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PHARMACOLOGY OF BENZILIC ACID DERIVATIVES

BY

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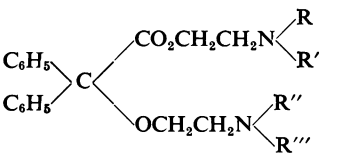
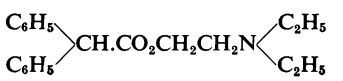
In this paper the results are presented of a pharmacological study of a new series of benzilic esters (" Ro- series ") of type I related in structure both to benadryl (II), one of the early antihistamine drugs (Loew, Kaiser, and Moore, 1945), and to trasentin (III), an established general spasmolytic drug (Meier, 1936 ; Johnson and Reynolds, 1937 ; Goodman and Gillman, 1941). They do not contain the *cyclo*hexane ring present in trasentin-6H. Table I shows the 11 members of the series. The synthesis of these compounds has been described elsewhere (Morrison, Königstein, and Cohen, 1950).



(I)

(II)



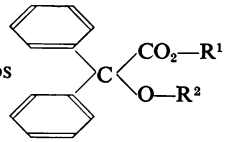
(III)

METHODS

Isolated intestine.—Isolated guinea-pig ileum, suspended in a 2.5-ml. bath (Barsoum and Gaddum, 1935), was used in screening for antihistamine and other spasmolytic activity. A Wellcome " Agla " all-glass micro-syringe, fitted with " Agla " micrometer attachment, was used to inject small volumes (0.005–0.01 ml.) of stimulating drug. Tests on isolated rabbit intestine were made in a 50-ml. organ bath. A light frontal writing isotonic lever was used, its total angle of excursion not exceeding 30 degrees.

Isolated lung.—Guinea-pig lung was used, the air passages being perfused with the medium described by McDowall and Thornton (1930). The animals were anaesthetized with ether to the point of respiratory arrest, and then killed by opening the thorax. Perfusion was made through an isolated heart cannula inserted into the trachea, fluid escaping through scarifications of the lung lobe. One lung only was perfused, the other being tied off at the bronchus. Preheating and perfusion were carried out by means of a mammalian heart perfusion apparatus. The temperature was maintained at 38° C. (±0.5°). Between each addition of drug, the lung was washed by forcing fluid through the alveoli under a pressure of 80 mm. Hg. For this purpose a 5-ml. " Record " syringe was attached to the upper end of the trachea by a glass T-piece. Records were made at a constant pressure of 40 mm. Hg. Antihistamine activity was

TABLE I

RO- COMPOUNDS 

Serial No.	R^1	R^2	Name of compound
3-0131	$CH_2CH_2N(C_2H_5)(C_2H_5)$	$CH_2CH_2N(CH_3)(CH_3)$	Diethylaminoethyl diphenyl-(dimethylaminoethoxy)-acetate
3-0190	$CH_2CH_2N(CH_3)(CH_3)$	$CH_2CH_2N(CH_3)(CH_3)$	Dimethylaminoethyl diphenyl-(dimethylaminoethoxy)-acetate
3-0255	CH_2CH_2N(morpholine)	CH_2CH_2N(morpholine)	β-morpholinoethyl diphenyl-(β′-morpholinoethoxy)-acetate
3-0275	$CH_2CH_2N(CH_3)(CH_2CH:CH_2)$	$CH_2CH_2N(CH_3)(CH_3)$	Allylmethylaminoethyl diphenyl-(dimethylaminoethoxy)-acetate
3-0257	$CH_2CH_2N(C_2H_5)(C_2H_5)$	CH_2CH_2N(morpholine)	Diethylaminoethyl diphenyl-(β-morpholinoethoxy)-acetate
3-0277	CH_2CH_2N(piperidine)	$CH_2CH_2N(CH_3)(CH_3)$	Piperidinoethyl diphenyl-(dimethylaminoethoxy)-acetate
3-0276	$CH_2CH_2N(CH_2CH:CH_2)(CH_2CH:CH_2)$	$CH_2CH_2N(CH_3)(CH_3)$	Diallylaminoethyl diphenyl-(dimethylaminoethoxy)-acetate
3-0280	CH_2CH_2N(morpholine)	$CH_2CH_2N(CH_3)(CH_3)$	β-morpholinoethyl diphenyl-(β-dimethylaminoethoxy)-acetate
3-0281	$CH(CH_3)CH_2N(C_2H_5)(C_2H_5)$	$CH_2CH_2N(CH_3)(CH_3)$	β-diethylamino*iso*propyl diphenyl-(β-dimethylaminoethoxy)-acetate
3-0282	$CH_2CH_2N(CH_3)(CH(CH_3)_2)$	$CH_2CH_2N(CH_3)(CH_3)$	β-*iso*propylmethylaminoethyl diphenyl-(β-dimethylaminoethoxy)-acetate
3-0289	$CH_2CH_2N(C_2H_5)(C_2H_5)$	$CH_2CH_2N(CH_3)(CH(CH_3)_2)$	Diethylaminoethyl diphenyl-(*iso*propylmethylaminoethoxy)-acetate

