THE PREPARATION AND PHARMACOLOGY OF SOME PHENOLIC CARBAMATES AND ALLOPHANATES

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A series of alkyl-substituted phenyl carbamates has been screened for analgesic activity by two methods and the ED50 of a representative number has been determined by one of them. An attempt has been made to correlate the analgesic activity with chemical constitution and the following points emerge: (i) at least one *m*-alkyl group is essential for significant activity, (ii) where only one substituent is present, maximal activity occurs when this is ethyl, (iii) two *o*-substituents abolish the activity, (iv) 2,4,5-trimethylphenyl carbamate (compound 686) possesses the highest activity, (v) the most active compounds are in general also the most toxic, compound 688 being a notable exception, (vi) *o*-substitution by a group larger than methyl tends to reduce activity, (vii) substitution on the carbamate N, except by hydroxyl, strongly reduces the activity. The toxicity of some of the compounds could not be attributed to their anticholinesterase activity.

SOME years ago the reassessment of salicylamide and its related compounds as analgesics was undertaken, in the hope that this might lead to the development of an improved drug of the aspirin type. This led to the appraisal of other amides, for example, phenoxyacetamide, and to the synthesis of a large series of phenolic carbamates, the first member of which, *o*-chlorophenyl carbamate, had shown significant activity by the screening test then employed by us. Most of the members of this series are carbamates of mono- and polyalkyl phenols.

CHEMICAL

The following are new intermediates which were obtained by standard methods.

3,5-*Dimethylphenol* n-*butyrate*. A solution of 3,5-dimethylphenol (122 g.) in pyridine (200 ml.) was cooled in ice and stirred whilst butyric anhydride (173·8 g., 1·1 mole) was added during 30 minutes. After standing overnight the mixture was heated for 1 hr. at 100°, cooled and poured on ice and hydrochloric acid. The ester was extracted with ether, and the extract washed with water, dried and evaporated. The residue was distilled, b.p. 127° at 14 mm. Found: C, 74·5; H, 8·2. $C_{12}H_{16}O_2$ requires C, 75·0; H, 8·3 per cent.

2-n-*Butyryl*-3,5-*dimethylphenol* was prepared by Fries re-arrangement (1 mole $AlCl_3$ in CS_2) of 3,5-dimethylphenol *n*-butyrate. It crystallised from light petroleum (b.p. 40–60°), m.p. 55–58·5°. Found: C, 74·3; H, 8·4. $C_{12}H_{16}O_2$ requires C, 75·0; H, 8·3 per cent.

2-n-*Butyl*-3,5-*dimethylphenol* was prepared by Clemmensen reduction of 2-*n*-butyryl-3,5-dimethylphenol. It crystallised from light petroleum

TABLE I

A SUMMARY OF THE NEW CHLOROFORMATES

Phenyl chloroformate	Boiling point	Formula	Found			Required		
			C	H	Cl	C	H	Cl
3,5-Dimethyl-	96–97° at 12 mm.	$C_9H_9ClO_2$	58·6	5·1	19·3	58·6	4·9	19·2
3-Ethyl-5-methyl	107–108° at 11 mm.	$C_{10}H_{11}ClO_2$	61·1	5·7	17·5	60·5	5·5	17·9
3,4-Dimethyl-	101° at 13 mm.	$C_9H_9ClO_2$	58·6	4·9	19·4	58·6	4·9	19·2
2,5-Dimethyl-	89–90° at 11 mm.	$C_9H_9ClO_2$	59·2	4·8	19·2	58·6	4·9	19·2
2,6-Dimethyl-	83–83·5° at 11 mm.	$C_9H_9ClO_2$	—	—	18·5	—	—	19·2
2,3-Dimethyl-	94° at 12 mm.	$C_9H_9ClO_2$	58·9	5·0	19·0	58·6	4·9	19·2
2,3,5-Trimethyl-	108° at 12 mm.	$C_{10}H_{11}ClO_2$	61·0	6·0	17·4	60·5	5·5	17·9
2-Phenyl-	86–90° at 0·05 mm., m.p. 59–62·5°, except light petroleum	$C_{13}H_9ClO_2$	—	—	15·8	—	—	15·3
p-Phenyl-	132–137° at 0·4 mm., m.p. 35–40°, except light petroleum	$C_{13}H_9ClO_2$	67·6	3·9	14·8	67·1	3·9	15·3

