Antibacterial Agents Not Presently Employed as Preservatives in Ophthalmic Preparations Found Effective Against *Pseudomonas aeruginosa*

By S. R. KOHN†, LOUIS GERSHENFELD, and MARTIN BARR

Fifty-one chemical substances, not heretofore employed as preservatives in ophthalmic solutions, were examined for their effectiveness as antibacterial agents against 13 different strains of *Pseudomonas aeruginosa*. *In vitro* and *in vivo* methods were employed. Eleven of these agents showed a satisfactory sterilizing time against these strains. Further study of the following is indicated to determine their practical use as effective preservatives in ophthalmic preparations: Intexsan LB, Intexsan MB, Dichloran, DMBC, Cetol, Arquad 16, Virac, Betadine, Biopal VRO, chlorhexidine, and colistin.

A COMMONLY FOUND and hazardous contaminant in ophthalmic solutions is *Ps. aeruginosa*. The latter causes a serious type of corneal ulcer encountered in ophthamology, often resulting in the loss of the infected eye.

The chemicals which are commonly employed as antibacterial agents in multidose ophthalmic solutions were investigated for their effectiveness against 13 strains of *Ps. aeruginosa*, utilizing in each instance inactivating media of determined neutralizing activity for subculturing (1). Only benzalkonium chloride had a sterilizing time of less than 1 hour against the 13 strains of bacteria (1). An antibacterial substance which has a sterilizing time greater than 1 hour may be arbitrarily considered to be too slow-acting for use as a preservative in multidose ophthalmic solutions. Accordingly, this investigation was undertaken to determine the length of time required for sterilization by various antibacterial agents not heretofore employed as preservatives in ophthalmic solutions against *Ps. aeruginosa*. The main purpose was to ascertain whether any of these chemicals were capable of sterilizing in less than 1 hour.

EXPERIMENTAL[1]

Antibacterial Agents.—The chemicals studied for their effectiveness as antibacterial agents for ophthalmic solutions against *Ps. aeruginosa* were: 37 quaternary ammonium compounds: myristyl gamma-picolinium chloride,[2] Hyamine 1622,[3] Hyamine 10-X,[4] Hyamine 3500,[5] Hyamine 2389,[6] Emcol E-11,[7] Emcol E-607,[8] Emcol E-6075,[9] Ruson,[10] Tetrosan 3, 4D,[11] Tetrosan 60%,[12] Onyxide 75%,[13] BTC-776,[14] Intexsan MB-50,[15] Intexsan LB-50,[16] Intexsan LCB-50,[17] Intexsan LQ-75,[18] Intexsan OE-75,[19] Dichloran,[20] Cetol,[21] Bromat,[22] Bretol,[23] Germitol,[24] SD-75,[25] CTA-Stearate,[26] cetyl pyridinium bromide,[27] Laurol,[28] DMBC,[29] Arquad 12,[30] Arquad 16,[31] Arquad 18,[32] Arquad C,[33] Arquad S,[34] Arquad T,[35] Arquad HT,[36] cetyl pyridinium chloride,[37] and lauryl pyridinium chloride;[38] eight amphoteric surface-active agents: Tego 103-S,[39] Janusol,[40] Miranol OM-SF Concentrate,[41] and Miranol C2M Concentrate,[42] Miranol MSA Modified,[43] Miranol 2MCA Modified,[44] Product BCO,[45] and Product BDO;[46] three iodophors: Biopal VRO,[47] Virac,[48] and Betadine;[49] and a group of miscellaneous agents including Agosan,[50] chlorhexidine,[51] and colistin.[52]

Test Bacteria.—Two dilutions (1:10 and 1:1000) of 24-hour-old cultures of 13 different strains of *Ps. aeruginosa* (1) were used in this study. The bacteria were identified and the cultures prepared as previously described (1).

In Vitro Method.—The chemicals were studied for their effectiveness as bactericidal agents against 13 strains of *Ps. aeruginosa* using a procedure as noted in *Experiment III*, described in a previous study (1). The inactivating media employed for the purpose of neutralizing the antibacterial agents were evaluated using experiments which were also previously described (1). The most effective neutralizing media for use in these studies are listed in Table I.

In Vivo Method.—The purpose and the method employed for verifying the *in vitro* results (sterilizing times) for each of the antibacterial agents using an *in vivo* procedure have been reported (1). This same technique was used in this study.

