RESEARCH PAPERS

STUDIES IN THE EFFECT OF SUBSTITUTION ON THE LOCAL ANÆSTHETIC ACTIVITY OF PYRAZOLINE DERIVATIVES

PART I. 1-PHENYL-5-(3′-METHOXY-4′-*n*-PROPOXYPHENYL)-3-β-DIALKYLAMINO (PIPERIDINO; MORPHOLINO)-PYRAZOLINES

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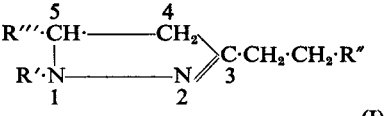
Received January 7, 1952

PYRAZOLINES of the type I are known to possess local anæsthetic activity (Nisbet et al.[1]) and it has been shown that when R‴ in position 5 is a phenyl group, alkoxy substitution on this group has a profound influence upon the physiological activity of the molecule (Levvy and Nisbet[2]). Similar effects have been observed by alkoxy substituents in the analogues of metycaine, benzoyl-γ-(2′-methyl-piperidino)-*n*-propanol hydrochloride (McElvain and Carney[3]).



(I)

In order to study the effect of alkoxy substitution and also the effect of varying the tertiary amino group — R″ in the pyrazolines of type I, a series of compounds have been prepared in which:

R′ = C_6H_5
R″ = N·$(CH_3)_2$, N·$(C_2H_5)_2$, N·$(n\text{-}C_3H_7)_2$, N·$(n\text{-}C_4H_9)_2$, morpholino and piperidino.
R‴ = 3-methoxy-4-*n*-propoxy-phenyl.

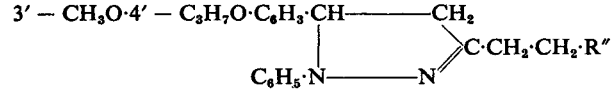
The local anæsthetic values of the compounds in the form of tartrates have been compared with procaine hydrochloride by intradermal injection in the back of the guinea-pig (Bülbring and Wajda[4]) and with cinchocaine hydrochloride on the cornea of the same animal. The relative toxicities of two of the most potent compounds and of procaine hydrochloride and cinchocaine hydrochloride were determined by intraperitoneal injection in mice.

The results of the pharmacological tests are summarised in Table I and consideration of the data indicates that variation of the tertiary amino group R″ substituted on the 3-ethyl group of the pyrazoline molecule causes a change in the local anæsthetic power of the compound. The order of descending potencies in the intradermal weal test is given in the series:—

N·$(C_2H_5)_2$ > N·C_5H_{10} > N·$(CH_3)_2$ > N·$(n\text{-}C_3H_7)_2$ > N·$(n\text{-}C_4H_9)_2$ > N·C_4H_8O

TABLE I

SUMMARY OF THE RESULTS OF THE PHARMACOLOGICAL TESTS ON CERTAIN PYRAZOLINE DERIVATIVES



R″	Guinea pig intradermal weal test		Corneal test on guinea-pig		Toxicity LD 50 (mice) mg./kg. i.p.
	Relative potency compared with procaine hydrochloride	Therapeutic value compared with procaine hydrochloride	Relative potency compared with cinchocaine hydrochloride	Therapeutic value compared with cinchocaine hydrochloride	
Dimethylamino	4·0	—	0·15	—	—
Diethylamino	7·0	3·5	0·25	0·95	125
Di-*n*-propylamino	2·5	—	0·06	—	—
Di-*n*-butylamino	3·5	—	0·05	—	—
Piperidino	6·0	4·5	0·1	0·52	170
Morpholino	1·5	—	—	—	—
Cinchocaine hydrochloride	—	—	1·0	1·0	33
Procaine hydrochloride ..	1·0	1·0	—	—	225
Cocaine hydrochloride ..	—	—	—	—	110

While in the corneal test the order of potency is given by the series:—

$$N{\cdot}(C_2H_5)_2 > N{\cdot}(CH_3)_2 > N{\cdot}(C_5H_{10} > N{\cdot}(n\text{-}C_3H_7)_2 > N{\cdot}(n\text{-}C_4H_9)_2 > N{\cdot}C_4H_8O$$

This variation in potency with change in the R″ group will perhaps be more easily appreciated by reference to Figures 1 and 2. Figure 1 illustrates graphically the results obtained in the intradermal weal test. The anæsthetic effect, measured in arbitary units to be described later, has been plotted against the concentration of the solution employed, using a logarithmic scale. The observation first made by Sinha[5] that a linear relationship exists between the two functions has again been confirmed. Since the slope of the regression lines are approximately equal to that of the procain hydrochloride, it is possible to compare with some degree of accuracy the relative potencies of the drugs with procaine hydrochloride as standard (Bülbring and Wajda[4]).