TABLE II

A SUMMARY OF THE NEW ALLOPHANATES

Phenyl allophanate	Method	M.p.	Crystallised from	Formula	Found			Required		
					C	H	N	C	H	N
3-Ethyl-	1	155–158° decomp.	Methanol	$C_{10}H_{12}N_2O_3$	57·8	5·8	13·7	57·7	5·8	13·5
2,5-Dimethyl- ..	1	183–184° decomp.	Ethyl acetate	$C_{10}H_{12}N_2O_3$	57·6	6·1	13·7	57·7	5·8	13·5
3,5-Dimethyl- ..	2	199° decomp.	Ethanol	$C_{10}H_{12}N_2O_3$	57·8	5·8	13·1	57·7	5·8	13·5
3,4-Dimethyl- ..	2	167° decomp.	Ethanol	$C_{10}H_{12}N_2O_3$	58·0	5·9	13·1	57·7	5·8	13·5
2,3-Dimethyl- ..	2	200° decomp.	Ethyl acetate	$C_{10}H_{12}N_2O_3$	57·8	5·8	13·1	57·7	5·8	13·5
3-Ethyl-5-methyl-	2	168° decomp.	Ethanol	$C_{11}H_{14}N_2O_3$	59·7	6·2	12·2	59·5	6·3	12·6

(b.p. 30–40°), m.p. 64·5–66°. Found: C, 80·6; H, 9·7. $C_{12}H_{18}O$ requires C, 80·9; H, 10·1 per cent.

2-n-*Propyl*-3,5-*dimethylphenol* was prepared by Clemmensen reduction of the corresponding propiophenone. It crystallised from *n*-hexane, m.p. 53·5–54°. Found: C, 80·6; H, 9·9. $C_{11}H_{16}O$ requires C, 80·5; H, 9·8 per cent.

4-n-*Propyl*-2,5-*dimethylphenol* was prepared by Clemmensen reduction of the corresponding propiophenone. It has b.p. 132° at 18 mm. Found: C, 80·5; H, 10·0. $C_{11}H_{16}O$ requires C, 80·5; H, 9·8 per cent.

Preparation of chloroformates. A 20 per cent w/v solution of sodium hydroxide (220 ml.) was added to a solution of the phenol (1 g. mole) and phosgene (100 g.) in carbon tetrachloride (500 ml.) at −5° with vigorous stirring for 45 min. The organic layer was separated, washed with N sodium hydroxide and water, and dried over anhydrous sodium sulphate. The solvent was removed on the steam bath and the residual chloroformate was distilled *in vacuo*.

Table I lists the new chloroformates.

Preparation of Allophanates

Method 1. Cyanic acid vapour, from the thermal depolymerisation of cyanuric acid, was passed into an ethereal solution of a phenol until the theoretical weight increase had occurred. The crystalline precipitate was collected the next day (Blohm and Becker, 1952).

Method 2. Blohm and Becker (1952) found that ethyl and 3-chloropropyl chloroformates yield allophanates on heating with urea. Aryl chloroformates gave good yields of allophanates by this method. A phenyl chloroformate (1 mole) and urea (2 moles) protected from moist air were heated on the water bath until the urea dissolved and the mass re-solidified. After a further 3 hr. heating the mass was extracted with water and ether. The residue was crude allophanate. 2,6-Dimethylphenyl chloroformate did not react with urea.

Table II lists the new allophanates prepared.

Preparation of Carbamates

Method 1. A solution of the crude chloroformate, prepared as previously described, in carbon tetrachloride was reacted with 2 moles of ammonia or the appropriate amine to give the carbamate.

Method 2. 0·2 mole of a phenol was dissolved in dry carbon tetrachloride (100 ml.), dry powdered sodium cyanate (13 g.) was added, and the mixture was stirred whilst a solution of trichloracetic acid (33 g.) in carbon tetrachloride (80 ml.) was added. Stirring was continued at 55° for 6 hr. After cooling, water was added with stirring and the organic layer was separated, washed with N sodium hydroxide and water, and dried over anhydrous sodium sulphate. The solvent was evaporated and the residue was crystallised from the appropriate solvent.

Table III lists the phenyl carbamates prepared.

A number of miscellaneous carbamates are given in Table IV.

