LOCAL ANAESTHETIC ACTIVITY OF A SERIES OF NEW SUBSTITUTED BASIC ANILIDES

By

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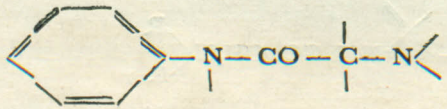
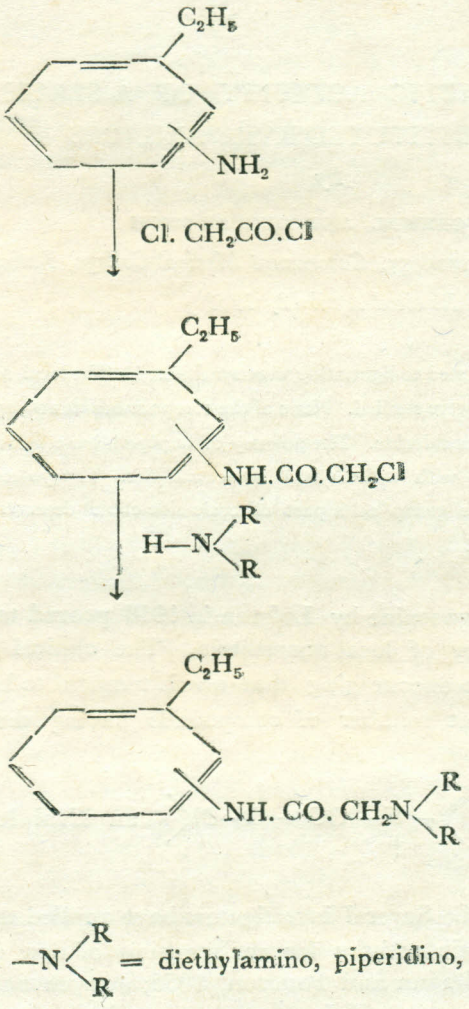
A series of compounds related to lignocaine were synthesized. The local anaesthetic activity and toxicity in mice were studied. None of the compouunds tested, approaches the potency of lignocaine hydrochloride. The potency of w-piperidino-ethylacetanilides may be favourably compared with w-diethyl-amino-acetanilides. The potentialities of compounds having piperidine group as a part of basic side chain deserves to be explored further.

The introduction of lignocaine by Lofgren in 1948 proved to be an important event in the history of local anaesthesia. The clinical experience with lignocaine has been so encouraging that one is tempted to believe that somewhere among the vast number of compounds having the chemical configuration  an ideal local anaesthetic might be discovered. Several investigators have applied this configuration of known anaesthetic activity for the synthesis of new compounds (Krantz *et al.*, 1954; Weidmann and Peterson, 1955; Astrom and Persson, 1960). To explore this avenue still further a series of compounds related to lignocaine were synthesized in the department. In the present study an attempt has been made to determine the local anaesthetic activity and toxicity of these compounds which are derivatives of ethylacetanilide and to compare them with lignocaine.

METHODS

In all eight compounds were prepared. The synthesis of these compounds was a two step process which is schematically shown in Fig. 1.



$-N\begin{matrix}R\\R\end{matrix}$ = diethylamino, piperidino, morpholino

Fig. 1.

The starting materials were O , m and p ethyl - anilines. These were reacted with chloro acetyl chloride leading to the corresponding ω-chloroethyl acetanilide derivatives. These were then condensed with secondary amines like diethylamine, piperidine and morpholine in an inert solvent medium, benzene. This resulted in the synthesis of compounds EA1 to EA8 (Table I).

TABLE I

Compound No.	*structure*	*General Physical characters*
EA1	ω--piperidino o—ethyl acetanilide	m.p. 202°C, white plates, moderately soluble. pH of 1% sol. 5·4.
EA2	ω—piperidino m—ethyl acetanilide	m.p. 144-145°C. White needles, moderately soluble pH 4·7.
EA3	ω—piperidino p—ethyl acetanilide	m.p. 170°C, colourless, coarse granular masses moderately soluble, pH 5.2.
EA4	ω—morpholino o—ethyl acetanilide	m.p. 189°—190°C, white, fine granules freely soluble, pH 4 8.
EA5	ω—morpholino m—ethyl acetanilide	m.p. 181°C, colourless plates, fairly soluble. pH 4 8.
EA6	ω—morpholino p—ethyl acetanilide	m.p. 185°-186°C,colourless plates, fairly soluble, pH 4·8.
EA7	ω—diethylamino m—ethyl acetanilide	m.p. 144·5°C, colourless, plates fairly soluble, pH 5·3.
EA8	ω—diethylamino p—ethyl acetanilide	m.p. 141°-142°C, fine, rounded or oblong crystals, pH 5·0, freely soluble.