RESULTS

Table II presents a summary of the results obtained. All of the antibacterial agents listed in this table gave results which indicate that they have a sterilizing time against 13 different strains of *Ps. aeruginosa* equal to or better than 1:5000 benzalkonium chloride. The sterilizing times (1) listed have been obtained under the severe *in vitro* test conditions and have been verified by an *in vivo* technique. In the latter procedure, those solutions of chemicals which produced growth in the subculture media produced ocular infections; those which did not produce growth did not produce ocular infections. The agents which gave longer sterilizing times than that given by 1:5000 benzalkonium chloride (45 minutes) are not listed in Table II.

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[1] See *Appendix* for footnotes to compounds listed.

TABLE I.—NEUTRALIZING MEDIA MOST EFFECTIVE

For "Quats"—Medium V (pH 6.7) (1)	
Lecithin[a]	0.5 Gm.
Tween 80[b]	3 Gm.
Nutrient broth, dehydrated[c]	0.8 Gm.
Purified water *q.s. ad.*	100 ml.
For Amphoteric Surfactants—Medium I (pH 6.8) (1)	
Nutrient broth, dehydrated[c]	0.8 Gm.
Purified water *q.s. ad.*	100 ml.
For Iodophors—Medium XIX (pH 6.7) (1)	
Sodium thiosulfate[d]	0.5 Gm.
Lecithin[a]	0.5 Gm.
Tween 80[b]	3 Gm.
Medium I *q.s. ad.*	100 ml.
For Agosan—Medium VIII (pH 7.1) (1)	
Fluid thioglycollate medium, dehydrated[c]	2.93 Gm.
Purified water *q.s. ad.*	100 ml.
For Chlorhexidine—Medium XX (pH 6.8) (1)	
Sodium lauryl sulfate[e]	0.5 Gm.
Medium I *q.s. ad.*	100 ml.
For Colistin—Medium III (pH 6.8) (1)	
Lecithin	0.5 Gm.
Glycerin	4 Gm.
Medium I *q.s. ad.*	100 ml.

[a] Lecithin, (Ex Ovo Soluble) Pfansteihl Laboratories, Inc., Waukegan, Ill. [b] Polyoxyethylene (20) sorbitan mono-oleate, Atlas Powder Co., Wilmington, Del. [c] Difco Laboratories, Inc., Detroit, Mich. [d] Sodium thiosulfate, reagent, Merck and Co., Rahway, N. J. [e] Marketed as Duponol C, by E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.

DISCUSSION

Among the 37 quaternary ammonium compounds studied, the following quats were found to be equal to or better than benzalkonium chloride in their bactericidal effectiveness against *Ps. aeruginosa:* Intexsan LB, Intexsan MB, DMBC, Cetol, Dichloran, and Arquad 16. Their sterilizing times were 45 minutes or less. However, before these compounds are employed as antibacterial preservatives in ophthalmic solutions, their toxicity, ocular irritability, stability, and compatibilities with the common ophthalmic drugs and vehicles must be determined. It should be mentioned that Medium V inactivated 1:1000 dilutions of the "quats," the strongest concentration studied.

The amphoteric surfactants possess either a positive or negative charge in solution depending on the pH of the latter. Based on the probability that an acid solution would cause the amphoteric surfactants to assume a positive charge, they were studied both in aqueous solutions and in aqueous solutions containing 1% boric acid. However, only one of these compounds, namely Tego 103-S, when used in a concentration of 1:1000, possessed antibacterial activity against *Ps. aeruginosa* after 24 hours of contact in either aqueous solutions or aqueous solutions containing 1% boric acid. Furthermore, the sterilizing time against *Ps. aeruginosa* was too long (9 hours), revealing no improvement over benzalkonium chloride.

Inasmuch as Virac is a quaternary-iodine complex, the inactivating medium employed in the investigation of the iodophors was the same medium as that employed for the quaternary ammonium compounds but with the addition of sodium thiosulfate to inactivate the iodine. The neutralizing medium, Medium XIX, was capable of neutralizing iodophors in concentrations of 500 p.p.m. of iodine, the strongest concentration tested.

