of histamine from tissues not containing mast cells (platelets, outer skin, intestine) in rats after 3 to 7 days (Cass & Marshall, 1962). Large doses of ACTH have a similar though less marked effect.

COMMENT

The results obtained by different workers *in vitro* are seldom comparable, particularly since the composition of the suspension fluid differs. When platelet-rich plasma is employed, the anticoagulants like EDTA may influence the results by removing calcium ions. As was mentioned earlier, calcium is necessary for the 5-HT releasing action of thrombin, trypsin, bacterial endotoxin, antigen-antibody reaction, glycogen or fatty acids. Heparin, on the other hand, prevents the action of thrombin, but the action of other factors of endogenous origin is not prevented at all or only by high concentrations. According to Zucker & Borrelli (1955b) platelets retain an abnormal spiney appearance during incubation in saline.

Incubation in plasma or in saline with 6% albumin promotes the disc shape again. The anticoagulants seem to have no fundamental effect on the 5-HT release induced by drugs from platelets in platelet-rich plasma. According to Born & Gillson (1959), the uptake of 5-HT is quicker in citrated plasma than in plasma treated with EDTA. Inhibition of the uptake by any agent does not necessarily mean that it also causes a release. However, any drug causing a release of a subcellular constituent will prevent the uptake of that substance.

From the work of Grette (1962) it can be concluded that thrombin acts on the surface membrane of the platelet and increases the permeability to calcium ions. Subsequently the intracellular calcium ions initiate contraction and the quick release of 5-HT and some additional components of the platelets. The release takes place without lysis of the cells but viscous metamorphosis is a parallel process at least with thrombin and trypsin.

There are certain similarities in the 5-HT release caused by endogenous factors. The involvement of proteolytic activation has been suggested as a common mechanism in most instances. The work done to explain the histamine liberation from mast cells (Uvnäs, 1958; West, 1959) is likely to be of value in studying the release of 5-HT. Some results indicate that histamine and 5-HT are localised in the same storage sites and that both amines are released *in vitro* by 48/80 (Schievelbein & Zitzelsberger, 1964) and by chlorpromazine.

Although reserpine is the best established 5-HT releaser, its mode of action is not yet clear. The only way to induce a pronounced depletion of 5-HT from platelets *in vivo* within a day is to give certain rauwolfia alkaloids. The mechanism of action of tetrabenazine is most probably similar, and a lack of effect by this drug *in vivo* is a question of dose and time of action.

The platelet damaging action of phenothiazines *in vitro* has similarities with the action of proteolytic enzymes (Paasonen & Solatunturi, 1965b). *In vivo* the lack of effect of chlorpromazine is probably again a matter of local concentration, and the membrane stabilising action of small concentrations as is suggested to occur in the brain by Gey & Pletscher (1961) may well be operating *in vivo* in platelets too. In the rat brain, however, Giarman & Schanberg (1962) found that chlorpromazine decreased the ratio of 5-HT present in the particles and in the supernatant fluid without decreasing the total amine. This finding is in keeping with the experiments showing *in vitro* platelet damage previously discussed in detail.

Not much is known about the 5-HT releasing action of the other drugs mentioned. Perhaps, with the exception of prenylamine, their influence on the platelets resembles that of chlorpromazine rather than reserpine. They act quicker than reserpine and at least some of them are known to liberate potassium, amino-acids and ATP from platelets. According to Buckingham & Maynert (1964), reserpine-treated platelets fail to accumulate 5-HT even after washing, whereas the effect of amphetamine can be washed out. The list of 5-HT releasers is growing but the list of mechanisms involved need not similarly increase.

There may be more than one storage site for noradrenaline at the nerve endings (Kopin, 1964), but there is no clear evidence that 5-HT is stored in more than one type of particle in platelets. Practically nothing is known about the release of 5-HT from the intra-platelet store(s). It is therefore impossible to discuss release at different cellular levels. An extensive discussion about the possible cellular mechanisms of amine binding has been presented by Green (1962).

Metabolism of 5-HT by platelets

In spite of the lack of synthesis, the platelets also possess the ability to metabolise 5-HT. When the platelet-rich plasma of rabbit is incubated *in vitro* with reserpine, about half of the platelet-amine will be liberated within 3 hr. Paasonen & Pletscher (1959) found no concomitant 5-HT increase in the plasma outside the platelets when the incubation was in air. Pretreatment of rabbits with a monoamine oxidase inhibitor (Paasonen & Pletscher, 1960), addition of such an inhibitor to the incubation material, or incubation in nitrogen (Paasonen, 1961a) all prevented the metabolism of the liberated amine. A minute amount of oxygen in the atmosphere is sufficient to metabolise the released 5-HT. Although the platelet-free plasma of rabbit has a weak 5-HT-metabolising activity *in vitro* (Paasonen & Airaksinen, 1965b), the plasma is not, or only to a small extent, responsible for the metabolism of the 5-HT liberated from the platelets. A large amount of 5-HT released from human platelets *in vitro* is also metabolised by the platelets, but only a small amount by platelets of the rat (Paasonen, 1961a).

Incubation of whole blood of the rabbit with reserpine causes a 5-hydroxyindoleacetic acid (5-HIAA)-like material to appear in plasma (Waalkes & Coburn, 1958). This requires the presence of the red blood cells, but these cells themselves are not able to metabolise 5-HT. A summary of the metabolism of the 5-HT released from platelets is presented in Fig. 3, taken from a report by Paasonen & Airaksinen (1965a). During

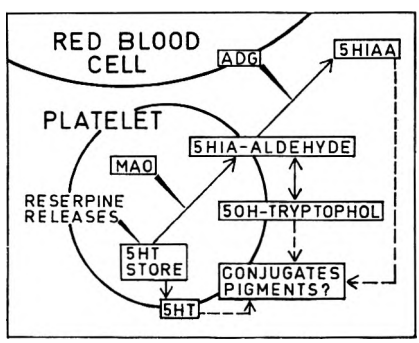

FIG. 3. The fate of 5-HT released from platelets by reserpine-like drugs. Mono amine oxidase converts 5-HT to 5-hydroxyindoleacet(5-HIA)-aldehyde which is then oxidised in the plasma to 5-hydroxyindoleacetic acid (5-HIAA) in the presence of red blood cells or their aldehyde dehydrogenase (ADG). For further explanation see text (Paasonen & Airaksinen, 1965a). By permission of Editors of *Ann. Med. exp. Fenn.*

the liberation from the platelets of 5-HT by reserpine, most of the amine is oxidized to 5-hydroxyindoleacetaldehyde and then reduced to 5-hydroxytryptophol [2-(5-hydroxyindol-3-yl)ethanol]. *O*-Sulphate conjugates of 5-hydroxytryptophol are probably also formed. If a small amount of blood or aldehyde dehydrogenase prepared from red blood cells is present in the platelet-rich plasma during the incubation, about 75% of the 5-HT liberated by reserpine is oxidised to 5-HIAA. Its phenolic sulphate is also formed. Bartholini, Pletscher & Bruderer (1964) have also reported the formation of 5-hydroxytryptophol by platelets. Monoamine oxidase inhibitors prevent this metabolism of 5-HT by platelets. According to Horita (1963), red blood cells prevent this action of hydrazine monoamine oxidase inhibitors like iproniazid and pheniprazine. Therefore, in experiments involving red blood cells and monoamine oxidase inhibitors, it is advisable to use non-hydrazine inhibitors, as for example pargyline, tranylcypromine or harmine.

The presence of monoamine oxidase in platelets is now well established (Paasonen, Solatunturi & Kivalo, 1964) and the activity of the enzyme per unit weight of homogenised platelets is about the same as that in the sympathetic ganglia. The monoamine oxidase activity of homogenised platelets of some mammalian species is listed in Table 2. Bone marrow

TABLE 2. THE MONOAMINE OXIDASE ACTIVITY IN PLATELETS OF SOME MAMMALIAN SPECIES EXPRESSED AS THE AMOUNT OF INDOLEACETIC ACID FORMED FROM TRYPTAMINE *in vitro* (Paasonen & Solatunturi, 1965a)

Species	nmol indoleacetic acid/mg/hr ± s.e.
Rabbit	1·250 ± 0·066 (9)
Human	0·700 ± 0·094 (5)
Cattle	0·157 ± 0·025 (4)
Dog	0·102 ± 0·002 (3)
Cat	0 (10)
Horse	0 (5)
Rat	0 (4)

No. of animals in parentheses.

of rabbit formed 1·000 ± 0·110(4) and that of cat 0·367 ± 0·092(3) nmol of indoleacetic acid/mg/hr. Because only a small part of bone marrow consists of megakaryocytes, the relatively high monoamine oxidase values obtained indicate high enzyme activity in these parent cells of platelets (or in other cells present). In our experiments where 5-HT was liberated by chlorpromazine from platelets in platelet-rich plasma, rabbit platelets metabolised their whole 5-HT content if the concentration of chlorpromazine was such that the time of total release was about one hr or more. The amine release during clotting occurs too quickly to be influenced by the monoamine oxidase of platelets.

It is likely that other pharmacologically active amines are also metabolised by the platelets. At least rabbit intact platelets *in vitro* oxidase adrenaline (Paasonen & Lahovaara, unpublished). The physiological and pharmacological significance of monoamine oxidase in platelets is unknown as also is the question of the extent to which the oxidative deamination occurs in these cells *in vivo*.

Platelet 5-HT and diseases

It is well known that an abnormally high amount of tryptophan is oxidized to 5-HTP and subsequently to 5-HT and 5-HIAA by malignant *carcinoid tumours*. Accordingly, relatively high values for 5-HT have been reported by several workers in the blood of these patients, sometimes associated with thrombocytosis. The increased level of 5-HT in platelets is probably due to an increased amount of free 5-HT in plasma available for absorption (Sjoerdsma & others, 1957).

In some *mentally defective patients*, for example those with cerebral palsy (with I.Q. <50) or with maternal rubella, Pare, Sandler & Stacey (1960) observed high levels of 5-HT in the serum and platelets. According to these workers the reason is not to be found in an increased uptake of 5-HT by platelets as studied *in vitro*, or in an increased ATP content in platelets. The volume and monoamine oxidase content of the platelets in these patients are normal (Paasonen & Kivalo, 1962; Paasonen & others, 1964). In this context it is interesting to note that lowered amounts of 5-HT were found by Krieger, Kolodny & Warner (1964) in the serum of patients suffering from certain brain tumours, especially those located in the hypothalamus. This might indicate that the function of certain brain areas may influence the structure of platelets, making them less capable of absorbing 5-HT *in vivo*, or may decrease the amount of 5-HT available in plasma for absorption, or alternatively impair the uptake and storage of 5-HT in some other way. It could be suggested that in mental deficiency with an elevated platelet 5-HT there is a hyperfunction of those brain areas whose injury leads to lowered platelet 5-HT values.

In *blood diseases* the most common finding is that platelets contain less 5-HT than normal. Hardisty & Stacey (1957) measured low values in myeloid and lymphatic leukaemia and pernicious anaemia as well as in iron deficiency, Hodgkin's disease and polycythaemia vera. The absorption of 5-HT *in vitro* was subnormal in many of these cases. In thrombocythaemias the 5-HT content of platelets can be normal, subnormal or above normal. The reason for the decrease may be connected with structural defects known to occur at least in some of these dyscrasias (Schulz, Jürgens & Hiepler, 1958).

Decreased amounts of 5-HT in platelets have been demonstrated in *rheumatoid arthritis* and other *inflammatory states* by Kerby & Taylor (1959a), who also noted (1959b) a decreased uptake of 5-HT by platelets of these patients *in vitro*.

Platelet 5-HT as a model in pharmacology

The concentration of 5-HT and other pharmacologically active substances in platelets as in some tissues of particular pharmacological interest such as nerve endings, brain cells and adrenal medulla, points to certain similarities between platelets and the other tissues. The release and uptake experiments are comparable in many respects. Only those rauwolfia alkaloids which have a sedative action and which cause amine depletion in the brain, cause a liberation of 5-HT from platelets (Brodie,

Shore & Pletscher, 1956). Of the phenothiazines, *N*-hydroxyethylpromethazine, a non-sedative agent which does not enter the brain, is not absorbed by the platelets and causes no 5-HT release (Ahtee & Paasonen, 1965 & unpublished). It differs in these respects from chlorpromazine. Thrombin, which releases 5-HT from platelets, has been shown by Markwardt & Nuernbergk (1965) to liberate catecholamines from perfused suprarenals.

It is conceivable that the absorption of 5-HT and other compounds from plasma by platelets is used as a means of detoxication. As has been discussed, it is likely that the mammalian body has facilities of its own for releasing 5-HT, and other amines, from the platelets and to metabolise the amine(s). Modification of either the uptake, the release, or the metabolism of the amine is expected to cause reactions whose nature remains undiscovered. In 5-HT research, platelets serve mainly as a model system for solving problems of general interest. The results obtained are also useful if they throw some light on the rôle of platelets themselves in health and disease.

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Research Papers

Some geometrical considerations concerning the design of tablets

J. P. CLEAVE

A theoretical examination of designs best suited to give a uniform rate of release of materials from solution tablets is presented.

THERE are circumstances in medical and industrial practice where solid material in the form of tablets has to pass into solution rather than to disintegrate. In certain circumstances it is desirable that the material should be released at a uniform rate. The purpose of this paper is to examine the theoretical considerations which underly the choice of a design to achieve this object.

Let a tablet be immersed in a fluid: consider a small region ABCD of area Δs on the surface of the tablet (Fig. 1). In a small interval of time

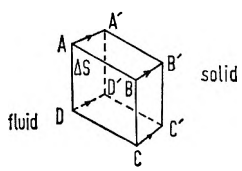

FIG. 1.

Δt a volume $\sigma \Delta s\, \Delta t$ of the tablet in the form of a layer ABCDA'B'C'D', where $AA' = BB' = CC' = DD' = \sigma \Delta t$(AA', BB', CC', DD' perpendicular to the plane ABCD) will pass into solution. It will be assumed that σ is a constant. This will be (approximately) so if the material of the tablet is of uniform composition and if certain obvious conditions on the solubility and rate of diffusion and on the relative volumes of tablet and fluid are met. Consider, then a tablet in the form of a parallelipiped, ABCDA'B'C'D' (Fig. 2) with $CD = DD' = l$, $AD = m$.

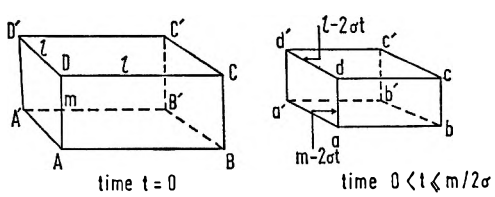

FIG. 2.

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From the preceding considerations we infer that in any interval Δt (preceding the tablet's final disappearance) each dimension of the tablet is reduced by a length $2\sigma\Delta t$. Hence, if the tablet is placed in fluid at time $t = 0$, at time t it will be reduced to the parallelipiped abcda'b'c'd' (Fig. 2) where $aa' = AA' - 2\sigma t$, $ab = AB - 2\sigma t$, ... Hence, supposing $m \leqslant l$, the tablet will be completely dissolved when $m - 2\sigma t = 0$, i.e. at time $\tau = m/2\sigma$. So by computing surface areas we find that the rate of solution $\Sigma(t)$ at time t, for $t < \tau$, is given by

$$\Sigma(t) = \sigma.2l(2m + l) - 8\sigma^2 t(m + 2l) + 24\sigma^3 t^2 \quad .. \quad (1)$$

Fig. 3 shows the graph of $\Sigma(t)$.

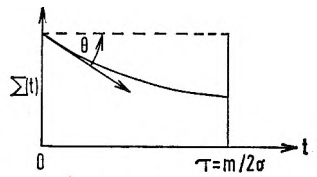

FIG. 3. Time variation of rate of solution.

The noteworthy feature here is that the rate of solution initially drops, the tangent to the curve at $t = 0$ being inclined at an angle θ to the t-axis, where $\tan\theta = 8\sigma^2 (m + 2l)$ = coefficient of t in $\Sigma(t)$. Further it is clear that no adjustment of dimensions of the tablet (short of putting $m = l = 0$!) can make $\theta = 0$. The mathematical problem we face can now be defined. For a given tablet, let $\Sigma(t)$ denote the rate of solution at time t after first being immersed in fluid and let τ denote the time at which the tablet is first completely dissolved. Assume that $\Sigma(t)$ can be expanded in the form

$$\Sigma(t) = \Sigma(0) + A_1 t + \phi(t) \quad .. \quad .. \quad (2)$$

where $*\phi(t) = a_0 t^2 + a_1 t^3 + \ldots$. The constant A_1 represents the initial rate of fall of $\Sigma(t)$, i.e. $\tan\theta = \left(\frac{d\Sigma}{dt}\right)_{t=0} = A_1$. In general A_1 is a function of the initial size of the tablet [c.f. (1)]. Thus our problem is to define some shapes of tablet for which $A_1 = 0$: then for such shapes

$$\Sigma(t) = \Sigma(0) + \phi(t), \quad \phi(t) = a_0 t^2 + a_1 t^3 + \quad .. \quad (3)$$

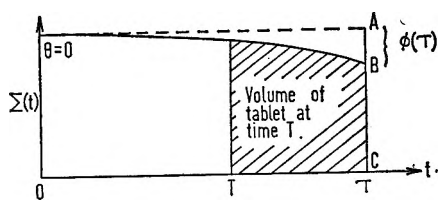

FIG. 4. Rate of solution of crs-tablets.

* Note $\int_0^\tau \Sigma(t)dt$ = initial volume of tablet
= area under graph of $\Sigma(t)$.

Tablets having such a shape we call *crs-tablets*: the graphs of their rate of solution have the form shown in Fig. 4. Of course, in general, the rate of change of $\Sigma(t)$, even for crs-tablets is non-zero because of the term $\phi(t)$ in (3). Hence as a measure of efficiency, ϵ, of a crs-tablet we take the proportionate drop in the rate of solution when the tablet is finally dissolved, i.e. referring to Fig. 4.

$$\epsilon = \frac{BA}{CA} = \frac{\Sigma(0) - \Sigma(\tau)}{\Sigma(0)} = \frac{\phi(\tau)}{\Sigma(0)} \quad .. \quad .. \quad (4)$$

Thus to get $\Sigma(t)$ as nearly constant as possible in a crs-tablet we aim, by adjusting the dimensions of the tablet, to make ϵ as small as possible.

The tablet shown in Fig. 2 is a non crs-tablet because the surface area decreases as the tablet dissolves. Our plan is to compensate for this decrease by incorporating in the tablet a surface whose area increases. Consider a cylindrical hole in a tablet (Fig. 5). If the hole has radius r

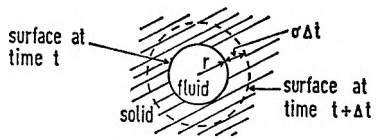

FIG. 5

at time t, then by the assumptions of the second paragraph, the radius will increase to $r + \sigma\Delta t$ at $t + \Delta t$. Hence the surface area of the hole will increase. We shall therefore consider tablets with holes in them: by appropriate choice of dimensions the tablets can be given the desired property.

Crs-tablets

In order to minimise computational complexity in finding surface areas we consider only tablets in the form of parallelipipeds.

1. One hole tablets (Fig. 6).

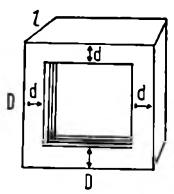

FIG. 6

Suppose $l \leqslant d$. Then $\tau = d/2\sigma$ and

$$\Sigma(0) = 8(l + d)(D - d)\sigma$$

$$\Sigma(t) = \Sigma(0) + 32\sigma^2 (d - D)t \text{ for } 0 \leqslant t \leqslant \tau \quad .. \quad (5)$$

(In this instance $\phi(t) = 0$, though this is not the case with the following examples). Now $D \geqslant 2d$. So comparing (5) and (2) we have $A_1 = 32\sigma^2 (d - D) < 0$. Thus a one-hole tablet of this type cannot be a crs-tablet.

2. Two hole tablets (Fig. 7).

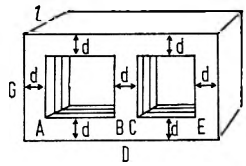

FIG. 7

Suppose $l \geqslant d$, AB = CE. Then $D \geqslant 3d$, $G \geqslant 2d$, $\tau = d/2\sigma$. Thus

$$\Sigma(0) = 2\sigma[l(3G + 2D - 7d) + d(3G + 2D - 6d)]$$

$$\Sigma(t) = \Sigma(0) - 8\sigma^2(3G + 2D - l - 7d)t + 24\sigma^3t^2 \text{ if } 0 \leqslant t \leqslant \tau \qquad (6)$$

Comparing (3) and (6) it can be seen that we can construct a crs-tablet by choosing

$$3G + 2D = l + 7d \qquad (7)$$

The efficiency, by (4) is then, using (7),

$$\epsilon = 3d^2/[(3G + 2D - 7d)^2 + d(3G + 2d - 6d)] \qquad (8)$$

Examples:

(i) D = 5d, G = 3d. By (7), $l = 12d$; (8) gives $\epsilon \cong \frac{1}{52}$.

(ii) D = 7d, G = 3d. Then $l = 16d$ and $\epsilon \cong \frac{1}{90}$.

Further information can be extracted from (8). In fact we can construct an upper bound for ϵ. Define μ by $(3G + 7d)/d = 12 + \mu$. Then since $D \geqslant 3d$, $G \geqslant 2d$ we have $\mu \geqslant 0$. Further, (8) can be rewritten as

$$\mu^2 + 11\mu + (31 - 3/\epsilon) = 0 \qquad (9)$$

The condition $\mu \geqslant 0$ requires that (9) have one positive solution.

Thus $$0 < \epsilon \leqslant 3/31,$$

i.e. it is impossible to get the efficiency worse than 3/31. For any ϵ such that $0 < \epsilon \leqslant 3/31$ a positive μ can be computed from (9) and hence G, D and l can be found.

3. Three-hole tablets (Fig. 8).

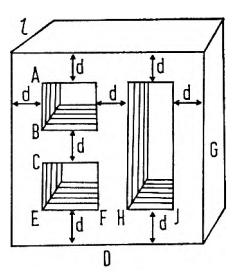

FIG. 8

Suppose $l \geqslant d$, AB = CE, EF = HJ. Then $G \geqslant 3d$, $D \geqslant 3d$, $\tau = d/2\sigma$. Hence,

$$\Sigma(0) = \sigma[l(6G + 5D - 19d) + d(6G + 5D - 15d)]$$

$$\Sigma(t) = \Sigma(0) - 4\sigma^2(6G + 5D - 19d - 4l)t - 48\sigma^3t^2 \text{ for } 0 \leqslant t \leqslant \tau \quad (10)$$

By putting

$$6G + 5D = 4l + 19d \quad .. \quad .. \quad .. \quad (11)$$

in (10) we have a crs-tablet whose efficiency, by (4) and (11) is

$$\epsilon = 48d^2/[(6G + 5D - 19d)^2 + 4d(6G + 5D - 15d)] \quad (12)$$

Examples:

(i) G = 5d, D = 5d. By (11), $l = 9d$. By (12), $\epsilon \cong 1/33$.

(ii) G = 5d, D = 7d. Then $l = 23d/2$ so that $\epsilon \cong 1/48$.

Next, by imposing the conditions G, D $\geqslant$ 3d on (12) we get

$$0 < \epsilon \leqslant 12/77.$$

4. Four-hole tablet (Fig. 9).

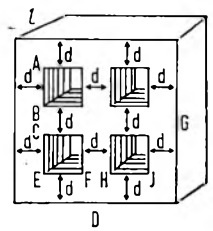

FIG. 9

Suppose $l \geqslant d$, AB = CE, EF = HJ. Then D, $G \geqslant 3d$, $\tau = d/2\sigma$.

$$\Sigma(0) = 6\sigma[l(D + G - 4d) + d(D + G - 3d)]$$

$$\Sigma(t) = \Sigma(0) - 24\sigma^2(D + G - 4d - l)t - 72\sigma^3t^2 \text{ for } 0 \leqslant t \leqslant \tau.$$

If

$$D + G = 4d + l$$

we have a crs-tablet whose efficiency is given by

$$\epsilon = 3d^2/[(D + G - 4d)^2 + d(D + G - 3d)] \quad .. \quad (13)$$

Examples:

(i) G = 5d, D = 5d; l = 6d and $\epsilon \cong 1/14$.

(ii) G = 7d, D = 5d; l = 8d and $\epsilon \cong 1/24$.

(iii) G = 9d, D = 5d; l = 10d and $\epsilon \cong 1/36$.

