so as to elicit noises of low intensity and pitch, comparable to the clicking sound of the counters (7). The present results indicate that the greater coverage of the peripheral portion of the arena, with the parallel beams, enhanced the natural tendency to stay near the periphery, and thus reduced the number of counts on the middle beam. With the crisscross arrangement, the more uniform coverage of all quadrants of the arena by the noise-producing beams apparently elicited a more consistent pattern of activity and dose–response relationship.

The higher counts produced by beam D than beam B, for the aggregated animals tested in the crisscross arrangement, indicates that the aggregated animals tended to clump together near the periphery, either near the counters (at the base of beam D) or away from them, thus activating beam D more often than beam B. The lack of any difference between the two peripheral beams (A and C), in the parallel arrangement, indicates that there was no consistent tendency either to approach or avoid the counters. A further comparison of the parallel beams also shows that the peripheral beams recorded higher counts than the middle beam for the animals under placebo and the low CPZ doses, but not for those under the highest dose. Apparently, the high doses of CPZ reduced the tendency to stay at the periphery of the arena.

With both beam arrangements there was a large dose–aggregation interaction, with CPZ producing a greater depression in activity of grouped than single mice. This indicates a greater tranquilizing effect of this compound in the stimulating social situation, in agreement with prior findings (3, 4). In the photocell activity cage with six crosscross beams recording on a single counter, the less consistent dose–aggregation interaction (4) may be due to failure of the single counter to record fully the high rate of beam interruptions during the intense activity of grouped animals in the placebo condition.

The six-beam Actophotometer is closely similar in dimensions and appearance to the independent beam instrument used in the present study. The counter which recorded activity in the six-beam unit was placed in a separate room and was inaudible to the animals. Apparently this condition of silence does not necessarily improve the delineation of drug effects; the present crisscross arrangement compares favorably with the six-beam unit in detecting the effects of low doses of CPZ. However, a separate experiment, with all other conditions equalized, would be necessary to test the effect of the audible counter clicks on spontaneous activity.

The superiority of the crisscross arrangement in detecting effects of small doses of CPZ, plus the greater drug effect with the peripheral than middle beam in the parallel arrangement, suggest that the most sensitive measure of effect of CPZ would be with an arrangement of two pairs of peripheral beams at right angles to each other, forming a tic-tac-toe pattern. The optimal conditions should probably include the use of animals in aggregations of five rather than singly as well as a separate counter for each beam.

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Distribution of Quaternary Ammonium Salts Between Chloroform and Water

By JOHN A. BILES, FOTIOS M. PLAKOGIANNIS, BEVERLY J. WONG, and PAULA M. BILES

The apparent partition coefficients, $K_{app.}$, of some alkylsulfate salts of six quaternary ammonium compounds and one tertiary amine are reported. The $K_{app.}$ of the corresponding bisulfate salts were determined by extrapolation. Some comparisons of molecular structures to the $K_{app.}$ are discussed. A method of analysis of the quaternary cations in the presence of long chain anions is reported. The relationship of longer crystal spacings of the sodium salts of alkylsulfates to the molecular weight is shown.

IN PREVIOUS communications (1, 2) it was reported that the partition of organic salts or complexes was determined by the molecular weight of the organic ions, the branching effect of the aliphatic amine cations, and the nature of the organic solvent system used. The authors showed that partitioning into the organic layer from the aqueous layer could be increased by the addition of proton donor molecules.

