Brit. J. Pharmacol. (1957), **12**, 447.

PHARMACOLOGICAL PROPERTIES OF ESTERS OF 1-ALKYL-2-HYDROXYALKYLPYRROLIDINE AND THEIR QUATERNARY DERIVATIVES

BY

P. ACRED, E. M. ATKINS, J. G. BAINBRIDGE, D. M. BROWN, R. M. QUINTON AND D. TURNER

From the Pharmacology Department, Beecham Research Laboratories Ltd., Betchworth, Surrey, and C. L. Bencard Ltd., Park Royal, London

(RECEIVED MAY 13, 1957)

A series of esters of 1-alkyl-2-hydroxyalkylpyrrolidine and their quaternary derivatives have been shown to possess significant anti-acetylcholine activity. The benzilic acid esters were the most active, followed by xanthene-9-carboxylic acid, fluorene-9-carboxylic acid and diphenylacetic acid esters in that order. The quaternary derivatives were more active than their corresponding tertiary compounds both *in vivo* and *in vitro*. The most active compound of the series tested *in vivo* was (1-methylpyrrolid-2-yl)methyl benzilate methiodide and was as potent as atropine. There was a progressive decrease in anti-acetylcholine activity and a proportional increase in local anaesthetic activity as the number of carbon atoms was increased from 1 to 3 in the pyrrolidyl side-chain of the tertiary salts of the benzilic acid ester series. Likewise increasing the size of the group on the nitrogen atom led to a decrease in anti-acetylcholine activity and an increase in local anaesthetic activity. Quaternization of the tertiary salts resulted in a loss of local anaesthetic activity. Most of the compounds tested possessed some antihistamine properties, while papaverine-like activity was confined to the tertiary salts only. No significant neuromuscular blocking activity was evident.

There have been numerous attempts to modify the structure of atropine with a view to minimizing its undesirable side effects, and many simple esters have been prepared which have shown anti-acetylcholine activity. This paper describes the pharmacology of the esters of pyrrolidyl alcohols synthesized by Doyle, Mehta, and Sach (unpublished observations). Of these compounds only the benzilic and diphenylacetic acid esters of 1-methyl-2-hydroxymethylpyrrolidine have previously been reported (Blicke and Lu, 1955).

METHODS

Tests in Vitro

Anti-acetylcholine Action. — Segments, approximately 2 cm. long, were removed from the lower ileum of young guinea-pigs weighing not more than 250 g. and suspended in oxygenated Tyrode solution at 37° in a 5 ml. bath. Acetylcholine (ACh), which was allowed to act for 30 sec., was added at 2 min. intervals until constant responses were obtained. The doses of atropine and test compound were added 30 sec. before ACh and were adjusted so that the ACh response was reduced to approximately 30% and 70% of the original. Between the successive doses of antagonist the ACh response was allowed to return to normal. A 16-point assay was then performed using a Latin square design (Fisher and Yates, 1953) and the results analysed by the procedure of Schild (1942).

Segments 2 to 3 cm. long, cut from the mid-portion of the jejunum of young rabbits weighing approximately 1.5 kg., were suspended in Tyrode solution at 37°. An identical statistical procedure to that used for the guinea-pig ileum anti-ACh assay was employed. A 4 min. cycle was used. The antagonist was added 60 sec. before the ACh. Fresh rabbit jejunum often gave irregular responses to ACh for the first 1 or 2 hr.; regular responses were obtained more rapidly, without loss in sensitivity or accuracy, with gut which had been stored for 18 hr. at 7°.

Antihistamine Action.—Preparations of guinea-pig ileum were made as for the anti-acetylcholine test. Histamine was added at 90 sec. intervals until the responses were regular. The antagonist was then added to the bath 30 sec. before the histamine and the depression in responses compared with that obtained by a standard antihistamine drug. If the activity of the unknown exceeded that of the standard, a 16-point Latin square design assay was performed.

