THE LOCAL ANÆSTHETIC ACTIVITY OF 4-ALKOXYBENZOATES

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THE investigation of substances suitable for the preparation of sparingly soluble penicillin salts[1], has led to the preparation of several *p*-alkoxybenzoates including the *p*-hydroxybenzoates of dialkylaminoethanol. It was soon recognised that the substances possess a powerful local anæsthetic activity, a property which has already been described for some of them. Substances I, III, V, X, have been synthesised by Rohmann and Scheurle[2], who examined their local anæsthetic activity. At about the same time, the compounds I, III, V were prepared in the United States[3] and their properties investigated by McIntyre and Sievers[4]; diethylaminoethyl *p*-ethoxybenzoate (III) has later been used clinically under the name of intracaine.

Because of the recent interest in the influence of alkoxy groups on local anæsthetic activity[5,6,7] it was decided to submit the homologous series of alkoxy benzoates to a more detailed pharmacological examination. The compounds examined are listed in Table I.

METHODS OF PREPARATION

4-*Alkoxybenzoic Acids.*

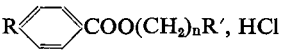
The *p*-alkoxybenzoic acids have been prepared by the reaction of an alkyl bromide on methyl *p*-hydroxy benzoate in the presence of potassium hydroxide in acetone, or sodium methylate in methanol, followed by hydrolysis of the esters so obtained[2,5,7,8,9].

$$HOC_6H_4COOCH_3 + RBr \longrightarrow ROC_6H_4COOCH_3 \longrightarrow ROC_6H_4COOH$$

For the preparation of *p-cyclo*hexyloxybenzoic acid, we tried the recently described method[10] of condensation of *cyclo*hexene with ethyl *p*-hydroxybenzoate in the presence of boron trifluoride. We had at our disposal only 48 per cent. boron trifluoride in ether, and we could not obtain the desired product. For this reason we used the following, less satisfactory, method: to a solution of potassium methylate, obtained by dissolving 4 g. of potassium metal in 60 ml. of absolute methanol, 15 g. of methyl *p*-hydroxybenoate was added. When the product was in solution, the methanol was completely removed *in vacuo*. To the residue 34 g. of *cyclo*hexyl bromide and 25 ml. of *cyclo*hexanol were added, and the mixture was heated in an oil bath at 180° C. for 30 hours. After cooling, the liquid was washed twice with 125 ml. of 2·5 per cent. sodium hydroxide solution. To the aqueous layer 10 g. of potassium hydroxide was added, and, after refluxing for 2 hours 8·3 g. *p*-hydroxybenzoic acid (m.pt. 210° to 212° C.) was recovered by acidification with hydrochloric acid. The lower layer was dried over sodium sulphate, and the excess of *cyclo*hexyl bromide and *cyclo*hexanol was distilled off *in vacuo*. The residue

was hydrolysed by refluxing with 10 g. of potassium hydroxide in 50 ml. of water and 50 ml. of ethanol during 4 hours. After cooling, the solution was acidified with hydrochloric acid, and 2·4 g. (10 per cent.) of *p-cyclo*hexyloxybenzoic acid m.pt. 182° to 184° C. was obtained.

