RESEARCH PAPERS

DIKETOPIPERAZINES—A NEW GROUP OF CENTRAL NERVOUS SYSTEM-DEPRESSING AGENTS

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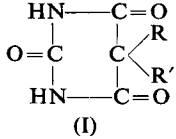
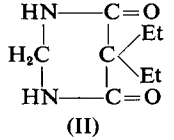
The pharmacology of Ph. 481, *N*(3′:4′dimethoxyphenylethyl)-2:6-diketopiperazine HCl is presented. The compound is shown to augment the effect of barbiturates in mice and of ether in rats. It specifically inhibits the conditioned escape response in rats. It is practically devoid of anticonvulsive, analgesic or antitussive activity, and it diminishes the tone of the skeletal muscles without interfering with neuromuscular transmission or polysynaptic spinal reflexes. It has slight antispasmodic activity on smooth muscles.

SOME one hundred hitherto unknown *NN*′-substituted 2:6- and 2:4-diketopiperazines were synthesized. The starting point of this programme was the barbiturate molecule (I), especially its 2-dihydrogenated congener mysoline (II). We decided to investigate the extent to which the pharmacological properties in this type of molecule would be preserved if its main features were introduced into the piperazine instead of the pyrimidine nucleus, the position of two keto groups being 2:5- (III) or 2:6- (IV). The derivatives of type III with two alkyl-, aryl- or aralkyl-groups, attached to one of the carbon atoms were found to be hardly if at all active. The activity on the central nervous system was as a rule higher in the compounds with one or two *N*-atoms alkylated. Details of the chemistry of the whole series of compounds are given elsewhere.[1] Several of them were shown to possess central depressant properties. In this paper the pharmacological activities of a representative compound *N* (3′:4′dimethoxyphenylethyl)-2:6-diketopiperazine HCl (Ph. 481) are given in detail (V).



(I)



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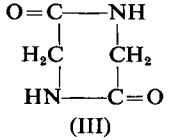

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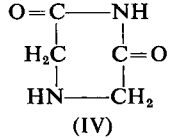

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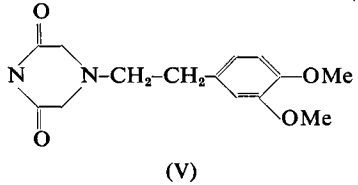

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Experimental Methods

Toxicity in Mice and Rats

The volume of the intravenous, intraperitoneal and oral doses in mice amounted to 0·1 ml./10 g., that of the intraperitoneal injection in rats to 0·1 ml./100 g. of body weight. To estimate the intravenous toxicity, the mice were first subjected for ten minutes to a temperature of 36 ± 1°. The injections were then given into the tail vein, the duration of injection being kept constant at 20 ± 2 seconds. The oral doses were given in a gum solution by stomach tube. After administering the drugs the animals were kept at a temperature of 24 ± 2° for an observation period of three days. The LD50 values and their 95 per cent confidence limits were computed with the graphical method described by Litchfield and Wilcoxon.[2]

Central Nervous System Activity

Observations on non-narcotised mice, rats, rabbits and dogs. We studied the influence of the drug on behaviour and over-all motility.

Anticonvulsive activity in mice. We investigated the influence of Ph. 481 on leptazol convulsions (60 mg./kg. i.v.), on electroconvulsions according to the method described by de Jongh,[3] on nicotine convulsions (0·5 mg./kg. i.v. injected nicotine bitartrate), on picrotoxin convulsions (4 mg./kg. i.v.), and on strychnine convulsions (1 mg./kg. i.v.).

Analgesic activity. For the experiments in rats we used a modification of the method of D'Amour and Smith[4] as described by de Jongh and Knoppers.[5] A modification of the hot plate method of Eddy and Leimbach[6] was used for observations in mice. The criteria of analgesic activity were described in a previous paper from our laboratory.[7]

Augmentation of hynoptic action of pentobarbitone sodium in mice (TNO strain). The investigation was carried out according to the method described previously[8]. After an intravenous dose of 20 mg./kg. of pentobarbitone sodium, only five out of 300 mice slept. Ph. 481 was given orally 30 minutes before the barbiturate injection.