Papaverine-like Activity.—A strip of rabbit jejunum 2 to 3 cm. long was suspended in Tyrode solu-

tion at 37° in a 5 ml. bath. Spasm was induced with barium chloride (250 μg./ml.) and the spasmolytic added 2 min. later, while the barium chloride was still present. The compound was allowed to act for a further 2 min. and then washed out. The degree of relaxation of the barium spasm produced by the compound under test was compared with that produced by papaverine HCl. Two further washings were performed at 5 min. intervals during the 15 min. cycle employed.

Curare-like Activity.—The rat phrenic nerve-diaphragm preparation, as described by Bülbring (1946), was used.

Langendorff Heart.—The heart from a guinea-pig weighing approximately 400 g. was removed under ether anaesthesia and connected by an aortic cannula to a perfusion system. Oxygenated Locke solution containing $NaHCO_3$ 0.5 g./l. was allowed to flow through a warming coil to the heart. The perfusion pressure was maintained between 60 and 70 cm. water. Drugs were injected in 0.05 to 0.1 ml. Locke solution close to the heart. The amplitude and frequency were recorded and the rate of coronary outflow was measured by a Gaddum drop recorder.

Tests in Vivo

Acute Toxicity.—Acute intravenous toxicities were estimated in male albino mice (18 to 22 g.); 10 mice were used with each dose and the results were analysed according to the method described by Finney (1952).

Anti-acetylcholine Action on Cat Blood Pressure.—The method of Kühl (1925) was used. Cats were anaesthetized with ether followed by chloralose-urethane mixture (40 mg./kg. chloralose, 160 mg./kg. urethane intravenously). Blood pressure recordings were made from the carotid artery and injections were made through a polythene cannula inserted into the femoral vein. The compounds were dissolved in saline.

A dose of ACh (0.5 to 1.0 μg.), which gave a just submaximal depression in blood pressure, was administered intravenously at 2 min. intervals until a regular depression of the blood pressure was obtained. The % depression of the ACh response caused by a small intravenous dose of spasmolytic given 1 min. previously was measured and matched with that caused by a dose of atropine (0.2 to 5.0 μg.). Small doses were employed so that recovery was rapid and several compounds could be assayed on each animal. Each drug was tested on several animals and the results averaged.

Intestinal Motility.—Decerebrate cats were used. A midline stab incision was made in the abdomen. The intestine was drawn out through the incision and glass cannulae were inserted into the duodenum and jejunum or ileum. The cannulae were bent at right angles so that when in position the longer arm was vertical. A ligature was tied round the intestine 5 to 10 cm. from the cannula and the loop was filled with liquid paraffin to give an internal pressure of about 10 cm. water. The cannula was connected to an air tambour and the intestine replaced in the abdominal cavity. Handling of the intestine was reduced to a minimum. After 2 to 3 hr., when the effects of the anaesthetic had worn off, kymographic recordings of intestinal movements were made. Compounds were injected intravenously in saline through a cannula inserted in the femoral vein.

Anti-acetylcholine Activity on Guinea-pig Colon.—Guinea-pigs were anaesthetized with urethane (2 g./kg. subcutaneously), and a cannula was tied into the colon. Carbachol (1 to 2 μg./guinea-pig) was injected intravenously, at 3 to 5 min. intervals, through a cannula in the jugular vein until the intestine responded regularly by contraction. A single dose of compound was administered intravenously and two further injections of carbachol were made. If there was no response to these, the drug was considered to be active at the dose given. From 2 to 6 animals were used at each dose level. The activity was expressed in terms of minimal effective dose.

Mydriatic Activity.—Assays for mydriatic activity were carried out on the mouse according to the method of Pulewka as used by Ing, Dawes, and Wajda (1945). The pupil diameter was measured in arbitrary units with the aid of a micrometer scale set in the eyepiece of a dissecting microscope (×10). Compounds were given subcutaneously or orally to groups of 5 mice (18 to 22 g.). The pupil diameter was measured at 5 min. intervals over a period of 30 min. Atropine was used as a standard. The results were assessed at the time of maximum activity, which was usually 20 min. after administration.

Local Anaesthetic Activity.—The intradermal weal method described by Bülbring and Wajda (1945) and modified by Somers and Edge (1947) was used to determine local anaesthetic activity. Guinea-pigs weighing 400 to 450 g. were employed.