In several communications Levine and co-

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workers (3–7) have discussed the results of their studies concerning the intestinal absorption of quaternary ammonium compounds. It was shown that the poor absorption of quaternary ammonium compounds was attributable to the formation of nonabsorbable complexes with mucin (3). Later Levine reported that, although intestinal mucus can form nonabsorbable complexes with quaternary ammonium compounds, removal of the mucus by washing the intestine resulted in a decrease rather than an increase in the absorption of these ions (4). This led to Levine's study of the absorption of a mixture of quaternary ammonium compound and a phosphatido-peptide fraction (PPF). In two separate reports Levine reported that a mixture of PPF and either benzomethamine or *d*-tubocurarine caused more efficient absorption of either quaternary ammonium compound (5, 6). Cavallito and O'Dell reported that the administration of certain sterol acids improved the oral responses to a quaternary hypotensive agent (8). Schanker has suggested that organic ions might penetrate the gastrointestinal-blood barrier by the diffusion of the ions through the barrier in the form of a less polar complex formed with some material normally present in the lumen. He also suggested that the absorption might occur by a specialized transport process analogous to those which transport certain inorganic cations (9).

These data indicate that it is possible that a cation–anion complex is formed which may facilitate the absorption of the quaternary ammonium compound. Therefore, a program was initiated to determine to what extent the presence of anions in aqueous solution containing quaternary ammonium compounds would become more soluble in lipoid solvents and determine to what extent, if any, these salts or complexes would affect the rate of intestinal absorption of the quaternary ammonium compound. The initial communication deals with the *in vitro* study of the partitioning of the alkylsulfate salts of quaternary ammonium compounds between chloroform and water. The *in vivo* studies will be the subject of a future communication.

EXPERIMENTAL

Reagents.—The sodium alkylsulfates were furnished by E. I. DuPont Co. Additional batches were synthesized. Benzomethamine was furnished by Squibb, oxyphenonium bromide by Ciba, methantheline bromide and propantheline bromide by Searle, isopropamide by Smith Kline & French, tridihexethyl iodide by Lederle, and 2-PAM chloride by A. Kondritzer. Homatropine HBr was purchased from Mallinckrodt Chemical Works. Chloroform U.S.P. and distilled water were used as partitioning solvents.

A cationic resin,[1] was cleaned and charged by first treating the resin with 5 *M* HCl and then washing repeatedly with distilled water to remove all traces of excess HCl. The resin was stored in distilled water.

Synthesis.—The sodium alkylsulfates were synthesized by refluxing the aliphatic alcohol (Matheson, Coleman and Bell) with a moderate excess of concentrated sulfuric acid. After refluxing and cooling, the oil was neutralized with sodium bicarbonate. Excess sodium chloride was added to precipitate the sodium alkylsulfate salt (10). The salt was recrystallized several times from alcohol–ether solutions. The degree of purity of the alkylsulfates was followed using X-ray powder diffraction. Carbon and hydrogen analyses were performed.

Preparation of Solutions.—Stock solutions of each of the sodium alkylsulfates were prepared by dissolving enough of the salt in distilled water to make a 0.0005 *M* solution. Also 0.0005 *M* concentrations of each of the quaternary ammonium compounds and homatropine hydrobromide were prepared by dissolving the required amount of solute in sufficient distilled water. The tropaeolin 00 stock solution was prepared as previously described (1).

Determination of the Apparent Partition Coefficients ($K_{app.}$).—The procedures previously described were modified (1, 2). It was concluded from initial studies that association of the cations and anions in aqueous solutions was very low when using very dilute concentrations. Initial studies also indicated the salt existed as ion–ion paired monomers in the organic phase (1). Therefore, a log–log plot of the concentrations of the salt in the chloroform *versus* the concentrations of the ions in the aqueous phase would yield a slope of 2 with the y intercept being equal to $pK_{app.}$

In applying these previous observations, equivalent concentrations of a specific sodium alkylsulfate and quaternary ammonium compound or homatropine were added to 4-oz. amber bottles and enough distilled water added to bring to 40 ml. (considered to be 40 Gm.). The concentrations of each solute were either 1.5, 2.5, 3.5, or 4.5 μm./40 Gm. To the 40 Gm. of aqueous solution was added 40 Gm. of chloroform. All stoppered bottles were shaken for at least 30 min. in an Eberbach horizontal shaker. Following the shaking, the liquids were separated by decantation or by using separators. Blank solutions were also prepared.