The results obtained from the corneal test are shown graphically in Figure 2 which records the relationship between duration of anæsthesia and the concentration of the solution employed, using a logarithmic scale.

The therapeutic values of two of the most potent compounds have been determined, the therapeutic value being defined as the ratio of the relative potency to the relative toxicity. By intradermal injection 1-phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-piperidino-ethyl-pyrazoline tartrate has a therapeutic value of 4·5 and 1-phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-diethylamino-pyrazoline tartrate has a value of 3·5 with procaine hydrochloride as standard. The corresponding values for the corneal test in the guinea-pig being 0·52 for the piperidino derivative and 0·95 for the diethylamino derivative with cinchocaine hydrochloride as standard.

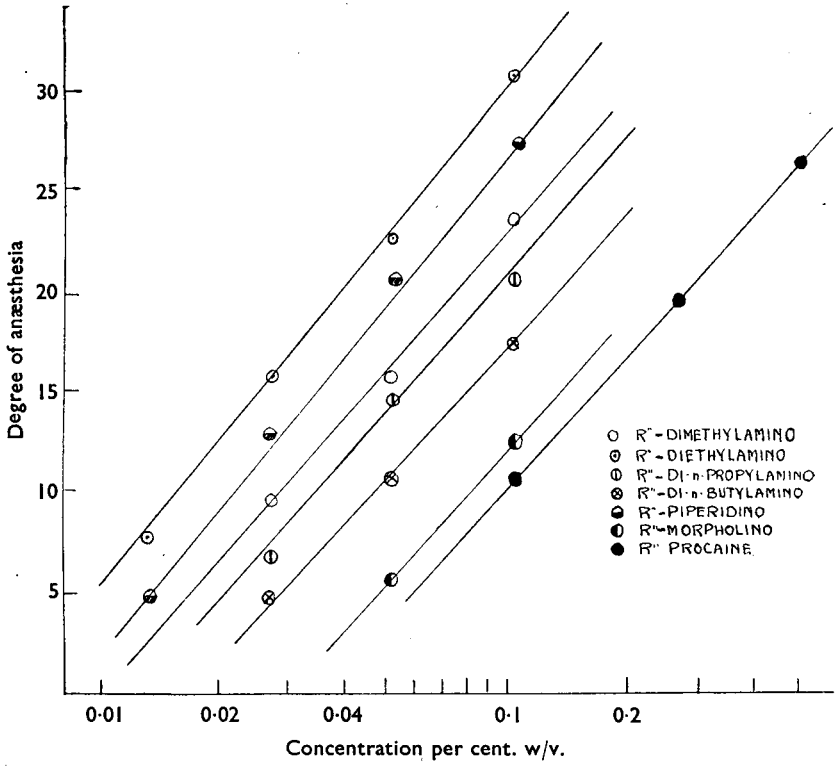
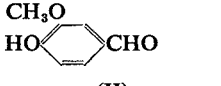


FIG. 1. Intradermal weal test in guinea-pigs.

SYNTHESIS OF THE PYRAZOLINE COMPOUNDS

The compounds used in this investigation were prepared by general methods described by Nisbet *et al.*[1] Vanillin (II) is converted to vanillidene acetone (III) and the latter by treatment with *n*-propyl-*p*-toluenesulphonate and sodium hydroxide to 3-methoxy-4-*n*-propoxy-benzylidene acetone (IV). The alkylated benzylidene acetone (IV) on treatment with formaldehyde and the hydrochlorides of various secondary bases (Mannich reaction) yields the unsaturated ketones (V). The phenylhydrazones of the ketones (VI) on treatment with dilute hydrochloric acid are isomerised to the pyrazoline hydrochlorides (VII), the bases of which are converted to the tartrates.