Finally, the conditions D, G $\geqslant$ 3d and (13) require

$$0 < \epsilon \leqslant 3/7.$$

Conclusion

Crs-tablets can be constructed with two or more holes—a single hole is unable to compensate the decrease in the outer surface area. The 2, 3, 4-hole csr-tablets have a natural ratio above which the proportionate decrease in the rate of solution cannot rise; $\frac{3}{39}, \frac{12}{77}, \frac{3}{7}$ respectively. If we take these as figures of merit we see that the two-hole tablet is basically a better structure than the others.

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Pharmacological studies on modaline sulphate (W 3207)

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Modaline sulphate (W 3207) has pharmacological effects which probably belong both to the group of monoamine oxidase inhibitors and the imipramine-like drugs. It differs from the monoamine oxidase inhibitors by producing an increase in body temperature in fully reserpinized animals. It differs from imipramine-like drugs in not potentiating the hyperthermic effect of isoprenaline. The effects of modaline are probably related to the formation of a metabolite because they are prevented by the administration of SKF 525 A.

MODALINE sulphate, 2-methyl-3-piperidinopyrazine monosulphate (W 3207), is a new compound with an original chemical structure, unrelated to hydrazine, iminodibenzyl derivatives or other known antidepressant agents (Gylys & Osborne, 1962), showing therapeutic antidepressive action (Feldman, 1963; Dunlop, De Felice, Bergen & Resnick, 1964). This compound has pharmacological effects which are typical both of monoamine oxidase inhibitors and imipramine-like substances (Dubnick, Morgan & Phillips, 1963; Gylys, Muccia & Taylor, 1963). The purpose of this paper is to report investigations aimed at resolving these effects and to establish whether the reported pharmacological effects are induced by the compound itself or by its metabolic products.

Experimental

MATERIALS AND METHODS

Female Sprague-Dawley rats, average weight 100 g, and female Swiss mice, average weight 22 g, were used. Modaline was given intraperitoneally to all animals. Isoprenaline was infused into the rat tail vein for 15 min at a dose of 400 or 40 μg/rat/min. In these experiments rats were kept restrained in individual cylindrical cages (diameter 4·7 cm, length 15·5 cm) of galvanised wire; the thermometer electrode cable was retained in the rectum throughout the experiment. In other experiments, body temperature was recorded by inserting the thermometer at the moment of determination. Monoamine oxidase inhibition was estimated *in vitro* according to Weissbach, Smith, Daly, Witkop & Udenfriend (1960) by measuring the oxidation of kinuramine in brain homogenates of rats pretreated *in vivo* with modaline.

Drugs used for comparison were pheniprazine (JB 516) (Horita, 1958; 1959; Spector, Prockop, Shore & Brodie, 1958), *N*-benzoyl-*N'*-phenylethylhydrazine (T-3) (Bettinetti, 1961; Jori, Bonaccorsi, Valzelli & Garattini, 1963) and desipramine (Dubnick, Leeson & Phillips, 1962; Garattini, Giachetti, Jori, Pieri & Valzelli, 1962). Other details of the experimental conditions appear in the various tables. All doses are expressed as salts. Drugs used were kindly supplied by the following

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sources: modaline (W 3207 B) (Warner-Lambert), desipramine (Geigy), nortriptyline (Pharmacia), pheniprazine (JB 516) (Farber), *N*-benzoyl-*N'*-phenylethylhydrazine (T-3) (supplied by Dr. S. Pietra), L(−)-dihydroxyphenylalanine (dopa) (Hoffmann-LaRoche), reserpine (Serpasil) (Ciba), phentolamine (Regitin) (Ciba), propranolol (Inderal) (Imperial Chemical Industries), dibenamine (Smith Kline & French), isoprenaline (Isoproterenol) (Biosintex).

Results

INHIBITION OF MONOAMINE OXIDASE ACTIVITY

Brain homogenates obtained from rats treated with various drugs were analysed for their monoamine oxidase activity. Table 1 demonstrates

TABLE 1. MONOAMINE OXIDASE INHIBITION AFTER TREATMENT WITH VARIOUS DRUGS *IN VIVO* AND POTENTIATION OF TRYPTAMINE EFFECTS BY VARIOUS DRUGS

Compound*	Dose (mg/kg i.p.)	% inhibition of brain monoamine oxidase	Compound †	ED50; mg/kg i.p. fiducial limits P <0·05)
Modaline	4	56	Modaline	4·7 (10·30–2·10)
"	8	89	Modaline**	6·60 (12·20–3·50)
"	16	93	Pheniprazine	0·55 (0·96–0·31)
Pheniprazine	5	82	T-3	1·85 (0·90–3·70)
Desipramine	15	−15	Desipramine	>15·0

* Given 4 hr before assessing monoamine oxidase activity.
** Given 30 min before tryptamine.
† Compounds were given 4 hr before tryptamine (5 mg/kg i.v.) ED50 = dose enhancing the effects induced by tryptamine to 50% of the maximum possible increase.

that modaline shows an effect similar to pheniprazine in blocking the activity of this enzyme, whereas desipramine, in agreement with the observations of Pulver, Exer & Hermann (1960) and Usdin & Usdin (1961), is inactive.

POTENTIATION OF THE PHARMACOLOGICAL EFFECTS OF TRYPTAMINE

Monoamine oxidase inhibitors enhanced the convulsive or tonic actions induced by intravenous injection of tryptamine (Tedeschi, Tedeschi & Fellows, 1959) which included hunching of the back, backward locomotion, Straub tail, salivation and clonic convulsions of the anterior paws. These symptoms were scored according to an arbitrary scale (1 + to 3 + for clonic convulsions and 1 + for each of the other symptoms; maximum 7+).

Table 1 also reports the ED50 of modaline, pheniprazine, and T-3 in the potentiation of tryptamine. Desipramine had no effect at doses active in other tests. Modaline was often very active when injected 30 min before tryptamine. The potentiation it induces was inhibited by pretreatment with SKF 525 A (see Table 2), a compound known to inhibit several microsomal enzymes responsible for the metabolism of various drugs (Brodie, Gillette & La Du, 1958; Gillette, 1963).

POTENTIATION OF HYPERTHERMIA INDUCED BY DOPA

In mice treated with monoamine oxidase inhibitors, dopa induced an increase in the motor activity and in the body temperature (Everett,

TABLE 2. EFFECT OF SKF 525 A ON THE POTENTIATION OF TRYPTAMINE INDUCED BY MODALINE

Treatment	Dose of modaline mg/kg	%increase of tryptamine effects after	
		30 min*	4 hr*
SKF 525 A + Modaline	16	76 ± 3	—
— "	16	100 ± 0·01	—
SKF 525 A + "	8	21 ± 3	76 ± 4
— "	8	91 ± 2	92 ± 5
SKF 525 A + saline	—	—	37 ± 8

All the animals received tryptamine (5 mg/kg i.v.).
SKF was given orally at a dose of 50 mg/kg 30 min before modaline.
* Time between modaline and tryptamine.

Davin & Toman, 1959; Van der Wende & Spoerlein, 1962). The data reported in Table 3 show that all the tested antidepressant drugs increased the hyperthermic effect of dopa.

TABLE 3. POTENTIATION OF DOPA HYPERTHERMIA

Compound	Dose mg/kg	Body temperature (°C) ± s.e. after dopa	
		30 min	60 min
Saline	—	38·2 ± 0·1	35·5 ± 0·3
Modaline	4	39·5 ± 0·5*	38·4 ± 0·5*
"	8	39·2 ± 0·2*	39·6 ± 0·1*
"	16	40·2 ± 0·2*	39·9 ± 0·2*
T-3	10	38·7 ± 0·4	39·9 ± 0·3*
Pheniprazine	2·5	39·3 ± 0·4*	38·5 ± 0·2*
Desipramine	7·5	39·6 ± 0·2*	35·4 ± 0·5

The compounds were given 12 hr after pheniprazine (10 mg/kg i.p.) and 30 min before dopa (50 mg/kg i.p.).
* Difference statistically significant ($P < 0·01$) in respect to the group treated with saline.

RESERPINE INHIBITION

Pretreatment with modaline inhibited the hypothermia induced by intravenous administration of reserpine. Table 4 summarizes the results

TABLE 4. EFFECT OF VARIOUS DRUGS ON THE HYPOTHERMIA INDUCED BY RESERPINE

Treatment (mg/kg)		Changes of body temperature (°C) ± s.e. after reserpine			
		40 min	4 hr	6 hr	24 hr
Saline	—	+1·2 ± 0·08	−1·7 ± 0·2	−2·9 ± 0·2	−1·3 ± 3
Modaline*	16	+1·9 ± 0·1	+1·0 ± 0·7	+0·5 ± 0·1	—
"	8	+1·2 ± 0·3	−0·3 ± 0·2	−0·7 ± 0·3	−0·5 ± 0·4
"	4	+1·3 ± 0·4	−0·9 ± 0·3	−1·2 ± 0·2	−1·3 ± 0·5
"	2	+0·8 ± 0·3	−2·5 ± 0·3	−2·7 ± 0·5	−0·3 ± 0·6
Desipramine	15	+1·7 ± 0·1	+0·7 ± 0·2	+0·8 ± 0·1	+0·3 ± 0·4
Saline	—	+1·5 ± 0·1	−1·3 ± 0·2	−2·3 ± 0·2	−0·8 ± 0·2
Modaline**	16	+2·4 ± 0·1	−0·1 ± 0·1	−0·3 ± 0·1	−0·6 ± 0·2
"	8	+2·0 ± 0·4	−0·5 ± 0·2	−0·9 ± 0·5	−1·0 ± 0·3
"	4	+0·8 ± 0·9	−3·1 ± 1·4	−2·7 ± 0·7	−0·2 ± 0·6
"	2	+1·5 ± 0·3	−2·6 ± 0·5	−3·3 ± 0·6	−1·4 ± 0·8
Desipramine	15	+1·5 ± 0·3	+0·4 ± 0·4	−1·1 ± 0·5	−0·5 ± 0·2
Pheniprazine	10	+2·8 ± 0·6	−0·2 ± 0·3	+0·5 ± 0·5	−1·1 ± 0·3

Reserpine was given intravenously (2·5 mg/kg). Drugs were given intraperitoneally 30 min* and 18 hr** before reserpine.

obtained with various doses of modaline when it was injected 30 min and 18 hr before reserpine. Modaline antagonised hypothermia in fully

reserpinised rats when it was injected 18 hr after reserpine. The data in Fig. 1 show that it paralleled the effect induced by desipramine at the

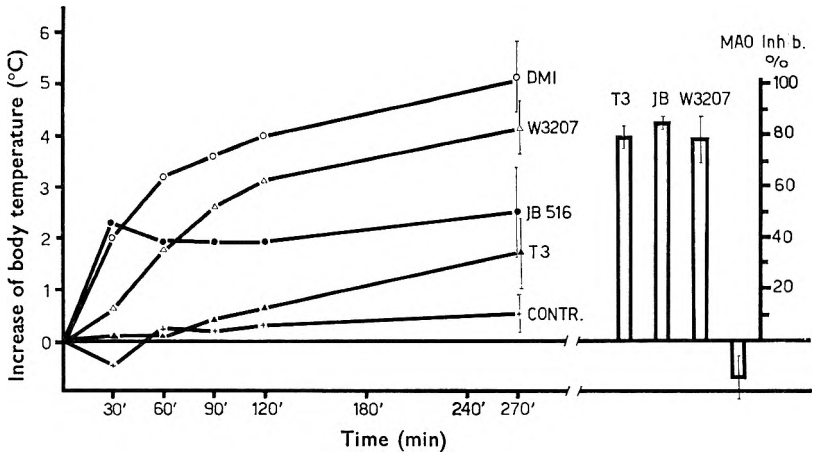

FIG. 1. Rats were treated 18 hr before the test with reserpine (5 mg/kg i.v.) at a room temperature of 20°. Drugs were injected i.p. at the beginning of the experiment: desipramine (DMI) 15 mg/kg; modaline sulphate (W3207) 16 mg/kg; pheniprazine (JB516) 2·5 mg/kg and T-3 10 mg/kg. The columns represent the % inhibition of brain monoamine oxidase. The vertical bars show the s.e. of the mean. Each point represents at least 5 determinations.

same dose level of 16 mg/kg. T-3 had no significant effect on reserpine-induced hypothermia at a dose that exhibited strong monoamine oxidase inhibition.

The reversal of reserpine hypothermia induced by modaline was almost completely inhibited by pretreatment with SKF 525 A (Fig. 2). The latter compound has no effect when injected alone in reserpinised rats.

POTENTIATION OF THE HYPERTHERMIA INDUCED BY ISOPRENALINE

Infusion with isoprenaline induced a rise in the body temperature (see Fig. 3). When infusion was at a rate of 40 μg/rat/min the peak was attained after about 90 min. No differences were noticed between the effect of a dose of 400 or 40 μg/rat/min. Desipramine and nortriptyline enhance this hyperthermia while the monoamine oxidase inhibitor pheniprazine reduced this effect (see Fig. 3).

Adrenalectomy as well as α- and β-adrenergic blocking agents, reduce the hyperthermia evoked by high concentration of isoprenaline (Table 5). Modaline slightly inhibited this hyperthermia (see Fig. 3 and Table 5).

Discussion

Our results confirm that modaline is a strong inhibitor of monoamine oxidase (Dubnick & others, 1963; Gylys & others, 1963) as shown by direct measurement of the enzymatic activity and by indirect evaluation of the pharmacological consequences of this effect (potentiation of tryptamine and dopa, inhibition of reserpine hypothermia).

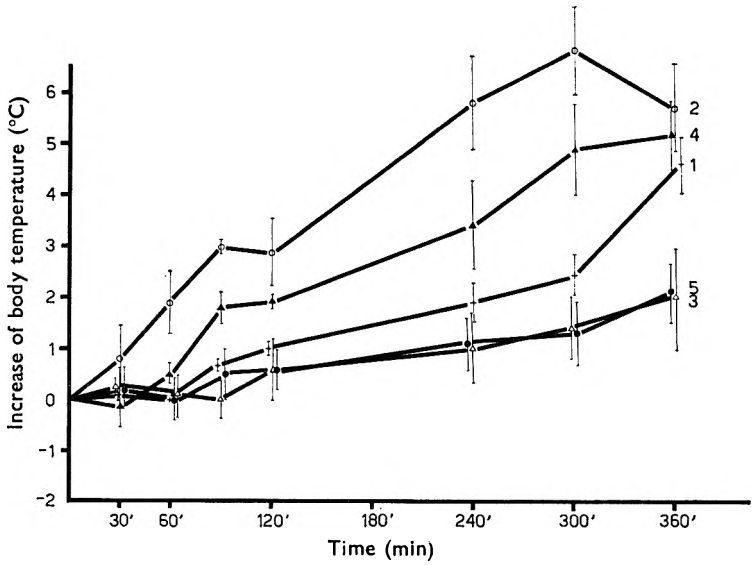

FIG. 2. Rats were treated 18 hr before the test with reserpine (5 mg/kg i.v.) at a room temperature of 20°. At the time 0 the following treatments were given: 1. SKF 525 A 50 mg/kg oral + modaline sulphate 16 mg/kg i.p. 2. Modaline sulphate 16 mg/kg. i.p. 3. SKF 525 A 50 mg/kg oral + modaline sulphate 8 mg./kg i.p. 4. modaline sulphate 8 mg/kg i.p. 5. Saline. The vertical bars show the s.e. of the mean. Each point consists of at least 5 determinations.

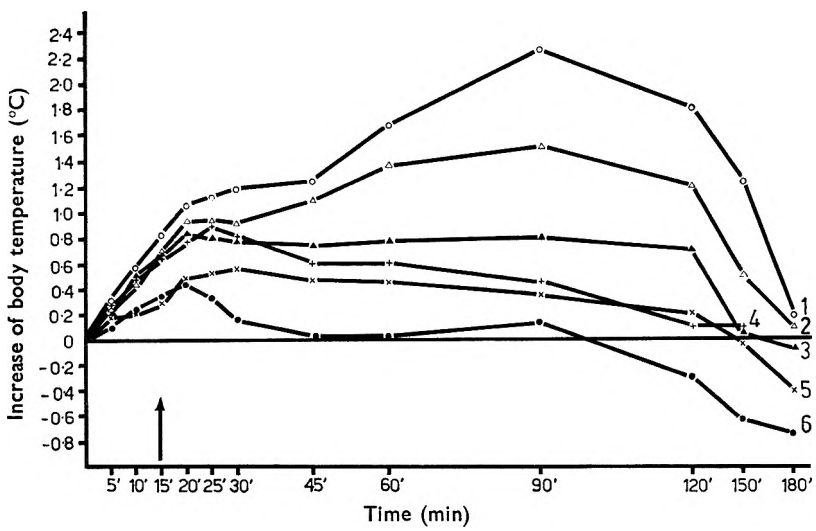

FIG. 3. Rats were infused with isoprenaline 40 μg/rat/min for 15 min. Drugs were given 1 hr before test at the following doses in mg/kg: 1. Desipramine 15. 2. Nortriptyline 10. 3. Saline. 4. Modaline sulphate 16. 5. Modaline sulphate 4. 6. Pheniprazine 10.

TABLE 5. EFFECT OF VARIOUS DRUGS ON THE HYPERTHERMIA INDUCED BY AN INFUSION OF ISOPRENALINE

Treatment	Dose (mg/kg i.p.)	Thermic index (°C) ± s.e.
Saline	—	7·4 ± 1
Desipramine	15	12·7 ± 1·4
Modaline	16	5·6 ± 1·8
Dibenamine	15	5·1 ± 1·7
Phentolamine	10	2·4 ± 0·8
Propranolol	5	2·8 ± 1·3
Adrenalectomy ..	—	2·9 ± 2·2

Thermic index is the sum of temperature difference (° C) before treatment and after: 5, 10, 15, 20, 25 30, 45, 60, 90, 120 min.
Desipramine and modaline were given 1 hr before isoprenaline; phentolamine, propranolol and dibenamine were given 15 min before the infusion. Adrenalectomy was performed three days before the test. Isoprenaline (400 μg/ml) was infused at the rate of 0·1 ml/rat/min for 15 min.

The inhibition of monoamine oxidase by modaline is characterised by a rapid onset (see Table 1) and by a long duration of action (see Table 4).

The attempts to reveal an imipramine-like component in the pharmacological effects of modaline have been successful under one experimental condition only. Like desipramine, modaline raised the body temperature in fully reserpinised animals. This effect was not related to an action on monoamine oxidase because similar blockade of the enzyme induced by compound T-3 or by pheniprazine did not affect the body temperature of reserpinised rats.

Because of the observations in reserpinised animals, we cannot exclude the possibility that the prevention of the reserpine hypothermia obtained with modaline is the result of a separate imipramine-like effect. With the enhancement of the hyperthermic response induced by dopa in monoamine oxidase blocked animals, it was not possible to distinguish an imipramine-like effect from the monoamine oxidase inhibition. In fact a second dose of a monoamine oxidase inhibitor potentiated the dopa hyperthermia like imipramine. These results may be related to the observation that a second administration of a monoamine oxidase inhibitor further increases the level of brain amines despite the already existing block of the enzyme (Dubnick & others, 1962). Modaline did not potentiate the hyperthermia after isoprenaline infusion, a test considered to be specific for imipramine-like drugs (Jori & Garattini, 1965). It is possible that the schedule of treatment for modaline was not the most suitable because it might produce either an increase or a reduction in the intensity of a sympathetic response depending on the dose as demonstrated for imipramine (Thoenen, Huerlimann & Haefely, 1964).

Attempts to distinguish between imipramine-like effects and monoamine oxidase inhibition by inhibiting the metabolism of modaline were not successful. The use of an inhibitor (SKF 525 A) of microsomal enzymes responsible for drug metabolism resulted in the blockade of the potentiation of tryptamine (see Table 2) and of the reversal of reserpine hypothermia (see Fig. 2).

These results therefore suggest that monoamine oxidase inhibition and imipramine-like effects are both present in the metabolic products of

modaline. The limitations and importance of the imipramine-like component in explaining the antidepressive action of modaline remain to be established.

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A direct and an indirect action of 5-hydroxytryptamine on the distal part of the isolated colon of the rat

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The motor response of 5-hydroxytryptamine (5-HT) on the distal part of the isolated rat colon was investigated by constructing dose: response curves to 5-HT, acetylcholine and nicotine; these were repeated in the presence of different antagonists and an anticholinesterase. Hyoscine abolished the responses to acetylcholine, almost completely blocked the effect of nicotine, but reduced the contractions to 5-HT only to about half of the original level. The anticholinesterase *NN'*-diisopropylphosphorodiamidic fluoride (mipafox) potentiated the responses to acetylcholine, 5-HT or nicotine. Procaine and cocaine inhibited to the same extent the large doses of 5-HT, but had no effect on the small doses. Both drugs reduced the contractions with nicotine. Three ganglion-blocking agents were used. Hexamethonium had no effect on acetylcholine or 5-HT, but antagonised nicotine. Mecamylamine had no effect on acetylcholine; it blocked the responses to nicotine and reduced the large doses of 5-HT. The action of dimethylphenylpiperazinium on the three agonists was similar to that of mecamylamine. 2-Bromolysergic acid diethylamide had no effect on the responses to acetylcholine, but reduced equally the contractions due to 5-HT and nicotine. It was concluded that 5-HT acted indirectly by stimulating the intramural parasympathetic ganglia and directly by an action on the muscle fibres. The direct action was pronounced with small doses, the indirect action with higher doses of 5-HT.

SMOOTH muscle is contracted by 5-hydroxytryptamine (5-HT) in two known ways. One is by a direct action on the muscle fibres, the second is via the nerves and involves a cholinergic pathway.

An indirect action was demonstrated by Rocha e Silva, Valle & Picarelli (1953), Robertson (1953) and Gaddum & Hameed (1954). Gaddum & Picarelli (1957) found both a direct and an indirect action of 5-HT on the terminal part of the guinea-pig ileum, while the evidence of an indirect mechanism was submitted by Day & Vane (1963), who concluded that the amine acted mainly on receptors in the nervous tissue. Brownlee & Johnson (1963), in a formal analysis of the mechanism of action of 5-HT in the guinea-pig ileum, located the precise site on the intramural parasympathetic ganglia.

A direct action of 5-HT was reported by Vane (1957) and by Paton & Vane (1963) on fundal strips of the guinea-pig, kitten and rat stomach.

This paper describes experiments on the distal part of the rat isolated colon and provides evidence of both a direct and an indirect action of 5-HT in this tissue.

Experimental

METHODS

Adult albino male rats, 175 to 325 g, were killed by a blow on the head and bled. The colon was excised, and a 2 cm segment from the descending part was removed after discarding 2 cm nearest the rectal junction.

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The oral end of the tissue was tied to a supporting hook and the other end to the recording lever. Organ baths containing 20 or 60 ml Krebs solution at 37° and aerated with a mixture of 95% oxygen and 5% carbon dioxide were used. Longitudinal contractions were recorded with isotonic frontal writing balsa levers loaded with 0·5 g. The magnification was ten times.

The preparation was set up and allowed to stabilise for 30 min. Dose: response curves were then constructed using five to seven doses of the three agonists. A contact time of 30 sec was used within a dose cycle of 4 min; between the doses the bath fluid was changed four times. The antagonist was then added to the bath and after 45 min the dose: response curves repeated; antagonist concentrations were re-established after each washing cycle. Subsequently the tissue was washed for an hour and the dose: response curves established again. Control experiments were made using preparations in which dose: response curves were repeated in the absence of the antagonist.

In the experiments with mipafox, this anticholinesterase was kept in contact with the tissue for 90 min; the tissue was then washed with Krebs solution for 30 min and the dose: response curves to the agonists repeated. Each agonist: antagonist experiment was made on preparations from at least four rats.

Most tissues were spontaneously active; those with marked and irregular spontaneous activity were not used. Frequently there was a change in tone during the experiments; if this change became too great the experiment was discontinued.

The composition of the Krebs solution (in g/litre of distilled water) was: 6·92 NaCl, 0·35 KCl, 0·28 $CaCl_2$, 2·1 $NaHCO_3$, 0·16 KH_2PO_4, 0·29 $MgSO_4.7\,H_2O$, and 2·0 glucose.

DRUGS

Agonists. These were acetylcholine chloride (Hopkins & Williams), histamine acid phosphate (L. Lights), 5-hydroxytryptamine creatinine sulphate (May and Baker), nicotine hydrogen tartrate (British Drug Houses).