Analyses.—Dilute aqueous solutions of the quaternary ammonium compounds and homatropine were used to prepare standard curves. An appropriate amount, usually 0.5 to 1.0 μm., of each solute was mixed with 25 ml. of a saturated solution of tropaeolin 00. The mixed solution was shaken with aliquots of chloroform for extraction. A total of 50 ml. of the chloroform extracts was collected in a 50-ml. volumetric flask. No change in the standard curve was obtained if a slurry of the standard solution of the organic cationic agent and resin was filtered and then treated with tropaeolin 00 and extracted with chloroform.

The procedure for assay of the quaternary ammonium compounds and homatropine in the presence of equivalent concentrations of alkylsulfate was

[1] Marketed as Dowex by the Dow Chemical Co., Midland, Mich.

TABLE I.—TWO LONGER CRYSTAL SPACINGS OF THE SODIUM SALTS OF ALKYLSULFATES

Alkylsulfate	d_2	d_3
Octyl	14.97 Å.	9.86 Å.
Nonyl	16.30	10.75
Decyl	17.59	11.61
Undecyl	19.07	12.46
Dodecyl	20.21	13.32
Tetradecyl	22.50	14.79

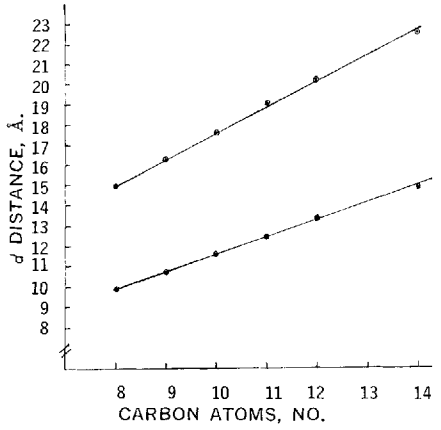


Fig. 1.—Two longer crystal spacings of the sodium salts of alkylsulfates.

modified because the anion interfered with the extraction of the "dyesalt" of the cation and tropaeolin 00. For successful analysis of the cation, enough solution containing approximately 1 μm. of cation was added to a 150-ml. beaker. The resin was then added to remove the anion. After standing for a short period of time, the solution was filtered into a 125-ml. separator. The resin collected in the funnel above the flask was washed with several small portions of distilled water. When washing was complete, 25 ml. of the saturated solution of tropaeolin 00 was added to the filtrate and the quaternary ammonium "dyesalt" was extracted with aliquots of chloroform. The chloroform extract was collected in a 50-ml. volumetric flask. All chloroform solutions were read at 425 mμ using a Beckman DU spectrophotometer.

Temperature Variation Studies.—The apparent partition coefficients of the quaternary ammonium alkylsulfates were determined at various temperatures ranging from 4° to 45°.

RESULTS AND DISCUSSION

Powder Diffraction Data.—The purification of the sodium salts of the alkylsulfates was followed by the change in the X-ray powder diffraction patterns.[2] Copper K-α radiation source was used. In general, it was found that the differences in peaks of diffraction became consistent as the alkylsulfates became pure. The d distances in angstroms for the second and third peaks are recorded in Table I and plotted in Fig. 1. The spacings obtained in these laboratories were not in complete agreement with those reported by Boyd *et al.* (11). It was also noted that distinctly different lines for the even-numbered and odd-numbered carbon atom chains were not observed as found with the fatty acids (12). This is illustrated in Fig. 1.

Apparent Partition Coefficients ($K_{app.}$).—Benzomethamine chloride was subjected to a detailed analysis since the studies of the intestinal absorption of this compound have been rather extensive (3). Both the homologous series of fatty acid salts and the alkylsulfates were studied. The fatty acids of molecular weight greater than decanoic acid were studied. The 0.0005 M solutions were prepared by adjusting the pH of the aqueous solution to 7.4 with NaOH to insure complete dissociation of the acid. It was found that partitioning did not occur with any of the fatty acid ions studied even when three or more equivalents of anion were mixed with one equivalent of cation. These observations can perhaps be explained by assuming that the partition constant of the fatty acid (even considering the almost total dissociation of acid) between chloroform and water is very large so that the fatty acid partitions in favor of the ion-ion pair of the fatty acid–ion and quaternary ammonium compound (13).