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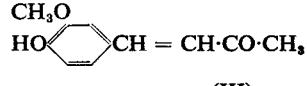

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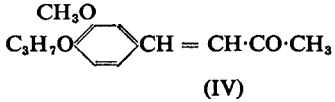

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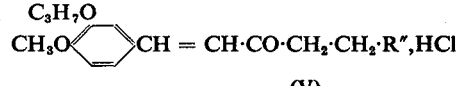
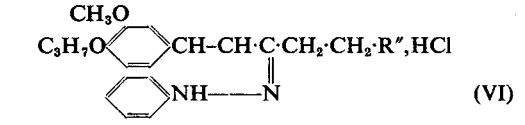
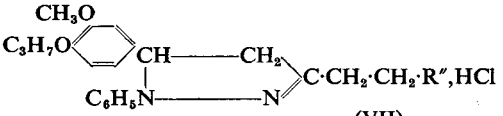


(V)



(VI)



(VII)

3-*Methoxy*-4-n-*propoxy-benzylidene acetone.* IV. Vanillidene acetone (105 g.) and *n*-propyl-*p*-toluene sulphonate (107 g.) are dissolved in ethanol (205 ml.) by heating under a reflux condenser. Potassium hydroxide (35 g.) dissolved in the minimum of water is added and the refluxing continued for 1½ hours. The contents of the flask are poured into water (2·5 l.) and the oil which separates allowed to crystallise.

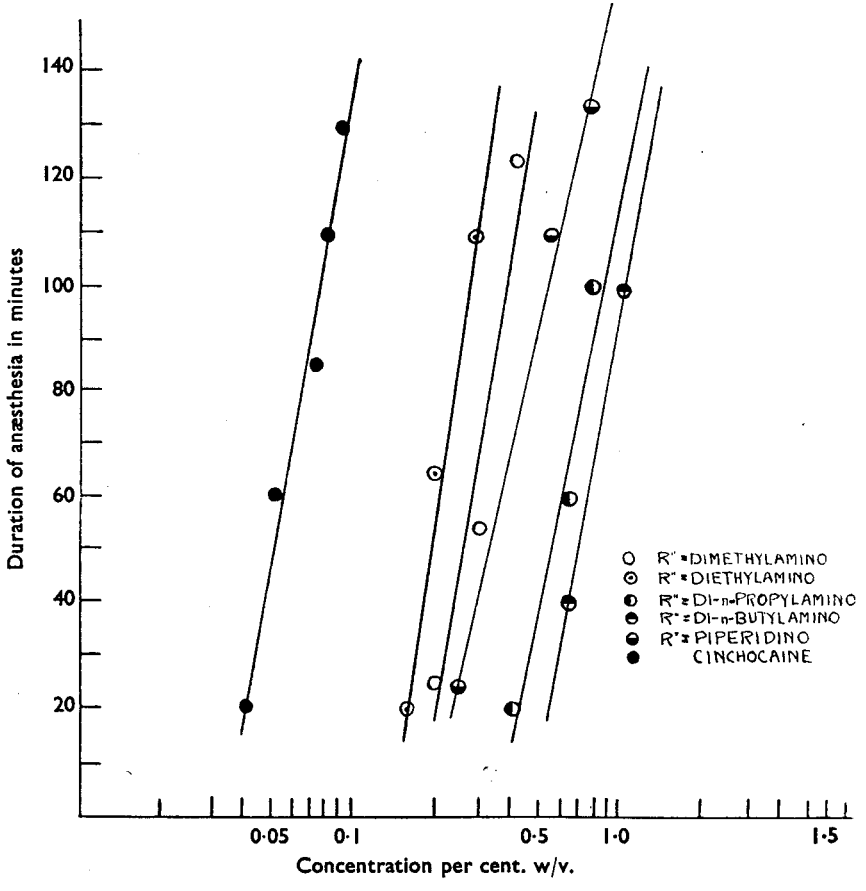


FIG. 2. Corneal test on guinea-pigs.

The solid is collected on a filter, washed, dried and recrystallised from acetone to give pale yellow crystals m.pt. 92° to 93° C. Yield 51 per cent. Found: C, 71·06; H, 7·59; $C_{14}H_{18}O_3$ requires C, 71·8; H, 7·69 per cent.