TABLE III

A LIST OF THE PREPARED PHENYLCARBAMATES

Phenyl carbamate	Method	M.p.	Crystallised from	Formula	Found			Required		
					C	H	N	C	H	N
3-Methyl-*	1	139°	Ethyl acetate	$C_8H_9NO_2$	64·2	6·0	9·1	63·6	6·0	9·3
3-n-Propyl-	1	108–9°	Cyclohexane	$C_{10}H_{13}NO_2$	67·0	7·1	7·9	67·1	7·3	7·8
3-Ethyl-	2	103–4°	50 per cent. aq. methanol	$C_9H_{11}NO_2$	65·5	6·7	8·8	65·5	6·7	8·5
3-n-Butyl-	2	110–112°	Cyclohexane	$C_{11}H_{15}NO_2$	69·0	8·0	7·1	68·4	7·8	7·3
3-n-Pentadecyl-	2	97°	Benzene	$C_{23}H_{37}NO_2$	76·1	10·6	4·3	76·2	10·7	4·0
2-n-Propyl-	2	104–6°	Cyclohexane	$C_{10}H_{13}NO_2$	67·1	7·5	7·9	67·1	7·3	7·8
2-Allyl-	2	117–8°	Cyclohexane	$C_{10}H_{11}NO_2$	68·1	6·0	8·0	67·8	6·2	7·9
2,3-Dimethyl-	1	138–141°	Benzene	$C_9H_{11}NO_2$	66·0	6·6	8·2	65·5	6·7	8·5
2,5-Dimethyl-	2	109–110°	Cyclohexane	$C_9H_{11}NO_2$	65·9	6·6	8·5	65·5	6·7	8·5
2,6-Dimethyl-	1	177° decomp.	Ethanol	$C_9H_{11}NO_2$	65·7	6·7	8·8	65·5	6·7	8·5
2-Ethyl-5-methyl-	2	106–7°	Cyclohexane	$C_{10}H_{13}NO_2$	67·5	7·6	8·0	67·1	7·3	7·8
2-n-Propyl-5-methyl	2	105–6°	Cyclohexane	$C_{11}H_{15}NO_2$	68·5	7·7	7·0	68·4	7·8	7·3
2-n-Butyl-5-methyl-	2	87–87·5°	Cyclohexane	$C_{12}H_{17}NO_2$	69·5	8·0	7·0	69·6	8·2	6·8
3-Methyl-4-ethyl-	2	99–100°	Cyclohexane	$C_{10}H_{13}NO_2$	67·0	7·5	7·9	67·1	7·3	7·8
3-Methyl-5-ethyl	2	100–101°	Benzene	$C_{10}H_{13}NO_2$	67·1	6·9	7·5	67·1	7·3	7·8
3-Methyl-4-n-propyl-	2	117–118°	Cyclohexane	$C_{11}H_{15}NO_2$	68·4	7·8	—	68·4	7·8	—
2,3,4-Trimethyl-	2	162–164°	Benzene	$C_{10}H_{13}NO_2$	67·7	7·2	7·8	67·1	7·3	7·8
2,3,5-Trimethyl-	1	137–139°	Benzene, light petroleum	$C_{10}H_{13}NO_2$	66·9	7·0	8·2	67·1	7·3	7·8
3,4,5-Trimethyl-	1	163–166°	Ethanol	$C_{10}H_{13}NO_2$	67·0	7·3	8·2	67·1	7·3	7·8
2,5-Dimethyl-4-ethyl	2	138–140°	Benzene	$C_{11}H_{15}NO_2$	68·4	7·5	6·7	68·4	7·8	7·3
2,5-Dimethyl-4-n-propyl	2	132–133·5°	Cyclohexane	$C_{12}H_{17}NO_2$	69·4	7·8	7·0	69·6	8·2	6·8
2,3-Dimethyl-6-ethyl-	2	132·5–134·5°	Carbon tetrachloride	$C_{11}H_{15}NO_2$	68·7	8·2	7·0	68·4	7·8	7·3
2-Ethyl-3,5-dimethyl-	1	110·5–112°	Cyclohexane	$C_{11}H_{15}NO_2$	68·3	7·5	7·3	68·4	7·8	7·3