TABLE I



	R	n	R′	m.pt. ° C.	Nitrogen*	
					Calculated per cent.	Found per cent.
I	OCH_3	2	$N(C_2H_5)_2$	145 to 146	—	—
II	OCH_3	2	$N(CH_3)_2$	157 to 158	5·39	5·55 5·58
III	OC_2H_5	2	$N(C_2H_5)_2$	173 to 174	—	—
IV	OC_2H_5	2	$N(CH_3)_2$	153 to 155	5·11	5·09 5·11
V	OC_3H_7 (*n*)	2	$N(C_2H_5)_2$	136 to 137	—	—
VI	OC_3H_7 (*n*)	2	$N(CH_3)_2$	130 to 131	4·86	4·76 4·75
VII	OC_3H_7 (*n*)	2		148·5 to 149·5	4·46	4·44 4·46
VIII	OC_3H_7 (*n*)	3	$N(C_2H_5)_2$	137 to 139	4·24	4·24 4·26
IX	OC_3H_7 (*n*)	3		121 to 123	4·27	4·10 4·09
X	OC_4H_9 (*n*)	2	$N(C_2H_5)_2$	137 to 140	—	—
XI	OC_4H_9 (*n*)	2	$N(CH_3)_2$	124 to 127	4·63	4·77 4·75
XII	OC_4H_9 (*n*)	2		156 to 157	4·27	4·27 4·29
XIII	OC_4H_9 (*n*)	3	$N(C_2H_5)_2$	127 to 128	4·07	4·07 4·08
XIV	OC_4H_9 (*n*)	3		140 to 141	4·09	4·06 4·08
XV	OC_4H_9 (*iso*)	2	$N(C_2H_5)_2$	128 to 130	4·25	4·25 4·25
XVI	OC_4H_9 (*iso*)	2	$N(CH_3)_2$	110 to 112	4·63	4·63 4·64
XVII	OC_4H_9 (*sec.*)	2	$N(C_2H_5)_2$	105 to 107	4·25	4·24 4·24
XVIII	OC_4H_9 (*sec.*)	2	$N(CH_3)_2$	92 to 94	4·63	4·62 4·64
XIX	OC_5H_{11} (*n*)	2	$N(C_2H_5)_2$	123 to 124	4·07	4·08 4·07
XX	OC_5H_{11} (*n*)	2	$N(CH_3)_2$	130 to 131·5	4·43	4·41 4·44
XXI	OC_6H_{13} (*n*)	2	$N(C_2H_5)_2$	144 to 145·5	4·02	4·04 4·03
XXII	OC_6H_{13} (*n*)	2	$N(CH_3)_2$	131 to 132·5	4·24	4·38 4·38
XXIII	OC_6H_{11} (*cyclo*)	2	$N(C_2H_5)_2$	141 to 143	4·04	4·04 4·02
XXIV	OC_6H_{11} (*cyclo*)	2	$N(CH_3)_2$	174·5 to 176	4·27	4·28 4·28
XXV	OC_8H_{17} (*n*)	2	$N(C_2H_5)_2$	130 to 131·5	3·63	3·65 3·64
XXVI	OC_8H_{17} (*n*)	2	$N(CH_3)_2$	129 to 131 and 136	3·92	3·92 3·93
XXVII	$OC_{16}H_{33}$ (*n*)	2	$N(C_2H_5)_2$	114 to 116 and 140	2·81	2·83 2·82
XXVIII	$OC_{16}H_{33}$ (*n*)	2	$N(CH_3)_2$	126 and 194	2·98	2·98 2·96
XXIX	NH_2	2	$N(C_2H_5)_2$	procaine		
XXX	NHC_4H_9	2	$N(CH_3)_2$	tetracaine,		
XXXI				lidocaine,		
XXXII				cocaine		

* The elementary analyses were made by Dr. A. Konovalow in the Laboratory of General Chemistry, University of Louvain.

Acid Chlorides.

The acid is refluxed with thionyl chloride; the excess of reagent is distilled off, and the acid chloride obtained by distillation *in vacuo*.

Other Compounds.

β-diethylamino- and β-dimethylaminoethanol are commercial products. γ-(1-Pyrrolidyl) propanol was prepared by the method of Kolloff *et al.*[11] and γ-diethylaminopropanol was prepared by the same method[11,12].

β (1-*Pyrrolidyl*) *Ethanol.*

To 85 g. of pyrrolidine, warmed on a water bath, 80·5 g. of ethylene-chlorohydrin was added (1 hour) and heating was continued for 8 hours. After cooling, the liquid was treated with a solution of 60 g. of sodium hydroxide in 200 ml. of water, and extracted 4 times with 100 ml. of benzene. The solution was dried over potassium carbonate, the solvent was removed, and the product was distilled *in vacuo*; yield, 50 g.; b.pt. 85°/20 mm. Hg.

TABLE II

ROC_6H_4COCl

R	Acid g.	Thionyl chloride ml.	Duration of heating hours	Acid chloride obtained g.	B.pt. °C.
CH_3	20	75	1	16	142/25 mm. Hg.
C_2H_5	10	50	1	9	142 to 145/20 mm. Hg.
n C_3H_7	35	50	1	26	154 to 158/25 mm. Hg.
n C_4H_9	39	50	3	29	162 to 163/25 mm. Hg.
iso C_4H_9	20	35	2	6	170 to 180/25 mm. Hg.
sec. C_4H_9	21	35	1	19	165/25 mm. Hg.
n C_5H_{11}	22	35	2·5	20	182/25 mm. Hg.
n C_6H_{13}	25	40	1·5	24	195/25 mm. Hg.
cyclo C_6H_{11}	15	25	1·5	12	190/25 mm. Hg.
n C_8H_{17}	27	35	1	24	180/2 to 3 mm. Hg.
n $C_{16}H_{33}$	20	35	1	17	250/2 to 3 mm. Hg.

Condensation Products.