1-*Dimethylamino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride*, (V). 3-Methoxy-4-*n*-propoxy-benzylidene acetone (10 g.) and dimethylamine hydrochloride (3·25 g.) are dissolved in ethanol (15·0 ml.) by heating. Paraformaldehyde (2·2 g.) is added in small portions and the heating, under a reflux condenser, continued for 30 minutes. The crystalline solid which forms on cooling and scratching the internal surface of the flask is recrystallised from ethanol to give white needles m.pt. 133° to 134° C. Yield 51 per cent. Found: N, 4·1 per cent.; $C_{17}H_{25}O_3N$,HCl requires N, 4·27 per cent.

Phenylhydrazone of 1-*dimethylamino*-5-(3′*methoxy*-4′-n-*propoxy*-*phenyl*) Δ^4-*penten*-3-*one hydrochloride* (VI). The unsaturated ketone $C_{17}H_{25}O_3N$, HCl (4·0 g.) is dissolved in ethanol (20·0 ml.) and phenylhydrazine (1·25 g.) in acetic acid (1·25 g.) added. The phenylhydrazone crystallises on standing and is recrystallised from ethanol to give yellow needles m.pt. 163° to 164° C. Yield 71 per cent. Found: N, 10·1 per cent.; $C_{23}H_{31}O_2N_3$,HCl requires N, 10·06 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-3-*β*-*dimethylamino*-*ethyl*-*pyrazoline tartrate* (VII). The phenylhydrazone $C_{23}H_{31}N_3O_3$,HCl (2·5 g.) is heated under a reflux condenser on a steam-bath with 0·1 N hydrochloric acid (25·0 ml.) for 30 minutes. On cooling, the pyrazoline separates as a green oil and is isolated by making the solution slightly alkaline with sodium hydroxide and extracting with ether. The tartrate is formed by the addition of an ethanolic solution of tartaric acid to the base and recrystallising from ethanol-light petroleum (1-1) to give pale yellow crystals m.pt. 79° to 80° C. Yield 44 per cent. Found: N, 7·5 per cent.; $C_{23}H_{31}O_2N_3$,$C_4H_6O_6$ requires N, 7·9 per cent.

The other pyrazoline compounds of the series were prepared in a similar manner from 3-methoxy-4-*n*-propoxy-benzylidene acetone (IV).

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-3-*β*-*diethylamino*-*ethyl*-*pyrazoline tartrate* (VII). M.pt. 47° to 48° C. Yield 38 per cent. Found: N, 7·3 per cent.; $C_{25}H_{35}O_2N_3$,$C_4H_6O_6$ requires N, 7·5 per cent.

1-*Diethylamino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 103° to 104° C. Yield 43 per cent. Found: N, 3·5 per cent.; $C_{19}H_{29}O_3N$,HCl requires N, 3·9 per cent.

Phenylhydrazone of 1-*diethylamino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 143° to 144° C. Yield 72 per cent. Found: N, 9·42 per cent.; $C_{25}H_{35}O_2N_3$,HCl requires N, 9·43 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-3-*β*-*di*-n-*propylamino ethyl pyrazoline tartrate* (VII). M.pt. 54° to 55° C. Yield 28 per cent. Found: N, 7·3 per cent.; $C_{27}H_{39}O_2N_3$,$C_4H_6O_6$ requires N, 6·96 per cent.

1-*Di*-n-*propylamino*-5-(3′-*methoxy*-4′-n-*propoxy*-*phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 124° to 125° C. Yield 46 per cent. Found: N, 3·48 per cent.; $C_{21}H_{33}O_3N$,HCl requires N, 3·65 per cent.

Phenylhydrazone of 1-*di*-n-*propylamino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 154° to 155° C. Yield 84 per cent. Found: N, 9·02 per cent.; $C_{27}H_{39}O_2N_3$,HCl requires N, 8·87 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-3-β-*di*-n-*butylamino-ethyl-pyrazoline tartrate* (VII). M.pt. 58° to 59° C. Yield 29 per cent. Found N, 7·04 per cent.; $C_{29}H_{43}O_2N_3,C_4H_6O_6$ requires N, 6·83 per cent.

1-*Di*-n-*butylamino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 93° to 94° C. Yield 43 per cent. Found: N, 3·42; $C_{23}H_{37}O_3N$,HCl requires N, 3·4 per cent.