expressed as percentage diminution of a standard histamine bronchospasm occurring in the presence of 0.5 mg. of each test compound.

Isolated heart.—Isolated rabbit hearts, perfused through the coronary vessels with Ringer-Locke solution at 38° C., were used.

Anaphylactic shock.—Guinea-pigs of varying age, weight, and sex were sensitized to egg-white as described by Landau, Marriott, and Gay (1948), and compounds were tested for their ability to protect them against anaphylactic shock.

General pharmacology.—Cats under chloralose anaesthesia were used in studying the effects of the drugs on blood pressure, spleen volume, and knee-jerk. Blood pressure was recorded from the carotid artery by mercury manometer and changes in spleen volume by plethysmograph and piston recorder. Knee-jerk was recorded by the method of Schweitzer and Wright (1937). Local anaesthetic activity was tested on the skin of the guinea-pig (Bülbring and Wajda, 1945); curariform activity on the isolated rat phrenic nerve-diaphragm preparation (Bülbring, 1946); analgesic activity in mice by the method described for rats by Davies, Raventos, and Walpole (1946); toxicity was determined by subcutaneous injection.

Assay of spasmolytic and antihistamine action.—Quantitative assays of spasmolytic activity were made on guinea-pig ileum and comparative activities against histamine, acetylcholine, potassium, and barium expressed in terms of the pA_2 scale (Schild, 1947). Schild's experimental procedure, however, was not applicable when potassium or barium was used as spasmogen, and was not therefore generally used for antihistamine and anti-acetylcholine assays, as it was considered essential to compare all forms of spasmolytic action on the basis of a common assay. The pA value was computed as $pA = -\log[A]$, where [A] is the molar concentration of antagonistic drug which produces a 50 per cent inhibition of a standard contraction induced by the spasmogen. A fixed time of contact (1 minute) was allowed between the introduction of the antagonist and of the spasmogen into the bath. Benadryl was used as standard antihistamine, atropine as standard anti-acetylcholine, and trasentin and papaverine as standards for anti-potassium and anti-barium activities respectively.

In the assay of antihistamine activity on the guinea-pig intestine, Schild's assay procedure was used for some of the compounds.

RESULTS

Anti-acetylcholine activity

The compounds were all active in diminishing acetylcholine spasm in the guinea-pig ileum, though much less active than atropine. The results are shown in Table II, in which values for anti-acetylcholine activity measured at *p*H 8.0 are given in descending order. It was later found that the anti-acetylcholine activity of 3-0131 was very sensitive to alkali, storage of its solutions at *p*H 8.0 leading to rapid breakdown and inactivation. Its relative activity as recorded here was therefore not in accord with subsequent experiments. The tracings shown in Fig. 1 demonstrate yet another effect of *p*H upon the activity of 3-0131. It will be seen that, as *p*H diminished from 6.8 to 4.8, the same dose of compound produced an effect varying from slight to considerable inhibition of the acetylcholine spasm. Here the *p*H values refer to that of the drug solution *before* injection into the bath. The bath *p*H, after adding the drug solution, was not altered within the range of 0.1 *p*H unit. This effect was not related to storage, since the solutions were freshly prepared and were acidic, in which condition only very slow decomposition occurred.