Antagonists. These were hyoscine hydrobromide (Burroughs Wellcome and Co.), cocaine hydrochloride (British Drug Houses), procaine hydrochloride (British Drug Houses), hexamethonium bromide (L. Lights), mecamylamine hydrochloride (Merck, Sharp and Dohme), 1,1-dimethyl-4-phenylpiperazinium iodide (Fluka), 2-bromolysergic acid diethylamide tartrate (Sandoz).

The anticholinesterase used was *NN'*-diisopropylphosphorodiamidic fluoride (mipafox) (L. Lights).

All concentrations are quoted as final bath concentrations in μg/ml in terms of base except those of 2-bromolysergic acid diethylamide, which are expressed in terms of the salt.

Results

Acetylcholine. Acetylcholine (0·001 to 0·256 μg/ml) contracted the tissue and gave reproducible contractions with repeated doses.

5-Hydroxytryptamine. 5-HT (0·003 to 12·8 μg/ml) contracted the tissue. Frequently, tachyphylaxis occurred when it was administered repeatedly despite washing the preparation several times for 15 min between the doses. The sensitivity of the tissue to 5-HT could be maintained by interpolating 2 to 3 doses of acetylcholine between each dose of 5-HT.

Nicotine. The dose: response curves to nicotine were constructed in a manner similar to those to 5-HT, because tachyphylaxis was often observed with repeated doses. Nicotine gave a motor response within the dose range used (1·0 to 32·0 μg/ml). In a few experiments nicotine gave either no response or a relaxation.

Histamine was used in concentrations of 1 μg to 5 mg/ml, all of which were inactive.

Hyoscine. After a treatment for 45 min with hyoscine (0·1 μg/ml) the dose: response curves to acetylcholine, 5-HT and nicotine were repeated (Fig. 1). Hyoscine blocked the responses to previously effective

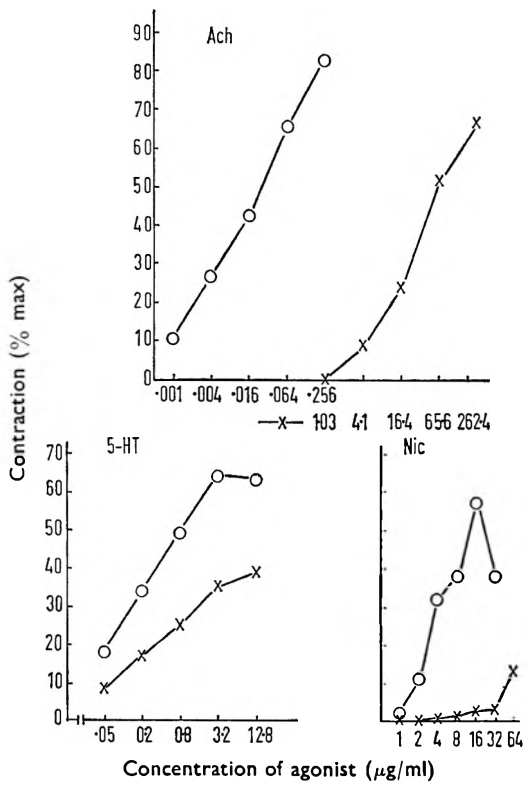

FIG. 1. The effect of hyoscine (0·1 μg/ml) on the dose: response curves to acetylcholine, 5-hydroxytryptamine and nicotine, on the distal part of the isolated colon of the rat. The responses to the agonists are plotted as per cent maximal contraction to acetylcholine against the log dose in μg/ml. Each curve represents the mean results from four experiments. The open circles show the responses to the agonists, the crosses show the responses in the presence of hyoscine. The previously effective doses of acetylcholine were blocked. The responses to 5-hydroxytryptamine were inhibited and those to nicotine nearly abolished.

doses of acetylcholine; higher concentrations of acetylcholine gave a dose: response curve parallel to the original curve (competitive blockade).

In the presence of the same concentration of hyoscine the responses to 5-HT and nicotine were opposed. The dose: response curve to 5-HT was shifted to the right, but the contractions due to nicotine were abolished except at the highest dose used.

Mipafox. Concentrations lower than 20 μg/ml of mipafox had no effect on the responses to acetylcholine. A concentration of 20 μg/ml of mipafox which slightly potentiated the contractions due to acetylcholine, also potentiated 5-HT and nicotine (Fig. 2). A higher concentration of mipafox (100 μg/ml) induced a sustained spasm of the tissue, so it became unworkable.

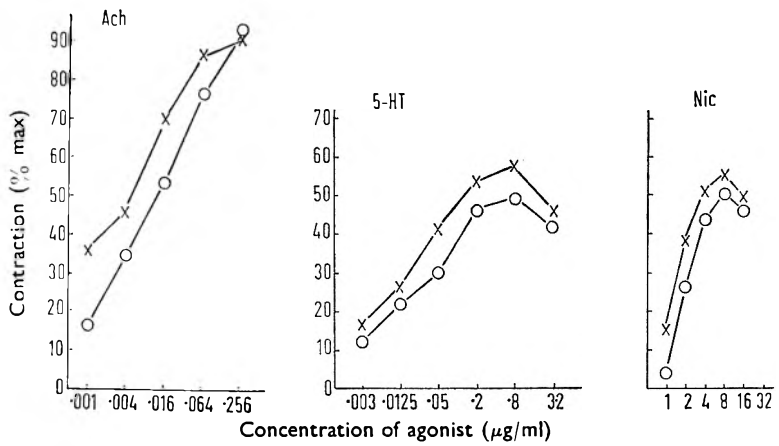

FIG. 2. The effect of mipafox (20 μg/ml) on the responses to acetylcholine, 5-hydroxytryptamine and nicotine, on the distal part of the isolated colon of the rat The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses after treatment with mipafox for 90 min. The dose: response curves to acetylcholine, 5-hydroxytryptamine and nicotine were shifted to the left and to the same extent. Each curve represents the mean of four experiments.

Cocaine. Fig. 3 shows the effect of cocaine, 5 and 20 μg/ml, on the dose: response curves to acetylcholine, 5-HT, and nicotine. Cocaine, 5 μg/ml, had no effect on the responses to acetylcholine and no significant effect on the dose: response curve to 5-HT, but it reduced the contractions due to nicotine to about one third of the maximal contraction to nicotine.

Cocaine, 20 μg/ml, shifted the dose: response curve to acetylcholine to the left (potentiation) and depressed the higher doses to 5-HT to about one half of its maximal response. This concentration of cocaine caused no further reduction of the responses to nicotine.

Procaine. Procaine, 10 μg/ml, displaced the dose: response curve to acetylcholine slightly to the right; it caused a pronounced depression of the responses to the higher doses of 5-HT and almost completely abolished the contractions due to nicotine (Fig. 4).

Hexamethonium. In concentrations of 20 and 60 μg/ml, hexamethonium caused no shift in the dose: response curves due to acetylcholine and 5-HT. With both concentrations the dose: response

curves to nicotine were shifted to the right; 60 μg/ml of hexamethonium was more effective than 20 μg/ml (Fig. 5).

Mecamylamine. Mecamylamine, 5 and 10 μg/ml, did not affect the dose: response curves to acetylcholine, while the low concentration

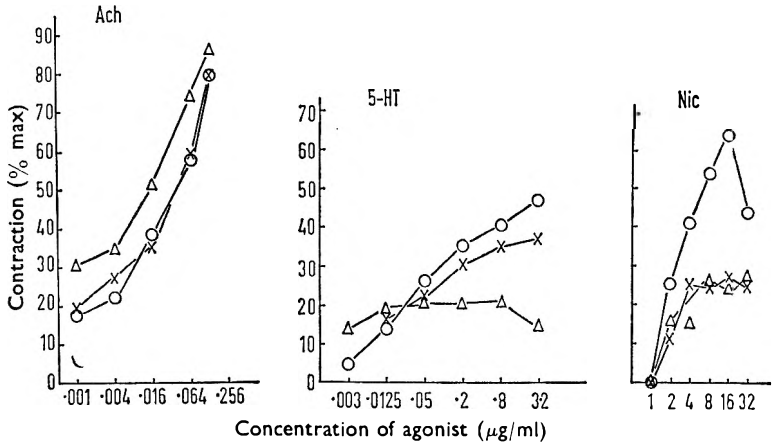

FIG. 3. The effect of cocaine (5 and 20 μg/ml) on the responses to the three agonists. The ordinates and abscissae are as in Fig. 1. The open circles show the responses to the agonists, the crosses represent these responses in the presence of cocaine 5 μg/ml, and the triangles the responses in the presence of cocaine 20 μg/ml. The low concentration of cocaine had no significant effect on the dose: response curves to acetylcholine and 5-hydroxytryptamine, but it inhibited the responses to nicotine. 20 μg/ml of cocaine had no further effect on the nicotine contractions, but it inhibited the upper part of the dose: response curve to 5-hydroxytryptamine and displaced the dose: response curve to acetylcholine to the left. Each curve represents the mean of six experiments.

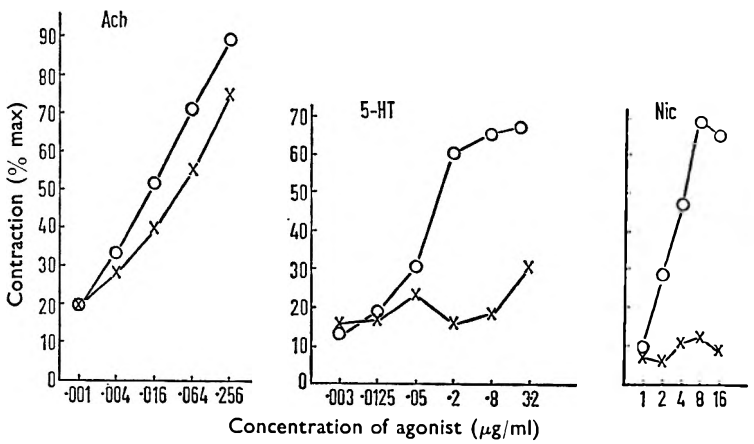

FIG. 4. The effect of treating the distal part of the isolated colon of the rat with procaine (10 μg/ml). The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists and the crosses show these responses in the presence of procaine. The dose: response curve to acetylcholine was displaced slightly to the right, while the responses to nicotine and the higher doses of 5-hydroxytryptamine were inhibited. Each curve represents the mean of five experiments.

abolished the responses to nicotine. Both concentrations depressed the dose: response curve to 5-HT due to concentrations higher than 0·05 μg/ml to about one half of its maximal response (Fig. 6). In one experiment mecamylamine, 5 μg/ml, failed to reduce the contractions due to 5-HT.

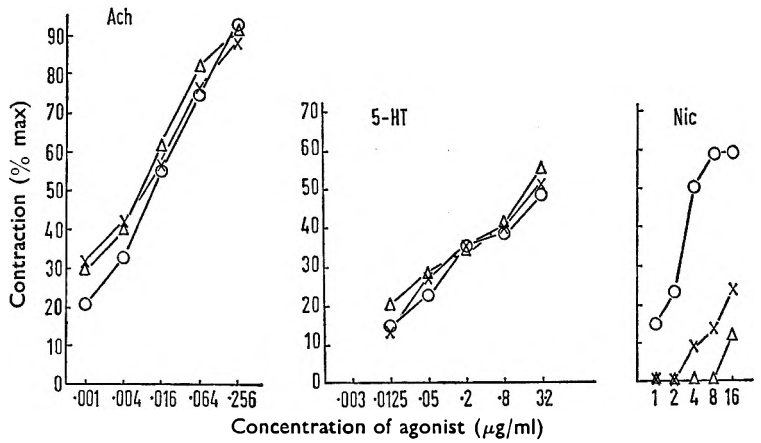

FIG. 5. The effect of hexamethonium (20 and 60 μg/ml) on the responses to the agonists. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses of the agonists, the crosses show these responses in the presence of hexamethonium 20 μg/ml and the triangles the responses in the presence of hexamethonium 60 μg/ml. Neither concentration of hexamethonium affected the dose: response curves to acetylcholine and 5-hydroxytryptamine, but inhibited the responses to nicotine; 60 μg/ml of hexamethonium was more effective than 20 μg/ml on the dose: response curve to nicotine. Each curve represents the mean of eight experiments.

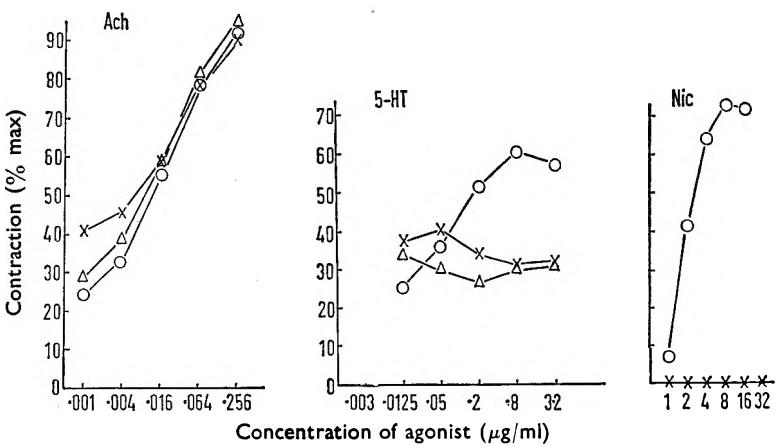

FIG. 6. The effect of mecamylamine (5 and 10 μg/ml) on the responses to the three agonists. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists, the crosses represent these responses in the presence of mecamylamine 5 μg/ml, and the triangles the same responses in the presence of mecamylamine 10 μg/ml. The dose: response curves to acetylcholine were not affected. Both concentrations depressed the upper part of the dose: response curve to 5-hydroxytryptamine, while 5 μg/ml of mecamylamine abolished the responses to nicotine. Each curve represents the mean of four experiments.

Dimethylphenylpiperazinium. After a concentration of 5 μg/ml of dimethylphenylpiperazinium, the dose: response curve to acetylcholine was little changed or slightly moved to the right, and all responses due to nicotine were reduced. This concentration of dimethylphenylpiperazinium depressed only the contractions caused by the higher doses of 5-HT to about one half of the maximal response to the amine (Fig. 7).

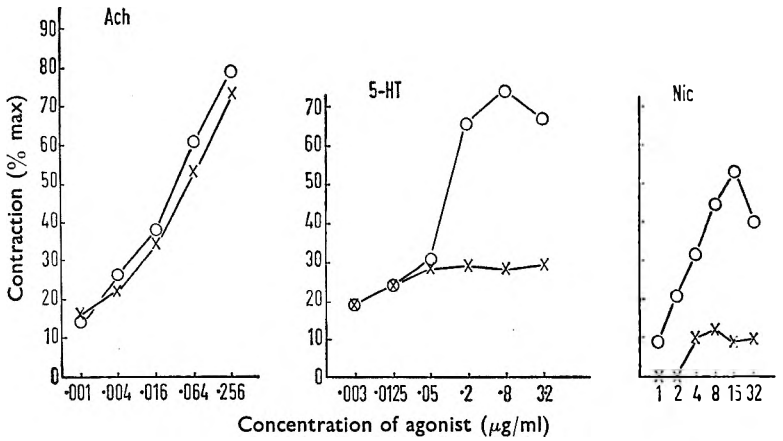

FIG. 7. The effect of dimethylphenylpiperazinium (5 μg/ml) on the responses to acetylcholine, 5-hydroxytryptamine and nicotine. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the agonists, the crosses represent these responses in the presence of dimethylphenylpiperazinium. The dose: response curve to acetylcholine was slightly shifted to the right, all the responses due to nicotine and the contractions caused by higher doses of 5-hydroxytryptamine were depressed. Each curve represents the mean of four experiments.

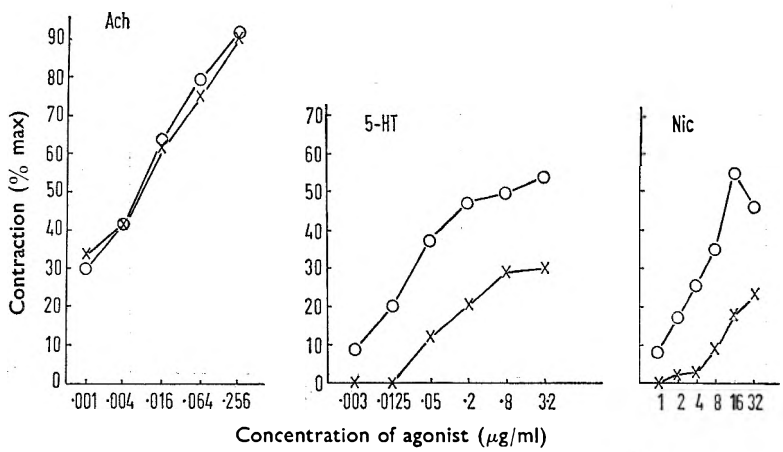

FIG. 8. The effect of 2-bromolysergic acid diethylamide (0·2 μg/ml) on the responses to acetylcholine, 5-hydroxytryptamine and nicotine. The ordinates and abscissae are as in Fig. 1. The open circles represent the responses to the three agonists, the crosses show these responses in the presence of 2-bromolysergic acid diethylamide. The dose: response curve to acetylcholine was not affected, but the contractions due to 5-hydroxytryptamine and nicotine were inhibited to the same extent. Each curve represents the mean of five experiments.

2-Bromolysergic acid diethylamide. A concentration of 0·2 μg/ml of 2-bromolysergic acid diethylamide did not abolish the contractions due to acetylcholine, but shifted the dose: response curves to 5-HT and nicotine to the right. A higher concentration (0·5 μg/ml) of bromolysergic acid diethylamide did not block the responses to 5-HT completely, but the interpretation of these results was complicated by an increase in tone caused by the antagonist.

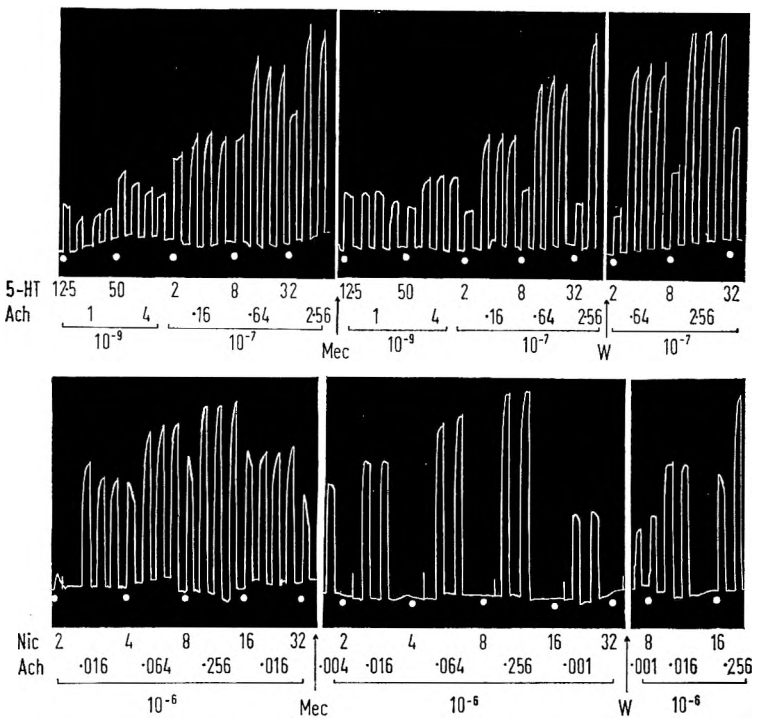

FIG. 9. The upper records show dose: response curves to 5-hydroxytryptamine and acetylcholine. Each dose of 5-hydroxytryptamine was spaced with three doses of acetylcholine. The responses due to 5-hydroxytryptamine are represented by the white dots. At Mec, mecamylamine (10 μg/ml) was added to the bath, and after a contact time of 45 min the dose: response curves were repeated in the presence of mecamylamine. At W the preparation was washed for an hour, and the dose: response curves were established again. The lower records show a similar experiment with nicotine in which mecamylamine (5 μg/ml) was added at Mec. The responses to nicotine are shown by the white dots.

Discussion

These experiments are concerned with a quantitative agonist: antagonist analysis of the site of action of 5-HT on the isolated colon of the rat. The agonists acetylcholine, 5-HT and nicotine all produced contraction of the preparation, while histamine even at concentrations as high as 5 mg/ml had no effect, suggesting the absence of histamine receptors in the tissue.

In the presence of a concentration of hyoscine which abolished the responses to acetylcholine and nearly blocked the effect of nicotine the contractions due to 5-HT were reduced to about half of the original level.

From these results it may be suggested that 5-HT contracts the tissue partly by an action involving a cholinergic mechanism—either an action on the muscarinic acetylcholine receptors or, like nicotine and dimethylphenylpiperazinium, by release of acetylcholine through the nerve pathway, and also partly by an action directly on the smooth muscle.

In the guinea-pig ileum the action of 5-HT is abolished by muscarinic blocking agents, as shown by Rocha e Silva & others (1953) and Brownlee & Johnson (1963). On the terminal part of the guinea-pig ileum Cambridge & Holgate (1955) and Gaddum & Picarelli (1957) found that atropine blocked only 50% of the responses to 5-HT. In other tissues the muscarinic blocking agents had no effect on the action of 5-HT; this was reported also by Vane (1957) on the fundal strip of the rat stomach and by Sleisenger, Law, Smith, Pert & Lewis (1955) on the distal colon of the dog *in vivo*.

The fact that the anticholinesterase mipafox potentiated the contractions due to acetylcholine, 5-HT or nicotine and to the same extent, provided further evidence of the involvement of a cholinergic link.

The accepted challenge for the presence of a nervous involvement is the effect of cooling to 20°, the use of cocaine or procaine, the use of botulinum toxin, the reduction in calcium ions or the increase of magnesium ions (Harry, 1962). Each of these has the effect of reducing the amount of acetylcholine set free at postganglionic nerve terminals. The one chosen here was the use of cocaine or procaine.

The results obtained with the local anaesthetic drugs, procaine and cocaine, site part of the action of 5-HT on the cholinergic nerve pathway and part of the action as a direct stimulation of muscle fibres. A concentration of procaine which abolished the contractions due to nicotine caused a reduction of the responses to the higher doses of 5-HT, but did not affect the contractions to the smaller doses. A reduction similar to that produced by procaine of the higher doses of 5-HT was obtained with the high concentration of cocaine. The specificity of these results may be questioned, because cocaine in both concentrations failed to block the contractions due to nicotine completely and the high concentration of cocaine potentiated the responses to acetylcholine. Trendelenburg (1962) analysed this potentiation of acetylcholine by cocaine on the nictitating membrane and found that cocaine potentiated only the lower part of the dose: response curve to acetylcholine. Moreover, Trendelenburg (1956) showed that cocaine potentiated the direct action of 5-HT on the nictitating membrane and abolished the stimulating effect on the superior cervical ganglion.

Whether the indirect action of 5-HT involved a ganglionic synapse was challenged by the use of ganglionic blocking agents. Hexamethonium, in producing a competitive blockade of the ganglia, did not antagonise 5-HT or acetylcholine in concentrations which antagonised nicotine. These results excluded the possibility of a preganglionic action of 5-HT.

Similar results were obtained by Feldberg (1951), Kosterlitz & Robinson (1958), and Brownlee & Johnson (1963).

Dimethylphenylpiperazinium, 5 μg/ml, which in a high concentration is considered to block ganglia by depolarisation (Ling, 1959), shifted the dose: response curve to acetylcholine slightly to the right, but produced a pronounced reduction to the response of nicotine. This concentration of dimethylphenylpiperazinium depressed only the responses to the higher doses of 5-HT to about one half of their maximal contraction.

Mecamylamine which produces an action intracellularly (Bennet, Tyler & Zaimis, 1957) reduces the responses to 5-HT to an extent similar to those of procaine, cocaine, and dimethylphenylpiperazinium in concentrations which blocked nicotine.

The persistence of contractions due to 5-HT in the presence of hexamethonium also excluded an action on the nicotinic ganglionic receptors, while the results obtained with dimethylphenylpiperazinium and mecamylamine site the indirect cholinergic part of the action of 5-HT on or in autonomic ganglia. These results are similar to those obtained by Brownlee & Johnson (1963), who sited the action of 5-HT in the guinea-pig ileum on the intramural parasympathetic ganglia. Furthermore, Trendelenburg (1956) gave evidence of specific tryptamine receptors in the cat superior cervical ganglion; these receptors were not blocked by hexamethonium. Similar results were reported by Bindler & Gyermek (1961) in the cat inferior mesenteric ganglion. Moreover, the results, together with the evidence of hyoscine and the local anaesthetic drugs, show that small doses of 5-HT acted directly on 5-HT receptors in the muscle while large doses acted indirectly.