Phenylhydrazone of 1-*di*-n-*butylamino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 143° to 144° C. Yield 74 per cent. Found: N, 8·81 per cent.; $C_{29}H_{43}O_2N_3$,HCl requires N, 8·4 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-3-β-*piperidino-ethyl pyrazoline tartrate* (VII). M.pt. 65° to 67° C. Yield 42 per cent. Found: N, 7·2 per cent.; $C_{26}H_{35}O_2N_3,C_4H_6O_6$ requires N, 7·35 per cent.

1-*Piperidino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 146° to 147° C. Yield 46 per cent. Found: N, 4·2 per cent.; $C_{20}H_{29}O_3N$,HCl requires N, 3·8 per cent.

Phenylhydrazone of 1-*piperidino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 168° to 169° C. Yield 64 per cent. Found: N, 9·2 per cent.; $C_{26}H_{35}O_2N_3$,HCl requires N, 9·18 per cent.

1-*Phenyl*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-3-β-*morpholino-ethyl-pyrazoline tartrate* (VII). M.pt. 64° to 65° C. Yield 32 per cent. Found: N, 8·52 per cent.; $C_{25}H_{33}O_3N_3,C_2H_3O_3$ requires N, 8·43 per cent.

1-*Morpholino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (V). M.pt. 164° to 165° C. Yield 55 per cent. Found: N, 3·8 per cent.; $C_{19}H_{27}O_3N$,HCl requires N, 4·0 per cent.

Phenylhydrazone of 1-*morpholino*-5-(3′-*methoxy*-4′-n-*propoxy-phenyl*)-Δ^4-*penten*-3-*one hydrochloride* (VI). M.pt. 158° to 159° C. Yield 85 per cent. Found: N, 8·96 per cent.; $C_{25}H_{33}O_3N_3$,HCl requires N, 9·14 per cent.

Pharmacological Tests

The local anæsthetic potency of each compound was compared with procaine hydrochloride by intradermal injection on the back of the guinea-pig and with cinchocaine hydrochloride by local application to the cornea of the same animal.

Intradermal Weal Test. In the guinea-pig weal test, the method described by Bülbring and Wajda[4] with some slight modification was used. Procaine hydrochloride was taken as standard in preference to cocaine hydrochloride, as Bülbring and Wajda[4] have shown that the vaso-constrictor action of cocaine makes it unsuitable as a standard for the determination of the relative local anæsthetic potency of new compounds which possess no such vaso-constrictor action. 4 guinea-pigs were used for each test. The hair was removed by means of hand clippers 24 hours previous to the test, during which time any irritation

resulting from the clipping had disappeared. Solutions of the drug for test were prepared in 0·9 per cent. saline solution and a volume of 0·25 ml. of each of 4 concentrations of the compound under test and of procaine hydrochloride was injected intradermally along each side of, and at the same distance from the midline of the back of, each animal. Bülbring and Wajda[4] found that the sensitivity of the skin increased from the back to the front. In order to overcome the errors which would arise from this variation, it was the practice in these experiments to inject each concentration at a different relative site on the back of the animal in each of the 4 guinea-pigs as indicated in Table II. The weal formed from each injection was marked out in ink and the enclosed area tested for anæsthesia by pricking with a pin. After observing the animal's normal reaction to the prick, the marked area was tested 6 times at intervals of 3 to 5 seconds. This test was applied every 5 minutes for half an hour and the number of times the pricks failed to produce a response were added up for each concentration and out of a possible 36 gave an indication of the degree of anæsthesia. The results of the test are given in Table III and shown graphically in Figure 1.

TABLE II

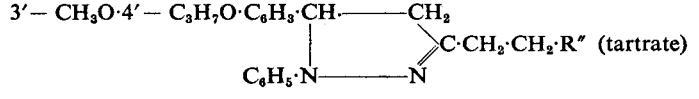
POSITION OF THE INTRADERMAL INJECTIONS ON THE BACK OF THE GUINEA-PIG. 0·25 ML. OF EACH SOLUTION INJECTED INTRADERMALLY

Animal 1	Animal 2	Animal 3	Animal 4
T_4 P_1	P_4 T_3	T_1 P_2	P_3 T_2 Head
P_3 T_2	T_1 P_3	P_4 T_3	T_4 P_1
T_1 P_2	P_2 T_2	T_4 P_1	P_4 T_3
P_4 T_3	T_4 P_1	P_2 T_2	T_1 P_2 Tail

T_1, T_2, T_3 and T_4, Test solutions 0·0125, 0·025, 0·05 and 0·1 per cent.
P_1, P_2, P_3 and P_4, Procaine solutions 0·125, 0·25, 0·5 and 1·0 per cent.