A modification of the corneal reflex method described by Chance and Lobstein (1944) was also used. Three dose levels of both test and standard compound were employed and 2 guinea-pigs were used for each dose. The solutions of the compounds were made in phosphate buffer at *p*H 7.3. The standard (procaine HCl) solution was applied to one eye and the test solution to the other. The cornea was then touched lightly with a thin bristle 6 times every 2 min. for 20 min., and the number of times that the animal failed to blink was noted. After a 4 hr. period, the same animals were used again in a crossover test. The results, expressed as % response, were plotted against log concentration and analysed according to the method described by Burn, Finney, and Goodwin (1950).

Salivary Flow.—The effect of compounds on salivary flow was estimated by the method employed by Issekutz (1917), as modified by Brown and Quinton (1957).

Gastric Secretion.—The method used was essentially that of Shay, Komarov, and Fels (1945). Six male albino rats weighing 100 to 200 g. were starved for 18 to 24 hr. in cages with wide-mesh wire bottoms to

prevent coprophagy. The duodenum was then ligated under ether anaesthesia and 4 ml. of warm saline was given intraperitoneally. Three rats were given 5 mg./kg. of the test compound subcutaneously, while the remaining three were given 1.0 ml. saline. The animals were returned to their cages and killed 6 hr. later. The oesophagus was ligated and the stomach was removed and cleared of adhering mesentery, dried with blotting paper, and the contents drained through a slit into the centrifuge tube. The gastric contents were centrifuged for 10 min. at approximately 4,000 rev./min. and the total volume and the volume of solid contents measured. 1 ml. of the supernatant was titrated with N/100 NaOH for free and total acid, Topfer's reagent being used as indicator.

Neuromuscular Blocking Action.—The decerebrate cat gastrocnemius-sciatic nerve preparation was used. Stimuli of 2 to 3 V., frequency 7/min., were applied to the sciatic nerve. Compounds at 1/20th and 1/10th of their respective intravenous LD50 values were administered in saline at 10 min. intervals through a cannula in the femoral vein.

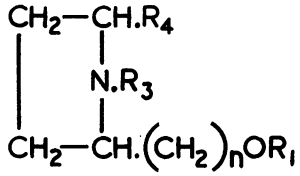
Results

Anti-acetylcholine Activity on Isolated Guinea-pig Ileum and Rabbit Jejunum.—The result of the anti-ACh activity of each compound on the isolated guinea-pig ileum (Table I) represents the weighted mean of at least four separate assays. The standard error of the combined mean values was calculated, giving on average a standard error of the order of 5% ($P=0.05$). At least two anti-ACh assays were also performed on the rabbit jejunum, and, as a rule, the relative potencies were less, generally by a factor of about 25%, than

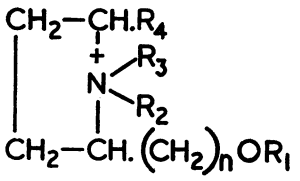
TABLE I

INTRAVENOUS TOXICITIES, ANTI-ACETYLCHOLINE AND SPASMOLYTIC ACTIVITIES OF A SERIES OF TERTIARY AND QUATERNARY SALTS OF PYRROLIDYL ALCOHOLS ESTERIFIED WITH VARIOUS ACIDS. ACID RADICAL R_1 DERIVED FROM DIPHENYLACETIC ACID (GROUP A); BENZILIC ACID (GROUP B); XANTHENE-9-CARBOXYLIC ACID (GROUP C); FLUORENE-9-CARBOXYLIC ACID (GROUP D)

The asterisk indicates an ester of the secondary base.



