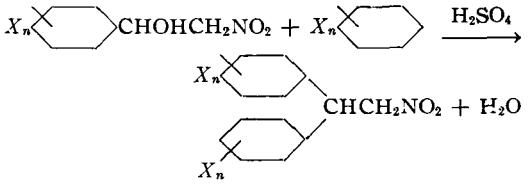
Antibacterial Properties of a Series of Diarylalkylammonium Chlorides*,†

By E. B. HODGE, M. C. BACHMAN, and M. B. NEHER‡

The preparation and properties (including bacteriostatic activity) of a series of diarylalkylammonium chlorides are described. Several of these compounds are active bacteriostatic agents with wide bacterial spectrums. Small changes in the structures of the compounds in the series cause marked changes in antibacterial activity.

CONDENSATIONS between 1-aryl-2-nitroethanols and some aromatic compounds have been disclosed by Müller (1). These reactions take place as shown in the equation below:



where X = halogen, hydrogen, or methyl; n = 1 or 2. Extension of this reaction by Hass and Neher (2), by Hass, Neher, and Blickenstaff (3), and by the senior author has made available an extensive series of diarylalkyl nitro compounds.

It has been found that the hydrochlorides of the amino compounds formed by reducing some of these nitro compounds have pronounced antibacterial activity, and it has been found possible to correlate their structures and antibacterial activities to a certain extent

The formulas, melting points, and analyses of the series of amine hydrochlorides formed as indicated above are given in Table I, and the bacteriostatic activities of these compounds are shown in Table II.

In order to study further the relationship between structure and activity in compounds of this type, N,N-dimethyl-2-*o*-chlorophenyl-2-*p*-chlorophenylisopropylammonium chloride and 1,1-bis-(*p*-chlorophenyl)-2-acetamidopropane were prepared and tested. These two compounds showed no antibacterial activity under the conditions of the test. Likewise 2-nitro-1,1-bis-(*p*-chlorophenyl)propane, the parent compound of one of the most active amine hydrochlorides showed no antibacterial activity.

It is evident that slight changes in structure in compounds of the type studied cause marked changes in antibacterial activity. The introduction of substituents on the aromatic groups causes changes in antibacterial activity which vary with the nature and position of the substituents. In general, chlorine seems to be one of the most effective substituents for increasing antibacterial activity.

The organisms selected represent a rather wide variety of types and compounds such as 2,2-bis-(*p*-chlorophenyl)isopropylammonium chloride, which showed activity against every organism tested, would be expected to have a rather wide bacterial spectrum. This compound (P-21) was selected for further tests. It was found to be irritating when injected subcutaneously into guinea pigs in amounts as low as 20 mg. per day and was not tested further *in vivo*. Since it appeared that the most likely field of application of P-21 would be as a surface antiseptic, further tests were made to explore this possibility. Without going into detail on the results of these tests, they can be summarized by the statement that P-21 is very similar both in bacteriostatic and bactericidal activity to at least two widely used quaternary ammonium compounds, but in a few cases is slightly less active than these compounds.

EXPERIMENTAL

Preparation of Amine Hydrochlorides.—The amine hydrochlorides were all prepared in good yields by substantially the same procedure. Their preparation will be illustrated by a description of the conditions used for the preparation of 2,2-bis-(*p*-chlorophenyl)isopropylammonium chloride.

A solution of 75 Gm. (0.242 mole) of 2-nitro-1,1-bis(*p*-chlorophenyl)propane in 800 ml. of methanol was reduced at 40° and 1,500 lb. per square inch hydrogen pressure in the presence of 15 Gm. of Raney nickel for about six hours. Then the catalyst was filtered out, 30 ml. of concentrated hydrochloric acid was added, and the solution was concentrated until crystals appeared. It was then cooled, stirred with 500 ml. of cyclohexane to give 60 Gm. (78%) of nearly white product, m. p. 262–266°. The purified product (recrystallized from ethanol-cyclohexane) melted at 269–270° (corr.) with slight decomposition.

Preparation of 2-*o*-Chlorophenyl-2-*p*-chlorophenyl-N,N-dimethylisopropylammonium Chloride.—Fifty-eight grams (0.183 mole) of 2-*o*-chlorophenyl-2-*p*-chlorophenylisopropylammonium chloride was

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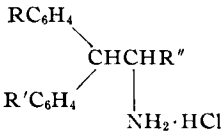
‡ Purdue University. Present address: Department of Chemistry, Ohio University, Athens, Ohio.