The 0.0005 M solutions of the sodium alkylsulfate salts were also used to study their effect on the partition of benzomethamine between chloroform and

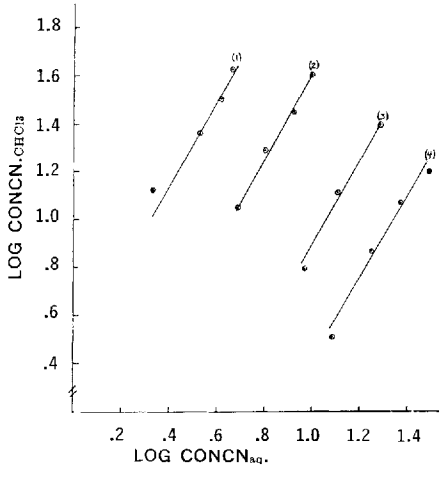


Fig. 2.—Log-log plot of the distribution of benzomethamine with 4 different concentrations of dodecyl sulfate (*1*), undecylsulfate (*2*), decylsulfate (*3*), and nonylsulfate (*4*).

TABLE II.—EXTENT OF ASSOCIATION OF ORGANIC CATIONS WITH ALKYLSULFATES IN CHLOROFORM–WATER MIXTURES

Organic Cation	Alkylsulfate Anion[a] C_9	C_{10}	C_{11}	C_{12}
Benzomethamine	1.89	1.75	1.83	1.70
Isopropamide	1.60	1.70	...	1.92
Oxyphenonium	1.84	1.40	1.50	1.60
Homatropine	...	1.78	1.85	1.80

[2] An XRD-5 spectrometer was used. The instrument was purchased with the aid of an Augustus P. Pfeiffer Foundation grant.

[a] Recorded values indicate the slope obtained by plotting log chloroform concentration against log aqueous concentration.

TABLE III.—APPARENT PARTITION COEFFICIENTS OF THE ALKYLSULFATES OF SOME ORGANIC CATIONS, EXPRESSED AS $pK_{app.}$

Organic Cation	Alkylsulfate Anion[a] C_8	C_9	C_{10}	C_{11}	C_{12}
Benzomethamine	...	3.95	4.48	5.22	5.87
Isopropamide	3.93	4.49	4.96	5.60	6.20
Oxyphenonium	4.30	4.66	5.52	5.88	6.33
Methantheline	...	4.07	...	5.86	6.68
Propantheline	...	5.60	6.63	7.54	8.56
Tridihexethyl	...	4.90	5.61	6.38	7.29
Homatropine	...	3.42	3.88	4.69	5.25

[a] $K_{app.}$ expressed as liters/mole.

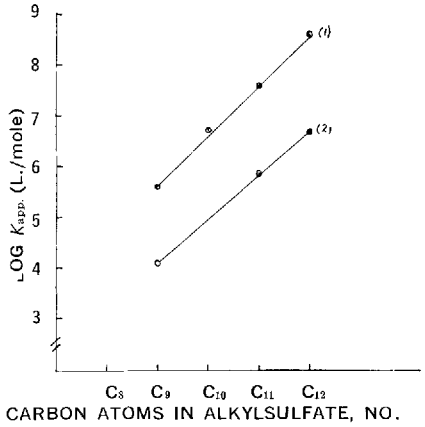


Fig. 3.—The apparent partition coefficients of alkylsulfates of propantheline (*1*) and methantheline (*2*) between chloroform and water.

water. The highest concentration of alkylsulfate used in the study was less than the concentration at which dimerization of anion has been observed (14). The sodium salts of nonyl-, decyl-, undecyl-, and dodecylsulfate were each used to determine their effect on the partition of benzomethamine. The log chloroformic concentration of benzomethamine was plotted against the log aqueous concentration of benzomethamine. The data obtained are plotted in Fig. 2.