There is a certain similarity between these results and those obtained by Gaddum & Picarelli (1957) on the terminal part of the guinea-pig ileum. They concluded that 5-HT activated two different receptors, one sited in the intramural nervous system (M-receptors) which was blocked by morphine, the other one sited on the intestinal muscle (D-receptors) which could be blocked by phenoxybenzamine and lysergic acid diethylamide.

Gaddum & Hameed (1954) showed that lysergic acid diethylamide was a specific antagonist of 5-HT on the rabbit ear and rat uterus. In the experiments described in this paper its bromo-derivative abolished the effects of small doses of 5-HT and reduced those of large doses; no conclusion can be drawn from this result because nicotine was similarly affected by bromolysergic acid diethylamide.

Brownlee & Johnson (1963) found that 5-HT stimulated the guinea-pig ileum mainly by an indirect action, while Gaddum & Picarelli (1957) concluded that only 50% of the action of 5-HT was due to an indirect action on the terminal part of the guinea-pig ileum. The experiments described in this paper suggest that about 30% of the action of 5-HT is due to a direct action. Furthermore, Dalgliesh, Toh & Work (1953) and Feldberg & Toh (1953) have used the atropinised proximal part of the isolated rat colon for the assay of 5-HT in the tissue extracts showing that a direct action is involved in this preparation.

Brownlee & Johnson (1963) found that, with the guinea-pig ileum,

the dose: response line for 5-HT was steep. On the isolated colon of the rat the dose: response line was more shallow. This is consistent with the dual nature of the action involving both indirect and direct effects.

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A comparison of the anti-anaphylactic properties of ethanolamine and hydrocortisone

P. GOADBY AND W. G. SMITH

Both ethanolamine and hydrocortisone potentiate the anti-anaphylactic activity of mepyramine in actively sensitised guinea-pigs subjected to anaphylactic shock by exposure to aerosols of antigen solution. The effects of these two substances have now been compared. After intramuscular injection, the peak effect with ethanolamine occurred 1 hr later whereas with hydrocortisone it occurred 18 hr later. Both substances were also effective, after 45 min and 12 hr respectively, as aerosols. The optimum intramuscular dose of ethanolamine was 10 mg/kg and that of hydrocortisone 100 mg/kg. After aerosol administration, optimum effects were observed when 5% solutions of either drug were used.

CORTISONE and synthetic analogues have been used successfully in the treatment of asthma and more particularly in status asthmaticus (Bordley, Carey, Harvey, Howard, Kattus, Newman & Winkwerder, 1949; Carryer, Koelsche, Prickman, Maytum, Lake & Williams, 1950; M.R.C., 1956).

Though several investigators have been unable to prevent the death of guinea-pigs in anaphylactic shock by pretreatment with cortisone (Leger, Leith & Rose, 1948; Dworetsky, Code & Higgins, 1950; Friedlander & Friedlander, 1950). Feinberg, Malkiel & McIntyre (1953) reported that pretreatment with cortisone 18 hr before exposure to aerosolised antigen increased the time passively sensitised guinea-pigs could withstand exposure to antigen.

We found that soluble hydrocortisone potentiated the anti-anaphylactic effects of mepyramine in actively sensitised guinea-pigs and diminished the release of SRS-A (slow reacting substance of anaphylaxis) from a sensitised guinea-pig lung shocked *in vitro* (Goadby & Smith, 1963). The results with hydrocortisone were similar to those of Smith (1961) for ethanolamine. However, although the *in vitro* conditions for the ethanolamine experiments were similar to those for the hydrocortisone study, the *in vivo* conditions were different, so that direct comparisons were not possible.

Aerosolised hydrocortisone in low dosage has produced encouraging results in the treatment of human bronchial asthma (Foulds, Greaves, Herxheimer & Kingdom, 1955; Helm & Heyworth, 1958), the most satisfactory results being in patients with allergic asthma without hypersecretion. A comparison of the anti-anaphylactic effects of ethanolamine and hydrocortisone administered by aerosol to guinea-pigs was therefore made.

Experimental

Guinea-pigs, 250–350 g, were sensitised to egg albumin by the intraperitoneal injection of 2 ml of a 5% solution. After three weeks they were exposed to an aerosol produced by applying air at 15 lb/in^2 to a

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Wright nebuliser (Wright, 1958) containing a 1% solution of egg albumin. The time to onset of dyspnoea and cough was noted for each animal and termed the "collapse time". Exposure to albumin was repeated at weekly intervals for three weeks and the mean "collapse time" for the last two weeks was termed the "normal collapse time" (Goadby & Smith, 1962). The animals were then divided into groups of ten such that the mean "collapse time" for the groups was between 60 and 100 sec.

One hr before the fourth weekly exposure to antigen each animal was injected with 1 mg/kg of mepyramine (as maleate) intramuscularly and the ratio of the "treated collapse time" to the "normal collapse time" was termed the "protection ratio" (Smith, 1961). The next week the animals were exposed to antigen without pretreatment to ascertain that mepyramine had produced no lasting effect on their sensitivity.

One week later, the potentiating effect of ethanolamine or hydrocortisone on the protection afforded by mepyramine 1 mg/kg given 1 hr before exposure to antigen, was determined. Ethanolamine was used as a solution of the hydrochloride, the doses being expressed as ethanolamine base. Hydrocortisone was used as a solution of the sodium hemisuccinate, the doses being expressed as the alcohol. The times between intramuscular injection for potentiating drug and exposure to aerosolised antigen and the doses by the intramuscular route giving optimum results were investigated.

The effect of a prior inhalation of an aerosol of ethanolamine or hydrocortisone on the anti-anaphylactic effects of mepyramine was also examined. The rate of aerosolisation of the solution was 8·0 ml/hr. Animals were exposed for 15 min. The pretreatment time was taken as the time from the removal of the animals from the drug aerosol to the time of entry into the chamber for exposure to aerosolised antigen. The effect of varying dosage was achieved by altering the concentration of the drug solution and keeping the other conditions constant.

The histamine aerosol used to study the irritant effects of ethanolamine and hydrocortisone aerosols was produced by applying air at 15 lb/in^2 to a Wright nebuliser containing a 0·1% solution of histamine (as acid phosphate). The rate of aerosolisation was 8·0 ml/hr. Groups of ten unsensitised guinea-pigs were used. Partial protection was obtained by giving 0·05 mg/kg adrenaline, intramuscularly, 15 min before exposure to the histamine aerosol. Hydrocortisone and ethanolamine aerosols were given 12 hr and 45 min respectively before exposure to histamine aerosol.

Results

The results are expressed as the mean "protection ratio" ($\pm$ s.d.) for the group of ten animals. An animal which tolerated antigen for twenty times its "normal collapse time" was considered to be fully protected. The number of fully protected animals in a group is shown separately from the mean "protection ration" ($\pm$ s.d.) of the remainder.

Table 1 shows that potentiation of the anti-anaphylactic effects of mepyramine was maximal when ethanolamine was given 1 hr before

TABLE 1. TIME SEQUENCE OF THE PROTECTIVE EFFECTS OF ETHANOLAMINE AND HYDROCORTISONE IN GROUPS OF TEN ANIMALS

Group mean "Normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + ethanolamine[2] pretreatment		Hr between ethanolamine pretreatment and shock	Group mean "normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + hydrocortisone[3] preatment		Hr between hydrocortisone pretreatment and shock
	Group mean protection ratio ± s.d.	fully protected animals	Mean protection ratio of remainder ± s.d.			Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.	
90·1	4·38 ± 1·28	0	6·27 ± 3·65	0·25	83·9	3·43 ± 1·23	1	4·33 ± 1·78	1
82·9	4·28 ± 1·65	3	7·34 ± 0·81	0·50	88·2	5·30 ± 1·88	2	6·44 ± 1·79	6
92·1	4·72 ± 1·18	3	5·63 ± 2·31	0·75	84·9	4·21 ± 1·27	3	7·36 ± 4·60	12
99·8	4·15 ± 2·23	5	11·30 ± 4·58	1·0	64·8	3·14 ± 1·98	4	5·45 ± 1·88	18
93·6	5·31 ± 2·50	1	6·62 ± 1·88	2·0	80·5	4·54 ± 1·81	0	4·97 ± 1·12	24
93·1	6·15 ± 1·98	2	8·19 ± 3·31	4·0					

[1] 1 mg/kg mepyramine (as maleate) intramuscularly 1 hr before shock. [2] 10 mg/kg ethanolamine (as hydrochloride) intramuscularly.
[3] 100 mg/kg hydrocortisone (as sodium hemisuccinate) intramuscularly.

TABLE 2. DOSE-EFFECT RELATIONSHIP OF ETHANOLAMINE AND HYDROCORTISONE IN GROUPS OF TEN ANIMALS

Group mean "Normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + ethanolamine[2] pretreatment		Dose of ethanolamine (mg/kg)	Group mean "normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + hydrocortisone[3] preatment		Dose of hydrocortisone (mg/kg)
	Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.			Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.	
89·4	3·74 ± 1·10	0	6·27 ± 1·69	5	87·5	4·87 ± 0·93	0	5·24 ± 1·37	25
99·8	4·15 ± 2·23	5	11·30 ± 4·58	10	89·8	5·13 ± 2·26	0	4·41 ± 1·56	50
74·7	3·81 ± 1·98	5	5·93 ± 3·27	20	64·8	3·14 ± 1·98	4	5·45 ± 1·88	100
86·9	3·76 ± 1·29	2	6·94 ± 3·60	40	94·7	3·85 ± 2·06	3	4·39 ± 2·23	200

[1] 1 mg/kg mepyramine (as maleate) intramuscularly 1 hr before shock. [2] Ethanolamine (as hydrochloride) intramuscularly 1 hr before shock.
[3] Hydrocortisone (as sodium hemisuccinate) intramuscularly 18 hr before shock.

TABLE 3. TIME SEQUENCE OF PROTECTION OBSERVED AFTER AEROSOL ADMINISTRATION IN GROUPS OF TEN ANIMALS

Group mean "Normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + ethanolamine[2] pretreatment		Hr between ethanolamine pretreatment and shock	Group mean "normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + hydrocortisone[3] preatment		Hr between hydrocortisone pretreatment and shock
	Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.			Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.	
65·9	5·57 ± 2·18	0	5·94 ± 2·60	0·25	81·3	4·02 ± 1·41	0	4·13 ± 1·55	1
94·2	2·82 ± 1·26	2	4·69 ± 2·86	0·50	90·4	3·94 ± 1·28	1	5·95 ± 2·41	6
92·9	4·67 ± 1·09	6	7·01 ± 3·97	0·75	98·7	4·41 ± 2·14	4	4·78 ± 2·35	12
96·0	4·07 ± 1·67	1	5·35 ± 3·79	1·0	88·7	4·02 ± 1·59	1	7·50 ± 4·34	18
90·0	3·61 ± 1·96	2	5·53 ± 2·15	2·0					

[1] 1 mg/kg mepyramine (as maleate) intramuscularly 1 hr before shock. [2] Ethanolamine aerosol 5% given for 15 min ending at time quoted before shock.
[3] Hydrocortisone aerosol 5% given for 15 min ending at time quoted before shock.

TABLE 4. EFFECT OF INCREASING THE CONCENTRATIONS OF ETHANOLAMINE AND HYDROCORTISONE ON THE PROTECTION OBSERVED AFTER AEROSOL ADMINISTRATION IN GROUPS OF TEN ANIMALS

Group mean "Normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + ethanolamine[2] pretreatment		Conc. ethanolamine aerosol %	Group mean "normal collapse time" (sec)	Mepyramine[1] pretreatment	Mepyramine + hydrocortisone[3] preatment		Conc. of hydrocortisone aerosol %
	Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.			Group mean protection ratio ± s.d.	Fully protected animals	Mean protection ratio of remainder ± s.d.	
99·0	3·08 ± 1·21	1	3·67 ± 1·38	2·5	95·8	4·21 ± 3·20	3	6·44 ± 3·28	2·5
92·9	4·67 ± 1·09	6	7·01 ± 3·97	5	98·7	4·41 ± 2·14	4	4·78 ± 2·33	5
90·6	4·46 ± 1·42	1	5·72 ± 3·85	10	100·8	4·05 ± 1·93	2	6·55 ± 3·96	10

[1] 1 mg/kg mepyramine (as maleate) intramuscularly 1 hr before shock. [2] Ethanolamine aerosol for 15 min ending 45 min before shock.
[3] Hydrocortisone aerosol for 15 min ending 12 hr before shock.

exposure to antigen though some protection was observed at five of the six time intervals used. The potentiation of mepyramine by hydrocortisone was maximal when the animals were exposed to antigen 18 hr after administration of the steroid. Some protection was observed at four of the five time intervals tested.

Ethanolamine in the presence of mepyramine was found to confer greater protection from the effects of anaphylaxis than mepyramine alone at three of the four dose levels used (see Table 2). The same number of animals was fully protected by both 10 mg/kg and 20 mg/kg, but of those animals not fully protected a greater level ($P > 0.05$) of protection was seen at 10 mg/kg. Hydrocortisone potentiated the anti-anaphylactic effects of mepyramine at two of the three dose levels tested. The maximum effect was shown by 100 mg/kg, four animals being fully protected. The response of the animals not fully protected did not differ from that of the mepyramine controls.

Ethanolamine aerosol showed the greatest potentiation of the anti-anapylactic effects of mepyramine when it was administered 45 min before exposure to antigen (Table 3). Six animals were fully protected and the remaining four showed a greater protection ($P > 0.05$) than the mepyramine controls. Hydrocortisone aerosol was most effective when given 12 hr before exposure to antigen, four animals being fully protected. The remaining animals did not differ significantly from the mepyramine controls.

Ethanolamine showed marked potentiation of the anti-anaphylactic effects when administered as an aerosol of a 5% solution (Table 4). Very little protection was observed with aerosols of 2·5% and 10% solutions. Hydrocortisone also showed maximum protection as a 5% solution. Those animals not fully protected gave responses which were not significantly different from the mepyramine controls. Some protection was observed with 2·5% and 10% solutions.

Aerosols of 10% solutions of hydrocortisone and ethanolamine did not affect the collapse times of unsensitised animals exposed to histamine aerosol (Table 5). Ethanolamine 10% did reduce the protection afforded by 0·05 mg/kg adrenaline against the histamine aerosol although hydrocortisone 10% did not.

TABLE 5. EFFECT OF PREVIOUS EXPOSURE TO AEROSOLS OF CONCENTRATED SOLUTIONS OF ETHANOLAMINE AND HYDROCORTISONE ON GROUPS OF TEN ANIMALS EXPOSED TO HISTAMINE AEROSOL, 0·1%

Group mean "normal collapse time" (sec)	Pretreatment	Fully protected animals	Protection ratio of remainder ±s. d.
66·7	Ethanolamine aerosol 10%, 45 min before shock	0	0·91 ± 0·35
100·7	Hydrocortisone aerosol 10%, 12 hr before shock	0	1·64 ± 0·53
83·3	Adrenaline 0·05 mg/kg intramuscular 15 min before shock ..	3	4·91 ± 2·36
99·4	Adrenaline 0·05 mg/kg i.m. + ethanolamine aerosol 10% ..	0	2·04 ± 0·97
99·6	Adrenaline 0·05 mg/kg i.m. + hydrocortisone aerosol 10% ..	2	3·96 ± 1·89

Discussion

The experiments confirm the previous results (Smith, 1961; Goadby & Smith, 1963) that whilst hydrocortisone and ethanolamine do not protect

actively sensitised guinea-pigs from the effects of aerosolised antigen, they potentiate the anti-anaphylactic effects of mepyramine in some animals. There are, however, differences in the pattern of activity of the two drugs.

The onset of the protective effect of ethanolamine was between 15 and 30 min and the effect was maximal 1 hr after intramuscular administration. The effect of hydrocortisone developed slowly and was not maximal until 18 hr after intramuscular injection. The time to onset of the effect of ethanolamine is probably that required for absorption, transport and binding of the drug at the site of action. The slow development of the effect of hydrocortisone probably involves an alteration of tissue metabolism, as suggested by Goadby & Smith (1962). It was not possible to obtain full protection from anaphylaxis by pretreatment with ethanolamine and mepyramine (as reported by Smith, 1961). With ethanolamine, some animals were fully protected and some others were partly protected, whilst with hydrocortisone some animals were protected but the remainder were not.

These results contrast with those of Herxheimer & Stresemann (1965). The differences between their method and the one reported here are small but important. Herxheimer & Stresemann did not use the same animals both for the mepyramine control and for the ethanolamine plus mepyramine study. Also, when our animals received mepyramine plus ethanolamine they were receiving their second protective pretreatment in three weeks. Other differences have been reviewed by Smith (1965). Administration of the drugs by aerosol showed the same general pattern as that for intramuscular injection, except the time to onset of the effect was shorter in both instances. No greater level of protection was obtained but the amount of drug to obtain protection is smaller. Animals were exposed to 100 mg of drug in 90 litres of air. The minute volume of a guinea-pig is 0·16 litres (Spector, 1956). Therefore, without allowing for losses on the apparatus, in 15 min the maximum dose it received is 2·66 mg.

The failure to produce increased protection by increasing the concentration of the aerosolised solutions above 5% has received further investigation. Aerosols of 10% solutions of both drugs were found to be irritant to the respiratory passages of guinea-pigs and caused a characteristic scratching of the nose with the front paws. This might account for the result obtained with 10% ethanolamine aerosol since, although this solution did not affect the collapse times of sensitised guinea-pigs subjected to aerosolised antigen or unsensitised guinea-pigs exposed to histamine aerosol, it reduced the protection afforded by a small dose of adrenaline against histamine aerosol. However, it is unlikely that an irritant effect would explain the result obtained with hydrocortisone 12 hr after administration. Also, 10% aerosols of hydrocortisone failed to reduce the collapse times of sensitised guinea-pigs subjected to aerosolised antigen, of unsensitised guinea-pigs exposed to histamine aerosol and of guinea-pigs partially protected by adrenaline against the effects of histamine aerosol.

Ethanolamine produces a similar protection to that of hydrocortisone in

sensitised guinea-pigs subjected to aerosolised antigen. The onset of action of ethanolamine is quicker and its duration is shorter. The dose of ethanolamine is less critical than that of hydrocortisone when the drug is given parenterally, but the irritant nature of concentrated solutions should be considered when using aerosol administration.

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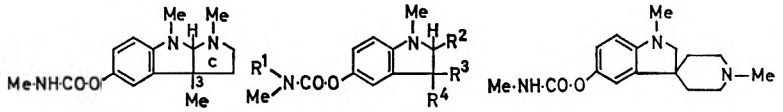
Synthesis of 1,3,3-trimethyl- and 1,2,3,3-tetramethyl-5-(methyl- and dimethyl-carbamoyloxy) indolines and their methiodides

M. AHMED AND B. ROBINSON

The title compounds have been synthesised for testing as anticholinesterases.

FOLLOWING the observation that the anticholinesterase activity of the alkaloid physostigmine (eserine) (I) is dependent upon the



I II III

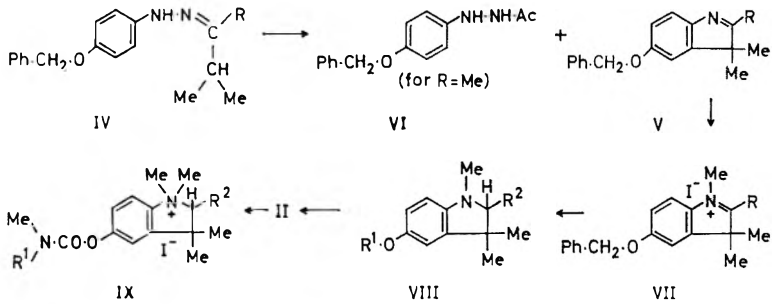
presence of the methylcarbamoyloxy group and the basic nitrogen atom, many compounds incorporating these two features, and also those with a dimethylcarbamoyloxy group in place of a methylcarbamoyloxy group, and with a quaternary instead of a tertiary nitrogen atom, were synthesised and their anticholinesterase activity investigated (for a review of synthetic analogues of physostigmine see Stempel & Aeschlimann, 1956).

The first synthetic anticholinesterases incorporating a 5-hydroxyindoline nucleus and omitting ring C of physostigmine (I), 5-dimethylcarbamoyloxy-1,2,3-trimethylindoline (II; $R^1 = R^2 = R^3 = Me$, $R^4 = H$) and its methiodide, were prepared by Gardner & Stevens (1947). Later, Kolosov & Preobrazhenskii (1953) synthesised 1-methyl-5-methylcarbamoyloxy (II; $R^1 = R^2 = R^3 = R^4 = H$) and 1,3-dimethyl-5(methyl- and dimethyl-carbamoyloxy)indoline (II; $R^2 = R^4 = H$, $R^3 = Me$, $R^1 = H$ and Me respectively) and their methiodides. Pharmacological tests indicated that all these 5-hydroxyindoline derivatives are anticholinesterases. Another 5-hydroxyindoline derivative (III) has also been synthesised and found to possess only weak anticholinesterase activity (Kretz, Müller & Schlittler, 1952).

Whereas C-3 in the indoline nucleus of physostigmine (I) is quaternary, the corresponding atom in the above-mentioned synthetic analogues of structure (II) is secondary or tertiary. We have now prepared, by the route shown in scheme 1, the corresponding 5-hydroxyindoline derivatives (mentioned in the title) in which C-3 of the indoline nucleus is quaternary.

Fischer indolisation (for a recent review see Robinson, 1963) of isobutyraldehyde *p*-benzyloxyphenylhydrazone (IV; R = H) and isopropyl methyl ketone *p*-benzyloxyphenylhydrazone (IV; R = Me) using 25% aqueous ethanolic acetic acid as catalyst gave respectively the 3*H*-indoles (V; R = H) and (V; R = Me). As expected from previous observations

From the Department of Pharmacy, The University, Manchester 13.



Scheme 1

(for references see Robinson, 1963), no 5-benzyloxy-2-isopropylindole, which would result from the alternative direction of indolisation in IV (R = Me), could be isolated from the second indolisation. A by-product was, however, isolated from this reaction and was shown (see Experimental) to be *N'*-*p*-benzyloxyphenylacethydrazide (VI). Quaternisation of (V; R = H and Me) with methyl iodide gave the corresponding methiodides (VII; R = H and Me), which upon reduction with sodium borohydride gave the corresponding indolines (VIII; R^1 = Ph·CH_2, R^2 = H and Me). Debenzylation was then effected by hydrogenolysis at room temperature and pressure using 10% palladium-upon-charcoal as catalyst to give (VIII; R^1 = H, R^2 = H and Me). Treatment of these two phenols with methyl isocyanate in the presence of a trace of sodium (cf. Kolosov & Preobrazhenskii, 1957) then gave the required methylurethanes (II; R^1 = H, R^3 = R^4 = Me, R^2 = H and Me), which with methyl iodide were converted into the corresponding methiodides (IX; R^1 = H, R^2 = H and Me). Reaction of the phenols with dimethylcarbamoyl chloride in dry pyridine (cf. Kolosov & Preobrazenskii, 1957) gave the dimethylurethanes (II; R^1 = R^3 = R^4 = Me, R^2 = H and Me), isolated as the crystalline hydrochlorides. Reaction of the free bases, liberated from the hydrochlorides, with methyl iodide afforded the methiodides (IX; R^1 = Me, R^2 = H and Me).

Experimental

Melting-points were recorded on a Kofler hot-stage apparatus and are uncorrected. Ultraviolet spectra were measured in ethanolic solution, unless otherwise stated, on a Unicam SP 800 spectrophotometer, and infrared spectra as Nujol mulls on a Unicam SP 200 spectrophotometer. The proton magnetic resonance spectrum was recorded in deuterochloroform solution on a Varian A 60 spectrometer using tetramethylsilane as internal standard. Solutions were dried with anhydrous magnesium sulphate, and solvents were removed on a steam-bath under reduced pressure (water-pump).