TABLE II

ANTI-ACETYLCHOLINE ACTIVITY OF RO- COMPOUNDS COMPARED WITH ATROPINE ON GUINEA-PIG ILEUM

Ro- compound No.	Dose μg. $\equiv$ 0.005 μg. atropine sulphate	Activity: atropine = 1.0
3-0190	2.0	0.0025
-0275	2.0	0.0025
-0277	3.0	0.0017
-0281	3.0	0.0017
-0289	3.0	0.0017
-0257	3.5	0.0014
-0276	4.0	0.0013
-0282	5.0	0.0010
-0131	13.0	0.0004
-0280	20.0	0.0003
-0265	>100.0	<0.0001

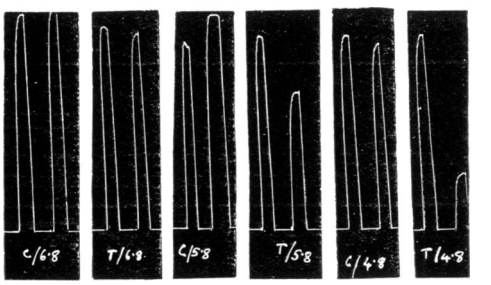


FIG. 1.—Effect of *p*H upon anti-acetylcholine action of Ro-0131. Read from left to right. In each group of two contractions, the first represents an ordinary acetylcholine contraction, the second an acetylcholine contraction in the presence of injected saline (controls marked "C") or in the presence of 5 μg. 3-0131 (tests marked "T"). Numbers refer to *p*H of the added solution.

pA values

Since it has been shown (Fig. 1) that spasmolytic activity in the compounds was greater when the *p*H of the solution was on the acid side, pA_2 values were determined with solutions of the hydrochlorides at *p*H 5.0–6.0. Doses of spasmogens were: acetylcholine, 0.1 μg. base; histamine, 0.1 μg. base; potassium chloride, 2.0 mg.; barium chloride, 1.0 mg. Results of these assays are given in Table III. All the Ro- compounds show some spasmolytic activity, including antihistamine action, on the isolated guinea-pig ileum. As a spasmolytic agent 3-0131 compares favourably with trasentin, but is a poor antihistamine compared with benadryl.

Antihistamine activity of the most active compounds was more accurately assayed by the method of Schild (1947). Results are given in Table IV, values for pA_2 representing the average of four determinations. All the Ro- compounds tested were considerably less active as antihistamines than benadryl and mepyramine. Trasentin, given a sufficiently long time of contact, is also more active as an antihistamine than the Ro- compounds tested similarly.

Further studies of antihistamine action

The values in Table V represent percentage reduction of histamine bronchospasm (due to 0.2 mg. histamine) produced by 0.5 mg. of each compound. The test substance in 0.05 ml. at *p*H 1.0 was introduced into the perfusion stream.

TABLE III

SPASMOLYTIC ACTIVITY OF RO- COMPOUNDS ON GUINEA-PIG ILEUM, COMPARED WITH TRASENTIN, BENADRYL, AND PAPAVERINE, EXPRESSED AS pA_2 UNITS

Compound No.	Anti-acetylcholine pA_2	Anti-histamine pA_2	Anti-potassium pA_2	Anti-barium pA_2
3-0131	6.2	5.7	4.8	4.6
-0190	6.0	5.1	4.5	4.1
-0265	4.8	3.8	4.0	3.8
-0275	6.0	5.0	4.8	5.0
-0257	5.7	4.6	4.6	4.3
-0277	6.1	5.5	4.7	5.1
-0276	5.6	5.4	5.0	4.7
-0280	4.8	4.5	4.1	4.0
-0281	5.8	5.4	5.0	4.7
-0282	6.0	5.0	4.4	4.4
-0289	5.9	5.4	4.9	4.3
Trasentin	5.9	5.4	4.4	4.5
Benadryl	5.7	7.4	4.3	4.5
Papaverine	—	—	—	3.9

TABLE IV

ANTIHISTAMINE ACTIVITY OF SOME RO- COMPOUNDS COMPARED WITH THAT OF BENADRYL, TRASENTIN, AND NEOANTERGAN USING SCHILD'S METHOD OF ASSAY

Compound No. or name	Time for maximum action (min.)	pA_2 (anti-histamine)
3-0131	5	6.9 (±0.1)
-0190	5	6.9 (±0.1)
-0275	10	5.7 (±0.2)
-0277	15	6.6 (±0.2)
-0276	10	6.0 (±0.4)
-0280	12	6.2 (±0.1)
-0282	7	5.9 (±0.1)
Trasentin	15	7.7 (±0.1)
Benadryl	11	8.6 (±0.1)
Neoantergan	7	9.8 (±0.1)

An equal volume of 0.1 N-HCl similarly injected did not affect the action of histamine on the lung. A tenth the quantity of benadryl (0.05 mg.) produced 67 per cent inhibition of the histamine effect; from this it has been calculated that benadryl is 7.6 times as active as 3-0131, the most active member of the series under test.

