Substituting $K_w/[OH^-]$ for $[H^+]$ and dividing numerator and denominator by $(k_4 + k_6)K_T$:

$$\frac{\text{rate}}{[P][C^-]} = \frac{\frac{k_1k_5K_w}{(k_4 + k_6)K_T} + \frac{k_1k_6K_T + k_3k_5K_{2c}}{(k_4 + k_6)K_T}[OH^-] + \frac{k_3k_6K_{2c}}{(k_4 + k_6)K_w}[OH^-]^2}{\frac{(k_2 + k_5)K_w}{(k_4 + k_6)K_T} + [OH^-]}$$

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Bactericidal Properties of Straight-Chained Alkyltrimethylammonium Bromides in a Simple Emulsion System

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The individual constituents of the potent bactericidal agent cetrimide B.P. are among those alkyltrimethylammonium bromides (C_8 through C_{18}). which have been synthesized by classical methods. Each of these quaternary compounds was then emulsified with six (C_8 through C_{18}) fatty alcohols. The bactericidal properties of these emulsions are compared by the critical killing dilution method. When emulsified, only the C_{12} and C_{14} quaternary compounds exhibited any appreciable bactericidal action. All of the quaternary compounds in the series are inactivated when emulsified with myristyl alcohol.

THE OFFICIAL COMPOSITION of the quaternary ammonium bactericidal agent cetrimide B.P. has gradually changed over the last decade (1–3). Other investigators (4, 5) have recorded significant effects on their studies brought about by these changes.

Similar effects were noted in this laboratory during *in vitro* bactericidal testing of an experimental product. This product, a medicated cream, was an oil-in-water emulsion which utilized cetrimide B.P. as its bactericidal agent. The disperse phase was comprised mainly of stearyl alcohol.

The erratic bactericidal results were discovered to have been caused by a shift in the predominant constituent in the cetrimide utilized. Cetrimide conforming to the 1963 B.P. monograph, *i.e.*, "comprised mainly of tetradecyltrimethylammonium bromide . . ." afforded excellent bactericidal activity. Cetrimide which conformed to the 1953 B.P. monograph, *i.e.*, "comprised mainly of hexadecyltrimethylammonium bromide . . ." was completely inactive in the product. Some anomalous bactericidal results were also obtained when alcohols other than stearyl were introduced into the formula.

The present study was initiated to determine: (*a*) the bactericidal properties of the component compounds which comprise cetrimide when they are incorporated into a simple emulsion system, and (*b*) if a pattern of relationship can be deter-

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TABLE I—ALKYLTRIMETHYLAMMONIUM BROMIDES, $R(CH_3)_3NBr$

R	Critical Killing Dilution Data[a] CKD *S. aureus*	Lit. CKD[c] *S. aureus*	CKD *S. typhosa*	Lit. CKD[c] *S. typhosa*	Physical Constants and Analyses[b] M.p.,[d] °C.	Lit. M.p., °C.	Formula	Br, % Calcd.	Found
n-Octyl	1/100	1/30	1/150	1/75	212–220°	215°[e]	$C_{11}H_{26}BrN$	31.68	31.46
n-Decyl	1/1,000	...	1/1,200	...	238–242°	239–242°[e]	$C_{13}H_{30}BrN$	28.51	28.31
n-Dodecyl (lauryl)	1/6,500	1/4,000	1/9,000	1/9,000	243–247°	243°[e]	$C_{15}H_{34}BrN$	25.91	25.47
n-Tetradecyl (myristyl)	1/50,000	1/38,000	1/90,000	1/36,000	243–245°	244–255°[c]	$C_{17}H_{38}BrN$	23.75	23.44
n-Hexadecyl (cetyl)	1/90,000	1/80,000	1/130,000	1/40,000	248–253°	237–243°[c]	$C_{19}H_{42}BrN$	21.92	21.43
n-Octadecyl (stearyl)	1/55,000	1/64,000	1/80,000	1/8,000	238–245° dec.	230–240°[c] dec.	$C_{21}H_{46}BrN$	20.36	20.01

[a] Critical killing dilution data were obtained by the Microbiology Laboratory, Operating Services Division of Miles Laboratories, Inc. [b] Analyses were performed by the Corporate Pharmacy Research Laboratory, Miles Laboratories, Inc. [c] See *Reference 9*. [d] Melting points are uncorrected. [e] See *Reference 15*.

mined among these constituents when fatty alcohols of varying chain lengths are used as the oil phase in the emulsion.