5-*Benzyloxy*-3,3-*dimethyl*-3H-*indole* (V; R = H). *p*-Benzyloxyphenylhydrazine hydrochloride (Mentzer, Beaudet & Bory, 1953) (15 g) was suspended in 25% aqueous acetic acid (600 ml) and isobutyraldehyde (5 g) was added. The mixture was then boiled under reflux for 1 hr when

a dark-brown oil separated. Ethanol (300 ml) was then added and the resulting clear brown solution was boiled under reflux for a further 5 hr. The ethanol was removed, and water (2·5 litres) was added to the aqueous acidic residue. The liberated oil was taken into ether (4 × 500 ml), the combined ethereal solutions were extracted with 3N hydrochloric acid (3 × 300 ml), the combined acidic extracts were basified by the addition of sodium hydroxide pellets (ice also added) and the liberated oil was taken into ether (3 × 300 ml). After drying the combined ethereal solutions, removal of the solvent afforded the 3H-*indole* (V; R = H) as a tan-coloured crystalline solid (5·53 g; 37%), m.p. 98–99°. A small sample was recrystallised from aqueous ethanol to afford cream-coloured needles, m.p. 98–100°. Found: C, 81·3; H, 6·6. $C_{17}H_{17}NO$ requires C, 81·2; H, 6·7%. λ_{max} 218, 278 λ_{min} 244 mμ (log ε 4·09, 3·97 and 3·51 respectively); λ_{max} 248·5, 322 λ_{min} 235, 268–269 mμ (log ε 4·00, 3·97, 3·85 and 3·39 respectively in concentrated hydrochloric acid) (ultraviolet absorption of 3*H*-indole and 3*H*-indole cation chromophores respectively).

The above 3*H*-indole (5·3 g), without purification by recrystallisation, was dissolved in methyl iodide (20 ml). After standing at room temperature for 6 days, the crystals which had been deposited from the reaction mixture during this time were collected and washed with ether to afford the *methiodide* (VII; R = H) as pale-yellow prisms (2·45 g; 30%), m.p. 142–144°. Recrystallisation from ethanol-ether gave pale-yellow plates, m.p. 146–147°. Found: C, 55·2; H, 5·1. $C_{18}H_{20}INO$ requires C, 54·9; H, 5·1%.

5-*Benzyloxy*-2,3,3-*trimethyl*-3H-*indole* (V; R = Me). This was prepared from *p*-benzyloxyphenylhydrazine hydrochloride (Mentzer, Beaudet & Bory, 1953) (15 g) and isopropyl methyl ketone (6·0 g) following a method analogous to that described above for the preparation of 5-benzyloxy-3,3-dimethyl-3*H*-indole (V; R = H). On removal of the solvent from the dried ethereal solution of the basic product, the 3H-*indole* (V; R = Me) was obtained as a grey-brown solid (9·85 g, 62%), m.p. 94–96°. Recrystallisation of a small sample from aqueous ethanol afforded buff-coloured needles, m.p. 96–98°. Found: C 81·7; H 7·3. $C_{18}H_{19}NO$ requires C, 81·5; H, 7·1%. λ_{max} 219, 272 λ_{min} 241 mμ (log ε 4·06, 4·00, and 3·49 respectively); λ_{max} 244, 312 λ_{min} 235, 263·5 mμ (log ε 3·79, 3·78, 3·71 and 3·28 respectively in concentrated hydrochloric acid) (ultraviolet absorption of 3*H*-indole and 3*H*-indole cation chromophores respectively).

The ethereal solution remaining after extraction with 3N hydrochloric acid was washed with aqueous sodium carbonate solution, dried, evaporated to about 5 ml, and cooled. The crystalline deposit was collected and washed with a little ether to give VI as golden-yellow plates (0·49 g; 3·5%), m.p. 156–160°. Recrystallisation from ethanol afforded glistening yellow plates, m.p. 158–160°. Found: C, 70·05; H, 6·2. $C_{15}H_{15}N_2O_2$ requires C, 70·3; H, 6·2%. ν_{max} 3290 ± 10 m and 3200 ± 10 m (N–H stretching) and 1660 ± 3 ms (amide C = O stretching) cm^{-1}. τ = 5·03 (2-proton singlet) (benzyloxy methylene protons) and 8·00 (3-proton singlet) (acetyl group methyl protons).

The 3*H*-indole (V; R = Me) (9·65 g), without purification by recrystallisation, was dissolved in ether (100 ml), methyl iodide (20 ml) was added, and the solution was stood at room temperature. After 3 days the crystalline precipitate was collected and washed with ether to give the *methiodide* (VII; R = Me) as tan-coloured needles (12·0 g; 81·5%), m.p. 206–207°. Recrystallisation from ethanol-ether gave pale-yellow plates, m.p. 207–208°. Found: C, 56·0; H, 5·3. $C_{19}H_{22}INO$ requires C, 56·0; H, 5·4%.

5-*Benzyloxy*-1,3,3-*trimethylindoline* (VIII; $R^1 = Ph{\cdot}CH_2$, $R^2 = H$). To a solution of 5-benzyloxy-3,3-dimethyl-3*H*-indole methiodide (VII; R = H) (2·3 g) in methanol (70 ml) was added finely powdered sodium borohydride (1·5 g) in small quantities over a period of about 5 min with gentle swirling. After addition was complete the reaction mixture was kept at room temperature for 2 hr, water (100 ml) was added, and the crystalline precipitate was collected, washed with water and dried (P_2O_5–vacuum desiccator) to give the *indoline* (VIII; $R^1 = Ph{\cdot}CH_2$, $R^2 = H$) as white plates (1·45 g; 93%), m.p. 46–48°, unchanged by recrystallisation from aqueous ethanol. Found: C, 81·2; H, 8·2. $C_{18}H_{21}NO$ requires C, 80·9; H, 7·85%. λ_{max} 250, 311–312 λ_{min} 225·5, 285 mμ (log ε 3·98, 3·46, 3·64 and 3·10 respectively) (ultraviolet absorption of indoline chromophore).

5-*Benzyloxy*-1,2,3,3-*tetramethylindoline* (VIII; $R^1 = Ph{\cdot}CH_2$, $R^2 = Me$). 5-Benzyloxy-2,3,3-trimethyl-3*H*-indole methiodide (VII; R = Me) (10·0 g) was similarly reduced with sodium borohydride (5·0 g) in methanol (220 ml), the *indoline* (VIII; $R^1 = Ph{\cdot}CH_2$, $R^2 = Me$) being obtained as white plates (6·8 g; 98%), m.p. 58–59°, unchanged by recrystallisation from aqueous ethanol. Found: C, 81·25; H, 8·2. $C_{19}H_{23}NO$ requires C, 81·1; H, 8·2%. λ_{max} 249, 312–313 λ_{min} 226, 285 mμ (log ε 4·02, 3·47, 3·66 and 3·10 respectively) (ultraviolet absorption of indoline chromophore).

1,3,3-*Trimethyl*-5-*methylcarbamoyloxyindoline* (II; $R^1 = R^2 = H$, $R^3 = R^4 = Me$). 5-Benzyloxy-1,3,3-trimethylindoline (VIII; $R^1 = Ph{\cdot}CH_2$, $R^2 = H$) (1·55 g) dissolved in commercial absolute ethanol (100 ml) was hydrogenolysed at room temperature and pressure in the presence of 10% palladium-on-charcoal catalyst (0·5 g). After 7 hr, when hydrogenolysis was complete, the catalyst was removed by rapid filtration and the filtrate evaporated to give 5-*hydroxy*-1,3,3-*trimethylindoline* (VIII; $R^1 = R^2 = H$) as a viscous gum (0·95 g; 95%) which soon completely crystallised (recrystallisation and characterisation was not effected owing to the rapid decomposition of the compound on exposure to air). The phenolic product (0·80 g) was dissolved in dry ether (60 ml) and sodium (about 1 mg) was added, followed by methyl isocyanate (3 ml). After standing at room temperature for 3 days with occasional shaking, the reaction mixture was filtered and the filtrate evaporated to give the *methylurethane* (II; $R^1 = R^2 = H$, $R^3 = R^4 = Me$) as a red-coloured gum (0·95 g; 89·5%) which soon completely crystallised. Recrystallisation from ether afforded grey-white prisms (0·55 g), m.p. 122–124°. Found: C, 66·55; H, 7·4. $C_{13}H_{18}N_2O_2$ requires C, 66·6; H, 7·7%. ν_{max} 1703 ± 3 ms (C = O stretching) and 3370 ± 10 m (N–H stretching) cm^{-1}.

To the mother liquor from the recrystallisation in ether (15 ml) was added methyl iodide (4 ml). After standing for 6 days at room temperature the crystalline deposit which had slowly formed was collected and washed with ether to give the *methiodide* (IX; $R^1 = R^2 = H$) as tan-coloured prisms (0·61 g; 94·5%), m.p. 186–189°. Recrystallisation from ethanol afforded buff-coloured prisms, m.p. 196–197°. Found: C, 44·9; H, 5·7. $C_{14}H_{21}IN_2O_2$ requires C, 44·7; H, 5·6%.

1,2,3,3-*Tetramethyl*-5-*methylcarbamoyloxyindoline* (II; $R^1 = H$, $R^2 = R^3 = R^4 = Me$). 5-Benzyloxy-1,2,3,3-tetramethylindoline (VIII; $R^1 = Ph{\cdot}CH_2$, $R^2 = Me$) (7·3 g) dissolved in commercial absolute ethanol (150 ml) was hydrogenolysed, by the method described above for the hydrogenolysis of 5-benzyloxy-1,3,3-trimethylindoline (VIII; $R^1 = Ph{\cdot}CH_2$ $R^2 = H$), to give a quantitative yield of 5-*hydroxy*-1,2,3,3-*tetramethylindoline* (VIII; $R^1 = H$, $R^2 = Me$) as a light-brown viscous oil which completely crystallised. A small sample was recrystallised from ether to give tan-coloured prisms, m.p. 104–109°. Found: C, 75·2; H, 9·0. $C_{12}H_{17}NO$ requires C, 75·4; H, 8·9%. The phenol was then converted into the *methylurethane* (II; $R^1 = H$, $R^2 = R^3 = R^4 = Me$) in 94% yield, following a procedure similar to that described above, the product being recrystallised from ether-light petroleum (b.p. 40–60°) to give cream-coloured prisms, m.p. 110–112°. Found: C, 67·8; H, 8·4. $C_{14}H_{20}N_2O_2$ requires C, 67·75; H, 8·05%. ν_{max} 1704 ± 3 s (C = O stretching) and 3410 ± 10 m (N—H stretching) cm^{-1}.

The methylurethane (3·2 g) was dissolved in ether (150 ml), methyl iodide (15 ml) was added, and after standing at room temperature for 8 days, the *methiodide* (IX; $R^1 = H$, $R^2 = Me$) (2·67 g; 53%) which had deposited was collected. Recrystallisation from ethanol-ether afforded cream-coloured prisms, m.p. 168–171°. Found: C, 46·25; H, 6·2; $C_{15}H_{23}IN_2O_2$ requires C, 46·15; H, 5·9%.

5-*Dimethylcarbamoyloxy*-1,3,3-*trimethylindoline hydrochloride* (II; $R^1 = R^3 = R^4 = Me$, $R^2 = H$) HCl. 5-Hydroxy-1,3,3-trimethylindoline (VIII; $R^1 = R^2 = H$) (1·50 g) (prepared as described above) was dissolved in dry pyridine (10 ml), dimethylcarbamoyl chloride (4·0 g) was added, and the mixture was heated at 140–150° for 3 hr. After cooling, the reaction mixture was evaporated to dryness, water (10 ml) was added, and the mixture again evaporated. After repeating this operation a further three times, the brown gummy residue was partitioned between ether (100 ml) and 10% aqueous potassium hydroxide solution (15 ml). After drying, the ethereal solution was evaporated to leave a brown oil. This was dissolved in ethanol (10 ml), 20% hydrochloric acid (2·5 ml) was added, and the solution was evaporated to afford a viscous gum which crystallised on trituration with methanol-ether to give the hydrochloride of the *dimethylurethane* (II; $R^1 = R^3 = R^4 = Me$, $R^2 = H$) as yellow prisms (1·15 g; 45%), m.p. 110–112°. Recrystallisation from methanol-ether afforded pale-yellow prisms, m.p. 111–112°. Found: C, 58·85; H, 7·6. $C_{14}H_{21}ClN_2O_2$ requires C, 59·1; H, 7·3%. ν_{max} 1736 ± 3 ms (C = O stretching) and 2250 ± 10 m, broad (N^+ — H stretching) cm^{-1}.

The hydrochloride (0·50 g) was treated with an excess of cold aqueous

saturated sodium bicarbonate solution, the liberated base was taken into ether (2 × 100 ml), the combined ethereal solutions were dried, and evaporated to about 10 ml. Methyl iodide (10 ml) was added and after 6 days the crystalline deposit (0·60 g; 88%) which had gradually formed was collected. Recrystallisation from ethanol-ether afforded the *methiodide* (IX; R^1 = Me, R^2 = H) as pale-yellow needles, m.p. 172–176°. Found: C, 46·0; H, 6·0. $C_{15}H_{23}IN_2O_2$ requires C, 46·1; H, 5·8%.

5-Dimethylcarbamoyloxy-1,2,3,3-tetramethylindoline hydrochloride (II; R^1 = R^2 = R^3 = R^4 = Me) HCl. Similarly, 5-hydroxy-1,2,3,3-tetramethylindoline (VIII; R^1 = H, R^2 = Me) was converted into the hydrochloride of its *dimethylurethane* (II; R^1 = R^2 = R^3 = R^4 = Me) in 66% yield. The *hydrochloride*, initially obtained as a gum, completely crystallised on standing *in vacuo* overnight over potassium hydroxide pellets. Recrystallisation from commercial absolute ethanol afforded white needles, m.p. 138–140°. Found: C, 60·15; H, 7·5. $C_{15}H_{23}ClN_2O_2$ requires C, 60·25; H, 7·7%. ν_{max} 1727 ± 3 s (C = O stretching) and 2130 ± 10 s, broad (N^+ — H stretching) cm^{-1}.

The *free base*, liberated from the hydrochloride, was converted into the *methiodide* (IX; R^1 = R^2 = Me) in 57% yield by the method described above to prepare the methiodide (IX; R^1 = Me, R^2 = H) from the corresponding hydrochloride. Recrystallisation from ethanol-ether afforded buff-coloured prisms, m.p. 180–181°. Found: C, 47·8; H, 6·4. $C_{16}H_{25}IN_2O_2$ requires C, 47·5; H, 6·1%.

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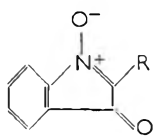
Preparation and antibacterial activity of isatogens and related compounds

M. HOOPER, D. A. PATTERSON AND D. G. WIBBERLEY

A number of 2-substituted isatogens are shown to possess moderate *in vitro* antibacterial properties. The syntheses of closely related compounds in the pyrrolo-[2,3-*c*]pyridine and pyrrolo[3,2-*b*]pyridine series are described. Antibacterial activities of these compounds, of the structurally similar indolones, *N*-hydroxy-indoles, indoxyls and indoles are recorded, together with those of certain styryl compounds required as intermediates.

IN routine *in vitro* antibacterial screening tests we found that 2-pyrid-2'-ylisatogen (I; R = pyrid-2-yl) showed interesting activity. This led us to prepare a short series of closely related isatogens and some aza-analogues (II and III). 2-Phenylisatogen (I; R = Ph) (Pfeiffer, 1916),



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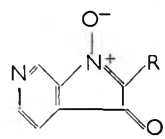

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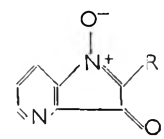
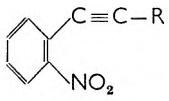


(III)

methyl isatogenate (I; R=CO·O·Me) (Alessandrai, 1928) and 2-pyrid-4'-ylisatogen (I; R=pyrid-4-yl) (Patterson & Wibberley, 1965) were obtained from the isomeric acetylenes (IV) under conditions previously described in the literature. Closely related to these acetylenes is 3-nitro-4- phenylethynylpyridine (V; R=Ph), which was derived from 3-nitro-4-styrylpyridine by chlorination followed by dehydrochlorination, and which yielded 3-oxo-2-phenyl-3*H*-pyrrolo[2,3-*c*]pyridine 1-oxide (II; R=Ph) on treatment with nitrosobenzene. The dehydrohalogenation of 4-(1,2-dichloro-2-pyrid-2'-ylethyl)-3-nitropyridine failed to yield any 3-nitro-4-pyrid-2'-ylethynylpyridine (V; R=pyrid-2-yl) and thus frustrated our attempts to prepare the 2-pyrid-2'-ylpyrrolopyridine 1-oxide (II; R=pyrid-2-yl). Several other attempted dehydrohalogenations also failed and in the case of 4-($\alpha\beta$-dibromophenethyl)-3-nitropyridine the main product of reaction was 3-nitro-4-styrylpyridine.

Splitter & Calvin (1955) showed that isatogens may be synthesised from the corresponding styryl compounds, in certain instances, on exposure to sunlight of their solutions in benzene. We have now demonstrated that the method is also successful for the synthesis of 2-*p*-dimethylamino-phenyl-3-oxo-3*H*-pyrrolo[2,3-*c*]pyridine 1-oxide (II; R=*p*-$Me_2N{\cdot}C_6H_4$) from 4-*p*-dimethylaminostyryl-3-nitropyridine (VI; R=Me_2N) and for the synthesis of 2-*p*-dimethylaminophenyl-3-oxo-3*H*-pyrrolo[3,2-*b*]pyridine 1-oxide (III; R=*p*-$Me_2N{\cdot}C_6H_4$) from 2-*p*-dimethylaminostyryl-3-nitro pyridine (VII; R=Me_2N).

From the School of Pharmacy, The Technical College, Sunderland.



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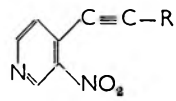

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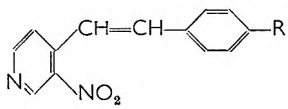

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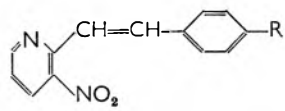
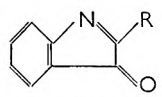


(VII)

Traces of a product, suggested by the evidence of its infrared spectrum to be 2-*p*-hydroxyphenyl-3-oxo-3*H*-pyrrolo-[2,3-*c*]pyridine 1-oxide (II; R=*p*-HO·C_6H_4), were obtained by irradiation of the sparingly soluble hydroxy-compound (VI; R=OH), but attempts to prepare 3-oxo-3*H*-pyrrolo[3,2-*c*]pyridine 1,5-dioxides by irradiation of solutions of 4-nitro-3-styrylpyridine *N*-oxides were unsuccessful.

Indole derivatives such as the 3*H*-indolones (VIII), the *N*-hydroxyindoles (X) and the indoxyls (XI; R_1=OH) may all be considered to be derived theoretically from the corresponding isatogens by reduction. However, we have recently shown that the compound previously thought to be 2-pyrid-2′-yl-3*H*-indolone (VIII; R = pyrid-2-yl) is in fact 2-pyrid-2′-yl-indoxyl (Patterson & Wibberley, 1965). We also find that the compound hitherto thought to be 2-methoxycarbonyl-3*H*-indolone (VIII; R = CO·O·Me) shows NH absorption in its infrared spectrum, has an ultraviolet spectrum closely similar to that of other 2,2′-disubstituted di-indoxyls (Hassner & Haddadin, 1963), and is therefore presumably 2,2′-dimethoxycarbonyl-2,2′-di-indoxyl (IX; R=CO·O·Me). 2-Phenyl-3*H*-indolone (VIII; R=Ph) was the only authentic indolone we were able



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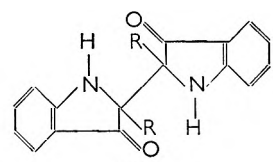

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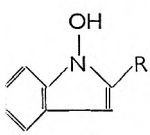

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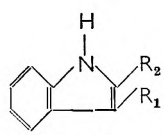

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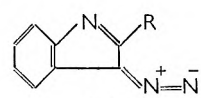

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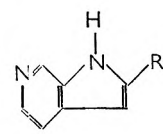

(XIII)

to test. The four *N*-hydroxyindoles recorded in Table 1 were prepared by methods described in the literature. The four known indoxyls were prepared by reduction of the corresponding isatogens with phenylhydrazine.

The literature contains several references to the antibacterial activity of indole itself (e.g. Sandholzer & Tittsler, 1934); it was of interest therefore to prepare 2-phenyl-, 2-pyrid-2′-yl-, and 2-pyrid-4′-yl-indoles for

comparison of their activity with the corresponding isatogens. The three compounds were obtained by a Fischer-type synthesis using polyphosphoric acid (Sugasawa & others, 1957). Such methods are not readily applicable in the pyridine series and the aza-analogues 2-phenyl-, 2-pyrid-2′-yl and 2-pyrid-4′-yl-pyrrolo[2,3-*c*]pyridine (XIII) were prepared by the Madelung-type cyclisation of the corresponding amides (cf. Clayton & Kenyon, 1950). Treatment of 3-amino-2-pyrid-2′-ylindole with nitrous acid had yielded 3-diazo-2-pyrid-2′-yl-3*H*-indole (Patterson & Wibberley, 1965) and the corresponding derivatives of 2-phenyl- and 2-pyrid-4′-ylindole have now been prepared.

ANTIBACTERIAL ACTIVITIES

The results of antibacterial screening tests are in Table 1. The isatogens and 3-oxo-3*H*-pyrrolopyridine 1-oxides were all effective against Gram-positive organisms, but only 2-phenylisatogen and 2-pyrid-2′-ylisatogen

TABLE 1. MINIMAL INHIBITORY CONCENTRATION (MG/100 ML) OF ISATOGENS AND RELATED COMPOUNDS

Compound	Formula	Test organisms				
		Staph. aureus	*B. subtilis*	*E. coli*	*S. typhi*	*P. vulgaris*
Methyl isatogenate	I; R = CO·O·Me	2·5	10	>40	20	>40
2-Phenylisatogen	I; R = Ph	10	10	20*	20*	20*
2-Pyrid-2′-ylisatogen	I; R = pyrid-2-yl	2·5	2·5	10	10	20
2-Pyrid-4′-ylisatogen	I; R = pyrid-4-yl	5	5	>40	>40	>40
3-Oxo-2-phenyl-3*H*-pyrrolo[2,3-*c*]pyridine 1-oxide	II; R = Ph	1·25	5	2·5	>40	>40
2-*p*-Dimethylaminophenyl-3-oxo-3*H*-pyrrolo[2,3-*c*]pyridine 1-oxide	II; R = $Me_2N{\cdot}C_6H_4$	2·5*	0·6*	20*	>40*	>40*
2-*p*-Dimethylaminophenyl-3-oxo-3*H*-pyrrolo[2,3-*b*]pyridine 1-oxide	III; R = $Me_2N{\cdot}C_6H_4$	>40	>40	20	>40	10
1-Hydroxy-2-phenylindole	X; R = Ph	0·31	0·31	1·25*	10*	40*
Methyl indoxylate	XI; R_1 = OH, R_2 = CO·OMe	2·5	10	5	10	40
2-Pyrid-2′-ylindole	XI; R_1 = H, R_2 = pyrid-2-yl	<0·31	0·31	>40	5	>40
2-Pyrid-4′-ylindole	XI R_1 = H, R_2 = pyrid-4-yl	1·25	2·5	>5*	>5*	>5*
3-Nitroso-2-pyrid-4′-ylindole	XI; R_1 = NO, R_2 = pyrid-4-yl	10	10	10	>40	>40
3-Amino-2-pyrid-2′-ylindole	XI; R_1 = NH_2, R_2 = pyrid-2-yl	10	>40	5	>40	2·5
3-Amino-2-pyrid-4′-ylindole	XI; R_1 = NH_2, R_2 = pyrid-4-yl	<1·25	10	10	10	>40
3-Diazo-2-phenyl-3*H*-indole	XII; R = Ph	<0·31	<0·31	1·25	1·25	5
3-Diazo-2-pyrid-2′-yl-3*H*-indole	XII; R = pyrid-2-yl	0·31	0·31	1·25	0·62	5
3-Diazo-2-pyrid-4′-yl-3*H*-indole	XII; R = pyrid-4-yl	1·25	1·25	1·25	1·25	1·25
2-Pyrid-2′-ylpyrrolo[2,3-c] pyridine	XIII; R = pyrid-2-yl	1·25	1·25	10	10	20
2-Pyrid-4′-ylpyrrolo[2,3-*c*] pyridine	XIII; R = pyrid-4-yl	2·5	10	5	10	10
4-Nitro-3-styryl-pyridine 1-oxide		5	5	5	5	5

*Tested as a suspension in ethanol.