Equimolecular amounts of the acylchloride and the alkylaminoalkanol in dry benzene are heated for 1 to 2 hours on the water bath. After cooling the solid which separated is filtered off and recrystallised in acetone or absolute ethanol-ether mixtures. The different products are listed in Table I. It will be noticed that compounds XXVI, XXVII, XXVIII present the phenomenon of mesomorphism, already described for the acids[9].

Pharmacological Activity

(1) *Surface Anæsthesia.*

Since the method was introduced by Regnier[13], the disappearance of the corneal reflex has been widely used as a method to test the surface anæsthetic activity. Because of the different results observed in different animal species[14] our experiments have been performed on both rabbits and guinea-pigs.

The hair was clipped around the eyes of the animals. The solution of the anæsthetic, made up in 0·65 per cent. saline solution, was applied to only one eye, the other serving as control. 4 drops of the test solution were instilled into the conjunctival sac of the rabbit's eye and 2 drops into the guinea-pig's eye. After 1 minute, the eye was washed with 0·65 per cent. saline solution, and the anæsthesia was examined by stimulating the cornea 100 times in rapid succession with horsehair mounted on a glass rod. This was repeated every 3 minutes, until the corneal reflex reappeared. The eyes were also observed for evidence of irritation or mydriasis.

This method was used to determine (1) the threshold anæsthetic concentration (TAC100), i.e., the minimum concentration that would produce anæsthesia of the cornea in all animals; (2) the duration of the anæsthesia when a 1 per cent. solution was used. This value was obtained by using the actual results obtained with different animals, and checking them on the curve obtained by plotting the duration of anæsthesia against the logarithm of the concentration. We found an approximately linear relation between these two variables, as also observed by several other investigators[15]. The activities of all the substances are summarised in Tables III and IV. It will be seen that the duration of anæsthesia is usually

TABLE III

CORNEAL ANÆSTHESIA

	Rabbits			Guinea-pigs		
	Percentage strength of solution giving TAC100	Duration with 1 per cent. solution		Percentage strength of solution giving TAC100	Duration with 1 per cent. solution	
		minutes	Potency ratio, cocaine = 1		minutes	Potency ratio, cocaine = 1
I	>15	0	—	>15	0	—
II	>15	0	—	>15	0	—
III	1/4	9	1·3	1/4	24	1·3
IV	4	0	—	2	0	—
V	1/8	12	1·7	1/8	26	1·4
VI	1/8	16	2·3	1/8	36	2·0
VII	1/4	17	2·4	1/2	42	2·3
VIII	1/8	23	3·3	1/8	27	1·5
IX	1/32	25	3·6	1/32	51	2·8
X	1/4	19	2·7	1/4	42	2·3
XI	1/8	18	2·6	1/4	36	2·0
XII	1/8	14	2·0	1/16	36	2·0
XIII	1/16	25	3·6	1/8	66	3·5
XIV	1/32	29	4·1	1/16	66	3·7
XV	1/8	27	3·9	1/8	78	4·3
XVI	1/8	19	2·7	1/8	45	2·5
XVII	1/2	28	4·0	1/4	33	1·8
XVIII	1	23	3·3	1/2	30	1·7
XIX	1/16	25	3·6	1/8	45	2·5
XX	1/32	27	3·9	1/16	48	2·7
XXI	1/32	44	6·3	1/16	95	5·3
XXII	1/32	44	6·3	1/16	120	6·7
XXIII	1/8	12	1·7	1/8	45	2·5
XXIV	1	15	2·1	1	36	2·0
XXV	8	0	—	4	0	—
XXVI	10	0	—	5	0	—
XXVII	>15	0	—	>15	0	—
XXVIII	>15	0	—	>15	0	—
XXIX	8	0	—	5	0	—
XXX	1/32	75	10·7	1/32	126	7
XXXI	1/2	21	3	1/4	24	1·3
XXXII	1/2	7	1	1	18	1

All results refer to solutions of the hydrochlorides.

much longer in the guinea-pig than in the rabbit, but when relative values are examined (duration of anæsthesia with 1 per cent. solution/duration of anæsthesia with 1 per cent. cocaine solution) it appears that the results obtained with both animal species are similar or that the differences are irregular.

Substances XIX and XX produced an irritation of the rabbit's eye at a concentration of 1 per cent. ; substances XXV, XXVI, XXVII, XXVIII, had only a slightly irritating effect.

(2) *Infiltration Anæsthesia.*

The technique of Bülbring and Wajda[16] has been used. A volume of 0·2 ml. of 0·85 per cent. saline solution, containing the anæsthestic at different concentrations, was injected intradermally into the back of a guinea-pig. The weal thus formed was pricked 6 times with a needle, every 5 minutes, until all stimuli produced a reflex of the skin muscles.

