Synthesis, Purification, and Influence of Chain Length on Toxicity of Two Quaternary Ammonium Compounds

By THEODORE H. EICKHOLT, GARY K. BRASHIER*, MICHAEL D. HIGGINS, and U. B. MARTINEZ

The synthesis and purification of two quaternary ammonium compounds was accomplished. The influence of chain length upon toxicity of the two compounds was determined using oral LD_{50} values, in fasted animals, and intraperitoneal LD_{50} values in male Swiss-Webster mice and male Wistar rats except where limited by the critical micelle concentration and the volume required for administration. The larger molecular weight compound was more toxic i.p. in rats than mice while the lower molecular weight compound was more toxic orally in mice than rats. The i.p. LD_{50} values in mice for both compounds were nearly identical and these values in both rats and mice after 24 hr. for the larger weight compound were practically the same. The usual greater toxicity with i.p. administration *versus* oral was not seen with these compounds in mice but was observed in rats.

QUATERNARY AMMONIUM COMPOUNDS are capable of a variety of pharmacologic activities, from antihypertensive to antiseptic. Many surface-active agents are quaternary ammonium compounds and have received considerable investigation as bactericidal agents (1, 2). Cetylpyridinium chloride and benzalkonium chloride are common examples, the latter of which is not a pure, single compound. The LD_{50} of cetylpyridinium chloride i.p. in the mouse is stated to be 10 mg./kg. (3). The LD_{50} in the rat orally is 200 mg./kg., subcutaneously 250 mg./kg., intraperitoneally 6 mg./kg., and intravenously 30 mg./kg. (3). The LD_{50} of benzalkonium chloride, calculated as commercial solution, in the mouse i.p. is 10 mg./kg., and i.v. 10 mg./kg. (3). For none of these figures are times of death indicated.

A mixture of triethyl ammonium bromide homologs[1] is commercially available.

It was, therefore, the purpose of this study to synthesize in a pure form dodecatriethylammonium bromide (DTEAB) and tetradecatriethylammonium bromide (TTEAB) and to determine the influence of chain length on the toxicity of these two compounds, for which little work on the pure systems has been done.

EXPERIMENTAL

The quaternary ammonium bromides were prepared by a method similar to that of Scott and Tartar (4), an established synthesis procedure for making surfactants of the cationic type. The essential differences were ones of degree, *e.g.*, (*a*) reflux times were longer, up to 3 days, (*b*) solvents were not exclusively ethanol, (*c*) surfactants were not limited to only trimethyl head groups on the monomer. Varying the conditions in *a* and *b* the extent of reaction was controlled, thereby producing more surfactant by shifting the equilibrium to the right. The position of equilibrium is most certainly affected by the nature of the monomer formed (*c* above) consequently the reflux conditions were improved as dictated by the specific synthesis.

The long-chain alkyl bromide was mixed with a 35–40% excess of the appropriate trialkylamine and this solution was added to a particular volume of the reaction solvent (CH_3OH or CH_3CH_2OH). The resulting solution was heated under total reflux from 7 hr. to 3 days as, in general, reaction time increases as molecular weight of the quaternary increases.

After the reaction mixture cooled, most of the alcohol and excess amine were boiled off, in some cases, under reduced pressure. Ethyl ether was added to precipitate the quaternary crystals.

Purification was effected by (*a*) repeated recrystallization from alcohol (CH_3OH) by adding ether, (*b*) Soxhlet extraction using ether for as long as 20 hr., or (*c*) a combination of the first two methods. The ether was then filtered from the crystals. The last traces of ether were removed by heating under vacuum for about 24 hr. at approximately 35° (5).

When ethanol was substituted for methanol as the reaction solvent, the reflux temperature was increased sufficiently to enhance the quaternary yield, in some cases by as much as 100%.

Starting materials were reagent grade products of either Eastman Organic Chemicals or Matheson, Coleman, and Bell. In those cases in which the gas chromatogram of the alkyl bromide showed more than one large peak, the compound was purified by vacuum distillation.

The primary criterion for purity of the quaternary compounds was the absence of a minimum in the surface tension-concentration curve as reported by Miles and Shedlovsky (6, 7) and verified by others (8, 9).