TABLE III

THE MEAN NUMBER OF PRICKS (WITH STANDARD ERROR) OUT OF A POSSIBLE 36, FAILING TO ELICIT A RESPONSE AFTER INTRADERMAL INJECTION OF THE DRUG IN GUINEA-PIGS



Concentration of the solution injected (per cent. w/v)	0·1	0·05	0·025	0·0125
R″				
Dimethylamino	24 ± 4·5	16 ± 3·5	10 ± 3·5	3 ± 1·3
Diethylamino	31 ± 4·5	23 ± 1·3	16 ± 1·8	8 ± 1·7
Di-*n*-propylamino	18 ± 3·4	11 ± 2·1	7 ± 1·6	0
Di-*n*-butylamino	21 ± 2·3	15 ± 3·4	7 ± 1·8	0
Piperidino	28 ± 2·2	21 ± 2·8	13 ± 2·1	5 ± 1·7
Morpholino	13 ± 4·7	6 ± 2·5	0	0
Concentration of solution injected (per cent. w/v)	1·0	0·5	0·25	0·125
Procaine hydrochloride	36 ± 0	27 ± 2·5	20 ± 2·3	11 ± 4·2

Corneal Tests. The compounds were also examined for their topical local anæsthetic effect by application to the cornea of an animal. The cornea of the rabbit was first used for this purpose but it was subsequently

found that the cornea of the guinea-pig gave more consistent results. Chance and Lobstein[6] have also found the guinea-pig to be the better subject for this test.

Cocaine hydrochloride was first used as the standard but this was found unsatisfactory. On plotting the logarithm of the concentration against duration of anæsthesia, it was found that over a limited range of concentrations, the regression lines for the pyrazolines were parallel, but these were not parallel to that for cocaine hydrochloride. In order to find a suitable reference standard, various local anæsthetics were tested and it was found that cinchocaine hydrochloride had a regression line parallel to that of the pyrazolines (see Figure 3) and it was, therefore, selected as standard.

The local anæsthetic, dissolved in normal saline was applied by means of a dropping tube to the eye of the guinea-pig which was held in such a position as to allow the cornea to be covered by a film of the solution