It was noted that the iodophors, in either aqueous solutions or in aqueous solutions containing 1% boric acid, were effective against *Ps. aeruginosa* in concentrations as low as 25 p.p.m. of available iodine. It is known that iodophors are more stable in acid solutions and hence more effective. It should be emphasized that aqueous solutions containing 25 p.p.m. of available iodine had pH values in the acid range. Thus, there was no difference in the antibacterial effectiveness of the iodophors against *Ps. aeruginosa* in aqueous solutions or in aqueous solutions containing 1% boric acid. However, it was observed that the iodophors in aqueous solutions containing 10 p.p.m. of available iodine did not have pH values in the acidic range. Thus, the sterilizing times for the iodophors against *Ps. aeruginosa* in aqueous solutions (10 p.p.m. available iodine) required more than 1 hour of contact, while the same iodophors in aqueous solutions containing 1% boric acid were effective against *Ps. aeruginosa* after 30 minutes of contact.

From the findings in this study, it would appear that Virac, Betadine, and Biopal VRO may possibly be used as effective preservatives in ophthalmic solutions. However, as stated previously for the quaternary ammonium compounds, other properties of these iodophors, which are desired for antibacterial preservatives used in ophthalmic solutions, must be thoroughly investigated before employing the iodophors in such solutions. Medium VIII, fluid thioglycollate medium, was found to be an

TABLE II.—STERILIZING TIMES AGAINST *Ps. aeruginosa* (10^8) FOR CHEMICAL AGENTS NOT HERETOFORE EMPLOYED AS PRESERVATIVES IN OPHTHALMIC SOLUTIONS WHICH SHOW PROMISE FOR FURTHER STUDY

Chemical Agent	Concn.	Sterilizing Time, Min.
Intexsan LB	1:5000	45
Intexsan MB	1:5000	30
Dichloran	1:5000	45
DMBC	1:5000	45
Cetol	1:5000	30
Arquad 16	1:5000	45
Virac[a]	100 p.p.m. I_2	15
Virac[a]	50 p.p.m. I_2	15
Virac[a]	25 p.p.m. I_2	15
Betadine[a]	100 p.p.m. I_2	15
Betadine[a]	50 p.p.m. I_2	15
Betadine[a]	25 p.p.m. I_2	15
Biopal VRO[a]	100 p.p.m. I_2	15
Biopal VRO[a]	50 p.p.m. I_2	15
Biopal VRO[a]	25 p.p.m. I_2	15
Virac[b]	10 p.p.m. I_2	30
Betadine[b]	10 p.p.m. I_2	30
Biopal VRO[b]	10 p.p.m. I_2	30
Chlorhexidine	1:50,000	30
Chlorhexidine	1:25,000	15
Chlorhexidine	1:10,000	15
Chlorhexidine	1:5000	15
Colistin	1000 units/ml.	15
Colistin	500 units/ml.	15
Colistin	250 units/ml.	45

[a] In aqueous solution and in 1% boric acid solution. [b] In 1% boric acid solution only.

effective inactivating agent for Agosan, neutralizing 1% w/v of the substance, the highest concentration studied. This was to be expected, since the sulfhydryl groups in the thioglycollate are reactive not only with mercurial ions but also with other metallic ions.

Agosan, in a concentration of 0.5% possessed a sterilizing time against *Ps. aeruginosa* of 6 hours, a time period which is not an improvement over that for 1:5000 benzalkonium chloride.

Medium XX, which contained 0.5% sodium lauryl sulfate in nutrient broth medium, neutralized chlorhexidine in concentrations up to 1:1000, the strongest concentration studied.

Chlorhexidine in concentrations of 1:50,000 had a sterilizing time of 30 minutes for *Ps. aeruginosa*. Stronger concentrations had a faster sterilizing time. Further investigations are indicated for chlorhexidine as they concern toxicity, irritability, stability, and compatibility with the common medicaments and vehicles.

Medium III was found to be a good inactivating agent for colistin, neutralizing 2000 units/ml., the strongest concentration studied. Medium III was selected because colistin, like polymyxin B sulfate, is a polypeptide and carries a positive charge.

Colistin, in a concentration of 250 units/ml., had a sterilizing time of 45 minutes against *Ps. aeruginosa*. In stronger concentrations, the sterilizing time was reduced considerably.

SUMMARY

Fifty-one chemical substances, not heretofore employed as preservatives in ophthalmic solutions, were studied for their effectiveness against 13 different strains of *Ps. aeruginosa*. *In vitro* and *in vivo* methods were employed. Sterilizing times were determined in each instance and these were compared with that of benzalkonium chloride.