Tertiary esters



Quaternary esters

Ester	BRL No.	n	R_2	R_3	R_4	LD50 (intraven.) mg./kg.	Anti-ACh: Isol. G-Pig Ileum	Anti-ACh: Cat B.P.	Mydriasis (Atropine=1·0)	Saliv. Flow	Anti-Hist. (Antazoline SO_4=1·0)	Anti-$BaCl_2$ Spasm. (Papaverine HCl=1·0)
						Tertiary Esters						
A	373	2	—	CH_3	H	26·0	0·58	0·01	—	—	0·08	1·0
A	432	2	—	C_2H_5	H	15·0	0·22	0·01	—	—	0·04	1·0
B	485	1	—	CH_3	H	32·5	0·65	0·10	0·35	0·02	1·0	1·0
*B	539	2	—	H	H	27·0	0·61	0·01	<0·05	0·04	0·02	0·4
B	416	2	—	CH_3	H	28·5	0·99	0·10	0·19	0·06	0·13	0·75
B	433	2	—	C_2H_5	H	15·4	0·64	0·1	0·13	0·05	0·02	0·8
B	529	2	—	C_3H_7	H	10·5	0·18	0	<0·05	0·01	1·0	2·0
B	447	2	—	CH_3	CH_3	15·5	0·36	0·01	<0·05	0·01	0·15	1·0
B	490	3	—	CH_3	H	11·5	0·12	0	0·1	0	0·01	1·0
C	448	2	—	CH_3	H	24·5	0·93	0·01	—	—	0·15	1·0
D	417	2	—	CH_3	H	21·5	0·48	0·01	—	—	0·20	1·5
						Quaternary Esters						
A	424	2	CH_3	CH_3	H	3·0	0·45	0·03	—	—	0·1	0
B	499	1	CH_3	CH_3	H	21·5	1·02	1·0	1·09	2·03	0·08	0
B	526	1	CH_3	C_2H_5	H	14·0	1·29	0·5	1·03	0·42	0·06	0
B	425	2	CH_3	CH_3	H	22·0	2·02	0·15	0·17	0·69	0·1	0
B	481	2	CH_3	C_2H_5	H	13·0	2·72	0·40	1·33	0·95	0·15	0
B	494	2	C_2H_5	C_2H_5	H	10·0	0·23	0·06	0·25	0·06	0·03	0
B	521	2	CH_3	C_3H_7	H	8·9	0·24	0·12	0·28	0·19	0·01	0
B	454	2	CH_3	CH_3	CH_3	12·0	0·09	0	<0·05	0·01	0·01	0
B	564	3	CH_3	CH_3	H	5·4	0·53	0	<0·15	0·02	0	0
C	453	2	CH_3	CH_3	H	4·7	1·18	0·2	—	—	0·01	0
D	438	2	CH_3	CH_3	H	4·4	0·45	0·07	—	—	—	0
Atropine						82·0	1·00	1·0	1·0	1·0	0·03	0
Atropine methonitrate						8·8	0·87	1·5	—	1·65	0	0
Homatropine						84·0	0·26	—	—	0·01	0·01	0
Oxyphenonium						16·0	0·64	0·4	0·93	0·62	0	0
Methantheline						6·6	1·73	0·25	0·12	—	0·01	0
Propantheline						3·9	2·09	1·0	0·6	1·13	0·15	0

those obtained on the guinea-pig ileum. The most active compound tested on isolated guinea-pig ileum was β-(1-methylpyrrolid-2-yl)ethyl benzilate methiodide (BRL 481). On the other hand (1-methylpyrrolid-2-yl)methyl benzilate (BRL 485) was the most active compound of the series on the rabbit jejunum. On this tissue, however, methantheline was the most active of all the compounds tested. The quaternary derivatives were more active than their corresponding tertiary salts.

Antihistamine, Papaverine-like, and Curare-like Actions in Vitro.—Most of the compounds showed some detectable antihistamine action, compounds BRL 485 and BRL 529 having activities equal to that of antazoline sulphate. Most of the tertiary salts exhibited a papaverine-like action against barium-chloride-induced spasms of the rabbit jejunum equal to that of papaverine HCl. Compound BRL 529 was the most potent, being twice as active as papaverine. Quaternization destroyed or greatly reduced this property. Potencies less than 0.05 of papaverine HCl were taken as nil.

No significant curare-like action could be detected in any of the compounds.