Stoichiometric equation:

$$RBr + R_3'N \xrightarrow[\text{reflux}]{\text{alcohol}} R\overset{+}{N}R_3'\overset{-}{Br}$$

$(R = C_{12}, C_{14})$ $(R' = Me, Et, Pr, Bu)$

Example:

$$C_{12}H_{25}Br + Et_3N \rightarrow C_{12}H_{25}\overset{+}{N}Et_3\overset{-}{Br}$$
(DTEAB)

To determine the influence of chain length on the toxicity of the two pure compounds, animals were treated as follows.

Male mice of the Swiss-Webster strain received 7.5, 15, and 30 mg./kg. of DTEAB and TTEAB i.p. Thirty animals were used at each dosage and the LD_{50} after 24, 48, and 72 hr. was determined using the Reed-Muench method (10).

Male rats of the Wistar strain received 30, 60, and

Received July 17, 1967, from the Department of Pharmacology and Toxicology, School of Pharmacy, Northeast Louisiana State College, Monroe, LA 71201

Accepted for publication May 15, 1968.

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[1] Diaper Pure, Boyle-Midway, Division American Home Prod. Corp., New York, N. Y.

TABLE I—SUMMARY OF LD_{50} STUDIES OF DTEAB AND TTEAB ORALLY (IN FASTED ANIMALS) AND INTRAPERITONEALLY IN MALE SWISS-WEBSTER MICE AND MALE WISTAR RATS

		No. Dead/Total No. Treated After			LD_{50} Determined mg./kg.		
	Dose, mg./kg.	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.
				DTEAB			
Mice							
i.p	7.5	7/30	17/30	19/30	19.60 ± 2.07		
	15.0	4/30	13/30	19/30		11.25 ± 1.58	
	30.0	29/30	30/30	30/30			8.38 ± 1.16
p.o.	5.0	9/40	12/40	15/40	12.84 ± 1.42		
	10.0	14/40	25/40	29/40		7.95 ± 0.82	
	20.0	28/40	39/40	40/40			7.03 ± 0.61
Rats							
i.p.	30.0	5/10	8/10	9/10	30.00 ± 6.28		
	60.0	10/10	10/10	10/10		—	
	120.0	10/10	10/10	10/10			—
p.o.	62.5	0/20	0/20	0/20	—		
	125.0	2/20	3/20	5/20		—	
	250.0	2/20	2/20	2/20			—
				TTEAB			
Mice							
i.p.	7.5	6/30	18/30	20/30	20.07 ± 2.17		
	15.0	5/30	13/30	17/30		11.14 ± 1.61	
	30.0	27/30	29/30	30/30			8.45 ± 1.26
p.o.	12.5	18/35	27/35	29/35	15.63 ± 1.83		
	25.0	22/30	23/30	23/30		—	
	50.0	28/30	28/30	28/30			—
Rats							
i.p.	7.5	1/10	1/10	1/10	21.67 ± 3.49		
	15.0	1/10	1/10	1/10		20.83 ± 3.35	
	30.0	9/10	10/10	10/10			20.83 ± 3.35
p.o.	10.0	1/16	1/16	1/16	—		
	20.0	1/20	1/20	1/20		—	
	40.0	1/20	1/20	1/20			—

120 mg./kg. of DTEAB and 7.5, 15, and 30 mg./kg. of TTEAB i.p.

Ten animals were used at each dosage and the LD_{50} after 24, 48, and 72 hr. was determined where possible using the Reed-Muench method (10).

Male mice, Swiss-Webster strain, were fasted at least 20 hr. but not longer than 24 hr., water was given *ad libitum*. The animals were fasted in screen-bottom cages such that there was no access to feces or litter.

The fasted mice in groups of 30–40 received 5, 10, and 20 mg./kg. of DTEAB and 12.5, 25, and 50 mg./kg. of TTEAB orally by means of stomach intubation. The oral LD_{50} was calculated where possible after 24, 48, and 72 hr. using the Reed-Muench method (10).

Male rats, Wistar strain, were fasted similarly to the mice with water *ad libitum* in screen-bottom cages such that there was no access to feces or litter.