The results for benzomethamine shown in Fig. 2 indicate that there was no apparent change in association over the 1.5–4.5 μM/40 Gm. concentration range studied. The slope for each anion varied from 1.70 to 1.89 as shown by the first entry in Table II. The slope would theoretically be 2.00 if dissociation of ions was complete in the aqueous phase and the complex existed as an ion-ion paired monomer in the chloroform. Similar studies were done using other organic cationic agents. The slopes of each cation–anion studied were calculated. The values are recorded in Table II.

The $pK_{app.}$ for the various alkylsulfates and the quaternary ammonium compounds and homatropine were determined. Since the extent of dissociation in the aqueous phase and the association in the chloroformic phase was similar for the various quaternary ammonium compounds listed in Table II, it was decided that the $pK_{app.}$ could be determined for the alkylsulfates of methantheline bromide, propantheline bromide, and tridihexethyl iodide using but one concentration, namely 2.5 μm. of quaternary ammonium ion and 2.5 μm. of alkylsulfate. The $pK_{app.}$ for all values listed in Table III were calculated from the 2.5 μM concentrations. In calculating the $pK_{app.}$ it was assumed that the slope would ideally be two, that is, that the ions were completely dissociated in the aqueous and that the ions existed as paired monomers in the chloroform. Thus, calculations were made using Eq. 1

$$pK_{app.} + 2 \log \text{concn.}_{aq.} = \log \text{concn.}_{CHCl_3} \quad \text{(Eq. 1)}$$

The calculated values are listed in Table III.

Partition studies were also performed using 2-pyridinealdoxime methiodide (2-PAM). However, the partitioning using sodium laurylsulfate was too low to determine any apparent partition coefficient. The data from Table III are plotted in Figs. 3 and 4. The data for methantheline and propantheline are plotted separately to compare more readily the two structurally similar compounds. When the linear plots in Figs. 3 and 4 are extended through the ordinate, one may posssibly conclude the intercepts to be the apparent partition constants for the acid sulfate (bisulfate) salts. These possible apparent partition constants may also be calculated knowing the change in the $pK_{app.}$ per carbon atom of the alkylsulfate. This change represents the slope of the line in Figs. 3 and 4. The information is recorded in Table IV.

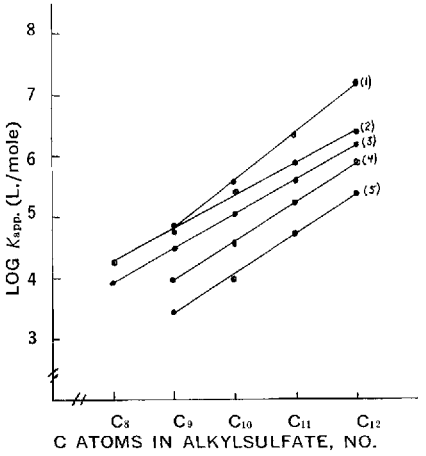
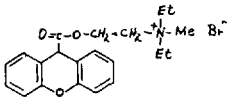


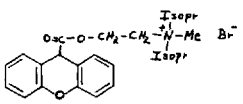
Fig. 4.—The apparent partition coefficients of alkylsulfates of tridihexethyl (*1*), oxyphenonium (*2*), isopropamide (*3*), benzomethamine (*4*), and homatropine (*5*) between chloroform and water.