2-Ethyl-4,5-dimethyl-	1	142·5–143·5°	Ethanol	$C_{11}H_{15}NO_2$	—	—	7·15	68·4	7·8	7·3
2-n-Propyl-3,5-dimethyl-	1	128–128·5°	Cyclohexane	$C_{12}H_{17}NO_2$	69·9	8·0	6·7	69·6	8·2	6·8
2,3,4,5-Tetrameth	2	177–178·5°	Toluene	$C_{11}H_{15}NO_2$	68·9	7·4	7·0	68·4	7·8	7·3
2,3,5,6-Tetrameth	2	191–192°	Toluene	$C_{11}H_{15}NO_2$	68·2	7·8	7·0	68·4	7·8	7·3
2,3,5-Trimethyl-6-ethyl-	2	150·5–152°	Benzene	$C_{12}H_{17}NO_2$	69·6	8·2	6·8	69·6	8·2	6·8
3-Methoxy-	2	136–138°	Ethyl acetate	$C_8H_9NO_3$	57·6	5·5	8·1	57·5	5·4	8·4
4-Methoxy-	2	127–129°	Ethyl acetate	$C_8H_9NO_3$	57·9	5·5	8·3	57·5	5·4	8·4
4-t-Butyl-	2	118–119°	Cyclohexane	$C_{11}H_{15}NO_2$	68·7	7·9	7·2	68·4	7·8	7·3
2-Phenyl-	1	143°	Ethanol	$C_{13}H_{11}NO_2$	73·7	5·1	6·4	73·3	5·2	6·6
3-Phenyl-	2	157–158°	Benzene	$C_{13}H_{11}NO_2$	72·8	5·1	6·9	73·3	5·2	6·6
4-Phenyl-	1	170°	Ethanol	$C_{13}H_{11}NO_2$	73·5	5·3	7·1	73·3	5·2	6·6
3-Chloro-	2	135–137°	Benzene	$C_7H_6ClNO_2$	49·0	3·6	7·9	49·0	3·5	8·2
4-Chloro-	2	142°	Ethyl acetate	$C_7H_6ClNO_2$	49·0	3·3	8·0	49·0	3·5	8·2
2-Methyl-4-chloro-	2	153°	Ethyl acetate	$C_8H_8ClNO_2$	51·5	4·0	7·8	51·8	4·3	7·6
3-Methyl-4-chloro-	2	150–152°	Ethyl acetate	$C_8H_8ClNO_2$	51·8	4·4	7·8	51·8	4·3	7·6
4-Methyl-2-chloro-	2	155°	Benzene	$C_8H_8ClNO_2$	52·4	4·5	7·4	51·8	4·3	7·6
2,4-Dichloro-	2	129°	Benzene	$C_7H_5Cl_2NO_2$	41·0	2·5	6·8	40·8	2·4	6·8
2,4,5-Trichloro-	2	131–132°	Benzene	$C_7H_4Cl_3NO_2$	35·5	1·5	5·9	35·0	1·7	5·8
3-Methyl-(*N*-methyl)	1	74–75°	Light petroleum (60–80°)	$C_9H_{11}NO_2$	65·9	6·8	8·1	65·5	6·7	8·5
3-Methyl-(*N*-ethyl)	1	b.p. 1140/0·2 mm.	—	$C_{10}H_{13}NO_2$	67·3	7·2	7·9	67·1	7·3	7·8
3-Methyl-(*N*-n-butyl)	1	52–53°	Light petroleum (40–60°)	$C_{12}H_{17}NO_2$	69·9	8·5	6·6	69·6	8·2	6·8
3-Methyl-(*N*-*m*-tolyl)	1	71–72°	Light petroleum (60–80°)	$C_{15}H_{15}NO_2$	74·7	6·3	6·1	74·7	6·2	5·8
3-Methyl-(*NN*-dimethyl)	1	b.p. 147°/16 mm.	—	$C_{10}H_{13}NO_2$	67·5	7·4	8·0	67·1	7·3	7·8
2,3-Dimethyl-(*N*-methyl)	1	110–112°	Cyclohexane	$C_{10}H_{13}NO_2$	67·3	7·6	7·9	67·1	7·3	7·8
2,3,5-Trimethyl-(*N*-hydroxy)	1	173·5–174·5°	Benzene	$C_{10}H_{13}NO_3$	62·0	6·6	6·8	61·6	6·7	7·2