Isolated Mammalian Heart.—Atropine in single doses up to 100 μg. usually had no effect on the amplitude, or was slightly stimulant; it had no effect on the rate or coronary flow of the perfused heart. In doses over 20 μg. the tertiary amine esters of benzilic acid appeared to have negative inotropic and chronotropic actions, which were particularly marked with BRL 416. A dose of 50 μg. of this compound usually produced a pronounced decrease in all cardiac functions leading to complete ventricular arrest. The secondary base BRL 539 and the ethyl-compound BRL 433 were equally depressant, whereas the short- and long-chain homologues BRL 485 and BRL 490 showed less activity in this respect. The quaternary amines had generally no action or a slight positive inotropic effect with high doses.

Toxicities.—The acute intravenous toxicities of the compounds are given in Table I. In general there was an increase in toxicity with increase in length of the side chain attached to the pyrrolidine nucleus and with the size of the group attached to the nitrogen atom. Quaternization of the tertiary compounds invariably led to greater toxicity, although the tertiary salts showed signs of possessing central stimulant properties absent from the quaternary derivatives.

BRL 485 and BRL 416 were the least toxic of the tertiary salts, while BRL 499 and BRL 425, their methyl quaternary analogues, were approximately one and a half times as toxic, but were the least toxic of the quaternary salts. On the other hand, atropine methonitrate was much more toxic than atropine.

Anti-acetylcholine Effect on Blood Pressure.—The results obtained are given in Table I. Compound BRL 499 was the most active of the compounds tested in the BRL series and was equal to propantheline in this respect. Compounds BRL 526 and BRL 481 had also considerable activity. The recovery from most of the compounds followed much the same pattern as after atropine.

Gastro-intestinal Motility.—There was no apparent relationship between the effect of drugs on the gastro-intestinal motility of the cat and their action in antagonizing acetylcholine on the blood pressure of the cat. An inhibitory response of the intestine was usually obtained from the first injection of the drug, but the response to a second injection was variable. In many experiments a second response was frequently not obtained and the gut became completely refractory to anti-ACh drugs. This made the interpretation of results and a direct comparison of activities difficult. We also found that the charcoal meal test in mice gave no clear guide to intestinal inhibition by anti-ACh drugs.

Anti-acetylcholine Action on Guinea-pig Colon.—In view of the wide variability obtained with the preceding method and the uncertainty of correct interpretation, it was decided to use carbachol as an intestinal stimulant, and to note the depressant effect of compounds on this response. Difficulties were again experienced in that the intestines of some animals were found to be completely resistant to carbachol, even though profuse salivation occurred in response to the injections. Furthermore, a response to carbachol tended to appear and disappear spontaneously during the course of the experiment. For this reason the experiments were designed to be of short duration, and all animals which failed to respond to an initial dose

TABLE II

MINIMUM EFFECTIVE INTRAVENOUS DOSE REQUIRED TO INHIBIT CARBACHOL-INDUCED CONTRACTION OF GUINEA-PIG COLON

Compound	Minimum Effective Dose (μg./kg.)
Atropine	8
Oxyphenonium	32
Propantheline	Variable 16 to 64
BRL 485	Variable
BRL 499	8
BRL 526	64
BRL 416	64
BRL 425	16
BRL 433	64
BRL 481	32
BRL 521	64

of carbachol were discarded. The results obtained with the more active compounds of the benzilic acid series (group B) are given in Table II. A typical response to carbachol and its inhibition by an anti-ACh drug (BRL 499) is seen in Fig. 1. This compound proved to be the most active synthetic compound tested.

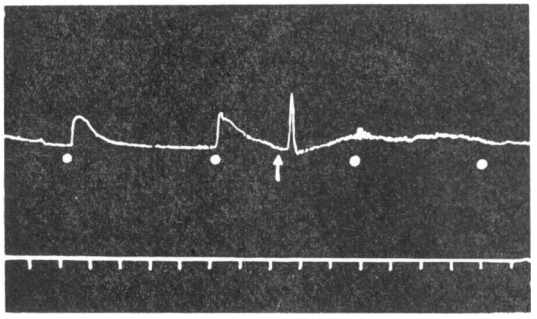


FIG. 1.—Recording of the response of the guinea-pig colon following 1 μg. carbachol administered intravenously at the dots and the inhibition produced by compound BRL 499 (16 μg./kg.) given at the arrow. Time, 1 min.