TABLE IV.—APPARENT PARTITION COEFFICIENTS OF SOME ORGANIC AMINE SULFATES

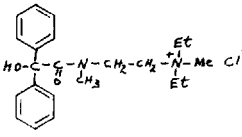
Organic Cation	$\Delta pK_{app.}$ per Carbon Atom in Alkylsulfate	$pK_{app.}$ for the Bisulfate Salt
Benzomethamine	0.64	−1.81
Isopropamide	0.55	0.50
Oxyphenonium	0.53	0.09
Methantheline	0.87	−3.76
Propantheline	0.99	−3.13
Tridihexethyl	0.81	−2.43
Homatropine	0.64	−2.37



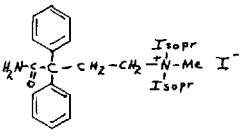
Methantheline Bromide



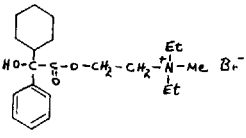
Propantheline Bromide



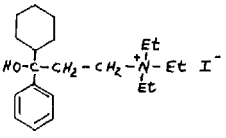
Benzomethamine



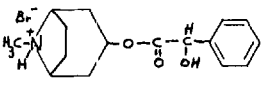
Isopropamide



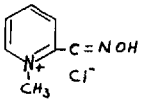
Oxyphenonium Bromide



Tridihexethyl Iodide



Homatropine HBr



2-PAM Chloride

Fig. 5.—Structures of quaternary ammonium compounds and one amine.

The $pK_{app.}$ values for the bisulfate salts were not determined because the ordinate intercept values were too small. Also the concentrations were too low in aqueous solutions for accurate determination.

The structures of the organic cationic agents listed in Table IV are illustrated in Fig. 5. When the data in Table IV are related to the structures given in Fig. 5, limited conclusions may be drawn. Some of the conclusions are made with the assumption that halide ions in the concentration present do not alter the apparent partition coefficient of the alkylsulfates. There is evidence that bromides and iodides in particular affect the distribution of organic cations between aqueous and organic solvents (7, 15, 16). A comparison of the partition studies of methantheline and propantheline bromides indicates that the $K_{app.}$ increases (*i.e.*, lipoid solubility increases) as the molecular weight increases. However, the change in $pK_{app.}$ per carbon atom change in the alkylsulfates is not the same for the two compounds. No conclusions can be drawn with respect to the $pK_{app.}$ of the alkylsulfates of tridihexethyl, benzomethamine, isopropamide, and oxyphenonium and their molecular structures. A comparison of tridihexethyl and oxyphenonium does indicate that an ester linkage and methyl group alter the $pK_{app.}$ considerably. A comparison of the structures of benzomethamine and oxphenonium does indicate that the degree of aromaticity and ester or amide alters the $pK_{app.}$ to a large extent. A comparison of the changes in structures of isopropamide and benzomethamine indicates that changes in functional groups alter the $pK_{app.}$ to a significant extent.

The apparent partition coefficients for the alkylsulfate salts of benzomethamine, tridihexethyl, and isopropamide were determined at various temperatures to determine to what extent, if any, iceberg structuring (17) around the nonpolar portion of the organic cation and anion occurred in aqueous solution. Studies were run at 4°, 23°, 32°, and 45°. No significant differences in $pK_{app.}$ values could be detected over this range of temperature. This was surprising; however, the solubility of water in chloroform may have contributed to a canceling effect. Nevertheless, it was of interest to find that significant differences occurred with one experimental study of benzomethamine and sodium laurylsulfate, but could not be duplicated with other batches of laurylsulfate. Carbon, hydrogen analysis indicated a quite small amount of adulteration of the one batch of sodium laurylsulfate. The adulterant present was not identified.

A useful analytical assay for quaternary ammonium compounds using the "dyesalt" method of analysis was developed when the aqueous solutions contained alkylsulfates. It was noted that very small amounts of the alkylsulfate would interfere with the extraction of the "dyesalt" even when huge amounts (relatively speaking) of the anionic dye was used. This indicated that the stability constant of the quaternary ammonium alkylsulfate was significantly high when compared to the tropaeolin 00 salt of the quaternary ammonium salt.

The intestinal absorption of the alkylsulfate salts of the various quaternary ammonium compounds illustrated in Fig. 5 is now being studied and will be the subject of a future communication.

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