Augmentation of the ether effect in rats. White rats weighing 170–200 g. were used. Ether was rapidly blown into the container through an injection needle fixed to the lid[9]. The rats were removed from the container after a fixed interval and then placed on their backs. The duration of the narcosis was determined by the return of the righting reflexes. Ph. 481 and the saline solution were injected intraperitoneally immediately before exposure to the ether.

Tremorine syndrome in mice. The following reactions could be observed in all animals 30 minutes after the intravenous injection of 28 mg./kg. tremorine: tremor, dacryorrhoea, salivation and diarrhoea. Ph. 481 was given orally one or two hours before the tremorine injection.

Mescaline intoxication in mice. An intravenous injection of 160 mg./kg. of mescaline caused death in 33 out of 45 animals (73·5 per cent). The typical responses are a kind of clonic seizure followed by respiratory

arrest within one or two minutes. Ph. 481 was administered by the oral route one or two hours before the mescaline injection.

Conditioned escape reaction in rats. The method of the pole-climbing response as described by Cook and Weidley[10] was slightly modified[11]. The drug was given by the intraperitoneal and oral routes and the influence on the conditioned and unconditioned responses was observed at 30 minutes, 1, 2, 4, 6 and 24 hours after the injection.

Mono- and Polysynaptic Reflexes in Cats

Decapitated cats were used. The knee jerk reflex was elicited with an electro-magnetically driven hammer as described in a previous paper[12]. The crossed extensor reflex was elicited by stimulating the femoral nerve on the other side with supramaximal condenser discharges. The stimuli were given alternately to the patellar tendon and the femoral nerve at 15 second intervals. The substances to be tested were injected into the femoral vein and the movements of the leg were recorded by an isotonic lever.

Blood Pressure and Respiration in Cats and Rats

Cats. Allobarbitone-narcotised, decerebrated and decapitated cats were used. The drugs were injected into the femoral vein.

Rats. The blood pressure was measured with a modified tail plethysmograph on unnarcotised animals[13]. Ph. 481 was injected intraperitoneally. The observation period was six hours.

The respiration was measured in unnarcotised rats by a method described previously[14]. The observation period was for two hours after intraperitoneal injection of the drug.

Body Temperature in Rats

The body temperature of rats weighing 150–200 g. was recorded by thermocouple at intervals of 15 minutes once before and ten times after intraperitoneal injection of the drug. The average fall during the observation period exceeded a value of 1·5° only once in 30 experiments. This value was used as an all-or-none criterion for a hypothermic effect to be used for estimating an ED50 value according to Litchfield and Wilcoxon[2].

Pupil Diameter in Mice

We used the method described by Pulewka[15] with white mice weighing 16–19 g. The pupil diameters were measured immediately before and 10, 30, 60, 120 and 240 minutes after the intravenous injection of the compound.

Antitussive Activity in Rats

We used a modification of the method of Winter and Flataker[16]. Rats weighing 120–160 g. were exposed to an air flow loaded with 0·02 per cent SO_2. Details of the method will be reported. The criterion of antitussive activity was the complete suppression of cough.

The Isolated Phrenic Diaphragm Preparation in the Rat

The Bülbring[17] preparation was used, with direct and indirect stimulation.

Motility of the Intestine in the Cat

Decerebrated cats were used. A rubber balloon filled with saline and connected to a polyethylene tube was inserted into an isolated loop of the ileum, at a pressure of 5–10 mm. of water, and movements recorded graphically.

RESULTS

Toxicity in Mice and Rats

The results are given in Table I.

TABLE I

LD50 VALUES WITH 95 PER CENT CONFIDENCE LIMITS (MG./KG.)