Table IV shows the duration of anæsthesia after infiltration with 0·1 per cent. solutions. (These results have been compared with those obtained with other concentrations.)

(3) *Sciatic Nerve Block.*

Guinea-pigs were tied on a board with the limbs in extension. The hair was shaved from the thigh on one hind leg, and a volume of 0·3 ml. was injected close to the sciatic nerve according to the method of Shackell[17]. The anæsthesia was tested by pricking the skin of the thigh with a needle. The initial concentration of the drug was 2 per cent. (hydrochloride), and subsequently decreased by one half.

In Table IV are shown (*a*) the concentration necessary to produce anæsthesia in all animals (TAC100), (*b*) the duration of sensory paralysis with the concentration of 1 per cent.

TABLE IV

INFILTRATION ANÆSTHESIA AND SCIATIC NERVE BLOCK IN GUINEA-PIGS

	Infiltration Anæsthesia		Sciatic Nerve Block		
	Duration with 1 per cent. solution		Percentage strength of solution giving TAC100	Duration with 1 per cent. solution	
	minutes	Potency ratio, procaine = 1		minutes	Potency ratio, procaine = 1
I	0	—	—	0	—
II	0	—	—	0	—
III	33	2·2	—	15	1·4
IV	12	0·8	—	6	0·6
V	24	1·6	½	48	4·4
VI	24	1·6	½	36	3·3
VII	27	1·8	½	28	2·5
VIII	36	2·4	½	39	3·5
IX	36	2·4	½	72	6·5
X	28	1·9	½	66	6·0
XI	23	1·5	½	63	5·7
XII	27	1·8	½	76	6·9
XIII	39	2·6	½	82	7·5
XIV	39	2·6	⅛	89	8·1
XV	42	2·8	½	33	3·0
XVI	34	2·3	½	28	2·4
XVII	31	2·1	1	36	3·3
XVIII	20	1·3	1	49	4·5
XIX	37	2·5	½	49	6·3
XX	36	2·4	½	69	6·1
XXI	56	3·7	½	81	7·4
XXII	49	3·3	½	100	9·1
XXIII	21	1·4	½	33	3·0
XXIV	18	1·2	½	26	2·4
XXV	9	0·6	—	6	0·6
XXVI	6	0·4	—	0	—
XXVII	0	—	—	0	—
XXVIII	0	—	—	0	—
XXIX	15	1·0	½	11	1·0
XXX	48	3·2	1/32	108	9·8
XXXI	30	2·0	—	15	1·4
XXXII	27	1·8	½	33	3·0

All results refer to solutions of the hydrochlorides.

RESULTS

The study of the results summarised in Tables III and IV obtained by topical application, by infiltration and by nerve block anæsthesia shows that there is a regular increase in the local anæsthetic activity with the size of the alkoxy group.

A maximum is obtained with the hexyl group; after that, the anæsthetic effect abruptly falls with the higher homologues. It must be noticed, however, that the octyl- and cetyl- derivatives are almost insoluble in water.

A similar increase in the activity has been observed with alkoxybenzoates of γ-(2-methylpiperidino) propanol[5] and with 4-amino-2-alkoxy benzoates of diethylaminoethanol and -propanol[7b]. The high activity which McElvain and Carney[5] found for the *cyclo*hexyl derivative has not been confirmed in these series.

The results obtained with the *iso*butoxy (XV, XVI) and the *sec.*-butoxy benzoates (XVII, XVIII) are similar to those which were obtained with the *n*-butoxy compounds (X, XI).

These experiments allow us to confirm that the propanol esters ($n = 3$) are more active than the corresponding ethanol esters ($n = 2$)[18]: V and VIII, VII and IX, X and XIII, XII and XIV.

The diethylamino compounds are usually slightly more active than the dimethylamino derivatives, and the pyrrolidino group is rather superior to the diethylamino group: V and VII, VIII and IX, X and XII, XII and XIV. The favourable influences of a pyrrolidino group on the anæsthetic effect had already been noticed in the propylbenzoate[19].

TOXICITY

(1) *General Toxicity.*

The acute toxicity was examined on white mice weighing between 18 and 24 g. After intraperitoneal injection of the different compounds, the animals were kept under observation for 48 hours at a temperature of approximately 18° C. The anæsthetics were dissolved in 0·85 per cent. sodium chloride solution, and the volume injected varied between 0·5 and 1·0 ml. After determination of the minimum lethal dose, 5 or 6 different dosages were injected to groups of 10 animals, in order to obtain mortality rates under and above 50 per cent. From these results, the LD50 has been calculated (Table V).