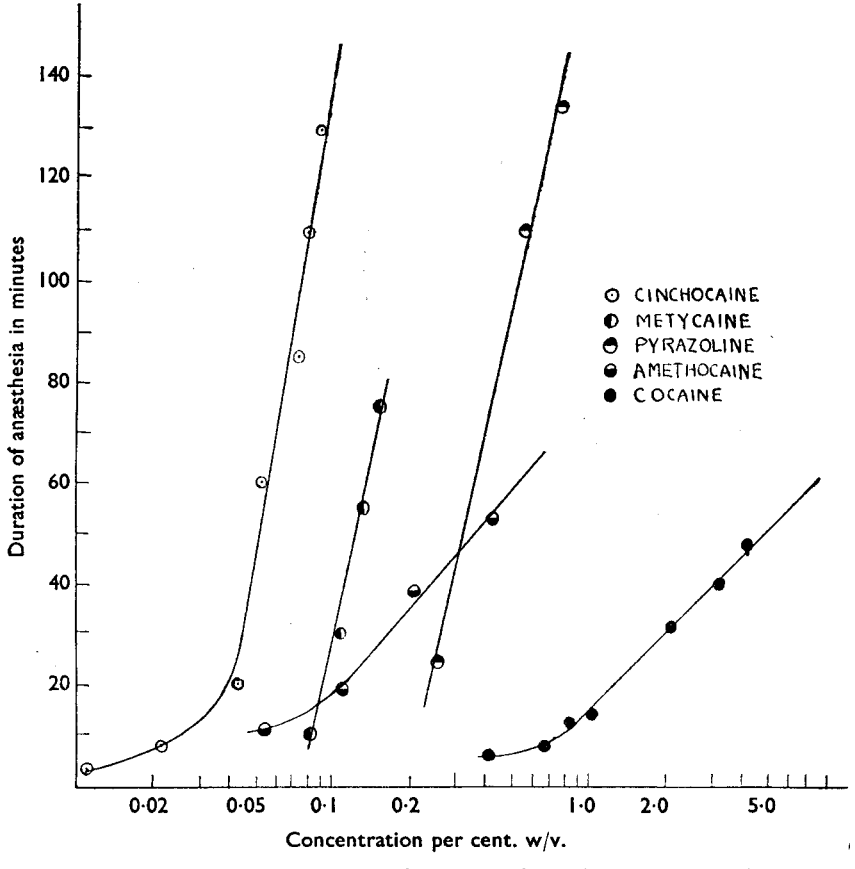


FIG. 3. Corneal test on guinea-pigs.

Pyrazoline = 1-phenyl-5-(3′-methoxy-4-*n*-propoxyphenyl)-3-β-piperidinoethylpyrazoline tartrate

for a period of 15 seconds. If the guinea-pig blinked during this period the film was renewed. The cornea was tested every 5 minutes by touching the centre 3 times with a hog's bristle attached to a glass rod. The duration for each concentration was obtained by noting the time when 2 or more positive responses were obtained from the 3 stimulations. The results are shown graphically in Figure 2 in which duration of anæsthesia has been plotted against the logarithm of the concentration of the solution. From these figures the approximate relative potency compared with cinchocaine hydrochloride as given in Table I was calculated.

Toxicity Tests. The approximate median lethal dose was calculated by intraperitoneal injection of mice weighing about 20 g. A suitable concentration of the drug was used and the dose, calculated in mg./20 g. mouse, was varied by varying the volume of the fluid injected. The volume injected did not at any time exceed 0·6 ml./20 g. of body weight. The results for these tests are summarised in Table IV and from these results the approximate values for the median lethal dose as given in the last column of the Table were found.

TABLE IV

RESULTS OF TOXICITY TESTS OBTAINED BY INTRAPERITONEAL INJECTION OF MICE

Drug	Dose mg. /20g.	Number of mice injected	Number of deaths	Mortality per cent.	LD 50 mg/20 g.
Cinchocaine HCl	0·4	6	0	0	
	0·5	7	0	0	
	0·6	6	1	18	0·66
	0·7	7	5	70	
	0·8	7	7	100	
Procaine HCl ..	1·0	10	0	0	
	2·0	20	0	0	
	4·0	20	8	40	4·5
	6·0	10	8	80	
	8·0	20	20	100	
R_2 = Diethylamino ..	2·0	10	0	0	
	3·0	10	9	90	
Compound A.	3·5	10	8	80	2·5
	4·0	20	16	80	
	4·5	8	8	100	
R_2 = Piperidino ..	3·0	10	3	30	
	4·0	10	8	80	
Compound B.	5·0	10	10	100	3·4
	6·0	10	10	100	

Compound A. 1-Phenyl-5-(3'-methoxy-4'-*n*-propoxy-phenyl)-3-β-diethylamino-ethyl-pyrazoline tartrate.

Compound B. 1-Phenyl-5-(3'-methoxy-4'-*n*-propoxy-phenyl)-3-β-piperidino-ethyl-pyrazoline tartrate.

SUMMARY

1. Several analogues of 1-phenyl-5-(3'-methoxy-4'-*n*-propoxy-phenyl)-3-β-dialkylamino (piperidino, morpholino)-ethyl-pyrazoline tartrate have been prepared.

2. The pharmacological tests, which were of a screening nature to obtain approximate values, indicate that the compounds are potent local anæsthetics both by intradermal injection and by absorption through mucous membrane.

3. From the pharmacological examination it appears that the activity of the molecule attains a maximum when the size of the secondary amino group in the 3-ethyl position is diethylamino.

4. Of the series 1-phenyl-5-(3′-methoxy-4′-*n*-propoxy-phenyl)-3-β-diethylamino-ethyl-pyrazoline tartrate was found to be the most potent having a therapeutic value of 3·5 with reference to procaine hydrochloride by intradermal injection on the guinea-pig and a value of 0·95 with reference to cinchocaine hydrochloride on the cornea of the guinea-pig, the relative toxicity having been determined by intraperitoneal injection in mice.

A grant from the Carnegie Trust for the Universities in Scotland towards the cost of the animals used in this research is gratefully acknowledged.

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