Further investigation of this compound was carried out on the guinea-pig in anaphylactic shock. The protection of guinea-pigs against death from anaphylactic shock was assessed for benadryl and for 3-0131 (Table VI). Though the number of animals used was small, it is concluded that 3-0131 is less active than benadryl in protecting guinea-pigs against anaphylactic shock. There are now available a number of antihistamines of greater activity than benadryl, so that 3-0131 may be classified as a weak antihistamine.

TABLE V

ANTIHISTAMINE ACTIVITY OF THE RO- COMPOUNDS AS ESTIMATED ON THE ISOLATED GUINEA-PIG LUNG

Ro- compound No. (in order of activity)	Percentage inhibition of histamine effect
3-0131	88 (±10)
-0277	59 (±10)
-0281	51 (±10)
-0276	47 (± 9)
-0289	46 (± 9)
-0190	25 (± 5)
-0282	24 (± 5)
-0280	23 (± 5)
-0275	No action
-0257	,, ,,
-0265	,, ,,

TABLE VI

COMPARISON OF RO-0131 WITH BENADRYL FOR ABILITY TO OPPOSE ANAPHYLACTIC SHOCK IN THE GUINEA-PIG

Compound	Dose in mg./kg. base	No. of animals shocked	No. of deaths	Percentage survival
None	—	10	9	10
Benadryl (as hydrochloride)	3	4	2	50
3-0131 (as hydrochloride)	3	4	4	0
3-0131 (as hydrochloride)	15	6	2	67
3-0131 (as salicylate)	15	10	3	70

TABLE VII

LOCAL ANAESTHETIC ACTIVITIES OF SOME RO- COMPOUNDS IN TERMS OF PROCAINE, ASSAYED BY GUINEA-PIG WEAL METHOD

Compound	Benadryl	Trasentin	3-0281	-0257	-0131	-0190	-0280	-0265	-0282
Local anaesthetic activity (procaine = 1) ..	0.68	0.86	>2.40	2.40	0.68	0.67	0.58	0.35	0.29

Local anaesthetic action

A representative number of the compounds were tested for local anaesthetic action, and the results are given in Table VII. The relative potencies were computed from dose-response curves. Since these were approximately parallel, the dose of each compound producing a median response (failure to respond to 18 out of 36 pricks) gave adequate comparison. Procaine was used as standard. All the compounds tested were local anaesthetics, 3-0281 and 3-0257 being more than twice as active as procaine.

Effects on blood pressure, spleen volume, and knee-jerk

Doses of 5 mg. of each compound injected into a cat under chloralose anaesthesia produced a steep but transient fall in blood pressure, which was not abolished by atropine. Trasentin and benadryl produced a similar effect. There were no significant effects on the knee-jerk response or on spleen volume. Blood pressure effects are shown in Table VIII. Benadryl differed from the Ro- compounds and trasentin in that it caused a transient fall in arterial pressure, followed by a sustained rise.

TABLE VIII

FALL IN BLOOD PRESSURE IN THE CAT (CHLORALOSE) DUE TO 2 MG./KG. OF EACH RO- COMPOUND INTRAVENOUSLY

Compound No.	B.P. fall in mm. Hg ± 10	Compound No.	B.P. fall in mm. Hg ± 10
3-0281, -0289	120	3-0190, -0282, -0276, -0257	50
-0131	80		
-0277, -0280	60	-0265	20

Effects on isolated rabbit heart

Compounds diminished the force of contraction of the isolated perfused rabbit heart, with no marked change in rate. The amplitude of the recorded beat greatly diminished, and cardiac action approached arrest in diastole. This effect was produced with relatively large amounts of each compound (0.5–1.0 mg.).