The fasted rats in groups of 16 or 20 received 62.5, 125, and 250 mg./kg. of DTEAB and 10, 20, and 40 mg./kg. of TTEAB orally by stomach intubation similarly to obtain the oral LD_{50}.

The doses in all animals were calculated on a mg./kg. basis and all animals used were approximately 10–12 weeks of age with average weights for mice and rats 19 and 128 g., respectively. All surviving animals were observed for a period of 30 days to include any possibility of latent effects.

RESULTS AND DISCUSSION

The synthesis of the expected products was indicated by elemental analysis. Similar confirmation was reported by Venable (11) on related compounds made by this method.

Anal.[2]—Calcd. for (DTEAB) $C_{18}H_{40}BrN$: C, 61.94; H, 11.50; N, 3.99. Found: C, 61.40; H, 11.73; N, 3.97.

Anal.[2]—Calcd. for (TTEAB) $C_{20}H_{44}BrN$: C, 63.46; H, 11.71; N, 3.70. Found: C, 61.81; H, 11.67; N, 3.86.

The possibility of isomers still exists; however, based on studies of organic syntheses of this nature and on the surface and micellar properties of aqueous solutions of these compounds, it would appear identity of the products was established.

The synthesis of the pure quaternary compounds was shown by the lack of a minimum in the surface-tension concentration curve. A purity of 99% plus was obtained. The absence of minima in these curves reflected a high degree of purity and agrees with earlier reports (6, 9)

The critical micelle concentration (CMC) in water at 30° for DTEAB was 5×10^{-3} g./ml. and for TTEAB was 1.06×10^{-3} g./ml.

No particular observance of any autonomic effects typical of other quaternary ammonium compounds was made. A few of the mice injected i.p. with DTEAB first appeared hyperexcitable but soon returned, as were all other animals, to their normal, grouped together, daytime positions.

The results of the LD_{50} studies of DTEAB i.p. and p.o. in mice and rats are listed in Table I. The LD_{50} values of DTEAB i.p. in mice calculated from the results in Table I using death after 24, 48, and 72 hr. as the end points are 19.60 ± 2.07, 11.25 ± 1.58, and 8.38 ± 1.16 mg./kg., respectively. Similarly, the LD_{50} values of DTEAB orally in mice

[2]Analyses performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

are 12.84 ± 1.42, 7.95 ± 0.82, and 7.03 ± 0.61 mg./kg. after 24, 48, and 72 hr., respectively.

In the rats, only the i.p. LD_{50} for DTEAB after 24 hr. could be calculated and was found to be 30.00 ± 6.28 mg./kg.

In the mice with DTEAB the i.p. and p.o. LD_{50} values were reversed from what is normally expected when comparing toxicities with these two routes of administration. Orally, DTEAB appeared more toxic than i.p.

In the rats, comparing the i.p. and p.o. DTEAB doses administered, the normal toxicity relationship between the routes of administration was seen. In preliminary work no deaths i.p. were obtained below 30 mg./kg., 50% mortality at 30 mg., and 100% mortality above this, making further calculations impossible. Orally, larger doses were prevented due to the CMC and the maximal volume allowable which could be intubated. In preliminary work, larger doses and volumes, were attempted with no greater mortality rate resulting.

Larger doses of DTEAB were required for toxicity in rats than in mice by either route of administration, being less toxic in the rat and especially appearing much less toxic orally.

The results of the LD_{50} studies of TTEAB i.p. and p.o. in mice and rats are listed in Table I. The LD_{50} values of TTEAB i.p. in mice calculated from the results in Table I using death after 24, 48, and 72 hr. as the end points are 20.07 ± 2.17, 11.14 ± 1.61, and 8.45 ± 1.26 mg./kg., respectively. Only the 24-hr. p.o. LD_{50} for TTEAB in mice could be calculated and was found to be 15.63 ± 1.83 mg./kg.

In the rats, the i.p. LD_{50} for TTEAB was found to be 21.67 ± 3.49, 20.83 ± 3.35, and 20.83 ± 3.35 after 24, 48, and 72 hr., respectively. No p.o. LD_{50} for TTEAB in rats could be obtained again due to CMC and volume limitations.