* cf. Avenarius (1923)

TABLE IV

A SUMMARY OF THE MISCELLANEOUS CARBAMATES

Carbamate	Method	M.p.	Crystallised from	Formula	Found			Required		
					C	H	N	C	H	N
4-*m*-Tolyloxycarbonyl-morpholine	1	82–83°	Cyclohexane	$C_{12}H_{15}NO_3$	65·5	7·1	6·2	65·2	6·8	6·3
1-*m*-Tolyloxycarbonyl-piperidine	1	63–63·5°	Light petroleum (60–80°)	$C_{13}H_{17}NO_2$	71·6	7·9	6·9	71·3	7·8	6·4
β-Naphthyl	2	156–157°	Benzene	$C_{11}H_9NO_2$	70·7	5·2	7·2	70·6	4·8	7·5
5-Hydroxytetralin ..	2	138–140°	Cyclohexane	$C_{11}H_{13}NO_2$	69·5	6·9	7·4	69·2	6·8	7·3
4-Hydroxyindane ..	2	139·5–140·5°	Benzene-cyclohexane	$C_{10}H_{11}NO_2$	68·0	6·1	8·3	67·9	6·2	7·9
5-Hydroxyindane ..	1	147–148°	Cyclohexane	$C_{10}H_{11}NO_2$	68·0	6·2	7·9	67·9	6·2	7·9

PHARMACOLOGY

Methods

All mice used were female albinos of Schofield strain.

Analgesic activity. Compounds were first screened by the hot-plate method of Woolfe and Macdonald (1944) with minor modifications. The mice were tested on the hot surface (temperature 54°) and any mice failing to respond before 15 sec. were discarded. Drugs were given orally as a suspension in 5 per cent acacia to groups of 20 mice, which were placed individually on the hot surface at intervals of 5, 10, 20 and 40 min. after dosing. The mean percentage increase in reaction time over the control time at each interval was calculated for each group and the final potency was expressed as the sum of these percentages. The numerical value obtained naturally varied according to the average control time, thus, the lower the control time the higher the possible numerical value. This test can therefore not be regarded as by any means quantitative, and the potency is described merely as negligible, low, moderate, or high.

For quantitative assessment of potency the tail-clip method decribed by Bianchi and Franceschini (1954) was used. By this means it was possible to determine the ED50 of a representative series of the most interesting compounds at 15 min. after oral administration.

Acute toxicity. Groups of not less than ten mice weighing between 20–25 g. were given three logarithmically graded doses in 5 per cent acacia. The percentage mortality was noted over a 5-day period. LD50 values were estimated by the method of Bliss (1938).

Chronic toxicity. Groups of 20 mice were given oral doses of the drug (compound 688), at doses of 1/20 and 1/60 of the lower limit of error of the LD50 daily excluding week-ends and public holidays for approximately 4 months. Weight gains were recorded, treated statistically and compared with the controls. Differential blood counts were made on the animals at the end of the experiment, and histological sections of a representative selection of organs from test and control animals were made after post-mortem examination.

Blood pressure and respiration. Guinea pigs weighing approximately 1 kg. were used, and anaesthetised with urethane, and the drug was injected in distilled water or saline into the cannulated jugular vein in a dose of 50 μg. for blood pressure effect and 100 μg. for effect on respiration.

Anticholinesterase activity. This was determined by the method of Buckles and Bullock (1956).

RESULTS

Analgesic tests. Table V gives ratings to the activities of selected compounds in the Woolfe-Macdonald test. These agree qualitatively with those obtained by the Bianchi tail-clip method. The latter are given in Table VI, which records the ED50 of a representative number of compounds 15 min. after oral administration. Observations on these two tests are made under the discussion of results.

Comparison of the activity of morphine, pethidine, and Compound 688. Woolfe and Macdonald found pethidine to show little activity in their test; in our test pethidine shows little activity and is less active than morphine, and much less so than compound 688.

The results are summarised in Table VII.

Toxicity Tests

Acute toxicity. The oral, and in some cases i.v., LD50 values for selected compounds are given in Table V.

