electroshock seizure elicited in mice with a 60-Hz alternating current of 50 mamp delivered for 0.2 sec *via* corneal electrodes. Activity in the subcutaneous pentylenetetrazol seizure threshold test is defined as failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 sec in duration) at a dose of 85 mg/kg.

RESULTS AND DISCUSSION

The results of the anticonvulsant activity tests (Table II) suggest that the substitution of a nitrile group for a chloromethyl group in beclamide leads to an increase in toxicity with a retention in activity against maximal electroshock and pentylenetetrazol-induced seizures. Compound III, having no carbon substitution, showed activity at a comparable or lower dose than beclamide (I). However, additional testing is required to determine whether the differences in potencies and toxicity are significant.

Of the four monosubstituted compounds tested, V, VII, and IX exhibited weak activity against maximal electroshock and pentylenetetrazol-induced seizures and neurotoxicity as evaluated by the rotorod test. Compound V appeared to be more toxic than VII or IX. Whether higher monosubstituted homologs would be active and/or toxic was not determined. No activity or toxicity was observed for the benzyl-substituted derivative (XI).

No activity was observed when the alkyl substituents in the symmetrically disubstituted derivatives contained six or more carbon atoms. Unlike the monosubstituted compounds, the active disubstituted derivatives (IV and VI) exhibited no toxicity at the dose levels tested.

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Direct Spectrophotometric Assay of Quaternary Ammonium Compounds Using Bromthymol Blue

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Abstract □ Benzalkonium chloride, benzethonium chloride, and chlorhexidine gluconate were assayed quantitatively by a direct spectrophotometric method with bromthymol blue buffered at pH 7.5. The method shows good results at concentrations of 0–300 μg/ml and in the presence of epinephrine bitartrate, phenylephrine hydrochloride, pilocarpine hydrochloride, and polyvinyl alcohol.

Keyphrases □ Quaternary ammonium compounds, various—spectrophotometric analyses in prepared and commercial solutions □ Spectrophotometry—analyses, various quaternary ammonium compounds in prepared and commercial solutions □ Bromthymol blue—used in spectrophotometric analyses of various quaternary ammonium compounds in prepared and commercial solutions □ Preservatives—various quaternary ammonium compounds, spectrophotometric analyses in prepared and commercial solutions

Low concentrations of quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, and chlorhexidine gluconate, which are used in clear ophthalmic solutions, are difficult to determine quantitatively and reproducibly. Most methods rely on complexing the quaternary ammonium compound with an acid dye such as methyl orange or bromphenol blue and extracting the complex with a chlorinated hydrocarbon solvent (1, 2). These methods have difficulties and inaccuracies arising from incomplete extraction or the emulsions formed with the hydrocarbon solvent and the quaternary ammonium compound-containing solution.

In response to the problems resulting from extraction of the dye complex, a direct method was developed using bromthymol blue buffered at pH 7.5; the reduction in absorbance was measured at 610 nm. The method was tested with benzalkonium chloride, benzethonium chloride, and chlorhexidine gluconate.

Table I—Standard Curves for Benzalkonium Chloride, Benzethonium Chloride, and Chlorhexidine Gluconate

Concentration, μg/ml	Absorbance		
	Benzalkonium Chloride	Benzethonium Chloride	Chlorhexidine Gluconate
0	0.804	0.804	0.804
100	0.593	0.659	0.667
200	0.370	0.471	0.538
300	0.225	0.325	0.438

EXPERIMENTAL

Apparatus—A pH meter and a spectrophotometer with 1-cm cells were used[1].

Reagents—Benzalkonium chloride USP, benzethonium chloride USP, epinephrine hydrochloride USP, polyvinyl alcohol, pilocarpine hydrochloride USP, phenylephrine hydrochloride USP, chlorhexidine gluconate BP, and hydroxyethylcellulose[2] were used as received. All other chemicals were reagent grade.

Buffer Solutions—Stock solutions of 0.05 and 0.25 *M* dibasic potassium phosphate and monobasic potassium phosphate were used in buffer preparation. A buffer of pH 7.5 was prepared by mixing either the 0.05 *M* solutions together or the 0.25 *M* solutions together until a pH of 7.5 was obtained.

[1] Beckman DU spectrophotometer.

[2] The 250 MR grade, Hercules Inc., Wilmington, Del.

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Table II—Assay of Benzalkonium Chloride with Various Compounds

Compound Present	Benzalkonium Chloride Found, μg/ml
Control	100
Epinephrine bitartrate, 10 mg/ml	104
Phenylephrine hydrochloride, 100 mg/ml	105
Pilocarpine hydrochloride, 40 mg/ml	102
Polyvinyl alcohol, 100 mg/ml	102

Dye Solutions—Bromthymol blue, 60 mg, was dissolved in 100 ml of ethanol.

Hydroxyethylcellulose Solution—Hydroxyethylcellulose, 500 mg, was dissolved in 100 ml of water.

Stock and Working Solutions—Stock benzalkonium chloride, benzethonium chloride, and chlorhexidine gluconate were prepared by dissolving accurately weighed amounts of the compounds in water. Working solutions were prepared by diluting aliquots of the stock solutions.