Comparing i.p. LD_{50} values in mice for DTEAB and TTEAB, the two-carbon increase in chain length and the resulting increase in molecular weight with TTEAB influenced the LD_{50} values obtained very little.

In mice with TTEAB the i.p. and p.o. LD_{50} values were again reversed from what is normally expected when comparing toxicities *via* these two routes of administration; oral administration being more toxic than i.p.

The oral LD_{50} in mice for TTEAB was larger than the oral LD_{50} for DTEAB indicating lesser toxicity for the larger molecular weight compound.

In rats, the i.p. LD_{50} for TTEAB did not change much over 24, 48, or 72 hr., whereas, in mice the i.p. LD_{50} for TTEAB changed considerably. However, the 24-hr. LD_{50} values for both rats and mice for TTEAB were nearly the same.

The i.p. LD_{50} for TTEAB in rats was smaller than the i.p. LD_{50} for DTEAB in rats indicating greater toxicity for the larger molecular weight compound.

The larger oral dose of TTEAB in rats indicates lesser toxicity than i.p. The variations observed in this study again demonstrate the problem of species variation although the data from the rats correlate with increase in molecular weight and toxicity increases. The lesser toxicity of these compounds i.p. than orally in mice is interesting to note as oral absorption of quaternary ammonium compounds is usually stated as difficult, only up to 15% of an oral dose being absorbed due possibly to formation of nonabsorbable complexes (12).

The surviving animals after the 30-day observation period appeared in good health and without deleterious effects.

SUMMARY AND CONCLUSIONS

Synthesis of very pure DTEAB and TTEAB was accomplished.

The influence of chain length or increase in molecular weight of the compounds on toxicity proved to be species variable.

The i.p. LD_{50} values of DTEAB in mice were found to be 19.60 ± 2.07, 11.25 ± 1.58, and 8.38 ± 1.16 mg./kg. after 24, 48, and 72 hr., respectively. The oral LD_{50} values of DTEAB in mice were 12.84 ± 1.42, 7.95 ± 0.82, and 7.03 ± 0.61 mg./kg. after 24, 48, and 72 hr., respectively. Only the 24-hr. i.p. LD_{50} value of DTEAB in rats could be determined and was found to be 30.00 ± 6.28 mg./kg. The usual greater toxicity from i.p. administration *versus* p.o. was not seen with DTEAB in mice but was in rats. DTEAB appeared more toxic in mice than rats, particularly orally.

The i.p. LD_{50} values of TTEAB in mice were found to be 20.07 ± 2.17, 11.14 ± 1.61, and 8.45 ± 1.26 mg./kg., after 24, 48, and 72 hr., respectively. Only the 24-hr. p.o. LD_{50} value of TTEAB in mice could be determined and was found to be 15.63 ± 1.83 mg./kg. The i.p. LD_{50} values of TTEAB in rats were found to be 21.67 ± 3.49, 20.83 ± 3.35, and 20.83 ± 3.35 mg./kg., after 24, 48, and 72 hr., respectively. No. p.o. LD_{50} of TTEAB in rats could be obtained due to limitations of critical micelle concentration and volumes required for administration. The usual greater toxicity from i.p. administration *versus* p.o. was again not seen with TTEAB in mice.

The i.p. LD_{50} values in mice for DTEAB and TTEAB were nearly identical. Chain length appeared to have little influence on toxicity in mice i.p. Orally, the LD_{50} values in mice indicated DTEAB was more toxic than TTEAB, the lesser molecular weight compound being the more toxic. The i.p. LD_{50} values in rats and mice after 24 hr. for TTEAB were practically the same. However, the i.p. LD_{50} values indicated that the higher molecular weight compound, TTEAB, in rats was more toxic than DTEAB which is what usually would be expected. TTEAB was less toxic p.o. than i.p. in rats again demonstrating the variability of absorption seen with quaternary compounds.

General comparisons of the LD_{50} values for DTEAB and TTEAB with those of cetylpyridinium chloride and benzalkonium chloride indicated lesser toxicity for the pure DTEAB and TTEAB compounds.