Thirty-seven quaternary ammonium compounds were investigated for their antibacterial effectiveness against *Ps. aeruginosa*. Of these, only six were found to possess equal or superior activity against *Ps. aeruginosa* compared to benzalkonium chloride. These included Intexsan LB, Intexsan MB, Dichloran, DMBC, Cetol, and Arquad 16.

Eight amphoteric surfactants were studied for their antibacterial effectiveness against *Ps. aeruginosa*. They were all found to be inadequate for use as antibacterial preservatives in ophthalmic solutions when compared to benzalkonium chloride.

Three iodophors, Virac, Betadine, and Biopal VRO were investigated and found to possess sterilizing times of less than 1 hour against *Ps. aeruginosa*.

Three other antibacterial agents, namely Agosan, chlorhexidine, and colistin, were investigated for their antibacterial activity against *Ps. aeruginosa*. Chlorhexidine and colistin were each found to possess sterilizing times of less than 1 hour against *Ps. aeruginosa*. Agosan required a longer sterilizing time.

REFERENCES

(1) Kohn, S. R., Gershenfeld, L., and Barr, M., THIS JOURNAL, **52**, 967(1963).

APPENDIX

[2] Marketed as Quatresin by The Upjohn Co., Kalamazoo, Mich.

[3] Di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride, Rohm and Haas Co., Philadelphia, Pa.

[4] Di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride, Rohm and Haas Co., Philadelphia, Pa.

[5] A 50% w/w hydroalcoholic solution of alkyl (C_{14}, 50%; C_{12}, 40%; C_{16}, 10%) dimethyl benzyl ammonium chloride, Rohm and Haas Co., Philadelphia, Pa.

[6] Fifty per cent w/w aqueous solution containing 40% methyl dodecyl benzyl trimethyl ammonium chloride and 10% methyl dodecyl xylene bis(trimethyl ammonium chloride), Rohm and Haas Co., Philadelphia, Pa.

[7] Fifty per cent w/w aqueous solution containing alkyl (C_8 to C_{18}, predominately C_{12}) benzyl trimethyl ammonium chloride, Witco Chemical Co., Inc., Chicago, Ill.

[8] N (lauroyl colaminoformyl methyl)pyridinium chloride Witco Chemical Co., Inc., Chicago, Ill.

[9] N(stearoyl colaminoformyl methyl)pyridinium chloride, Witco Chemical Co., Inc., Chicago, Ill.

[10] Aqueous solution containing 3.2% w/v of N(methyl heptyl colaminoformyl methyl)pyridinium chloride, Ruson Laboratories, Inc., Portland, Ore.

[11] Alkyl, dimethyl 3,4 dichloro-benzyl ammonium chloride, 60% w/w liquids, Onyx Chemical Corp., Jersey City, N. J.

[12] A 60% w/w liquid containing a mixture of alkyl dimethyl 3,4 dichlorobenzyl ammonium bromide and alkenyl dimethyl ethyl ammonium bromide, Onyx Chemical Corp., Jersey City, N. J.

[13] Alkenyl (predominately C_{18}) dimethyl ethyl ammonium bromide, a 75% w/w paste, Onyx Chemical Corp., Jersey City, N. J.

[14] Fifty per cent w/w liquid containing a mixture of alkyl dimethyl benzyl ammonium chlorides, Onyx Chemical Corp., Jersey City, N. J.

[15] Fifty per cent w/w liquid containing alkyl (predominately C_{14}, C_{16}, C_{12}) dimethyl benzyl ammonium chlorides, Intex Chemical Corp., Lodi, N. J.

[16] Fifty per cent w/w liquid containing alkyl (predominately C_{12}, C_{14}, C_{16}) dimethyl benzyl ammonium chlorides, Intex Chemical Corp., Lodi, N. J.

[17] Fifty per cent w/w liquid containing alkyl (predominately C_{12}, C_{14}, C_{16}) dichlorobenzyl ammonium chlorides, Intex Chemical Corp., Lodi, N. J.

[18] Seventy-five per cent w/w liquid containing alkyl (predominately C_{12}, C_{14}, C_{16}) isoquinolinium bromide, Intex Chemical Corp., Lodi, N. J.