(2) *Local Toxicity.*

The toxicity for the injected tissues has been examined after intradermal injection in the back of guinea-pigs, where the hair had been clipped away, 1 or 2 days before the experiment. Using one animal for each drug, injections of 0·2 ml. of at a concentration of 0·1, 0·25, 0·5, 1·0 and 2·0 per cent. solution, dissolved in 0·85 per cent. sodium chloride solution, were performed. After 24 hours the diameter of the irritated area was measured, and the degree of erythema, the presence of petechiæ or ulceration were noted. The tests were also carried out by adding sufficient

adrenaline to the solution to give a concentration of 1 in 100,000; except in the case of tetracaine very little or no modification was observed.

The results obtained with all the substances examined are summarised in Table V.

(3) *Hæmolysis.*

Because no direct relation could be found between hæmolytic activity and toxicity[20] it was necessary to investigate the hæmolytic activity of these compounds.

Solutions in 0·9 per cent. sodium chloride solution, in decreasing concentration of the various compounds were prepared, and added with 2 or 3 drops of defibrinated human blood. The degree of hæmolysis was examined after 24 hours at room temperature.

The results are given in Table V.

TABLE V

	LD50 for mice mg./kg.	Local irritation* diameter (mm.) of reaction zone[15]		Hæmolytic concentration
		With 0·5 per cent. solution	With 1 per cent. solution†	
I	575	+(3)	++(4)	>1/100
II	475	+(2)	++(3)	>1/100
III	300	0	0	>1/100
IV	475	+(4)	+(6)	>1/100
V	254	+(4)	++(7)	1/200
VI	242	0	+(7)	1/800
VII	208	0	+(4)	1/400
VIII	142	+(2)	+(6)	1/400
IX	73	+(3)	++(6)	1/600
X	192	0	++(6)	1/200
XI	258	+(3)	++(6)	1/800
XII	242	+(3)	++(7)	1/1000
XIII	192	+(5)	++(6)	1/1250
XIV	142	+(4)	++(9)	1/1500
XV	158	+(6)	+++(8)	1/1000
XVI	192	+(5)	+++(8)	1/800
XVII	442	+(6)	+++(8)	1/800
XVIII	542	+(4)	+++(5)	1/400
XIX	291	++(5)	+++(8)	1/2000
XX	208	++(5)	+++(9)	1/2000
XXI	258	+++(9)	+++(10)	1/3000
XXII	208	+++(9)	+++(11)	1/4000
XXIII	258	++(3)	+++(5)	1/2000
XXIV	275	+++(6)	+++(8)	1/1750
XXV	175	+++(5)	+++(6)	1/4000
XXVI	175	+++(4)	+++(5)	1/4000
XXVII	—	0	—	1/300
XXVIII	—	0	—	1/400
XXIX	202	0	+(3)	>1/100
XXX	52	+++(9)	+++(11)	1/300
XXXI	125	0	0	>1/100
XXXII	62	+(2)	++(5)	—

* + erythema
++ erythema with petechiæ
+++ erythema with ulceration
† between brackets the diameter of the reaction zone.

The results show that most of these compounds have a fairly low toxicity, even when compared with procaine (XXIX), and certainly more so in comparison with tetracaine (XXX) and lidocaine (XXXI). The symptoms of the acute intoxication were similar for all compounds. After injection, all the mice were deeply depressed and after a lethal dose the death, which occurred mostly within half an hour, was the result

of respiratory failure. No clonic convulsions, as with tetracaine, were observed. Substance XIII, with doses of 100 to 150 mg./kg. caused erection of the tail in sigmoid form (Straub reaction), which used to be considered characteristic for morphine derivatives. The Straub reaction has already been observed in other than morphine compounds[21,22,23,24,25].

Table V shows that substances III and VII (VI) are the only compounds which cause no irritation when effective anæsthetic concentrations are used. For the other anæsthetics, the irritation increases with the activity. However, the butoxy- (X to XVIII) and hexyloxy-derivative (XXI, XXII), which showed no irritating effect on the eye might perhaps, because of their activity, be used in surface anæsthesia. It must also be noticed that, although the parallelism is not perfect, the hæmolytic activity increases with the irritating effect.

Summary

1. A series of *p*-alkoxy benzoates of β-dimethylamino-, β-diethylamino-, β-(1-pyrrolidyl)-ethanol and of γ-diethylamino-; γ-(1-pyrrolidyl-)3 propanol has been prepared.

2. Some pharmacological activities of those compounds have been investigated.

3. A certain number of them show good local anæsthetic properties.

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