TABLE I.—MELTING POINTS AND ANALYSES OF A SERIES OF AMINE HYDROCHLORIDES,



Compound No.	R	R′	R″	M. p.[a] (° C.)	Analyses, % Chlorine Calcd.	Chlorine Found	Nitrogen Calcd.	Nitrogen Found
1	4-Cl	4-Cl	H	227–229	35.15	34.83	4.63	4.48
2	2-Cl	4-Cl	H	292–298 (d)[b]	35.15	35.28	4.63	4.62
3	2-Cl	4-CH(CH$_3$)$_2$	H	224	22.86	22.59	4.51	4.38
4	H	H	CH_3	276–278	14.32	14.61	5.65	5.46
5	H	2,4-DiCH_3	CH_3	300–303 (d)	12.86	12.90	5.08	4.88
6	2-Cl	H	CH_3	288–289 (d)	25.13	24.77	4.96	4.90
7	2-Cl	4-F	CH_3	303–304 (d)	23.62	23.23	4.67	4.53
8	2-Cl	4-Br	CH_3	276–282 (d)	19.64	19.25	3.88	3.71
9	2-Cl	4-CH_3	CH_3	291–293 (d)	23.94	23.86	4.73	4.63
10	2-Cl	4-Cl	CH_3	285–287 (d)	33.59	33.18	4.42	4.46
11	4-CH_3	4-CH_3	CH_3	242–247 (d)	12.86	13.02	5.08	5.14
12	4-Cl	4-CH(CH$_3$)$_2$	CH_3	295–300 (d)	21.86	22.18	4.32	4.19
13	4-Cl	4-C_2H_5	CH_3	244–258 (d)	22.85	22.99	4.51	4.51
14	4-Cl	4-CH_3	CH_3	265–267 (d)	23.93	24.13	4.73	4.64
15	4-Cl	4-Cl	CH_3	269–270 (d)	33.59	33.17	4.42	4.40
16	4-Cl	2,4-DiCl	CH_3	289–292 (d)	40.51	40.30	3.99	3.92
17	4-Cl	4-Cl	C_2H_5	225	32.17	31.82	4.24	3.96

[a] All melting points are corrected.
[b] (d) indicates decomposition, which varied from slight yellowing to considerable darkening.

TABLE II.—BACTERIOSTATIC ACTIVITIES OF COMPOUNDS SHOWN IN TABLE I

Compound No.	Minimum Inhibitory Concentrations in μg./Ml. Against: *S. aureus*[a]	*S. fecalis*[b]	*S. hemolyticus*[c]	*E. coli*[d]	*P. aeruginosa*[e]	*S. dysenteriae*[f]	*M. ranae*[g]	*P. pseudotuberculosis*[h]	*S. paradysenteriae*[i]	*M. Specialj*
1	100	75	>100	>100	...	50	...	50	...	25
2	>100	>100	>100	>100	>100	>100	10	...	...	...
3	75	50	75	75	...	...	...	10	50	...
4	>100	>100	>100	>100	...	...	>100	...	>100	>100
5	100	100	100	100	...	...	>100	25	100	...
6	>100	>100	>100	>100	...	100	...	>100	...	...
7	>100	>100	>100	>100	...	...	100	...	>100	100
8	100	100	>100	>100	...	>100	...	>100	...	...
9	100	>100	100	>100	...	>100	...	>100	...	...
10	50	25	50	100	...	...	>100	25	50	...
11	100	100	>100	>100	...	>100	...	>100	...	...
12	100	50	50	100	100	100	10	...	...	...
13	20	50	50	>100	50	50	10	...	...	...
14	75	100	50	75	...	...	100	25	100	...
15	50	50	50	100	50	50	10	...	...	...
16	25	25	25	>100	...	...	...	25	75	...
17	100	75	100	>100	...	>100	...	100	...	...

[a] *Staphylococcus aureus* (A.T.C.C. No. 9144). [b] *Streptococcus fecalis* (A.T.C.C. No. 6057). [c] *Streptococcus hemolyticus* (Lancefield Group O). [d] *Escherichia coli* (A.T.C.C. No. 167). [e] *Pseudomonas aeruginosa* (A.T.C.C. No. 9027). [f] *Shigella dysenteriae* (A.T.C.C. No. 8712). [g] *Mycobacterium ranae* (Kurung). [h] *Pasteurella pseudotuberculosis* (A.T.C.C. No. 6902). [i] *Shigella paradysenteriae* (A.T.C.C. 9199). [j] *Mycobacterium Special* (A.T.C.C. 607).

stirred and heated with a solution of 20 Gm. of sodium hydroxide in 200 ml. of water for thirty minutes. Then the mixture was cooled and extracted with 150 ml. of ether. The ether was dried over sodium sulfate and evaporated. To the residue was added 100 ml. of methanol and 11 Gm. (0.367 mole) of paraformaldehyde. This mixture was boiled for five minutes. It was then diluted with 500 ml. of methanol and reduced at 50° and 1,500 lb. per square inch hydrogen pressure in the presence of 10 Gm. of Raney nickel for five hours. After the catalyst had been filtered and the solvent had been evaporated, the residue was recrystallized from a benzene-ethanol mixture to give 15 Gm. (24%) of white crystals; m. p. (corr.), 211–212°.

Anal.—Calcd. for $C_{17}H_{20}Cl_3N$: Cl, 30.86; N, 4.06. Found: Cl, 30.69; N, 3.88.