The identity of the various intermediates and the final compounds was established by the determination of the physical constants and micro analysis carried out by the National Chemical Laboratory, Poona.

The hydrochlorides of the bases were used in all cases except in the case of compound EA4 where sulphate was used. The pH of 2 per cent solution of the compounds varied within a narrow range of 4·7 to 5·4. The solutions of the compounds were made in physiological saline and not in distilled water since the latter might itself show some local anaesthetic activity as reported by Beutner and Calesnick (1942). Lignocaine hydrochloride was used as reference standard.

The local anaesthetic potency was tested by the following techniques.

(i) *Surface anaesthetic action.*—The method first employed by Chance and Lobstein (1944) was used. Different concentrations of the salts of the compounds under study and lignocaine were instilled into one eye of ten guinea-pigs in such a way that it formed a clearly visible thin film and the lids were

held apart for 15 sec. Normal saline was instilled into the other eye which served as control. During the next five min. each eye was tested for blink reflex every minute using horse hair mounted on a glass rod as stimulator. Care was taken to approach the cornea from the side and with each application to the centre of the cornea the hair was bent to approximately the same degree every time, thus giving a consistent pressure. The degree of anaesthesia was expressed as the number of times out of ten the blink reflex remained absent.

The logarithm of concentrations was plotted against the probit of the percentage response. From this the concentration causing 50 per cent response (SAC 50) was estimated.

(ii) *Intradermal anaesthetic action.*—The technique advocated by Bulbring and Wajda (1945) was employed. Fully grown guineapigs were used. On the day preceding the test, the back of the animals was shaved. 0·25 ml. of solution of several different concentrations of the salts of each compound and lignocaine was injected intraderamally thus raising a wheal. Six pricks at intervals of 3 to 5 seconds were made to each wheal every five min. for 30 min. The number of pricks failing to cause a squeak (out of a total of 36) was taken as the degree of local anaesthesia.

The mean response for each concentration was plotted (ordinate) against the logarithm of the concentration (abscissa) and from the graph concentration producing 50 per cent response (IDAC 50) was calculated.

(iii) *Plexus anaesthesia in frog.*—The method introduced by Sollmann (1918) and developed by Bulbring and Wajda (1945) was employed using *Rana tigrina.* The frog was decapitated and spinal cord destroyed upto 2nd lumbar vertebra. Lumbar plexus was exposed after removing abdominal viscera. The solution of the respective compounds and lignocaine in 0 65 per cent sodium chloride solution was flooded into the pocket formed by the abdomen so as to completely submerge the plexus. N/20, N/10 and N/5 HCl was used as sensory stimulus in ascending order. End point was the abolition of withdrawl reflex in N/5 HCl. Both the feet of the frog were immersed in the acid solution for not longer than 10 sec, after which the feet were at once rinsed thoroughly with water. The results were plotted by taking log concentration as abscissa and the time which the drugs took to abolish the withdrawl reflex as the ordinate.

(iv) *Conduction anaesthesia.*—The method employed by Haining, Johnston and Scott (1960) was used. Adult albino mice of either sex weighing 20 to

30 g were tested by pinching the tail with rubber covered mosquito forceps. Only those which squeaked on the first or second application of this stimulus were employed. The respective compounds and lignocaine were dissolved in physiological saline and 0·05 ml of each drug was injected subcutaneously on each side of the root of the tail. The animals were retested at intervals and the proportion failing to respond to two stimuli recorded. The concentration causing 50 per cent response was found out graphically as in previous experiments (CAC 50).