Species	Route	LD50	Number of animals
Mouse	i.v.	520 ± 62	105
Mouse	i.p.	600 ± 282	95
Mouse	oral	> 1000	60
Rat	i.p.	414 ± 80	65

Central Nervous System Activity

Observations on non-narcotised mice, rats, rabbits and dogs. Mice and rats treated with the highest sublethal doses showed signs of muscular relaxation and highly reduced activity. There was a distinct prostration and atonia. With the more toxic doses the righting reflexes were lost, the animals showed signs of cyanosis and died gasping. These phenomena were especially evident after intravenous injection. Bleeding from the nose was observed in the rats after the highest doses (500 mg./kg.).

After intraperitoneal doses of 20–320 mg./kg. the activity in rabbits was reduced. This reduction was sometimes preceded by a short period of excitation. After the highest doses the animals had tremors and strong excitatory stimuli were needed to make them walk. All symptoms were reversible, even with the highest dose. With all doses a distinct acceleration of the respiration was seen soon after the injections, the respiration returning to normal after 30 minutes.

The compound was injected intravenously into dogs in a dose range of 2·5–160 mg./kg. Doses of 10 mg./kg. caused an inhibition of the spontaneous activity. Defecation and vomiting occurred after doses of 40 mg./kg. interfering with the "tranquillisation".

Anticonvulsive activity. The results of these experiments are given in Table II.

Analgesic activity. Ph. 481 was injected intraperitoneally into rats in a dose range of 20–160 mg./kg. After 80 mg./kg. two of ten animals showed a significant prolongation of the reaction time. After 160 mg./kg. this increased to three.

Occasional analgesic effects could be observed in mice after intraperitoneal injections of at least 80 mg./kg. (ED50 = 101 ± 17 mg./kg.)

Augmentation of the hypnotic action of pentobarbitone sodium in mice. Ph. 481 was given in oral doses of 10–80 mg./kg. The ED50 amounted to 32 ± 19 mg./kg., a dose which made 50 per cent of the animals sleep after the injection of a non-hypnotic quantity of pentobarbitone sodium. None of the animals died during the observation period of one day.

Augmentation of the ether effect in rats. Duration of anaesthesia was increased up to three times with doses of Ph. 481 from 10–40 mg./kg. According to Wilcoxon's test[18] the difference is already statistically significant at a dose level of 10 mg./kg. (P = 0·014).

TABLE II

INFLUENCE OF PH. 481 ON DIFFERENT TYPES OF CONVULSIONS IN MICE

Type of convulsions	Ph. 481 mg./kg.	Number of animals protected (Total number of animals) after 1 hour	after 2 hours	after 4 hours
Electro	up to 320	0/30	—	—
	640	7/40	1/10	1/10
	1280	9/30	12/20	10/20
Leptazol	up to 320	0/20	—	—
	640	1/29	0/20	2/10
	1280	2/22	6/19	4/19
Nicotine	up to 640	0/20	0/20	3/10
	1280	12/30	11/19	4/19
Picrotoxin	up to 320	0/20	1/10	0/20
	640	2/20	3/10	0/10
	1280	2/20	3/10	3/10
Strychnine	up to1280	0/20	0/20	—

Tremorine syndrome in mice. Ph. 481 was given in oral doses of 320, 640 and 1280 mg./kg. The tremorine syndrome was not affected even by the highest dose.

Mescaline intoxication in mice. Slight protection was observed with doses of Ph. 481 of 320–1280 mg./kg.

Conditioned escape reaction in rats. The lowest intraperitoneal dosage affecting the conditioned response was 25 mg./kg. 140 mg./kg. completely blocked the conditioned without affecting the unconditioned response. Larger quantities also affected the unconditioned response. A dose of 280 mg./kg. completely blocked the conditioned response for four hours when the unconditioned response was apparent again. The quantities needed for oral effects were higher. 100 mg./kg. was slightly effective. For the full effect of 1 g./kg. was needed. Comparative investigations showed that Ph. 481 is much more specific in this test than meprobamate. No dosage of the latter compound inhibits the conditioned response without depressing the unconditioned one.