Preparation of 1,1-bis-(*p*-Chlorophenyl)-2-acetamidopropane.—To a solution of 3.2 Gm. (0.01 mole) of 2,2-bis-(*p*-chlorophenyl)isopropylammonium chloride in 25 ml. of methanol was added 0.6 Gm. (0.011 mole) of sodium methoxide and then

3 ml. (0.03 mole) of acetic anhydride. The mixture was boiled gently for fifteen minutes, then cooled and poured into 50 ml. of water. Filtration gave 3.1 Gm. (97%) of white crystals, m. p., 170–174°. One recrystallization from cyclohexane, plus a little methanol, gave 2.3 Gm. (72%); m. p. 173–174° (corr.).

Anal.—Calcd. for $C_{17}H_{17}Cl_2NO$: Cl, 22.01; N, 4.35. Found: Cl, 21.70; N, 4.23.

Bacteriostatic Tests.—Each compound to be studied was incorporated in North Gelatin Agar (Difco) at the following levels: 100, 75, 50, 25, 5, 1.0, 0.1, and 0.01 μg./ml. (The highest level used for 1,1-bis-(*p*-chlorophenyl)-2-acetamidopropane was 50 μg./ml.). The plates were then streaked with saline suspensions of the test organisms washed from eighteen-hour agar slants. All plates except those containing mycobacteria were incubated for twenty-four hours at 37° before readings were taken. The acid-fast organisms were incubated for forty-eight hours. The inhibition readings were recorded as the lowest concentration at which no growth of the bacterial strain occurred.

REFERENCES

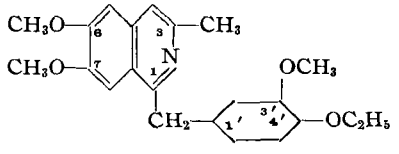
(1) Müller P., U. S. pat. 2,397,802, April 2, 1946.
(2) Hass, H. B., and Neher, M. B., abstracts of papers, 113th Meeting, American Chemical Society, 4L, 1948.
(3) Hass, H. B., Neher, M. B., and Blickenstaff, R. T., abstracts of papers, 115th Meeting, American Chemical Society, 40L, 1949.

Pharmacologic Studies of 6,7-Dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline*

By F. G. HENDERSON, R. E. SHIPLEY, and K. K. CHEN†

The synthetic analog of papaverine, 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl)-3-methyl-isoquinoline,[1] appears to be equal to papaverine in ability to dilate coronary and femoral arteries. This is true of the hydrochloride as well as the phosphate. It has very little effect on isolated or intact intestine. Acute toxicity studies show it to be less toxic than papaverine, both by intravenous injection in albino mice and oral administration in albino rats. Chronic toxicity studies failed to show any significant detrimental effect in rats receiving 100 mg. of the drug daily, or over 50 per cent of the LD_{50} daily. It is our belief that this compound warrants clinical trial.

PAPAVERINE is well known for its vasodilator effect. In recent years it has been used in the treatment of coronary artery disease (1), cerebral angiospasm (2), periarteritis nodosa (3), Raynaud's disease (4), thromboangiitis obliterans (5), pulmonary embolism (6), and other diseases involving spasm or obstruction of some portion of the arterial system. Recently our organic chemists have synthesized several compounds similar to papaverine in chemical structure and physiologic activity. One of these showed sufficient activity to warrant further study. Chemically it is 6,7-dimethoxy-1-(4′-ethoxy-3′-methoxybenzyl) - 3 - methyl - isoquinoline. The structural formula is as follows:



Two salts of this compound were prepared, the hydrochloride, m. p. 196–208°, and the phosphate, m. p. 197–199°. Both are water soluble, the phosphate to a greater degree than the hydrochloride. In our pharmacologic studies both salts were used.

PHARMACOLOGIC STUDIES

Effect on the Cardiovascular System

Coronary Artery Flow.—The effect of both salts was studied on the coronary arterial flow in mongrel dogs weighing 9–15 Kg. The animals were anesthetized with pentobarbital sodium by vein, and the thorax opened under artificial respiration. The descending, or circumflex, branch of the left coronary artery was isolated for cannulation. Heparin, Pontamine fast pink, or Paritol A was given intravenously to render the blood noncoagulable. The left carotid artery was cannulated, and connected to an optical recording rotameter (7, 8). From the outflow connection of the rotameter, flexible plastic tubing conducted the blood to a cannula inserted and tied into the peripheral portion of the coronary artery, the central end having been tied immediately prior to cannulation. In this manner the rate of blood flow from the carotid artery to the coronary vascular bed was continuously measured by the rotameter.

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† The authors are deeply indebted to Miss Eva Sommermeyer, Dr. P. N. Harris, and Messrs. R. M. Small, H. E. Roeder, and H. W. Worth for their invaluable assistance in this study.
[1] Eli Lilly and Company has registered Paveril phosphate (Dioxyline phosphate, Lilly) as the trade mark of this compound.