The following compounds had MIC 40 or >40 against all the Gram-negative organisms; any activity against Gram-positive organisms is indicated. 6-Nitro-2-phenylisatogen (*Staph. aureus* 10*, *B. subtilis* 10*), 2-phenyl-3*H*-indolone, 2,3-dihydro-2-hydroxy-2-pyrid-2′-ylindolone, 1-hydroxyindole-2-carboxylic acid, 1,2-dihydroxyindole, 1-hydroxy-2-pyrid-2′-ylindole (*Staph. aureus* 2·5), 2-phenylindoxyl (*Staph. aureus* 1·25), 6-nitro-2-phenylindoxyl, 2-pyrid-2′-ylindoxyl (*Staph. aureus* 0·62, *B. subtilis* 5), 2,2′-diphenyl-2,2′-di-indoxyl (*Staph. aureus* 2·5, *B. subtilis* 1·25), 2,2′-dimethoxycarbonyl-2,2′-di-indoxyl, 2-phenylindole, 3-nitroso-2-phenylindole (*Staph. aureus* 10), 3-nitroso-2-pyrid-2′-ylindole, 3-amino-2-phenylindole, 2-phenylpyrrolo-[2,3-*c*]pyridine, 5-methyl-2-phenylpyrrolo[3,2-*b*]pyridine, 3-*p*-dimethyl-aminostyryl-4-nitropyridine 1-oxide (*Staph. aureus* 10), 3-*p*-hydroxystyryl-4-nitropyridine 1-oxide, 3-nitro-2-styrylpyridine, 2-*p*-dimethylamino-styryl-3-nitropyridine, 3-nitro-4-styrylpyridine (*Staph. aureus* 2·5, *B. subtilis* 20), 4-*p*-dimethylaminostyryl-3-nitropyridine, 4-*p*-hydroxystyryl-3-nitropyridine.

showed a broad spectrum of activity. The inactivity of 2-phenyl-2*H*-indolone and the "hydrate" of 2-pyrid-2′-yl-3*H*-indolone suggests that the 1-oxide group is essential for growth inhibition. With the exception of 1-hydroxy-2-phenylindole the 1-hydroxyindoles and indoxyls were of little interest.

The pyrrolo[2,3-*c*]pyridines were generally more effective than the analogous indoles although the 3-diazo-group conferred a broad spectrum of activity in the 2-substituted indoles. The literature contains several references to the growth-inhibitory properties of styryl compounds (Rubtsov, Pershin, Novitskaya, Milovanova & Vichkanova, 1960) and nitrostyryl *N*-oxides (Buchmann & Kirstein, 1962). The activity of one of our intermediates, 4-nitro-3-styrylpyridine 1-oxide, is therefore not surprising.

Experimental

General method for the preparation of the styrylpyridines. A solution of the picoline (0·025 mole), the aldehyde (0·03 mole) and piperidine (0·005 mole) in methanol (10 ml) was refluxed for 24 hr. The solution was cooled, the styryl compound collected and crystallised from the stated solvent.

4-p-*Hydroxystyryl*-3-*nitropyridine* (VI; R=OH) (40%) separated from ethanol in orange needles, m.p. 257–259°. Found: C, 64·3; H, 4·1; N, 11·4. $C_{13}H_{10}N_2O_3$ requires C, 64·4; H, 4·2; N, 11·55%. A solution of the styryl compound (0·34 g) in benzene (250 ml) was exposed to sunlight for one week. The solution was concentrated to dryness and the residue triturated with ethanol to yield a maroon solid (0·006 g), m.p. > 360°. ν_{max} ($CHCl_3$) 1390s (*N*-oxide), 1710s cm^{-1} (C=O).

4-p-*Dimethylaminostyryl*-3-*nitropyridine* (VI; R=Me_2N) (82%) separated from ethanol as dark green needles, m.p. 162–163°. Found: C, 66·3; H, 5·6; N, 15·7. $C_{15}H_{15}N_3O_2$ requires C, 66·9; H, 5·6; N, 15·6%.

3-*Nitro*-4-(2-*pyrid*-2′-*ylvinyl*)*pyridine* (81%) separated from ethanol as yellow prisms, m.p. 110–111°. Found: C, 63·6; H, 4·0; N, 18·3. $C_{12}H_9N_3O_2$ requires C, 63·5; H, 4·0; N, 18·5%.

3-*Nitro*-2-*styrylpyridine* (VII; R=H) (12·2%) separated from aqueous methanol as yellow needles, m.p. 107–108°. A better yield (33%) was obtained by using acetic anhydride (0·038 mole) in place of the piperidine and methanol. Found: C, 68·8; H, 4·45; N, 12·35. $C_{13}H_{10}N_2O_2$ requires C, 69·0; H, 4·45; N, 12·4%.

2-p-*Dimethylaminostyryl*-3-*nitropyridine* (VII; R=Me_2N) (53%) was obtained after 4-days' reflux. It separated from methanol as dark-red needles, m.p. 148–149°. Found: C, 66·4; H, 5·4; N, 15·3. $C_{15}H_{15}N_3O_2$ requires C, 66·9; H, 5·6; N, 15·6%.

3-p-*Dimethylaminostyryl*-4-*nitropyridine* 1-*oxide* (28%) separated from 2-ethoxyethanol as dark-red needles, m.p. 207–208°. Found: C, 63·1; H, 5·5; N, 14·5. $C_{15}H_{15}N_3O_3$ requires C, 63·15; H, 5·3; N, 14·7%. Irradiation of a solution in benzene for 10 days yielded no pyrrolopyridine 1,5-dioxide.

3-p-*Hydroxystyryl*-4-*nitropyridine* 1-*oxide* (15·5%). Separated from acetic acid as orange needles, m.p. > 360°. Found: C, 60·2; H, 4·1; N, 10·7. $C_{13}H_{10}N_2O_4$ requires C, 60·5; H, 3·9; N, 10·8%.

4-(αβ-*Dichlorophenethyl*)-3-*nitropyridine*. A solution of 3-nitro-4-styrylpyridine (1·9 g) in glacial acetic acid was saturated with chlorine. The precipitate was collected and triturated with dilute aqueous ammonia to yield the *dichloro*-compound (1·3 g) as colourless prisms, m.p. 133–134° (from methanol). Found: C, 52·6; H, 3·3; Cl, 23·7; N, 9·4. $C_{13}H_{10}Cl_2N_2O_2$ requires C, 52·7; H, 3·4; Cl, 23·8; N, 9·4%.

4-(αβ-*Dibromophenethyl*)-3-*nitropyridine*. A solution of 3-nitro-4-styrylpyridine (3·8 g) in acetic acid (25 ml) and bromine (4·0 g) was maintained at 60–70° for 40 min. The product was collected and triturated with dilute aqueous ammonia to yield the *dibromo*-compound (3·1 g) as colourless prisms, m.p. 222–224° (from methanol). Found: C, 40·8; H, 2·6; Br, 40·9; N, 7·0. $C_{13}H_{10}Br_2N_2O_2$ requires C, 40·4; H, 2·6; Br, 41·4; N, 7·2%.

4-(1,2-*Dichloro*-2-*pyrid*-2′-*ylethyl*)-3-*nitropyridine*. Chlorination of 3-nitro-4-(2-pyrid-2′-ylvinyl)pyridine (2·0 g) under similar conditions yielded the *dichloro*-compound (0·6 g) as colourless prisms, m.p. 135–136° (from ethanol). Found: C, 48·0; H, 3·2; Cl, 23·5; N, 14·2. $C_{12}H_9Cl_2N_3O_2$ requires C, 48·3; H, 3·0; Cl, 23·8; N, 14·1%.

3-(αβ-*Dichlorophenethyl*)-4-*nitropyridine* 1-*oxide*. Chlorination of 4-nitro-3-styrylpyridine 1-oxide (2·0 g) under the above conditions yielded the *dichloro*-compound (1·2 g) as yellow prisms, m.p. 177–178° (from ethyl acetate). Found: C, 50·6; H, 3·4; N, 9·0. $C_{13}H_8Cl_2N_2O_3$ requires C, 50·3; H, 2·6; N, 9·0%.

3-*Oxo*-2-*phenyl*-3-*Hpyrrolo*[2,3-*c*]*pyridine* 1-*oxide* (II; R=Ph). A solution of 4-(αβ-dichlorophenethyl)-3-nitropyridine (1·3 g) and potassium hydroxide (0·52 g) in ethanol (5·0 ml) was refluxed for 2 hr and then concentrated to dryness. The residue was extracted with benzene (20 ml) and the extract passed down a column of silica gel (10 g) and Celite (5 g). The column was eluted with benzene and the eluate collected (500 ml) until its infrared spectrum no longer showed a peak at 2200 cm^{-1} (C≡C absorption of 3-nitro-4-phenylethynylpyridine). The solution was evaporated to yield the nitropyridine (V; R=Ph) (0·4 g) which, without further purification, was dissolved in chloroform (5·0 ml) and refluxed for 1 hr with nitrosobenzene (0·25 g). The solution was concentrated to low bulk when the *pyrrolopyridine* 1-*oxide* (0·32 g) crystallised out. It separated from ethanol as orange plates, m.p. 171–172°. Found: C, 69·5; H, 3·4; N, 12·4. $C_{13}H_8N_2O_2$ requires C, 69·6; H, 3·6; N, 12·5%. ν_{max} ($CHCl_3$) 1400s (*N*-oxide) 1710s cm^{-1} (C=C).

2-p-*Dimethylaminophenyl*-3-*oxo*-3H-*pyrrolo*[3,2-*b*]*pyridine* 1-*oxide* (III; R=$Me_2N{\cdot}C_6H_4$). A solution of 2-*p*-dimethylaminostyryl-3-nitropyridine (0·25 g) in benzene (250 ml) was exposed to sunlight for two weeks. The dark-blue solution was filtered and the filtrate evaporated to dryness. The residue was stirred with ethanol (5·0 ml) to yield the *pyrrolopyridine* 1-*oxide* (0·073 g) as dark-blue needles, m.p. 212° (decomp.) (from 2-ethoxyethanol). Found: C, 67·3; H, 5·0; N, 15·7. $C_{15}H_{13}N_3O_2$ requires

C, 67·5; H, 4·9; N, 15·7%. ν_{max} ($CHCl_3$) 1380s (*N*-oxide), 1715s cm^{-1} split (C=O).

2-p-*Dimethylaminophenyl*-3-*oxo*-3H-*pyrrolo*[2,3-c]*pyridine* 1-*oxide* (II; R=$Me_2N{\cdot}C_6H_4$). In a similar irradiation 4-*p*-dimethylaminostyryl-3-nitropyridine (VI; R=Me_2N) (0·5 g) after four days yielded the *pyrrolo*-[2,3-*c*]*pyridine* 1-*oxide* (0·145 g) as dark-blue needles, m.p. 206° (decomp.) (from ethanol). Found: C, 66·9; H, 5·0; N, 15·6. $C_{15}H_{13}N_3O_2$ requires C, 67·5; H, 4·9; N, 15·7%. ν_{max} ($CHCl_3$) 1380s (*N*-oxide), 1705s cm^{-1} (C=O).

2,2′-*Dimethoxycarbonyl*-2,2′-*di-indoxyl* (IX; R=CO·O·Me) (a) A solution of methyl indoxylate (0·3 g) and benzoyl peroxide (1·2 g) in acetone (50 ml) was allowed to evaporate to dryness at room temperature. The residue was boiled with benzene (10 ml), and the hot suspension filtered to yield the *di-indoxyl* (0·3 g). It separated from nitrobenzene as yellow needles, m.p. 255° (decomp.). Found: C, 63·4; H, 4·4; N, 7·7. $C_{20}H_{16}N_2O_6$ requires C, 63·2; H, 4·2; N, 7·4%. ν_{max} (Nujol) 1730s (ester C=O), 1700s (ring C=O), 3400m cm^{-1} (N–H). λ_{max} (ethanol) 235 mμ (log ϵ 4·27), 265 (3·75), 390 (3·40) (Hassner & Haddadin state λ_{max} 235, 255, 395 mμ for 2,2′-di-indoxyls). (b) Treatment of methyl isatogenate with phenylhydrazine under the conditions described by Ruggli & Bollinger (1921) for the preparation of 2-methoxycarbonyl-3*H*-indolone yielded the same di-indoxyl of identical infrared spectrum and undepressed mixed m.p.

3-*Picolinamido*-4-*picoline*. Picolinic acid (7·5 g) and thionyl chloride (20 ml) were refluxed together for 20 min and the excess thionyl chloride removed under vacuum. An extract in benzene (20 ml) of the residue was added to a solution of 3-amino-4-picoline (3·25 g) in benzene (20 ml) over 10 min. After 1 hr the precipitated amide hydrochloride was collected, treated with dilute ammonia solution until alkaline, and the mixture evaporated to dryness. The residue was extracted with boiling light petroleum (120°–160°) from which the *amide* (4·1 g) separated as pink needles, m.p. 141–142°. Found: C, 67·8; H, 5·3; N, 19·5. $C_{12}H_{11}N_3O$ requires C, 67·5; H, 5·2; N, 19·7%.

3-*Isonicotinamido*-4-*picoline*. A similar treatment of 3-amino-4-picoline (7·5 g) with freshly prepared isonicotinoyl chloride (from 24·7 g of isonicotinic acid) yielded the amide hydrochloride, which was converted into the *amide* (12·9 g) by basification of its aqueous solution. The amide crystallised as a *dihydrate*, m.p. 82–84° (from aqueous ethanol). Found: C, 57·5; H, 6·1; N, 18·5. $C_{12}H_{11}N_3O{\cdot}2H_2O$ requires C, 57·8; H, 6·0; N, 17·9%.

3-*Benzamido*-4-*picoline monohydrate*. A solution of 3-amino-4-picoline (5·6 g) and benzoyl chloride (8·0 ml) in pyridine (20 ml) was stirred at room temperature for 1 hr and then poured into water (125 ml). The oil which separated was extracted with chloroform and the extracts washed with water, dried, and evaporated to yield the amide monohydrate (6·9 g) as colourless needles, m.p. 80–82° (from aqueous ethanol). Found: C, 68·0; H, 6·0; N, 12·4. $C_{13}H_{12}N_2O.H_2O$ requires C, 67·8; H, 6·1; N, 12·2%. (Koenigs & Fulde, 1927, state m.p. 81° for the anhydrous compound).

2-*Pyrid*-2′-*ylpyrrolo*[2,3-c]*pyridine* (XIII; R=pyrid-2-yl). A solution of 3-picolinamido-4-picoline (1 g) and sodium ethoxide (0·7 g) in absolute ethanol (50 ml) was distilled during the passage of a stream of nitrogen. The bath temperature was raised to 325° where it was maintained for 10 min. Water (10 ml) was added to the cooled residue and the solution extracted with chloroform. Evaporation of the extracts followed by sublimation of the residue yielded the *pyrrolopyridine* (0·29 g) as prisms, m.p. 205–206° (from ethanol). Found: C, 73·7; H, 4·7; N, 21·6. $C_{12}H_9N_3$ requires C, 73·9; H, 4·6; N, 21·5%.

2-*Pyrid*-4′*ylpyrrolo*[2,3-c]*pyridine* (XIII; R=pyrid-4-yl). Similar treatment of 3-isonicotinamido-4-picoline at 300° for 10 min yielded the *pyrrolopyridine* (12%) as colourless prisms, m.p. 251–252° (from dioxan). Found: C, 73·5; H, 4·7; N, 21·7. $C_{12}H_9N_3$ requires C, 73·9; H, 4·6; N, 21·5%.

2-*Phenylpyrrolo*[2,3-c]*pyridine* (XIII; R=Ph). 3-Benzamido-4-picoline at 320° for 20 min yielded the 2-*phenylpyrrolopyridine* (24%) as colourless needles, m.p. 229–231° (from aqueous ethanol). Found: C, 79·6; H, 5·2; N, 13·9. $C_{13}H_{10}N_2$ requires C, 80·3; H, 5·2; N, 14·4%.

3-*Nitroso*-2-*pyrid*-4′-*ylindole* (XI; R_1=NO,R_2=pyrid-4-yl). 2-Pyrid-4′-ylindole (0·4 g), sodium nitrite (0·2 g) and acetic acid (10 ml) were stirred at room temperature for 10 min. Filtration yielded the nitrosoindole (0·43 g) as yellow needles, m.p. 249–250° (from 2-ethoxyethanol). Found: C, 70·1; H, 4·2; N, 18·75. $C_{13}H_9N_3O$ requires C, 69·9; H, 4·1; N, 18·8%.

3-*Amino*-2-*pyrid*-4′-*ylindole* (XI; R_1=NH_2,R_2=pyrid-4-yl). Sodium dithionite (0·75 g) was added to a solution of 3-nitroso-2-pyrid-4′-ylindole (0·35 g) in ethanol (2·0 ml) and 2N sodium hydroxide (4·0 ml) and the mixture heated on a water-bath for 4 min to yield the amine (0·25 g) as yellow-green prisms, m.p. 219–220° (from ethanol). Found: C, 74·8; H, 5·3; N, 19·9. $C_{13}H_{11}N_3$ requires C, 74·6; H, 5·3; N, 20·1%.

3-*Diazo*-2-*pyrid*-4′-*yl*-3H-*indole* (XII; R=pyrid-4-yl). A solution of sodium nitrite (0·2 g) in water (1·0 ml) was added dropwise to a suspension of 3-amino-2-pyrid-4′-ylindole (0·2 g) in water (10 ml) and concentrated sulphuric acid (0·75 ml) at 0° and the mixture stirred for 20 min. Neutralisation with sodium carbonate solution yielded the *diazoindole* (0·16 g) as yellow needles, m.p. 101–102° (from light petroleum). Found: C, 70·4; H, 4·0; N, 25·0. $C_{13}H_8N_4$ requires C, 70·9; H, 3·7; N, 25.5%. The solid discoloured on the surface after exposure to the air and light for several hours.

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Some tryptamine derivatives: 1-aryloxy-3-[(2-indol-3′-ylethyl)amino]propan-2-ols

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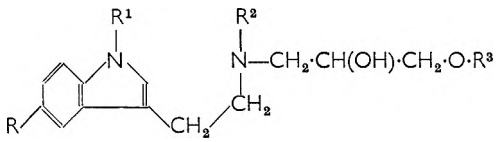
The preparation of some 1-aryloxy-3-[(2-indol-3′-ylethyl)amino]propan-2-ols is described. Their biological activity was insignificant.

BECAUSE many effective psycholytic drugs are substituted indoles related to 5-hydroxytryptamine (5-HT) we have prepared a series of substituted tryptamines of type (I) containing a 3-aryloxy-2-hydroxypropyl

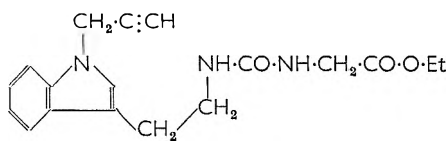


(I)

moiety, in the hope that these might possess useful central nervous system (CNS) activity possibly of the tranquilliser type (compare Beasley, Petrow & Stephenson, 1958).

5-Substituted tryptamines required in this work were prepared by the general method of Abramovitch & Shapiro (1955, 1956) and Abramovitch (1956), with slight variations of these methods as described by Pelchowicz & Bergmann (1959, 1960).

1-Alkyltryptamines were obtained as described by Potts & Saxton (1954) using sodamide in liquid ammonia as the alkylating medium. 1-Prop-2′-ynyltryptamine, prepared by this latter route, was characterised by the preparation of its *N*′-acetyl derivative and by reaction with ethyl isocyanatoacetate to yield the substituted hydantoate (II).



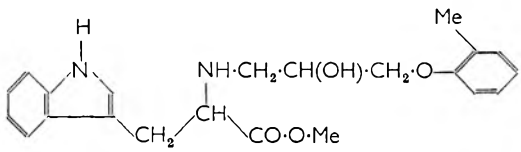
(II)

The required 1-aryloxy-3-[2-indol-3′ylethylamino]propan-2-ols (I) were obtained in moderate yield by reaction of a tryptamine with the appropriate 1-aryloxy-2,3-epoxypropane in refluxing alcoholic solution.

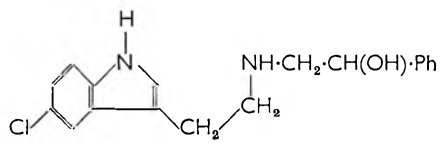
In preliminary experiments we had found that the methyl ester of tryptophan reacted with 1,2-epoxy-3-*o*-tolyloxypropane in methanolic solution to give the product (III), whilst reaction between 5-chlorotryptamine and styrene oxide yielded the required product (IV).

From the British Drug Houses Ltd., Graham Street, London, N.1.

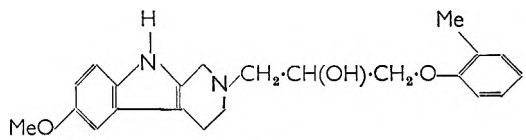
Finally, the β-carboline derivative (V) was prepared for direct comparison with the open-chain analogue (I; R = MeO, $R^1 = R^2 = H$, R^3 = *o*-tolyl). It was obtained readily by reaction of 1,2,3,4-tetrahydro-6-methoxy-β-carboline (Abramovitch & Shapiro, 1956) with 1,2-epoxy-3-*o*-tolyloxypropane.



(III)



(IV)



(V)

BIOLOGICAL STUDY

Biological study of the above products by Dr. D. K. Vallance and Mr. D. I. Barron (Biological Department, Godalming, Surrey) showed that only 1-{[2-(5-methoxy-1-prop-2′-ynylindol-3-yl)ethyl]amino}-3-*o*-tolyloxypropan-2-ol (I; R = MeO, $R^1 = R^2 = H$, R^3 = *o*-tolyl) possessed a moderate degree of CNS depressant activity. Thus, compared with chlorpromazine hydrochloride as standard, its activity was 1/9 in antagonising the toxicity of amphetamine in aggregated mice, 1/13 in preventing footshock-induced fighting in mice, 1/19 in anti-tremorine activity in mice and 1/24 in preventing head twitches induced in mice by the intraperitoneal injection of 5-hydroxytryptophan. Additionally, the compound had 1/26 of the activity of diphenylhydantoin in the maximal leptazol seizure test. The compound was not considered worthy of more detailed biological study.

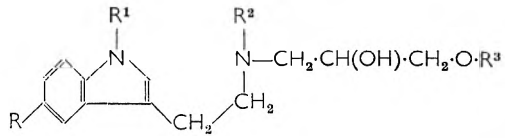
Experimental

Melting-points are uncorrected.

Some of the examples illustrate the preparative methods for the compounds listed in Table 1 which contains relevant analytical data.

Methyl ester of N-(2-*hydroxy*-3-*o*-*tolyloxypropyl*) *tryptophan*. A solution of the methyl ester of DL-tryptophan (4·4 g) and 1,2-epoxy-3-*o*-tolyloxypropane (3·3 g) in methanol (25 ml) was heated under reflux for 2 hr and the solvent then distilled off at reduced pressure. The residual

TABLE 1.