Effects on the isolated rabbit duodenum

The compounds arrested pendular movement and diminished tonus in the isolated rabbit duodenum. Concentrations of one part in 50,000 of all the Ro- compounds, excepting 3-0265, produced this effect.

Pharmacological tests with negative results

When tested for curariform activity, compounds 3-0131, -190, and -289 showed only a slight effect due to depression of the muscle itself. There was no demonstrable analgesic activity when compounds were injected subcutaneously into mice. When 3-0131 was combined with acetylsalicylic acid to form the corresponding salt, the analgesic activity of the product, injected intravenously into mice, was less than that of the acetylsalicylic acid alone.

Toxicity tests

The acute toxicity of 3-0131 was determined in some detail, as this drug was selected for clinical trial. The LD50 (subcutaneous in mice) was 475 mg./kg. Two other compounds—3-0190 and 3-0277—were tested elsewhere and found to be somewhat more toxic, the LD50 values being 300 and 350 mg./kg. respectively. Groups of 10 mice were used for each dose level, and log. dose-mortality curves were plotted. One half the LD50 of 3-0131, injected at intervals of 1–3 days over a period of 21 days, did not produce any signs of chronic toxicity.

Effects observed during administration of regular doses of 40 mg. three times daily in man for one week (two subjects) included nervousness, diminished appetite, and nausea, the latter after a period of 2–3 days. No other ill effects were observed. Single doses of 100 mg. were well tolerated by normal individuals.

In mice dying from the toxic action of this compound, the earliest signs were those of respiratory distress, with cyanosis visible about the mouth and nose. Very soon after there were convulsions and death would ensue, preceded by the animals leaping into the air. The trunk of the animal at death was usually in a state of extreme flexion, the mouth being wide open and jaws rigid. There was often marked exophthalmos and salivation. The general condition was suggestive of asphyxia, and toxic doses of the compound interfere with respiration in some manner that has not been determined.

Discussion

A series of compounds in which the chemical structure of benadryl and trasentin was combined has been found to possess similar pharmacological effects to these two drugs. The exact chemical hybrid of benadryl and trasentin (3-0131) shows a loss of specific antihistamine activity when compared with benadryl and a gain in general spasmolytic activity (anti-acetylcholine, anti-potassium, and anti-barium) by comparison with trasentin. The other homologues are in general less effective, either as antihistamines or as general spasmolytics.

The nature of the substituent on the nitrogen atoms does not greatly affect activity within the series, except in 3-0265. Here the presence of two morpholino-rings has rendered this compound less active than other members of the series, in all respects. More detailed study of 3-0131 has shown that this compound is unlikely to be of practical value purely as an antihistamine, but its low toxicity and high general spasmolytic activity make it worthy of clinical trial in allergy and in conditions involving smooth muscle spasm. The ability of the compound to lower arterial pressure in the cat, in contrast to benadryl, which raises arterial pressure, may prove to be a practical advantage if this difference holds true for man. Indications of the clinical value of 3-0131 have already been reported by Norman and Wrigley (1948).

On the basis of assays carried out on the guinea-pig gut, most of the compounds show higher anti-barium spasmolytic activity than papaverine. It is not, however, justifiable to argue from this that the Ro- compounds will be more useful than papaverine as spasmolytic drugs. Local anaesthetic activity is shared by these compounds in common with a number of other basic drugs. This is in accordance with the work of Burn and collaborators (Burn, 1948). Compounds 3-0257 and -281 are more active local anaesthetics than procaine. Curariform activity is absent, even with high concentrations of the drugs tested.

Summary

1. Eleven benzilic acid derivatives structurally related to benadryl and trasentin were found to possess varying degrees of general spasmolytic, antihistamine, and local anaesthetic properties. Their antihistamine activities were low compared with benadryl and mepyramine, but anti-acetylcholine activity was, in several compounds, higher than that of trasentin. Two compounds (3-0257 and 3-0281) are more potent local anaesthetics than procaine, being more than twice as active.

2. All compounds lower arterial blood pressure in the cat. The effect is not abolished by atropine.

3. The presence of a morpholino group in the molecule was found to diminish all types of pharmacological action, but no relationship of structure to activity was apparent for other substituents.

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