EXPERIMENTAL

Preparation of the Alkyltrimethylammonium Bromides—Straight-chained alkyltrimethylammonium bromides from C_8 to C_{18} were synthesized by classical methods (6). High purity synthetic fatty alcohols were used as starting materials.[1] The preparation of *n*-dodecyltrimethylammonium bromide is cited below for illustrative purposes.

In a 1-L. three-neck flask containing 102.3 Gm. (0.55 mole) of *n*-dodecyl alcohol, 62 Gm. (34 ml.) of concentrated sulfuric acid was slowly added along with 240 Gm. (1.4 moles) of 48% hydrobromic acid. This mixture was refluxed 7 hr. and allowed to cool to room temperature overnight. The mixture was diluted with 400 ml. water, then filtered through a sintered-glass funnel. The organic layer was separated mechanically and then was successively washed with 75-ml. portions of concentrated sulfuric acid, water, and 10% sodium carbonate solution. It was then dried over calcium chloride. The yield was 81 Gm.

The *n*-dodecylbromide, 81 Gm. (0.32 mole), was dissolved in 150 ml. dry acetone and 100 ml. of a 20% w/w solution of trimethylamine (0.33 mole) in dry acetone was added. After about 20 min. the crystalline *n*-dodecyltrimethylammonium bromide began to form. The crystals were collected after 5 hr., washed with dry acetone, dried, and weighed. The yield was 60 Gm. (60% of theory).

Recrystallization of the quaternary compounds with acetone A.R. resulted in products of excellent purity. The physical constants of these compounds are listed in Table I.

Establishment of the Critical Killing Dilution of the Quaternary Compounds in Solution—The complete technique for establishment of the critical killing dilution is outlined in the Association of Official Agricultural Chemists, Official Methods of Analysis (7). An excellent review of the method is offered by Reddish (8).

[1] These ethylene condensation compounds were generously supplied by the Continental Oil Co., Petroleum Chemicals Department, Ponca City, Okla., as Alfols 8 through 18.

Preparation of the 15% o/w Emulsion System Containing 0.5% Quaternary Compound—Each of the six quaternary compounds (C_8 through C_{18}) was emulsified with each of the six corresponding fatty alcohols (C_8 through C_{18}) in the following manner. The alkyltrimethylammonium bromide (0.5 Gm.) was dissolved in water (84.5 ml.) and heated on a water bath to 65°. The fatty alcohol (15.0 Gm.) was heated in a 250-ml. conical flask to 65°. The aqueous solution was then added to the alcohol and the flask was agitated for 5 min. on a wrist shaker. The emulsion was allowed to return to room temperature (about 1 hr.) and its physical appearance noted. These emulsions were left at ambient laboratory conditions and were examined after 24 hr. and again after 30 days for physical appearance.

Establishment of the Critical Killing Dilution for the Emulsions—To test the emulsions, the following modifications from the AOAC method (as cited above) are introduced. A 3.33% dilution of the emulsion is made in a glass-stoppered cylinder. Care is taken to insure that there is adequate uniform dispersion of the emulsion droplets throughout this system. Further dilutions are made from this stock solution. Five milliliters of the desired dilution is inoculated with the test organism. At time intervals of 5, 10, 15, and 20 min. a loopful of the seeded emulsion solution is withdrawn and subcultured in a nutrient broth. The subcultures are incubated at 37° for 48 hr. and examined macroscopically for growth. A standardized 5% phenol solution is used as a control to validate the results.