Acute toxicity studies were conducted on fully grown albino mice of either sex weighing 20 to 30 g. The animals were restrained in a special plastic mouse holder during the injections. Graded doses of the compounds and lignocaine dissolved in physiological saline were injected into the tail vein of a group of six animals. The volume of the dose was restricted in each case to 0·25 $\pm$ 0 1 ml. The animals were taken out of the mouse holder immediately after the injection and observed. Death of the animal was taken as the end point. At least three intermediate doses were injected between the one causing no fatality and that causing 100 per cent mortality. The LD50 of the compounds was determined by plotting the observations by the method of Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

The results of the tests are summed up in Table II. The compounds can be divided into three groups for the sake of discussion.

(i) ω-Piperidino-ethyl-acetanilides :—Compounds EA1, EA2 and EA3 ; substitution being at ortho, meta and para positions respectively.

(ii) ω-Morpholino-ethyl-acetanilides :— Compounds EA4, EA5, and EA6 ; substitution being at ortho, meta and para positions respectively,

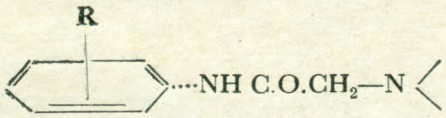
(iii) ω-Diethylamino—acetanilides :—Compounds EA7, EA8 substitution being at meta and para positions respectively.

ω-Piperidino-acetanilides were found to possess appreciable local anaesthetic activity and among these the substitution at para position yielded the strongest compound (EA3); the meta isomer (EA2) being the weakest and the ortho isomer (EA1) occupying an intermediary position. Even compound EA3 is a little weaker than lignocaine and shows activity approximately 80 per cent of the latter.

ω-Morpholino-acetanilides were the weakest of the series and among these the substitution at ortho position (compound EA4) leads to compara-

TABLE II

Local anaesthetic action of Ethyl acetanilides



Compound	R	—N<	SAC_{50}%	$Idac_{50}$%	CAC_{50}%	Plexus anaesthesia relative potency	LD_{50} mg/kg. I. V.
EA1	2—C_2H_5	—N	0·510	0·323	1·31	65·75	38·0
EA2	3—C_2H_5	—N	0·66	0 83	1·77	65·25	43·0
EA3	4—C_2H_5	—N	0·36	0·295	1·31	80·2	32·0
EA4	2—C_2H_5	—N O	0·75	1·51	3 87	56·3	48·0
EA5	3—C_2H_5	—N O	0·75	2·50	5·02	54·7	100 00
EA6	4—C_2H_5	—N O	1·60	2·81	3 87	53·3	115·0
EA7	3—C_2H_5	—N(C_2H_5)(C_2H_5)	0·515	0·525	1·37	73·7	29 0
EA8	4—C_2H_5	—N(C_2H_5)(C_2H_5)	0·545	0·645	1·69	64·5	48·0
Lignocaine 2:6—dimethyl		—N(C_2H_5)(C_2H_5)	0·267	0·24	0·99	100·0	28 0

SAC—Surface anaesthetic concentration.
IDAC—Intradermal anaesthesia concentration.
CAC—Conduction anaesthesia concentration.

tively stronger compound than substitution at meta and para positions (compounds EA5 and EA6). Even compound EA4 is approximately 1/4th as potent as lignocaine hydrochloride.

ω-Diethylamino-ethyl-acetanilides are more active than morpholino derivatives but are less active than the para isomer of piperidino derivatives (compound EA3). Among the diethylamino compounds the meta isomer (compound EA7) is slightly more potent than the para isomer (compound EA8). However, this slight increase in potency is achieved at the cost of marked increase in toxicity.

In all these compounds, except compound EA7, the toxicity and potency run hand in hand, the toxicity rising with increase in potency. The toxicity in the case of compound EA7 is disproportionately higher than its potency as a local anaesthesic As the pH of 1 per cent solution of the compounds used varied within a narrow range of pH 4·7 to 5·4, the difference in the activity of various compounds may be due to variation in structural configuration.

It is concluded that none of the compounds tested approaches the potency of lignocaine. The potency of ω-piperidino-ethyl-acetanilides can be favourably compared with ω-diethylamino-acetanilides hence the potentialities of compounds having piperidine group as a part of basic side chain deserve to be explored further.

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