[19] Seventy-five per cent w/w paste containing alkyl (predominately C_{13}) dimethyl ethyl ammonium bromides, Intex Chemical Corp., Lodi, N. J.

[20] Fifty per cent w/w liquid containing alkyl (C_8 to C_{18}) dimethyl dichlorobenzyl ammonium chloride, Fine Organics, Inc., Lodi, N. J.

[21] Cetyl dimethyl benzyl ammonium chloride, Fine Organics, Inc., Lodi, N. J.

[22] Cetyl trimethyl ammonium bromide, Fine Organics, Inc., Lodi, N. J.

[23] Cetyl dimethyl ethyl ammonium bromide, Fine Organics, Inc., Lodi, N. J.

[24] Sixty per cent w/w liquid containing lauryl dimethyl benzyl ammonium chloride, Fine Organics, Inc., Lodi, N. J.

[25] Seventy-five per cent w/w liquid containing alkenyl (predominately C_{18}) dimethyl ethyl ammonium bromides, Fine Organics, Inc., Lodi, N. J.

[26] Cetyl trimethyl ammonium stearate, Fine Organics, Inc., Lodi, N. J.

[27] Marketed by Fine Organics, Inc., Lodi, N. J.

[28] Lauryl dimethyl benzyl ammonium bromide, Fine Organics, Inc., Lodi, N. J.

[29] Fifty per cent w/w liquid containing lauryl dimethyl benzyl dimethyl ammonium chloride, Fine Organics, Inc., Lodi, N. J.

[30] Fifty per cent w/w liquid containing alkyl (C_{12}, 90%) trimethyl ammonium chloride, Armour Industrial Chemical Co., Chicago, Ill.

[31] Fifty per cent w/w liquid containing alkyl (C_{16}, 90%) trimethyl ammonium chloride, Armour Industrial Chemical Co., Chicago, Ill.

[32] Fifty per cent w/w liquid containing alkyl (C_{18}, 93%) trimethyl ammonium chloride, Armour Industrial Chemical Co., Chicago, Ill.

[33] Fifty per cent w/w liquid containing alkyl (C_8 to C_{18} predominately C_{12}) trimethyl ammonium chloride, Armour Industrial Chemical Co., Chicago, Ill.

[34] Fifty per cent w/w liquid containing alkyl (C_{16} to C_{18}, predominately unsaturated C_{18}) trimethyl ammonium chloride, Armour Industrial Chemical Co., Chicago, Ill.

[35] Fifty per cent w/w liquid containing alkyl (C_{14}, C_{16}, and C_{18}, predominately unsaturated C_{18}) trimethyl ammonium chlorides, Armour Industrial Chemical Co., Chicago, Ill.

[36] Fifty per cent w/w liquid containing alkyl (C_{14}, C_{16}, C_{18}, predominately saturated C_{18}) trimethyl ammonium chloride, Armour Industrial Chemical Co., Chicago, Ill.

[37] Marketed by Fine Organics, Inc., Lodi, N. J.

[38] Marketed by Fine Organics, Inc., Lodi, N. J.

[39] Salt of octyl amino ethyl glycine, Th. Goldschmidt AG, Essen, Germany.

[40] Mixture of lauryl and myristyl esters containing both primary amine and sulfate groups, Synthetic Chemicals, Inc., Paterson, N. J.

[41] Thirty-five per cent w/w liquid containing sodium, 1-oleyl 2-hydroxy 2 hydroxymethyl ethylene cycloimidinium 2-methylene carboxylate, Miranol Chemical Co., Inc., Irvington, N. J.

[42] Forty-nine per cent w/w liquid containing sodium, 1-undecyl 2-hydroxy 2-sodium ethoxymethylene carboxylate ethylene cycloimidinium 2-methylene carboxylate, Miranol Chemical Co., Inc., Irvington, N. J.

[43] Forty-seven per cent w/w liquid containing sodium 1-nonyl 2-lauryl sulfate 2-hydroxyethyl ethylene cycloimidinium 2-methylene carboxylate, Miranol Chemical Co., Inc., Irvington, N. J.

[44] Forty-six per cent w/w liquid containing 1-undecyl 2-undecyl sulfate 2-sodium ethoxymethylene carboxylate ethylene cycloimidinium 2-methylene carboxylate, Miranol Chemical Co., Inc., Irvington, N. J.