R	R¹	R²	R³	m.p. °C	Formula	Found C	Found H	Found Cl	Found N	Required C	Required H	Required Cl	Required N
MeO	H	H	Ph	109–111	$C_{20}H_{24}N_2O_3$	70·5	7·1		8·0	70·6	7·1		8·2
H	H	H	*o*-Me·C_6H_4	113–115	$C_{20}H_{24}N_2O_2$	73·5	7·2		9·1	74·0	7·5		8·6
MeO	H	H	*o*-Me·C_6H_4	128–130	$C_{21}H_{26}N_2O_3$	70·6	7·4		8·4	71·15	7·4		7·9
MeO	H	H	*o*-Me·C_6H_4	155–157†	$C_{21}H_{27}ClN_2O_3$†	64·3	6·9	9·6	7·5	64·5	7·0	9·1	7·2
Me	H	H	*o*-Me·C_6H_4	130–132	$C_{21}H_{26}N_2O_2$	74·8	7·6		8·1	74·5	7·7		8·3
Cl	H	H	*o*-Me·C_6H_4	148–150	$C_{20}H_{23}ClN_2O_2$	67·1	6·6	10·0	8·0	66·9	6·5	9·9	7·8
F	H	H	*o*-Me·C_6H_4	118–120	$C_{20}H_{23}FN_2O_2$	69·9	6·5	5·4*	8·3	70·2	6·8	5·6*	8·2
H	$CH_2{\cdot}C{:}CH$	H	*o*-Me·C_6H_4	94–96	$C_{23}H_{26}N_2O_2$	76·0	7·4		7·6	76·2	7·2		7·7
H	$Ph{\cdot}CH_2$	H	*o*-Me·C_6H_4	78–80	$C_{27}H_{30}N_2O_2$	78·0	7·4		6·6	78·2	7·3		6·8
H	H	Et	*o*-Me·C_6H_4	162–164†	$C_{22}H_{29}ClN_2O_2$†	67·5	7·7	9·0	7·2	67·9	7·5	9·1	7·2
EtO	H	H	*o*-Me·C_6H_4	121–122	$C_{22}H_{28}N_2O_3$	71·4	7·5		7·3	71·7	7·7		7·6
MeO	$CH_2{\cdot}C{:}CH$	H	*o*-Me·C_6H_4	94–96	$C_{24}H_{28}N_2O_3$	73·2	7·2		7·3	73·4	7·2		7·1
Cl	H	$CH_2{\cdot}C{:}CH$	*o*-Me·C_6H_4	186–188†	$C_{25}H_{27}Cl_3N_2O_2$†	63·6	6·0	16·7	6·7	63·7	6·1	16·4	6·5
MeO	H	$Ph{\cdot}CH_2$	*o*-Me·C_6H_4	118–120	$C_{28}H_{32}N_2O_3$	75·1	7·2		6·7	75·6	7·3		6·3
MeO	H	H	*m*-Me·C_6H_4	116–118	$C_{21}H_{26}N_2O_3$	70·8	7·7		7·8	71·15	7·4		7·9
H	H	H	*o*-MeO·C_6H_4	124–126	$C_{20}H_{24}N_2O_2$	70·2	7·1		8·1	70·6	7·1		8·2
MeO	H	H	*o*-MeO·C_6H_4	125–127	$C_{21}H_{26}N_2O_4$	67·6	7·1		7·8	68·1	7·1		7·6
MeO	H	H	*p*-MeO·C_6H_4	102–104	$C_{21}H_{26}N_2O_4$	68·4	7·3		7·1	68·1	7·1		7·6
MeO	H	H	*o*-Cl·C_6H_4	133–135	$C_{20}H_{23}ClN_2O_3$	64·4	6·3	9·1	7·4	64·1	6·2	9·5	7·5
MeO	H	H	*p*-Cl·C_6H_4	120–122	$C_{20}H_{23}ClN_2O_3$	64·0	6·1	9·1	7·2	64·1	6·2	9·5	7·5
H	Et	H	*p*-Cl·C_6H_4	110–112	$C_{21}H_{25}ClN_2O_2$	**67·7**	7·0	**9·5**	**7·3**	67·6	6·8	9·5	7·5
MeO	H	H	2,4-$Cl_2{\cdot}C_6H_4$	117–119	$C_{20}H_{22}Cl_2N_2O_3$	58·9	5·6	16·9	6·8	58·7	5·4	17·3	6·8
MeO	H	H	*o*-$C_6H_5{\cdot}C_6H_4$	122–124	$C_{26}H_{28}N_2O_3$	74·9	6·6		6·8	74·9	6·8		6·7

† = Hydrochloride
* = Fluorine

gum solidified on trituration with ether to yield the ester (2·4 g). m.p 125–127° (from methanol). Found: C, 69·5; H, 6·8; N, 7·0. $C_{22}H_{26}N_2O_4$ requires: C, 69·1; H, 6·9; N, 7·3%. The *hydrochloride* had m.p. 157–160° (decomp.) (from methanol-ether). Found: C, 63·2; H, 6·4; Cl, 8·7; N, 6·7. $C_{22}H_{27}ClN_2O_4$ requires: C, 63·1; H, 6·5; Cl, 8·5; N, 6·7%. The *phosphate* obtained when a solution of the base (3·5 g) in pyridine (20 ml) was treated with phosphoryl chloride (1·7 ml), and the mixture allowed to stand at room-temperature for 30 min and then poured into water, had m.p. 208–210° (decomp.) (from methanol). Found: C, 55·7; H, 5·7; N, 6·1; P, 7·0. $C_{22}H_{27}N_2O_7P$ requires: C,55·4; H, 5·7; N, 5·9; P, 6·5%.

2-{[2-(5-*Chloroindol*-3-*yl*)*ethyl*]*amino*}-1-*phenylethanol.* A suspension of 5-chlorotryptamine hydrochloride (4·6 g) in hot ethanol (15 ml) was treated with a solution of sodium hydroxide (0·8 g) in ethanol (10 ml). The mixture was filtered to remove sodium chloride, the filtrate treated with styrene oxide (2·3 ml), and the solution heated at reflux temperature for 1 hr. The ethanol was distilled off at reduced pressure to yield the alcohol (1·4 g), m.p. 129–131° [from benzene-light petroleum (b.p. 60–80°)]. Found: C, 68·9; H, 5·8; Cl, 11·4; N, 8·8. $C_{18}H_{19}ClN_2O$ requires: C, 68·7; H, 6·1; Cl, 11·3; N, 8·9%.

1-*Prop*-2′-*ynyltryptamine hydrochloride* (compare Potts & Saxton, 1954). To a solution of sodamide [prepared from sodium (1·7 g) in liquid ammonia (250 ml)], powdered tryptamine (11·7 g) was added in portions during 10 min with stirring at −60°. The mixture was stirred for a further 5 min then prop-2-ynyl bromide (9·2 g) was added dropwise; stirring was continued for 1 hr at −60° after which time the ammonia was allowed to evaporate freely. The residue was decomposed with water and the base isolated with ether; the base (12·1 g) had b.p. 150° at 0·2 mm. The *hydrochloride* had m.p. 191–193° (from ethanol-ether). Found: C, 67·0; H, 6·4; Cl, 14·5; N, 11·6. $C_{13}H_{15}ClN_2$ requires: C, 66·5; H, 6·5; Cl, 15·1; N, 11·9%.

N′-*Acetyl*-1-(*prop*-2-*ynyl*)*tryptamine* was obtained when the foregoing base (1·2 g) was heated with a mixture of acetic acid (3 ml) and acetic anhydride (2 ml) for 1 hr. It had m.p. 128–130° (from methanol). Found: C, 74·7; H, 6·7; N, 12·1. $C_{15}H_{16}N_2O$ requires: C, 75·0; H, 6·6; N, 11·7%.

N-*Ethoxycarbonylmethyl*-N′-{[2-(1-*prop*-2′-*ynylindol*-3-*yl*)*ethyl*]*amino*} *urea* was obtained when a solution of 1-prop-2′ynyltryptamine (2·0 g) in ether (30 ml) was treated dropwise with a solution of ethyl isocyanatoacetate (1·3 g) in ether (10 ml). It (2·4 g) had m.p. 121° (from benzene). Found: C, 65·8; H, 6·2; N, 12·7. $C_{18}H_{21}N_3O_3$ requires: C, 66·1; H, 6·5; N, 12·8%.

5-*Methoxy*-1-*prop*-2′-*ynyltryptamine hydrochloride* was prepared by alkylation of 5-methoxytryptamine with prop-2-ynyl bromide, using sodamide-liquid ammonia as described for 1-prop-2′-ynyltryptamine. The *base* (64% yield) had b.p. 180–190° at 0·1 mm. The *hydrochloride* had m.p. 200–202° (from ethanol-ether). Found: C, 63·6; H, 6·6; Cl, 13·4; N, 10·2. $C_{14}H_{17}ClN_2O$ requires: C, 63·6; H, 6·5; Cl, 13·4; N, 10·5%.

1-{[2-(5-*Methoxy*-1-*prop*-2′-*ynylindol*-3-*yl*)*ethyl*]*amino*}-3-*o*-*tolyloxypropan*-2-*ol*. A solution of the foregoing hydrochloride (1·6 g) in methanol (10 ml) was treated with a solution of potassium hydroxide (1 equiv.) in methanol (10 ml), followed by 1,2-epoxy-3-*o*-tolyloxypropane (0·9 ml). The mixture was heated under reflux for 2 hr and then filtered to remove potassium chloride. The filtrate was evaporated at reduced pressure to yield the alcohol, m.p. 94–96° [from benzene-light petroleum (b.p. 60–80°)].

1-{N-[2-(5-*Chloroindol*-3-*yl*)*ethyl*]-N-*prop*-2-*ynylamino*}-3-o-*tolyloxypropan*-2-*ol hydrochloride*. A solution of 1-{[2-(5-chloroindol-3-yl)-ethyl]amino}-3-*o*-tolyloxypropan-2-ol (3·6 g) in methanol (30 ml) was treated with prop-2-ynyl bromide (0·6 g). The mixture was heated under reflux for 15 hr. and then concentrated at reduced pressure. The residual gum was triturated with ether and the *hydrobromide* (2·2 g, m.p. 150–153°) of the starting base was then filtered off. The ethereal filtrate was washed with water, dried with anhydrous magnesium sulphate and then treated with dry hydrogen chloride to yield the hydrochloride, m.p. 186–188° (from ethanol).

3-[2-(2-*Hydroxy*-3-o-*tolyloxypropylamino*)*ethyl*]-5-*methoxyindole*-2-*carboxylic acid*. To a suspension of 3-ethyl-5-methoxyindole-2-carboxylic acid (2·4 g) in ethanol (25 ml) was added a solution of sodium hydroxide (0·4 g) in water (5 ml), followed by 1,2-epoxy-3-*o*-tolyloxypropane (1·65 g). The homogeneous mixture was heated under reflux for 3 hr, the ethanol distilled off at reduced pressure and replaced by water, and the solution acidified with acetic acid. The solids were collected, dried and extracted with boiling ethyl acetate, after which a small residue of insoluble starting material remained. The ethyl acetate extract deposited the acid (1·4 g) on cooling. It had m.p. 208–210° (from ethanol). Found: N, 6·8. $C_{22}H_{26}N_2O_5$ requires: N, 7·0%.

1-(1,2,3,4-*Tetrahydro*-6-*methoxy*-β-*carbolin*-3-*yl*)-3-o-*tolyloxypropan*-2-*ol*. To a solution of 1,2,3,4-tetrahydro-6-methoxy-β-carboline (4 g) in ethanol (100 ml) was added 1,2-epoxy-3-*o*-tolyloxypropane (3·3 g) and the mixture heated at reflux temperature for 3 hr. The alcohol (5·8 g) crystallised out on cooling and had m.p. 160–162° (from ethyl acetate). Found: C, 71·6; H, 7·4; N, 7·6. $C_{22}H_{26}N_2O_3$ requires: C, 72·1; H, 7·2; N, 7·6%.

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Antitrichomonal activity of 1-[(2, 4-dinitropyrryl)-ethyl]-2-methyl-5-nitroimidazole and related compounds

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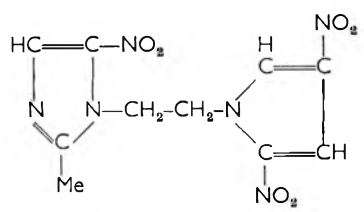
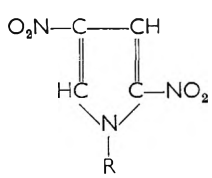
A series of 1-substituted dinitropyrroles was tested for trichomonacidal activity. The shorter the substituent the greater was the *in vitro* activity. No relationship was found between *in vivo* activity and substituent length. A comparison of the trichomonacidal activities of 1-[(2,4-dinitropyrryl)-ethyl]-2-methyl-5-nitroimidazole (ORF-2245) and 1-(2-hydroxymethyl)-2-methyl-5-nitroimidazole (8823 R.P.) was made. ORF-2245 exhibited activity equal to or greater than 8823 R.P. except in the treatment of established infections where its limited activity was attributed to poor absorption from the gut. Absorption, however, was apparently sufficient to combat or prevent the low-grade infections.

THE trichomonacidal activity of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (8823 R.P.) in mice experimentally infected with *Trichomonas vaginalis* was reported by Cosar & Julou (1959). They compared it with the antibiotic azomycin (Horie, 1956), and 2-acetamido-5-nitrothiazole (acinitrazole). Cuckler, Kupferberg & Millman (1955) had observed earlier that acinitrazole has therapeutic activity when administered orally to monkeys and mice.

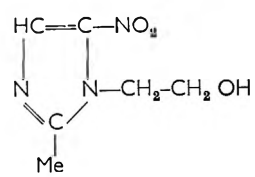
Recently we evaluated a series of nitro-derivatives of imidazoles and pyrroles particularly 1-[(2,4-dinitropyrryl)-ethyl]-2-methyl-5-nitroimidazole (ORF-2245) (I), the activity of which was compared with that of some other 2,4-dinitropyrrole derivatives (II) and with the compound 8823 R.P. (III) studied by Cosar & Julou.



ORF-2245 (I)



2,4-Dinitropyrroles (II)



8823 R.P. (III)

From the Ortho Research Foundation, Raritan, New Jersey, U.S.A.

Experimental

METHODS

Determination of *in vitro* trichomonacidal activity were made by testing the inhibitory effect of different concentrations of the compound against *Trichomonas foetus* (BrM_1) in simplified trypticase serum medium, pH 7·2, according to the procedure of Kupferberg, Johnson & Sprince (1948). Stock solutions of the drugs were made in acetone (no test tube contained more than 2% acetone). Inocula were prepared by incubating *T. foetus* (BrM_1) in simplified trypticase serum medium for 40 hr at 37·5°. Aliquots of 0·1 ml of these cultures were used to inoculate each test culture. The trichomonacidal level of the drug was the lowest concentration in which no viable cells were found after 3 days of incubation at 37·5°.*

For the determination of the PD50, white male mice (Barckman IS-32 strain), 18–22 g, were used. Cultures of *T. foetus* (BrM_1) grown for 40 hr in simplified trypticase serum medium at pH 7·2 and 37·5° were pooled and the number of the trichomonads per ml was determined with a Neubauer haemocytometer. The number of organisms was adjusted to approximately 10^6 ml by dilution with fresh serum medium and each test animal was injected intraperitoneally with 1 ml of the adjusted suspension.

For routine screening, the drug was suspended in a 0·25% sterile agar solution and administered orally on the day of infection. The drug concentration was adjusted so that no animal received more than 1 ml or less than 0·3 ml. Three groups of ten animals were each given varying doses of the drug on a mg/kg basis and ten untreated animals served as controls. Only tests in which the latter showed a mortality of at least 80% 7 to 10 days after infection were considered for analysis. The 50% end point was calculated according to the method of Reed & Muench (1938).

For the determination of therapeutic activity, the same procedure was used except that the drug was given 2 or 3 days after infection. Animals receiving multiple doses were given the drug on 3 successive days. Animals receiving only a single dose were treated on the second day after infection.

To determine drug activity against *T. vaginalis*, the drug was given orally and the organisms were injected subcutaneously. Several tubes of *T. vaginalis* (strain No.1†) were incubated at 37·5° in the serum medium (pH 6·0) for 40 hr and pooled. The number of organisms was adjusted to $1·5 \times 10^6$/ml and mice were infected subcutaneously (dorsally) with 1 ml of this suspension. The drugs were given by mouth within 1 hr of infection. All animals were killed 10 days after infection and examined for abscesses at the site of injection. Usually, 9 out of 10 untreated infected controls developed large abscesses during this period, and the presence of trichomonads was confirmed by inoculating Trichosel broth‡ with fluids from the abscesses.

**T. vaginalis* No. 2, simplified trypticase medium at pH 6·0 and an inoculum of 0·05 ml.

†*T. vaginalis* No. 1 is more pathogenic for mice than strain No. 2.

‡Baltimore Biological Laboratories.

For the determination of serum and urine levels the drug was administered orally to 10 mice at 500 mg/kg. After 5 hr the animals were killed with ether and blood samples were collected from the heart immediately. The blood was allowed to clot and was stored overnight at 10°. Urine samples were also collected from the bladder directly after the animals were killed.

Both serum and urine were assayed for trichomonacidal activity in 10 × 75 mm tubes using Trichosel broth (pH 7·2) and *T. foetus* as the test organism. The serially diluted urine and serum were inoculated with 0·02 ml of a 48 hr culture of *T. foetus*. Control tubes contained normal mouse serum or urine at concentrations of 1 : 10, 1 : 20 and 1 : 40.

For chromatography the urine, serum and faecal samples from 20 mice* were extracted with ethyl acetate; urine and serum were extracted with 2 volumes, faeces with 500 ml of ethyl acetate. The ethyl acetate was evaporated to dryness and reconstituted with 2 ml of acetone. This material was then used for chromatographic analysis. One to 4 μl of the acetone solution were applied to a thin layer chromatographic plate† containing alumina G and developed with isopropanol : hexane (6 : 4) for 50 min. The spots were observed at λ260 mμ.

Results and discussion

ACTIVITY OF 1-SUBSTITUTED 2,4-DINITROPYRROLES

This group of compounds was examined to determine possible relationships between the length of the substituted alkane group and the trichomonacidal activity of the compounds. Table 1 lists the comparative *in vitro* and *in vivo* activities of ten 1-substituted 2,4-dinitropyrroles against *T. foetus*. All the compounds exhibited either *in vitro* or *in vivo* activity but it was of interest that the compound with the greatest *in vitro* activity (ORF-1300) was without *in vivo* activity at the levels tested and two of the compounds with activity *in vivo* (ORF-1460 and ORF-1509) were inactive *in vitro*.

The results suggest a relationship between the number of carbon atoms (0–4) in the alkyl group and the *in vitro* activity of the compounds. When the dinitropyrrole substituent (R) was hydrogen, the activity was maximal. When the substituent group contained 4 carbon atoms, essentially all the activity was lost. When R was increased to C_5 or C_6 some activity was restored (see Table 1). No difference in activity was found between the normal or the iso configurations. Whereas the LD50 of the compound increased with the increase in chain length, there was no correlation between therapeutic activity *in vivo* and the length of the substituted group.

COMPARATIVE STUDIES ON ORF-2245 AND 8823 R.P.

In vitro. The *in vitro* experiments were conducted as described above except that both *T. vaginalis* and *T. foetus* were used as test organisms and 0·1 ml of the *T. foetus* culture was used in inoculating the Trichosel

*Swiss Webster mice.
†Custom Service Chemicals, Wilmington, Delaware.

TABLE 1. TRICHOMONACIDAL ACTIVITY OF 1-SUBSTITUTED 2,4-DINITROPYRROLES

ORF No.	R	*In vitro** vs. *T. foetus*	*In vivo* (mg/kg) orally vs. *T. foetus*	
			LD50	PD50
1300	H	1:800,000	20	Inactive at 16·5
1350	CH_3	1:200,000	220	67
1452	C_2H_5	1:100,000	68	Inactive at 53
1454	C_3H_7	1:50,000	900	30
1518	iso-C_3H_7	1:50,000	400	170
1460	n-C_4H_9	<1:10,000	1,200	53
1509	iso-C_4H_9	<1:10,000	1,200	75
1706	n-C_5H_{11}	1:10,000	1,500	50
1517	iso-C_5H_{11}	1:10,000	1,000	5·25
1516	n-C_6H_{13}	1:50,000	2,150	140

*Tube Dilution.

broth tubes that contained the serially diluted drugs. Both compounds had the same activity against *T. foetus*, i.e. 1:500,000. *T. vaginalis* was more sensitive to the action of these compounds, showing a greater susceptibility to ORF-2245 than to 8823 R.P. ($1:2 \times 10^6$ against $1:1 \times 10^6$).

Massive doses of the drugs were given in an attempt to determine the LD50. No end-point was reached with either drug although the animals were given as much as 4 g/kg in a single dose.

PD50 *T. foetus*. Table 2 shows the dose response of the two drugs when given in a single dose at the time of infection. The activity of ORF-2245 was significantly greater than that of 8823 R.P.

TABLE 2. THE RESPONSE OF MICE INFECTED I.P. WITH *Trichomonas foetus* AND TREATED WITH A SINGLE ORAL DOSE OF 8823 R.P. OR ORF-2245 ON THE DAY OF INFECTION

Dosage (mg/kg)	Mortality	Cumulative totals*		% Mortality
		survivors	dead	
8823 RP†				
105	2/9	19	2	9·4
87	3/9	12	5	29·4
72·9	7/9	6	12	66·6
60·7	8/9	4	20	83·3
50·6	7/9	3	27	90·0
42·1	8/9	1	35	97·2
Controls	10/10			100·0
ORF-2245‡				
67	1/10	29	1	3·3
33·5	0/10	20	1	5·0
16·75	4/10	10	5	33·3
8·37	7/10	4	12	75·0
4·18	9/10	1	21	95·0
2·09	10/10	0	31	100·0
Controls	10/10			100·0

* According to the procedure of Reed & Muench.
† PD50 = 78·7 mg/kg.
‡ PD50 = 12·6 mg/kg.

PD50 *T. vaginalis*. In combating abscess formation both drugs had approximately the same oral PD50, but the amount of drug required to prevent abscess formation differed markedly. For ORF-2245 in doses of 33·5; 21·0; 10·5 mg/kg the numbers of mice with abscesses were respectively 0/10; 0/10; 5/10. For 8823 R.P. corresponding figures were 135·0;

75·0; 42·0; 33·5; 10·5 giving 0/10; 1/10; 1/10; 2/10; 5/10 abscesses. Controls had 9/10 abscesses.

Therapeutic activity. Twenty-one days after infecting the animals intraperitonally with *T. foetus*, the survivors were killed and examined for lesions. When the drugs were given as 100 mg/kg in a single dose two days after infection, more than 66% of the animals treated with 8823 R.P. failed to survive (Table 3). At 300 mg/kg the mortality rates for 8823 R.P. and ORF-2245 respectively were 15 and 35%. The response to the drugs was about the same whether given as 300 mg/kg in a single dose on the second day of infection or given in three equal daily doses beginning on the second or third day of infection. The infecting organism was recovered from the survivors only when ORF-2245 was administered in a single dose (100 mg/kg) on the second day of infection.

TABLE 3. THERAPEUTIC EFFECT OF ORF-2245 AND 8823 R.P. IN MICE INFECTED INTRAPERITONEALLY WITH *T. foetus*

Compound	No. of mice	Dosage (mg/kg/day)	Doses*	Day of start of treatment	% Mortality	No. of survivors infected
ORF-2245	20	100	3	3rd	35	1/13
8823 R.P.	20	100	3	3rd	15	0/17
ORF-2245	20	100	3	2nd	35	1/13
8823 R.P.	10	100	3	2nd	10	0/9
ORF-2245	30	100	1	2nd	66·6	3/11
8823 R.P.	30	100	1	2nd	13·3	0/26
ORF-2245	20	300	1	2nd	35	0/13
8823 R.P.	20	300	1	2nd	15	0/17

* Given on successive days.

Since the therapeutic activity of ORF-2245 did not parallel the PD50 results, an experiment was designed to test directly the effect of the drug on the trichomonad while resident in the host. Four groups of 10 animals were infected intraperitoneally with 1 to 1·5 $\times$ 10^6 *T. foetus* organisms, as in the above studies. On the third day of the infection each of ten mice were given 6 mg orally (approximately 300 mg/kg) of either ORF-2245 or 8823 R.P. The fourth group was not treated and was retained as a control. The animals were killed with ether 24 hr after administration of the drugs and the peritoneal fluids were collected and pooled and a count made of the motile and non-motile organisms.

The untreated group had a viable count of more than 300 $\times$ 10^6 organisms/ml. ORF-2245 showed no lytic activity against the trichomonads. All organisms were actively motile. In the groups of animals treated with 8823 R.P. no viable organisms were observed. The mice treated with this drug had a count of 50 $\times$ 10^6 trichomonads per ml, all of which were non-motile.

Serum and urine levels in drug-exposed mice. A trichomonacidal urine level was reached after 500 mg/kg by mouth. Within 5 hr the 8823 R.P. produced urine levels substantially higher than those produced by ORF-2245. The level of activity of 8823 R.P. was greater than 1:2,560 in urine while that of ORF-2245 was 1:160 (Table 4). ORF-2245 showed no detectable trichomonacidal level in the serum while 8823 R.P. produced complete inhibition at 1:40.

TABLE 4. TRICHOMONACIDAL ACTIVITY OF URINE AND SERUM FROM MICE 5 HR AFTER ORF-2245 AND 8823 R.P. AT 500 MG/KG* ORALLY

Compound	Inhibitory activity of urine								
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560
ORF-2245	−	−	−	−	−	+	4+	4+	4+
8823 R.P.	−	−	−	−	−	−	−	−	−
Control (normal urine)	4+	4+	4+						
	Inhibitory activity of serum								
ORF-2245	4+	4+	4+	4+	4+	4+	4+	4+	4+
8823 R.P.	−	−	−	+	2+	4+	4+	4+	4+
Control (normal serum)	4+	4+	4+						

* The urine and serum were serially diluted using Trichosel broth and *T. foetus* as the test organism.
4+ Normal (2·5 to 3·5 million organisms/ml).
3+ Growth less than control.
2+ Sparse growth.
\+ No growth but inoculum not lysed.
− No organisms present.

Drug absorption. Because of the apparent disparity between protective and therapeutic activity of ORF-2245 either the material was being poorly absorbed or it was being degraded.

8823 R.P. and ORF-2245 were administered to two groups of 20 mice each. The urine and faeces were extracted and concentrates of the extracts were chromatographed on alumina thin-layer plates. No 8823 R.P. was isolated from the faeces but it was found unchanged in high concentration in the urine. ORF-2245 was not recovered in the urine but unchanged drug was found in large amounts in the faeces. These observations suggest that little ORF-2245 is absorbed.