RESULTS AND DISCUSSION

The bactericidal properties, in solution, of the alkyltrimethylammonium bromides which were prepared from synthetic alcohols as well as those made from naturally occurring alcohols (9, 10) are summarized in Table I. In general, it can be seen that the same over-all pattern of CKD's has been manifested. Because strains of bacteria exhibit varying resistance in tests such as these, it is not possible to say if there has been a significant improvement in bactericidal action due to the purity of the starting materials. The values obtained, particularly those for the C_{18} homolog, are somewhat higher than the literature values against the Gram-negative *S. typhosa*.

References to the behavior of cetrimide or its con-

stituent compounds in emulsion systems are somewhat limited. Christenson and Shelton (11) reported inactivation of cetyltrimethylammonium bromide (CTAB) by various grades of lanolin in some pectin lotions. They also reported that any additions to the oil phase, such as glyceryl monostearate, glyceryl monooleate, or vegetable oils, increased the killing time of the lotion. Prusak and Mattocks (12) reported inactivation of CTAB by hydrophilic ointment U.S.P. and hydrophilic petrolatum U.S.P. Of the 6 ointment bases which they studied, only a Carbowax ointment and pectin paste N.F. appeared suitable to obtain bactericidal action with CTAB. Davies (13) noted that a fatty glycol ethylene oxide condensate neutralized the bacteriostatic effect of CTAB. Frank and Stark (14) found cetrimide to be completely inactivated in hydrous emulsifying ointment B.P.

Table II—Emulsion Bactericidal and Pharmaceutical Properties

Emulsion No. Quaternary Chain Length	Emulsion No. Alcohol Chain Length	Emulsion Physical Appearance[a]	Critical Killing Dilutions Against *S. aureus*	Critical Killing Dilutions Against *Ps. aeruginosa*[b]
8	8	*A*	$<1/30$	...
8	10	*A*	$<1/30$	...
8	12	*A*	$<1/30$	...
8	14	*A*	$<1/30$	...
8	16	*A*	$<1/30$	...
8	18	*A*	$<1/30$	...
10	8	*A*	$<1/30$	...
10	10	*A*	$<1/30$	...
10	12	*A*	$<1/30$	...
10	14	*A*	$<1/30$	...
10	16	*A*	$<1/30$	...
10	18	*A*	$<1/30$	...
12	8	*A*	$1/75$	$1/90$
12	10	*B*	$1/90$	$1/30$
12	12	*B*	$1/60$	$1/30$
12	14	*B*	$<1/30$	...
12	16	*B*	$1/60$	$1/75$
12	18	*C*	$1/45$	$1/30$
14	8	*A*	$1/400$	$1/120$
14	10	*A*	$1/500$	$<1/10$
14	12	*B*	$1/30$	$<1/10$
14	14	*C*	$<1/30$	...
14	16	*C*	$1/45$	$<1/10$
14	18	*C*	$1/60$	$<1/10$
16	8	*B*	$1/120$	$1/120$
16	10	*B*	$<1/30$	...
16	12	*B*	$<1/30$	...
16	14	*C*	$<1/30$	...
16	16	*C*	$<1/30$	...
16	18	*C*	$<1/30$	...
18	8	*A*	$1/30$	$1/250$
18	10	*A*	$<1/30$	...
18	12	*B*	$<1/30$	...
18	14	*C*	$<1/30$	...
18	16	*C*	$<1/30$	...
18	18	*C*	$<1/30$	...

[a] Key: *A*, very poor; creamed or broken within 24 hr.; *B*, initially good but creamed or broken within 30 days; *C*, pharmaceutically elegant; stable for more than 30 days. [b] Emulsions whose CKD was less than $1/30$ against *S. aureus* were not tested against the Gram-negative *Ps. aeruginosa*.

Thirty-six 15% o/w emulsions with the quaternary compound present at 0.5% were prepared and were tested for bactericidal action by a modification of the AOAC phenol coefficient test. This test establishes the critical killing dilution (CKD) (that is, the maximum dilution which will kill a specified organism in 10 min. but not in 5). Any emulsion which achieved complete kill against *S. aureus* at a dilution of 1 to 30 or greater was then tested against the Gram-negative *Ps. aeruginosa*. Emulsions which failed to kill *S. aureus* at 1 to 30 were assessed as having no appreciable bactericidal activity. The critical killing dilutions for this series of emulsions are shown in Table II. Also shown in this table are the pharmaceutical properties of these emulsions based on their physical appearance.