[45] Thirty-three per cent w/w liquid containing cetyl betaine, E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.

[46] Thirty-three per cent w/w liquid containing a mixture of long chain betaines, E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.

[47] Iodine-nonyl phenoxypolyoxyethylene ethanol complex, in liquid form and containing 20% available iodine, Antara Chemicals, Division of General Aniline and Film Corp., New York, N. Y.

[48] N(methyl heptyl colamino-formyl methyl)pyridinium chloride containing coupled iodine, in liquid form and containing 0.6% available iodine, Ruson Laboratories, Inc., Portland, Ore.

[49] Polyvinylpyrrolidone-iodine complex, in liquid form and containing 1% available iodine, Tailby-Nason Co., Inc., Dover, Del.

[50] Partially polymerized silver mannuride, Ion-Exchange and Chemicals Corp., New York, N. Y.

[51] Diacetate salt of bis (*p*-chlorophenyl diquanido)-hexane. Marketed as Hibitane by Ayerst Laboratories, New York, N. Y.

[52] Marketed as Coli-Mycin S by Warner-Chilcott Laboratories, Morris Plains, N. J.

Comparative Hydrolytic Rates of Some Tropine Esters

By J. M. PATEL and A. P. LEMBERGER

A study has been carried out on the chemical kinetics of the hydroxyl ion catalyzed hydrolysis of nor-atropine, tropine phenylacetate, tropine phenoxyacetate, tropine *p*-nitrobenzoate, atropine ethylbromide, and atropine benzylchloride. The purpose of the study was to observe the effects of various acid moieties in tropine esters on overall reaction rate. The experimental results would seem to indicate that a number of factors may be involved, including steric, inductive, and hyperconjugation effects.

THE RELATIVE RATES of second-order alkaline hydrolysis of atropine (1), homatropine (2), atropine methylbromide, and homatropine methylbromide (3) have been reported previously. The purpose of this investigation was to study further the influence of inductive and steric factors on the hydrolytic cleavage of these esters. The kinetics of the hydroxyl ion catalyzed hydrolysis of nor-atropine were studied in order to establish hydrolytic rates for what might be considered the base compound. Tropine phenylacetate, tropine phenoxyacetate, and tropine *p*-nitrobenzoate were prepared and rates of hydrolysis determined. In addition, atropine ethylbromide and atropine benzylchloride were prepared and studied.

THEORY

Previous studies (1–3) have shown hydroxyl ion catalyzed hydrolysis to occur with tropine esters. Since nor-atropine, tropine phenylacetate, tropine phenoxyacetate, and tropine *p*-nitrobenzoate can exist as the free base or as the protonated form in solution, two hydrolytic pathways are possible. For convenience, hydrolysis of the free base will be designated Reaction 1, hydrolysis of the protonated form of the ester will be designated Reaction 2.

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In accord with the theoretical concepts employed by Higuchi, *et al.* (4), in their study on procaine, the reaction kinetics may be

$$-\log t_{1/2} = \log (OH^-) - \log [K_b + (OH^-)] + \log \left[\frac{k_1(OH^-)}{0.693} + \frac{k_2 \cdot K_b}{0.693}\right] \quad \text{(Eq. 1)}$$

where k_1 and k_2 are the second-order rate constants at high pH (Reaction 1) and low pH (Reaction 2), respectively, and K_b is the dissociation constant of the base.

At relatively high hydroxyl ion concentration, where $OH^- >> K_b$, Eq. 1 reduces to

$$-\log t_{1/2} = \log (OH^-) + \log \frac{k_1}{0.693} \quad \text{(Eq. 2)}$$

Conversely, at low hydroxyl ion concentration, where $K_b >> OH^-$ it simplifies to

$$-\log t_{1/2} = \log (OH^-) + \log \frac{k_2}{0.693} \quad \text{(Eq. 3)}$$

It is apparent, then, that at high pH and at low pH the rate of hydrolysis should be directly proportional to hydroxyl ion concentration even though the mechanism of hydrolysis changes.

In the case of esters containing quaternary nitrogen, such as atropine ethylbromide and atropine benzylchloride, the mechanism of hydrolysis remains the same throughout the entire pH range.

EXPERIMENTAL

Materials.—Nor-atropine sulfate (K and K Laboratories) was recrystallized from ethanol and water.