Mydriasis.—All the benzilic acid esters were tested for their mydriatic action. By the subcutaneous route compounds BRL 481, BRL 526 and BRL 499 were all of the same order of activity as atropine. Oxyphenonium and propantheline were somewhat less active and all the other members of the BRL series were much less active.

Given orally, only the tertiary salts showed regular absorption and gave repeatable results, but none was as active as atropine. The ratio of potencies of BRL 485 and BRL 416 to atropine between the subcutaneous and oral routes was, however, of the same order. The quaternary salts were absorbed erratically and required a dose of the order of 100 times the subcutaneous dose to show an effect. Even with such a dose, no significant absorption occurred in a considerable % of animals.

Local Anaesthetic Activity.—Selected compounds were tested for local anaesthetic activity, which was found in tertiary salts but not in quaternary salts. The results are given in Table III.

Salivary Flow.—The regression for inhibition of salivation by atropine against log dose was linear in two sets of 8 rabbits. Dose-response curves were also found for compounds BRL 416 and BRL 425 on the first set of rabbits and for compound BRL 499, oxyphenonium, propantheline and atropine methonitrate on the second set of rabbits. Since none of the curves obtained showed

TABLE III

COMPARISON OF LOCAL ANAESTHETIC ACTIVITY OF TERTIARY SALTS WITH PROCAINE HCl

The effect of increasing the number of carbon atoms in side chain, the size of group on the nitrogen atom, and of altering the acidic radical is recorded. See Table I for explanation of n, R_3, and R_4.

Compound	n	R_3	R_4	Corneal Reflex (Procaine HCl=1)	Intradermal Weal (Procaine HCl=1)
BRL 485 ..	1	CH_3	H	2·05	0·2 to 0·5
BRL 416 ..	2	CH_3	H	16·5	2·9
BRL 490 ..	3	CH_3	H	29·3	4·9
BRL 433 ..	2	C_2H_5	H	26·6	8·6
BRL 447 ..	2	CH_3	CH_3	28·6	
BRL 529 ..	2	C_3H_7	H	36·5	7·4
BRL 539 ..	2	H	H	0·2 to 0·5	
BRL 373 ..	2	CH_3	H	13·8	

significant deviation from parallelism or linear regression, it was decided to estimate activity at one dose level and to assume that the dose-response line passing through the point obtained would run parallel to the standard atropine curve. The dose which gave a 50% depression in response (ED50) was found by interpolating on the line so obtained. The relative potencies are given in Table I.

Gastric Secretion.—A quantitative assay was impractical owing to the large variation in the amount of acid secreted in untreated rats. The activities have, therefore, been measured in terms of an " all or none " response. All the compounds tested produced a significant reduction ($P=0.05$) in the volume of gastric secretion, except compounds BRL 485, BRL 447, BRL 454, BRL 481, and BRL 417.

Cat Gastrocnemius-sciatic Preparation.—None of the compounds had any significant action on the response of the gastrocnemius muscle to sciatic nerve stimulation.

DISCUSSION

All the compounds of the BRL series showed anti-ACh activity both *in vitro* and *in vivo*. On the basis of the *in vitro* comparative assays, the benzilic acid esters were the most active, followed by xanthene-9-carboxylic acid, fluorene-9-carboxylic acid and diphenylacetic acid esters, in that order.

Detailed study of the benzilic acid esters showed that maximum activity on the guinea-pig ileum occurred when the carbon side-chain attached to the pyrrolidine nucleus contained two carbon atoms. When the side-chain was kept constant, an increase in the size of the group on the nitrogen atom resulted in a proportional decrease in *in vitro* anti-ACh activity.

There was little difference between the tertiary compounds containing one or two carbon atoms in the side-chain in their antagonism of the vaso-depressor action of ACh, although the shorter chain compound appeared to be more active as a mydriatic and the two carbon chain compound more active as an anti-salivatory agent. The three carbon chain compound was, however, much less active as an anti-ACh agent. The short chain compound tended to be somewhat stimulatory and produced variable results against carbachol stimulation of the guinea-pig colon. The precise activity could not be measured, but appeared to be much less than that shown by the two carbon chain compound. The three carbon compound had little activity.