None of the emulsions made with the C_8 and C_{10} quaternary compounds met the 1 to 30 criterion for bactericidal activity. This is not surprising in view of their relatively weak bactericidal action in aqueous solution. Because of poor physical stability, none of these emulsions were acceptable from a pharmaceutical standpoint. However, all of the emulsions made with the C_{12} and C_{14} quaternary compounds had CKD's of at least 1 to 30 with the exception of those made with myristyl alcohol. The C_{12} emulsions exhibited surprising strength against the very resistant Gram-negative *Ps. aeruginosa*.

The inactivation of these compounds by myristyl alcohol is, as yet, unexplained. Emulsions have been made with three other commercial sources of this alcohol, all of which inactivate these quaternary compounds.

Table II also shows that cetyltrimethylammonium bromide, by far the most active of the series in aqueous solution, was completely inactivated in this system; except when combined with the C_8 alcohol. This activity is attributed to the fact that more of the quaternary compound is in the aqueous phase be-

Table III—Mixed Alcohol Emulsions

Emulsion System	Emulsion Physical Appearance	Critical Killing Dilution Against *S. aureus*	Critical Killing Dilution Against *Ps. aeruginosa*
0.5% C_{12} Quaternary 7.5% C_8 Alcohol 7.5% C_{16} Alcohol	Good initially but begins to separate within 30 days	$1/100$	$1/70$
0.5% C_{14} Quaternary 7.5% C_8 Alcohol 7.5% C_{16} Alcohol	Pharmaceutically elegant; stable for more than 30 days	$1/130$	$1/10$
0.5% C_{16} Quaternary 7.5% C_8 Alcohol 7.5% C_{16} Alcohol	Pharmaceutically elegant; stable for more than 30 days	$<1/30$	$1/10$
0.5% C_{18} Quaternary 7.5% C_8 Alcohol 7.5% C_{16} Alcohol	Pharmaceutically elegant; stable for more than 30 days	$<1/30$	$1/10$

cause of the relatively poor emulsion. The C_{18} analog parallels the C_{16} closely. Emulsions in these series have superior pharmaceutical properties and yet are inactive from a bactericidal standpoint.

Because emulsions made wih the C_8 alcohol did exhibit satisfactory bactericidal activity, although they were not pharmaceutically stable, a series of emulsions was made utilizing 7.5% of the C_8 alcohol and 7.5% of the C_{16} alcohol. It was postulated that they could have an acceptable bactericidal level as well as pharmaceutical stability. The CKD's for this series of emulsions are shown in Table III. Bactericidal activity is again found to be restricted to the C_{12} and C_{14} quaternary compounds. The C_{12} compound again shows its effectiveness against *Pseudomonas*. The C_{16} and C_{18} compounds again are inactivated in this system.

SUMMARY

Six straight-chained (C_8 to C_{18}) alkyltrimethylammonium bromides have been synthesized by classical methods, utilizing high purity synthetic alcohols as starting materials. Among these compounds are the individual constituents which comprise the bactericidal agent cetrimide B.P. The critical killing dilutions of these compounds, in solution, are comparable to those obtained from compounds prepared from naturally occurring alcohols. These quaternary compounds were then used as the emulsifying agent in a model oil-in-water system. Thirty-six emulsions were prepared and evaluated for bactericidal properties by the critical killing dilution method against *S. aureus* and *Ps. aeruginosa*. The results indicate that in addition to their poor bactericidal activity, the C_8 and C_{10} quaternary compounds lack the power to emulsify any of the alcohols in the selected system. The C_{12} and C_{14} quaternary compounds exhibit the greatest bactericidal activity. The C_{16} and C_{18} quaternary compounds are virtually inactivated by incorporation into this system. Emulsification with myristyl alcohol inactivates all of the quaternary compounds in this series.

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