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Keyphrases

Dodecatriethylammonium bromide—synthesis
Tetradecatriethylammonium bromide—synthesis
Quaternary ammonium compounds chain length—toxicity effect
Surface tension-concentration curves—purity determination
LD_{50} values—quaternary ammonium compounds

Determination of the pKa′ Value for 5,5-Diphenylhydantoin

By SURAJ P. AGARWAL and MARTIN I. BLAKE

The pKa′ value for diphenylhydantoin was found to be 8.31 by ultraviolet spectrophotometry and 8.33 by potentiometric titration.

A NONAQUEOUS titrimetric procedure for determining combinations of phenobarbital and sodium diphenylhydantoin was recently reported by Agarwal and Blake (1). In considering the feasibility of a differentiating titration for mixtures of phenobarbital and diphenylhydantoin, it was apparent that the pKa value for the latter component had not been reported in the literature. Preliminary studies (2) with a variety of solvents, titrants, and electrode systems indicated that a differentiating titration was not possible. It was concluded that the pKa values for the two components were probably too close together to permit an effective differentiating nonaqueous titration. This study was undertaken for the purpose of establishing the pKa′ for diphenylhydantoin. The spectrophotometric procedure described by Albert and Sergeant (3) was applied in this investigation. The pKa′ was also determined potentiometrically.

EXPERIMENTAL

Apparatus—All spectrophotometric measurements were made with a Carl Zeiss model PMQII spectrophotometer, equipped with matched 1.0-cm. silica cells. Potentiometric titrations were performed with a Beckman pH meter (Expandomatic) model 76A equipped with a glass electrode (Beckman No. 41263) and a calomel electrode (Beckman No. 39170). A 5-ml. buret (Kimax) graduated in 0.01 ml. was used for delivery of the titrant.

Reagents and Solutions—Reference standard diphenylhydantoin and sodium diphenylhydantoin were supplied by Parke-Davis and Co. Tris-(hydroxymethyl)aminomethane (THAM), primary standard grade, was obtained from Fisher Scientific Co. Buffer solutions were prepared by combining appropriate volumes of 1.0 *N* HCl and 0.01 *M* THAM solution to give the desired pH. All other chemicals were reagent grade.

Received March 18, 1968, from the Department of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612
Accepted for publication May 31, 1968.

Spectrophotometric Procedure—The absorption spectra of diphenylhydantoin were obtained in 0.01 *N* sodium hydroxide and in 0.01 *N* hydrochloric acid. Since the maximum difference in absorption for the ionized and unionized species occurs at 236 mμ, this was the wavelength selected for all absorbance measurements. The spectra are shown in Fig. 1.

A stock solution of diphenylhydantoin (0.01 *M*) in alcohol was prepared. One milliliter of this solution was transferred by pipet to each of seven 100-ml. volumetric flasks and the volume was brought to the mark with THAM buffer solutions having pH values of 7.7, 7.9, 8.1, 8.3, 8.5, 8.7, and 8.9, respectively. A similar series of solutions was prepared which contained 1 ml. of alcohol and the corresponding buffer solution. These served as blanks for the absorbance measurements. The average ionic strength was 0.005 (0.002–0.008).

Potentiometric Procedure—A series of stock solutions, 0.01 *M* in sodium diphenylhydantoin, was prepared in aqueous alcohol solution containing 20, 30, 40, and 50% alcohol by volume, respectively. Fifty milliliters of each solution was titrated potentiometrically with 1 *N* hydrochloric acid. The ionic strength was 0.01.

DISCUSSION

The pKa′ of diphenylhydantoin was determined spectrophotometrically by the procedure described by Albert and Sergeant (3). The absorbance of the

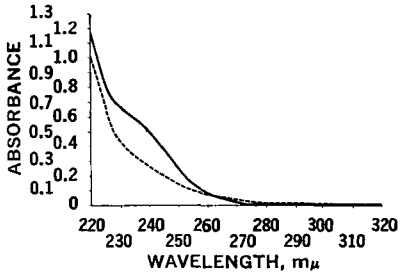

Fig. 1—Ultraviolet absorption spectra of 5,5-diphenylhydantoin in 0.01 N *HCl, solid line; and in 0.01* N *NaOH, broken line.*