ORF-2245 was dissolved in 99·5% dimethyl sulfoxide in an attempt to enhance absorption when given orally to mice at 500 mg/kg. There was no increase in the trichomonacidal level of the urine and the serum remained inactive.

Thus a relationship was found to exist between trichomonacidal activity and the length of the substituent in the 1 position of the dinitropyrrole. The shorter the alkyl grouping the greater was the *in vitro* trichomonacidal activity, the longer the length of the substituent, the lower the animal toxicity. There was no relationship between chain length and *in vivo* activity.

ORF-2245 appeared to be the drug with the greater trichomonacidal activity but almost complete inability to cope with an established infection greatly restricts its therapeutic value. The explanation of the relative therapeutic ineffectiveness may lie in the fact that the drug seems to be poorly absorbed by the gut.

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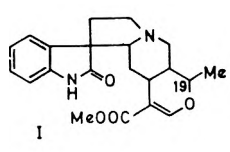
Letters to the Editor

Alkaloids from *Mitragyna speciosa* (Korth.)

SIR,—We have isolated from a methanolic extract of the leaves of *Mitragyna speciosa* Korth. a number of alkaloids, three of which have not been previously described (Table 1). The picrates prepared from the ether-extracted crude

TABLE 1. PROPERTIES AND SPECTRAL DATA OF SOME MITRAGYNA ALKALOIDS



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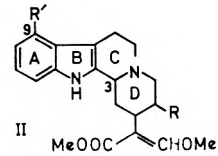

II

Analyses	C	H	N	OMe	Equiv. wt.
Structure I requires for $C_{21}H_{24}N_2O_4$	68·5	6·5	7·6	8·4	368
Speciophylline found	67·9	6·8	7·5	8·7	373†
Structure IIa requires for $C_{23}H_{30}N_2O_4$	69·3	7·6	7·0	23·4	398
Speciogynine found	68·9	7·8	7·0	23·2	400†
Structure IIb requires for $C_{23}H_{28}N_2O_4$	69·7	7·1	7·0	23·5	396
Paynantheine found	69·2	7·5	7·2	22·5	407†

	Speciophylline I	Speciogynine IIa R′=OMe, R=CH_2Me	Paynantheine IIb R′=OMe, R=CH=CH_2
Melting-point (Recryst. from . . .)	183° (dry ether)	214° (dry ether)	98° softens
$[\alpha]_{23\pm0.5°}^{D}$ ($CHCl_3$)	+91·3° (c, 0·21)	+28·4° (*c*, 0·26)	−28·9° (*c*, 0·27)
Approx. hRf* (a) alumina/$CHCl_3$	12	78	80
(b) silica gel/ether	3	40	52
Ultraviolet spectra (Abs. ethanol)	λ mμ log ε 224 (min) 3·98 224 4·14 283 3·34	λ mμ log ε 227 4·66 274s 3·92 284s 3·84 293 3·84	λ mμ log ε 227 4·63 272s 3·95 283s 3·86 292 3·84
Infrared spectra cm^{-1} (KCl) –N–H	3220	3372	3390
C/D Trans bands –CH	— *ca.* 2950	2750, 2808, 2850 2950	2755, 2800, 2853 2938
Ester/oxindole C=O	1713, 1685	1684	1706
C=C	1623	1614	1625, 1640
Picrate m.p. (From abs. ethanol)	215°	224°	202°
Picrate analyses	$C_{21}H_{24}N_2O_4$. $C_6H_3O_7N_3$ Req. Found C 54·3 54·3% H 4·5 4·9 N 11·7 11·85	$C_{23}H_{30}N_2O_4$. $C_6H_3O_7N_3$ Req. Found C 55·5 55·9% H 5·3 5·4 N 11·2 11·3	$C_{23}H_{26}N_2O_4$. $C_6H_3O_7N_3$ Req. Found C 55·7 55·3% H 5·0 5·15 N 11·2 10·4

* Thin-layer chromatography. Reference values: Mitraphylline (I), (a) 19, (b) 5; Mitragynine (II, R′ = OMe, R = CH_2Me), (a) 85, (b) 71.
† Titration in non-aqueous media.

bases yielded mitragynine (Beckett, Shellard & Tackie, 1965) and subsequently corynantheidine, isomitraphylline and a second isomer of mitraphylline which we have named *speciophylline* (I). The picrate mother liquors yielded ajmalicine, an isomer of mitragynine named *speciogynine* (IIa), a 9-methoxy derivative of corynantheine-like structure named *paynantheine* (IIb) and unidentified alkaloids. The ether-insoluble bases yielded mainly mitraphylline.

Mitraphylline, isomitraphylline, ajmalicine and corynantheidine were identified by comparison of the m.p., mixed m.p., $[\alpha]_D$,hRf values (thin-layer chromatography), ultraviolet, infrared and nmr spectra, with authentic samples of these alkaloids.

The data obtained indicate that speciophylline is an oxindole alkaloid of general structure similar to that of mitraphylline (I). The nmr spectrum of speciophylline (Table 2) shows that the ester methoxy group of the compound

TABLE 2. NMR SPECTRA OF SOME MITRAGYNA ALKALOIDS IN $CDCl_3$ 60 MC
(τ values from tetramethylsilane)

Protons	Speciophylline I	Speciogynine IIa	Paynantheine IIb
-C-Me	—	9·08 triplet	—
19 Me / -O R	8·74 doublet (J = 6·5 cps)	—	—
-CO-OMe	6·62 singlet	6·36 singlet	6·28 singlet
-OMe	—	6·27 singlet	6·23 singlet
C_6H-OMe	—	6·12 singlet	6·11 singlet
19 R / -O H	5·79 multiplet	—	—
$-CH=CH_2$	—	—	5·2–4·2 multiplets
aromatic	3·15–2·77 (4H) multiplet	3·54 (1H) 3·08 (2H) multiplets	3·50 (1H) 3·03 (2H) multiplets
olefinic	2·59 singlet	2·63 singlet	2·61 singlet
-N- H	0·50* singlet	2·00* singlet	2·07* singlet

* disappears upon deuteration.

(6·62τ) is shifted upfield compared with mitraphylline (6·40τ) and isomitraphylline (6·42τ). This is due to the position of the methoxyl group above the oxindole ring.

The alkaloids speciogynine and paynantheine were shown to be indoles by colour tests, ultraviolet, infrared and nmr data. These physical data, along with elemental analysis and equivalent weight determinations, indicate the structure of speciogynine to be generally similar to that of mitragynine (II; R′ = OMe, R = CH_2Me). A methoxy group in the 9-position of both speciogynine and paynantheine is suggested by the similarity of the splitting pattern of the aromatic protons for all these alkaloids [cf. mitragynine 3·52τ (1H), 3·08τ (2H)] and the three-proton aromatic methoxy singlet at about 6·1τ [mitragynine 6·14 τ] in the nmr spectrum (Table 2).

Infrared and nmr spectra of speciogynine and paynantheine indicate a trans C_3H junction for these alkaloids since bands are present between 2700–2800 cm^{-1} in the infrared spectrum (Wenkert & Roychaudhuri, 1956; Bohlmann, 1957; Rosen, 1961) and no C_3H multiplet in the nmr is present above 6·0τ (Wenkert, Wickbert & Leicht, 1961a,b; Uskokovic, Bruderer, von Planta, Williams & Brossi, 1964). The nmr spectrum of paynantheine is different from those of mitragynine and mitraciliatine in having no three-proton triplet for the C–CH_3 in the 9·1τ region. However, paynantheine has multiplets integrating for three protons in the olefinic region 5·2–4·2τ corresponding in chemical shift, multiplicity and integral with the vinyl signals of corynantheine (II; R′ = H, R = $CH=CH_2$). Paynantheine is therefore a 9-methoxy derivative of corynantheine-type structure and the first compound of this type to be reported.

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for extraction of the leaves and the provision of the crude picrates and extracts mentioned herein and for determination of the micro-rotations of the isolated alkaloids. We thank Professor M. M. Janot for providing a sample of corynantheine and corynantheidine. One of us (C.M.L.) was supported by a U.S. Public Health Service Fellowship 2-F2-GM-19, 473-02 from The National Institute of General Medical Sciences.

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The inhibition of adrenergic α-receptors and tryptamine receptors by macusine B

SIR,—Macusine B, an alkaloid isolated from *Strychnos toxifera*, blocks α-adrenergic receptors, stimulates β-adrenergic receptors and competitively inhibits tryptamine receptors in the guinea-pig isolated ileum (Leonard, 1965). A quantitative comparison has now been made with other compounds known to inhibit these receptors.

The antagonism of 5-hydroxytryptamine (5-HT) by macusine B was measured on the guinea-pig isolated ileum and the rat isolated uterus preparations. These tissues were suspended in an organ bath of Tyrode and De Jalon solutions respectively, as described previously (Leonard, 1965), and the pA_2 and pA_{10} values measured (Schild, 1947). The inhibition of adrenergic receptors was assessed using the guinea-pig isolated vas deferens preparation (Leach, 1956), and on the rabbit aortic strip preparation of Furchgott & Bhadrakom (1953). A modified aorta strip was prepared. A 3 cm length of aorta was first slit longitudinally, placed on a filter paper moistened with Krebs solution and then cut from each side in the horizontal plane so that the cuts alternated but did not extend to the mid-line. By leaving an uncut portion 1 mm wide down the midline and alternating the horizontal cuts by about 1 mm, a robust strip was achieved.

The aorta was then suspended in an organ bath of Krebs solution and attached to a frontal writing lever, backweighted by a load of 0·5 g, giving a × 10 magnification. Aorta prepared in this way gave regular responses to adrenaline ($2–4 \times 10^{-7}$M) for at least 12 hr.

In all experiments the pA values were measured on tissues from at least two animals and the pA_2 and pA_{10} values were measured separately on different pieces of tissue from the same animal. The pA_2 and pA_{10} values for macusine B on all the tissues were in the range 5·02–6·87 and 4·01–5·77 respectively; there was a slight increase in the value (0·08–0·65 pA units) when the contact time of the antagonist was increased from 2 to 10 min (Table 1). Schild (1957) calculated the pA_2–pA_{10} value for a first order competitive antagonist to be 0·95.

TABLE 1. ANTAGONISM OF ADRENALINE AND 5-HT BY MACUSINE B EXPRESSED AS pA_x VALUES

Tissue	Bath fluid and temperature	Concentration of agonist	Agonist contact time	Antagonist contact time	pA_2	pA_{10}	pA_2–pA_{10}
Rabbit aortic strip	Krebs; 37°	Adrenaline; $2–4 \times 10^{-7}$M	3 min	10 min	6·674	5·771	0·903
Guinea-pig vas deferens	Tyrode; 37°	Adrenaline; $4–8 \times 10^{-6}$M	30 sec ,,	2 min 10 min	5·569 5·905	4·011 4·627	0·578 0·778
Guinea-pig ileum ..	Tyrode; 37°	5-HT; $1·8–3·6 \times 10^{-7}$M	20 sec ,,	2 min 10 min	5·016 5·097	4·051 4·661	0·965 0·418
Rat uterus ..	De Jalon; 30°	5-HT; $1·8–3·6 \times 10^{-7}$M	20 sec ,,	2 min 10 min	6·220 6·870	5·647 5·068	0·573 0·802

As the pA_2–pA_{10} values for macusine B are much lower than this for the uterus and vas deferens it seems likely that the alkaloid is acting in a different way on the receptors in these tissues.

Table 2 gives a comparison of the pA_2 values for several antagonists of adrenaline and 5-HT. It is evident that macusine B is a weak inhibitor of

TABLE 2. COMPARISON OF THE pA_2 VALUES FOR AGONISTS OF ADRENALINE AND 5-HT

Tissue	Agonist	Antagonist	Contact time	pA_2	Reference
Rabbit aortic strip ..	Adrenaline	Dihydroergotamine	—	7·70	Calculated from data of Furchgott (1955)
		Phentolamine	—	7·52	
		Yohimbine	—	6·70	
		Macusine B	10 min	6·67	
		Piperoxan	10 min	6·28	Birmingham & Szolcsányi (1965)
Guinea-pig vas deferens ..	Noradrenaline	Dihydroergotamine	30 min	8·25	Birmingham & Szolcsányi (1965)
	,,	Piperoxan	5 min	6·47	Calculated from data of Leach (1956)
	Adrenaline	Macusine B	10 min	6·07	
Rat uterus ..	5-HT	Lysergic acid diethylamide	10 min	8·70	Gaddum (1953)
		Dibenamine	10 min	7·72	Gaddum, Hameed, Hathaway & Stephens (1955)
		Dihydroergotamine	10 min	6·90	
		Macusine B	10 min	6·87	Gaddum, Hameed Hathaway & Stephens (1955)
		5-Benzyloxygramine	10 min	6·79	

adrenergic and tryptamine receptors. On the rabbit aorta and the rat uterus this alkaloid is approximately equipotent with yohimbine and 5-benzyloxygramine respectively.

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The preparation of *O*-methylporphyroxine and its detection in opium

SIR,—Pfeifer (1965) has recently reported the detection of papaverrubin B (*O*-methylporphyroxine) in opium, but no experimental details were given. We have prepared *O*-methylporphyroxine and found it to differ from that described by Pfeifer & Teige (1962) in m.p. and infrared absorption spectrum. We have also detected *O*-methylporphyroxine in Japanese opium.

Porphyroxine was isolated from Japanese opium (2 kg) essentially by the method of Pfeifer & Teige (1962), although the later stages of their procedure (precipitation of thebaine hydrogen tartrate and column chromatography) were omitted since porphyroxine could be crystallised from methanol without these steps. Its m.p., 234–236°, was undepressed on admixture with a sample isolated from Indian opium by Genest & Farmilo (1963). The mother liquors were recycled through the extraction procedure until no more porphyroxine could be separated by crystallisation. The final filtrate was shown by thin-layer chromatography to contain, in addition to all the common opium alkaloids, porphyroxine and traces of several other compounds giving a red coloration when heated with phosphoric acid. Crude *O*-methylporphyroxine was separated from this mixture by preparative thin-layer chromatography (0·75 mm silica gel G /0·01N NaOH layers; solvent system, benzene : methanol 8 : 2), a zone of Rf 0·6–0·8 being eluted. Further purification was effected by extraction of an ether solution with aqueous 10% KOH to remove phenolics, crystallisation from methanol to remove the bulk of the narcotine, and countercurrent distribution (20 tubes) between chloroform and 2% aqueous tartaric acid. The material remaining after these treatments, some of which were repeated several times, was freed from residual traces of narcotine by repeated preparative thin-layer chromatography (0·25 mm silica gel G /0·01N NaOH layers; solvent system, benzene : ethyl acetate 75 : 25). A zone between Rf 0·1 and 0·3 was eluted, evaporation affording 2 mg of a light brown gum which could not be crystallised. It was shown to be homogeneous by thin-layer chromatography in six solvent systems (see Table 1), and to be identical with *O*-methylporphyroxine by thin-layer chromatography, colour reaction, infrared and ultraviolet spectra.

Authentic *O*-methylporphyroxine was prepared by methylation of porphyroxine (230 mg) with excess diazomethane in ether : ethanol at 0° for 6 hr. The

TABLE 1. THIN-LAYER CHROMATOGRAPHY OF *O*-METHYLPORPHYROXINE ON SILICA GEL G : 0·01 N NaOH LAYERS

System	Rf	$R_{porphyroxine}$	$R_{narcotine}$
Benzene : methanol 8 : 2	0·70	1·3	1·0
Benzene : diethylamine 95 : 5	0·68	2·1	1·0
Benzene : ethyl acetate 75 : 25	0·23	1·3	0·72
Benzene : ethyl acetate : diethylamine 90 : 5 : 5	0·59	2·4	1·0
Cyclohexane : chloroform : diethylamine 5 : 4 : 1	0·57	2·5	0·98
Cyclohexane : diethylamine 8 : 2	0·24	1·8	0·92

Spray reagents: porphyroxine and *O*-methylporphyroxine, 12% aqueous phosphoric acid; narcotine aqueous potassium iodoplatinate (Genest & Farmilo, 1963).

compound was purified by washing with 10% aqueous KOH, to remove unreacted starting material, and crystallised successively from methanol, ethyl acetate and methanol. Yield 180 mg (76%), m.p. 202–204°. (Pfeifer 1962, gives m.p. 241–243°). Found: C, 66·1; H, 6·25; N, 3·8; O, 23·9; active H, 0·2, 0·4%; *M* (mass spectrum), 385. Calc. for $C_{21}H_{23}O_6N$: C, 65·5; H, 6·0; N, 3·6; O, 24·9; one active H, 0·3%; *M* 385. The nmr spectrum ($CDCl_3$ solution) has singlets at 6·11, 6·15 and 6·32τ each integrating for three protons, and attributable to three methoxyl groups. In this region, the spectrum of porphyroxine [$(CD_3)_2SO$ solution] has two three-proton singlets (at 6·31 and 6·50τ). Ultraviolet data: *O*-methylporphyroxine in ethanol λ_{max} 285·5, 234 mμ (ϵ_{max} 6,400, 10,580) λ_{min} 258, 225 mμ (ϵ_{min} 1,180, 9,280). *O*-Methylporphyroxine gave the characteristic red coloration when heated with dilute mineral acid.

In the infrared spectrum (see Fig. 1), absorption due to the phenolic OH of porphyroxine (3,420 cm^{-1}) has disappeared, whilst the band at 3,300 cm^{-1}

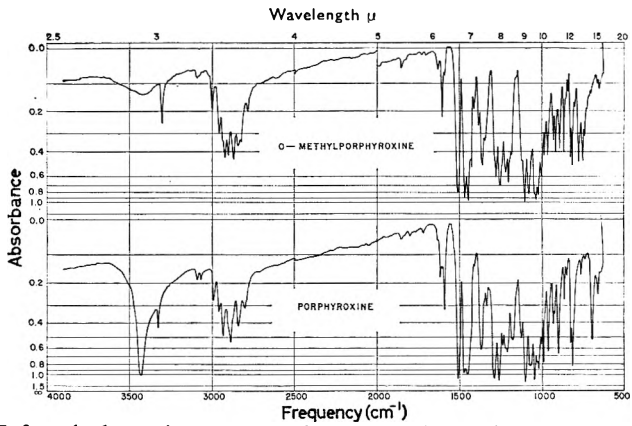

FIG. 1. Infrared absorption spectra of porphyroxine and *O*-methylporphyroxine (potassium bromide discs).

(attributable to :NH) remains. Pfeifer & Teige (1962) have published a spectrum for *O*-methylporphyroxine which differs markedly from ours, but which is identical with the spectrum of our porphyroxine. No analytical data were given for the compound and it seems probable that the conditions used effected change of the porphyroxine from one form to another. Klayman (1956) has reported the isolation of porphyroxine in two polymorphic forms, of m.p. 186–191° and 228–231°.

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Some pharmacological studies with 14-cinnamoyloxycodeinone

SIR,—In a recent communication to the British Pharmaceutical Conference (Buckett, 1964), 14-cinnamoyloxycodeinone was shown to be the most potent of a series of esters of 14-hydroxycodeinone. I now report a comparison of its pharmacological properties with those of morphine.

TABLE 1. THE ACUTE TOXICITY OF 14-CINNAMOYLOXYCODEINONE AND MORPHINE IN MICE

Route	Morphine LD50	14-Cinnamoyloxycodeinone LD50	Ratio (Morphine = 1·0)
Intravenous	199 (189–210)	31 (24–39)	6·4
Subcutaneous	330 (280–390)	530 (44–636)	0·62
Oral	745 (610–895)	1100 (900–1350)	0·68

The LD50 values are expressed in mg/kg in terms of base.
Limits of error (P, 0·95) are given in parentheses.

Table 1 shows the acute toxicity of 14-cinnamoyloxycodeinone in mice. The intravenous and subcutaneous values for morphine are similar to those determined by Randall & Lehmann (1950). There is a wide separation between LD50 values by intravenous and by other routes for 14-cinnamoyloxycodeinone but not for morphine. This may be due in part to the high lipid solubility of the ester facilitating rapid penetration into the central nervous system. In all instances, death was preceded by catalepsy and respiratory depression.

The analgesic potencies of 14-cinnamoyloxycodeinone relative to morphine in different tests are presented in Table 2. The compound is a potent analgesic

TABLE 2. THE ANALGESIC POTENCY OF 14-CINNAMOYLOXYCODEINONE IN RATS AND MICE

Analgesic test	Species	Route	ED50 mg/kg		Potency ratio (Morphine = 1·0)
			Morphine	14-Cinnamoyloxycodeinone	
Tail pressure*	Rat	s.c.	1·45 (1·1–1·9)	0·023 (0·017–0·03)	64 (43–94)
Tail pressure	Rat	Oral	5·76 (4·4–7·6)	0·036 (0·027–0·048)	160 (109–236)
Hot plate†	Mouse	s.c.	4·0 (3·3–4·7)	0·059 (0·048–0·073)	68 (51–87)
Tail clip‡	Mouse	s.c.	6·55 (4·8–8·9)	0·037 (0·024–0·059)	177 (101–310)

All values are based on measurement at times of maximum analgesic effect and are given in terms of base. Limits of error (P, 0·95) are given in parentheses.
* Buckett, Farquharson & Haining (1964); † Eddy & Leimbach (1953); ‡ Bianchi & Franceschini (1954).

and a comparison of subcutaneous and oral ED50 values shows it to be absorbed from the gastrointestinal tract. The onset (10 min) and duration (30 min) of analgesia after 14-cinnamoyloxycodeinone in mice were shorter than after equiactive doses of morphine, where the onset and duration were 20 and 90 min respectively. The compound was similarly potent in mice subjected to either a mechanical (tail clip) or a thermal stimulus (hot plate) and the analgesia produced by 14-cinnamoyloxycodeinone was more intense and depressant than that seen after morphine. This feature is generally associated with molecules in which a ketone group replaces the phenolic hydroxyl (Braenden, Eddy & Halbach, 1955).

At high doses morphine or 14-cinnamoyloxycodeinone increased the spontaneous activity of mice in a photocell box (Dews, 1953), the new compound being 100 times as potent as morphine subcutaneously. A similar ratio of activity was found for rectal hypothermia in mice after subcutaneous administration; but with a test for gastrointestinal motility in mice (Buckett, Farquharson & Haining, 1964), 14-cinnamoyloxycodeinone had 240 times the

potency of morphine. In an adaptation of a clinical method for assessment of respiratory depression (Campbell, Lister & McNicol, 1964) for anaesthetised dogs, 14-cinnamoyloxycodeinone was found to have 106 times the potency of morphine using the reduction of minute volume under 95% oxygen and 5% carbon dioxide and 90 times when the percentage change in pCO_2 under O_2 breathing was measured. The results of other comparative studies are given in Table 3. This evidence shows that 14-cinnamoyloxycodeinone differed from morphine in its ability to produce catalepsy at doses close to analgesic doses.

TABLE 3. THE RELATIVE POTENCY OF 14-CINNAMOYLOXYCODEINONE IN VARIOUS PHARMACOLOGICAL TESTS IN MICE

Test	Route	ED50 mg/kg		Potency ratio (Morphine = 1·0)
		Morphine	14-Cinnamoyl oxycodeinone	
Hind limb catalepsy	i.v.	> 10	0·066 (0·051–0·089)	>150
Grip on 45° plane	i.v.	> 10	<0·02	>500
Pentobarbitone (25 mg/kg i.p.) potentiation	s.c.	12·8 (11·2–14·8)	0·24 (0·20–0·28)	53
Anti-i.v. leptazol infusion	s.c.	>100	0·68 (0·45–1·03)	>147
Chimney*	s.c.	9·5 (5·9–15·2)	0·07 (0·055–0·09)	135
Lenticular opacity†	s.c.	32·6 (27–40)	0·45 (0·31–0·64)	72
		Index		
"Straub index"‡ (LD50/ED50 Straub tail)	i.v.	7·95	500	

All values are expressed in terms of base. Limits of error (P, 0·95) are given in parentheses.
* Boissier, Tardy & Diverres (1960); † Weinstock (1961); ‡ Shemano & Wendel (1964).

In addition the ability of the animals to remain on an inclined plane was lost. Anticonvulsant activity and barbiturate potentiation were exhibited only at high dosage and in common with all narcotic drugs lenticular opacity was observed.

The finding that the "Straub index" far exceeded that for any drug investigated by Shemano & Wendel (1964) suggested that 14-cinnamoyloxycodeinone would have a high capacity to produce physical dependence.

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