Mono- and Polysynaptic Reflexes in Cats

5 mg./kg. of mephenesin specifically affects the polysynaptic reflex, while the monosynaptic reflex is not influenced. Ph. 481 was given in

doses of 5–64 mg./kg. With the highest dose there was a reduction of both reflexes with a slight preference for the polysynaptic reflex.

Blood Pressure and Respiration in Cats and Rats

Cats. Allobarbitone narcotised cats. Doses upwards of 1 mg./kg. of body weight caused rapidly reversible hypotensive reactions. After 32 mg./kg. a fall in blood pressure of 70 mm. of Hg lasting 10–20 minutes was observed. The frequency of the respiration was distinctly augmented by dosages upwards of 16 mg./kg. A quantity of 64 mg./kg. caused death after acute hypotension and apnoea.

Decerebrated cats. Intravenous injections of 0·5–8 mg./kg. caused extremely short-lasting hypotensive reactions of 10–30 mm. of Hg. This hypotension increased with increasing doses and reached a value of 90 mm. of Hg after 128 mg./kg. Restoration to normal took about 6 minutes. The respiration is stimulated by dosages upwards of 4 mg./kg.

Decapitated cats. Intravenous doses of 0·5–8 mg./kg. caused reversible hypotensive reactions of about 20 mm. of Hg. Upwards of 8 mg./kg. this was preceded by acute hypertension, reaching a value of 20 mm. of Hg after 64 mg./kg. This dose also caused tachycardia.

Rats. The blood pressure was measured 1, 2, 4 and 6 hours after intraperitoneal injection of 20–160 mg./kg. No clear-cut changes of the blood pressure were seen.

The respiration was measured after 40–640 mg./kg. of Ph. 481 injected intraperitoneally. None of these doses caused a significant depression of the respiration.

Body Temperature in Rats

Ph. 481 was injected intraperitoneally in doses of 20–160 mg./kg. The ED50 for hypothermic potency amounted to 64 (40–120) mg./kg.

Pupil Diameter in Mice

Intravenous injections of 40, 80, 160 and 320 mg./kg. were given. Only with the highest dose did four of ten animals show a slight and fleeting mydriatic response.

Antitussive Activity in Mice

We gave intraperitoneal injections of 40–160 mg./kg. of Ph. 481. The ED50 = 70 (57–86) mg./kg. (the ED50 value of codeine being 26 (23–29) mg./kg.).

The Isolated Phrenic Diaphragm Preparation in the Rat

The volume of the bath was 125 ml. Doses of 25 mg. did not affect the response to direct and indirect stimulation of the diaphragm preparation.

Motility of the Intestine in the Cat

Doses upwards of 0·25 mg./kg. of Ph. 481 caused a reversible suppression of the movements of the intestine. This effect increased with increasing doses.

Discussion

It is largely a matter of opinion whether a substance such as Ph. 481 is to be considered a remote barbiturate analogue. It has some barbiturate-like properties, and in addition some new ones. Whereas the barbiturates do not specifically inhibit the conditioned avoidance response in rats, Ph. 481 does. It is remarkable that there is also an almost complete absence of anticonvulsive activity. Furthermore, the hypnotic anaesthetic potency is low, only sublethal doses suppressing the righting reflexes in experimental animals. Lastly, a remarkable hitherto unexplained pharmacological effect of the compound is the muscular relaxation which it causes. We were able to exclude curariform activity or specific inhibition of the polysynaptic spinal reflexes, but this is as far as we got in our search for an explanation.

Taking everything together, we feel inclined to include Ph. 481 in the category of the so-called tranquillisers. This is admittedly a rather poorly defined group, but it does seem that some importance can be attached to a specific inhibition of the escape response in rats. If this were taken as a sole criterion, the narcotics would be tranquillisers too. Absence of pronounced analgesic activity should therefore be another element in the definition of tranquillisers. As is shown in this paper, Ph. 481 meets this criterion.

We were struck by the observation that Ph. 481 has some anti-spasmodic activity, an action which may be related to the β-3:4-dimethoxyphenylethylamine moiety, which can be recognised in both the molecules of papaverine and Ph. 481.

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