A feature of the tertiary derivatives was their local anaesthetic activity, the quaternary compounds being completely inactive in this respect. As the anti-ACh action decreased, the local anaesthetic activity increased in proportion to the number of carbon atoms in the side-chain or attached to the nitrogen. Relative to procaine hydrochloride the compounds were much more active on the corneal reflex test than by the intradermal weal test, indicating that they penetrate mucous membranes. On the corneal reflex β-(1-ethylpyrrolid-2-yl)ethyl benzilate (BRL 433), however, appeared to be more active than β-(1-*n*-propylpyrrolid-2-yl)ethyl benzilate (BRL 529), which has a larger group on the nitrogen. This discrepancy is probably due to the error of the assay, which, by the nature of the test, was large.

All the tertiary salts were active orally. This was well shown by the oral mydriatic test. In mice, absorption from the alimentary tract was poor and erratic with all quaternary derivatives. Tests in human volunteers, however, indicated that BRL 499 was regularly absorbed at a dose of 5 mg. after oral administration.

The quaternary compounds having the same groups on the nitrogen atom showed maximum anti-ACh activity *in vitro* and *in vivo* when the side-chain contained only one carbon atom. (1-methyl-2-pyrrolid-2-yl)methyl benzilate methiodide (BRL 499) was the most active of all the compounds tested *in vivo*, and in every test was as potent as atropine. When the side-chain of the quaternary compounds was kept constant and the alkyl groups on the nitrogen atom increased in size, maximum *in vitro* activity occurred with the methyl-ethyl-derivatives (BRL 481 and BRL 526). In the two carbon chain series the corresponding methyl-propyl-compound (BRL 521) was comparatively much less active and had the same order of activity as the diethyl-compound (BRL 494). *In vivo*, the relative potencies of this series on salivary flow and mydriasis were greater than those for their action against ACh on the cat blood pressure and against carbachol-induced stimulation of the guinea-pig colon. In this latter test, however, activity was maximal with the dimethyl-compound (BRL 425).

The substitution of a methyl group in the 5-position in the pyrrolidine ring greatly reduced anti-ACh activity, but did not appear to influence local anaesthetic activity. Anti-ACh activity appears to depend, therefore, on the overall size of the group or groups on the nitrogen atom, on the length of the side-chain, and on the absence of other substituents in the pyrrolidine nucleus.

The authors wish to thank Dr. D. O. Holland for his interest and helpful discussions and Miss J. Sutcliffe and Mr. R. Burton for their assistance.

References

Blicke, F. F., and Lu, C. J. (1955). *J. Amer. chem. Soc.*, **77**, 29.
Brown, D. M., and Quinton, R. M. (1957). *Brit. J. Pharmacol.*, **12**, 63.
Bülbring, E. (1946). Ibid., **1**, 38.
—— and Wajda, I. (1945). *J. Pharmacol.*, **85**, 78.
Burn, J. H., Finney, D. J., and Goodwin, L. G. (1950). *Biological Standardisation*. London: Oxford University Press.
Chance, M. R. A., and Lobstein, H. (1944). *J. Pharmacol.*, **82**, 203.
Finney, D. J. (1952). *Probit Analysis*. London: Cambridge University Press.
Fisher, R. A., and Yates, F. (1953). *Statistical Tables for Agricultural, Biological and Medical Research*, 4th ed., p. 70. Edinburgh: Oliver and Boyd.
Ing, H. R., Dawes, G. S., and Wajda, I. (1945). *J. Pharmacol.*, **85**, 85.
Issekutz, B. (1917). *Zeitschr. f. Exp. Path. u. Therap.*, **19**, 99.
Kühl, G. (1925). *Arch. exp. Path. Pharmak.*, **109**, 295.
Schild, H. O. (1942). *J. Physiol.*, **101**, 115.
Shay, H., Komarov, S. A., and Fels, S. S. (1945). *Gastroenterology*, **5**, 43.
Somers, G. F., and Edge, N. D. (1947). *Quart. J. Pharm. Pharmacol.*, **20**, 380.