Test Solutions—A test solution was made by adding 1 g of epinephrine bitartrate and diluting it to 100 ml with the 100-μg/ml benzalkonium chloride working solution. This step was repeated using 10 g of phenylephrine hydrochloride and 4 g of pilocarpine hydrochloride and diluting to 100 ml with 100-μg/ml benzalkonium chloride working solution. For a contact lens wetting solution, a commercial product containing 10 mg of polyvinyl alcohol/ml was used.

Assay—In a 25-ml volumetric flask, 2.0 ml of hydroxyethylcellulose solution and 250 mg of sodium chloride were mixed with 5–10 ml of 0.05 *M* buffer solution. Then 50–300 μg/ml of a working quaternary ammonium solution and sufficient buffer to bring the volume to 25 ml were added. After mixing, the absorbance was measured at 610 nm on a spectrophotometer. A blank was prepared in the same manner but without the quaternary ammonium compound, and its absorbance was measured.

For the test solution, 2.0 ml of each was used. A 0.25 *M* buffer was substituted for the 0.05 *M* buffer when pilocarpine hydrochloride or epinephrine bitartrate was present since these compounds are acidic and a stronger buffer is needed to hold the pH at 7.5.

RESULTS AND DISCUSSION

Absorbances were measured between 400 and 700 nm using a blank solution and a 300-μg/ml working solution of each quaternary ammonium compound. The maximum reductions in absorbance occurred at 610 nm (Fig. 1). A standard linear curve was determined between 0 and 300 μg/ml at 610 nm (Table I).

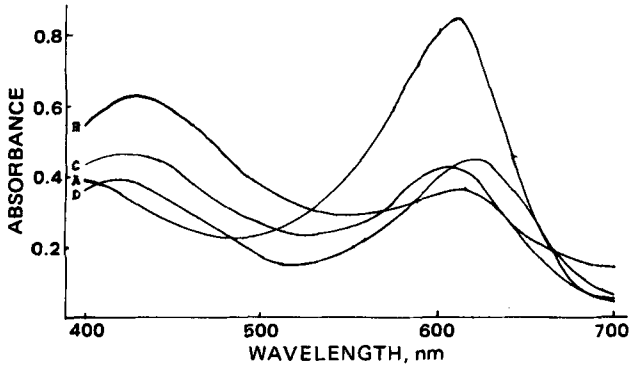
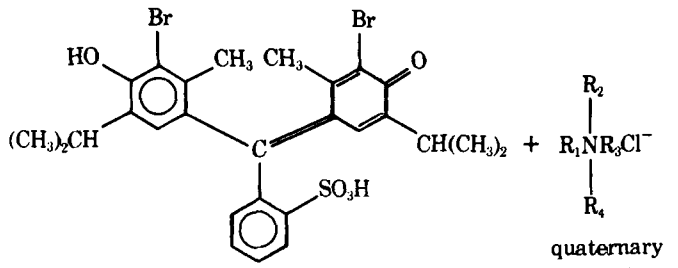
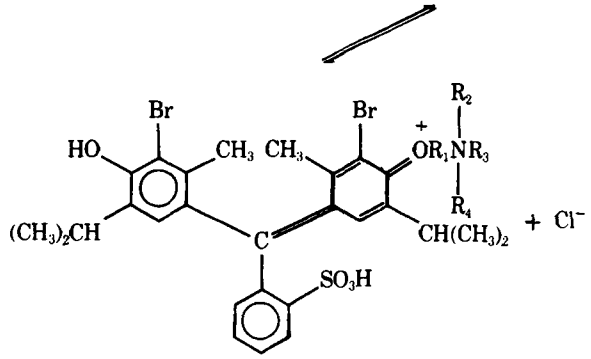


Figure 1—*Spectra of bromthymol blue (A), bromthymol blue and benzalkonium chloride (B), bromthymol blue and benzethonium chloride (C), and bromthymol blue and chlorhexidine gluconate (D).*





Scheme I

The method was then tested with some solutions that commonly contain benzalkonium chloride as a preservative. A sample of contact lens wetting solution containing 10 mg of polyvinyl alcohol/ml and 100 μg of benzalkonium chloride/ml was assayed. A value of 102 μg of benzalkonium chloride/ml was obtained by this method *versus* a value of 104 μg of benzalkonium chloride/ml obtained by a method using a methyl orange–benzalkonium chloride dye complex extracted with 1,2-dichloroethane (2).

The method was tried with a 10-mg/ml epinephrine bitartrate solution, a 100-mg/ml phenylephrine hydrochloride solution, and a 40-mg/ml pilocarpine hydrochloride solution, each containing 100 μg of benzalkonium chloride/ml. The results were compared to a 100-μg/ml benzalkonium chloride working solution. The results (Table II) showed little change from the working solution.

The mechanism of action is probably similar to the color change of bromthymol blue (I) in the presence of an acid or base (Scheme I).

This method offers advantages in determining quaternary ammonium compounds when extraction difficulties arise with other spectrophotometric methods.

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