TABLE V

ANALGESIC ACTIVITY OF A SERIES OF PHENOLIC CARBAMATES IN THE WOOLFE-MACDONALD SCREENING TEST

Compound No.	Phenol carbamate	Potency	Toxicity	
			Oral (LD50 mg./kg.)	Intravenous (LD50 mg./kg.)
613	3-Methyl-	Moderate	1116·0 (932·4–1337·0)	105·1 (97·2–113·5)
631	3-Ethyl-	High	143·6 (108·2–190·5)	20·88 (15·59–70·42)
641	4-Isopropyl-	Negligible	—	—
642	3,5-Dimethyl-	High	421·9 (337·3–527·8)	60·29 (51·59–70·42)
654	3,4-Dimethyl-	Very high	112·9 (85·0–149·8)	24·1 (20·4–28·5)
658	2,5-Dimethyl-	Very high	155·8 (142·8–169·9)	33·9 (23·12–49·71)
661	2,3,5-Trimethyl-	Very high	145·5 (100·3–211·2)	29·93 (24·77–36·17)
663	3-Ethyl-5-methyl-	High	374·4 (333·9–420·0)	—
666	3,5-Dimethyl-(allophanate)	Inactive	—	—
669	3-Methyl-4-ethyl-	High	39·82 (29·85–53·13)	—
678	2-n-Propyl-5-methyl-	Low	—	—
680	2,6-Dimethyl-	Low	—	—
682	2,3-Dimethyl-(allophanate)	Negligible	—	—
688	2,3-Dimethyl-	High	864·4 (645·7–1157·0)	—
686	2,4,5-Trimethyl-	Very High	35·8 (27·9–46·0)	—
690	2,5-Dimethyl-4-ethyl-	Very high	23·13 (20·49–26·10)	—
696	2,3,5-Trimethyl-6-ethyl-	Negligible	—	—
699	2,3,6-Trimethyl-	Negligible	—	—
700	2,3,5,6-Tetramethyl-	Negligible	—	—
703	2,4-Dimethyl-	Negligible	—	—
709	2,3,4,5-Tetramethyl-	Very high	—	—
714	2,3,4-Trimethyl-	High	—	—
727	2,3,5-Trimethyl-(*N*-methyl)	Negligible	—	—
729	2,3,5-Trimethyl-(*N*-hydroxy)	High	220·9 (161·0–302·9)	—
730	3-Methyl-4-ethyl-(*N*-acetyl)	Negligible	—	—
732	2,3-Dimethyl-(*N*-hydroxy)	High	—	—
734	2,4,5-Trimethyl-(*N*-hydroxy)	High	86·3 (71·64–104·0)	—

Chronic toxicity. Compound 688 (2,3-dimethylphenyl carbamate) was chosen for this test. The average weight gains showed no significant differences from those of the controls. There were three deaths at each dose level during the 17-week experimental period, apparently unconnected with the experimental conditions. The blood counts (average) and haemoglobin contents are given in Table VIII.

The cause of the apparently highly significant increase in leucocytes on the *low* dose of the drug is doubtful. However, since no abnormalities

attributable to the drug were reported in the histological examination of liver, kidney, and spleen, and owing to the frequency of the occurrence of subcutaneous abscesses, it is probable that these phenomena are unconnected with the drug. Moreover, persistently high leucocyte counts

TABLE VI

ORAL ANALGESIC ACTIVITY OF PHENOLIC CARBAMATES BY THE BIANCHI TAIL-CLIP METHOD

Compound*	Analgesic activity (ED50 mg./kg.)
613	58·0
631	12·2
642	17·4
654	3·5
658	10·6
661	2·58
663	16·0
686	1·34
688	8·8
690	1·76
696	No effect at 100
699	No effect at 100
700	No effect at 100
703	No effect at 100
709	1·60
714	16·6

* For chemical formulae see Table V.

TABLE VII

COMPARISON OF THE ANALGESIC ACTIVITY OF MORPHINE, PETHIDINE AND COMPOUND 688 ADMINISTERED BY MOUTH

Compound	Dose mg./kg.	No of tests	Index of potency
688*	10	8	343
688	5	4	100
Morphine HCl	10	4	206
Morphine HCl	5	2	92
Pethidine HCl	10	4	130
Pethidine HCl	5	2	51

* For chemical formula see Table V.

TABLE VIII

AVERAGE BLOOD COUNTS AND HAEMOGLOBIN CONTENTS OF MICE ON CHRONIC TOXICITY TEST OF COMPOUND 688*

	Erythrocytes millions/cu. mm.	Leucocytes thousands/cu. mm.	Haemoglobin (Sahli) per cent
Controls	12·72	14·28	113
Mice receiving 32 mg./kg. ..	11·52	14·70	120
Mice receiving 10 mg./kg. ..	11·82	23·50	123

* For chemical formula see Table V.

have been obtained in both control and test animals in other chronic toxicity tests, and may be associated with the particular mouse stock used.

Anticholinesterase activity. Table IX shows the results of a comparison of compounds 688 and 709 with neostigmine in the inhibition of the pseudocholinesterase of horse serum.

Compound 688, one of the less toxic carbamates selected for clinical trial, and compound 709 which was highly toxic and apparently one

of the most potent analgesics of the series, both possessed anticholinesterase activity. This activity is, however, negligible compared with that of neostigmine, and is much less than that of some *N*-substituted carbamates. The toxicity is therefore unlikely to be due to anticholinesterase activity.

Blood pressure and respiration. Intravenous injection of compound 688 produced a definite but inconsistent effect on the blood-pressure, the fall produced by 50 μg. being sometimes equivalent to that produced by 0·5 μg. of acetylcholine. The above doses of acetylcholine and compound 688 caused a slight rise in blood-pressure after injection of 0·25 mg. of atropine, further injection of which blocked the effect. Intravenous injection of 100 μg. produced no effect on respiration.

TABLE IX

ANTICHOLINESTERASE (PSEUDOCHOLINESTERASE) ACTIVITY OF TWO PHENOLIC CARBAMATES COMPARED WITH THAT OF NEOSTIGMINE

Compound	No. of tests	Inhibition per cent
Neostigmine (0·0002 mg./ml. in water) ..	6	87·8
709 (0·0002 mg./ml. suspended in water) ..	2	16·0
709 (0·5 mg./ml. in water)	1	86·3
709 (0·0002 mg./ml. in acetone)	1	7·2
709 (0·0004 mg./ml. in acetone)	1	10·3
709 (0·005 mg./ml. in acetone)	3	70·3
688 (0·005 mg./ml. in acetone)	2	23·6

For chemical formulae of compounds 709 and 688 see Table V. The activity of compound 709 is evidently $\geq$ 0·0004 of that of neostigmine.

DISCUSSION

The results of the analgesic tests by both the Woolfe–Macdonald and Bianchi tail-clip methods reveal that only the carbamates of 3-alkylphenols exhibit significant analgesic activity as determined by these tests. An attempt to correlate the effect of the orientation of substituent alkyl groups with analgesic activity was based on the activity of *m*-tolyl carbamate (compound 613). In 3-monoalkyl derivatives, maximum activity appeared at ethyl and thereafter activity fell off with increase in the size of the alkyl substituent until, with *m*-n-pentadecyl phenyl carbamate, only negligible activity remained. The activity of *m*-tolyl carbamate may be increased by the introduction of further alkyl substituents. Two *o*-substituents reduced the activity to negligible proportions, as in compounds 680, 696, 699 and 700. Introduction of two t-butyl groups (compound 701) also greatly depressed activity. The most active compound was 2,4,5-trimethylphenyl carbamate (compound 686); others with comparable activity are 2,5- and 3,4-dimethyl-, 2,3,5-trimethyl-, and 2,3,4,5-tetramethylphenyl carbamates, together with 2,5-dimethyl-4-ethylphenyl carbamate. Substitution of the carbamate group by any other radical than hydroxyl strongly reduced the activity. None of the allophanates exhibited significant activity. It is clear that under our conditions the Woolfe–Macdonald test is not adaptable to quantitative assays, but that the ED50 can be readily determined using the Bianchi test. In general the most active compounds are also very toxic, for example, compounds 686 and 690. The least toxic compound proved to be 613 with an

oral LD50 in mice of 1116 mg./kg. Compound 688, with an oral LD50 of 864 mg./kg., appeared to possess the best therapeutic ratio and was therefore selected for further pharmacological and clinical trial, to be reported elsewhere. Unfortunately it had little value in the treatment of pathological pain in man and this fact, combined with the results of a comparison with morphine and pethidine using the Woolfe–Macdonald test, suggests that this test alone is inadequate to assess a morphine-like analgesic. Our results were confirmed independently in another laboratory.

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