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PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITY: THERMODYNAMIC PROPERTIES OF COMPOUNDS RELATED TO ACETYLCHOLINE ASSESSED FROM DEPRESSION OF FREEZING-POINT AND ENTHALPIES OF DILUTION

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1 Measurements have been made of the osmotic coefficients and enthalpies of dilution of acetylcholine and of compounds related to it in which the carbonyl and ether groups have been replaced by methylene and the trimethylammonium group by triethylammonium. All were iodides. Measurements were also made with tetraethylammonium iodide and agree with published values.

2 Where necessary the affinities of the compounds for acetylcholine receptors in the guinea-pig ileum and frog rectus, or their activities relative to acetylcholine, have been measured.

3 The osmotic coefficients were used to calculate activity coefficients and excess free energies, which have been used with the excess enthalpies to calculate the excess entropies of the solutions. These indicate that the ester and carbonyl groups have a marked ordering effect on the ions in water compared with methylene groups; the ether group has an intermediate effect.

4 When the results are interpreted in terms of ion-pair formation they can be used to calculate the ion-association constants and enthalpies and entropies of formation of ion-pairs, and lead to similar conclusions: that the order associated with ion-pair formation is greater with the esters and ketones. There appears to be extensive ion-association in the concentrations (0.5 to 1 M) usually used in n.m.r. studies.

5 There is no obvious correlation between the effects of groups on water and their activity or affinity at muscarine-sensitive acetylcholine receptors but it is possible that ability to activate nicotine-sensitive receptors may be associated with an increase in order, though it would be necessary to study entropy changes in systems actually involving receptors in order to prove this. It is also necessary to suppose that ability to activate these receptors is limited to compounds with small onium groups.

Introduction

In attempts to account for the activity of drugs at receptors considerable attention has been given to their physicochemical properties. Activity at acetylcholine receptors has been postulated to be associated with particular conformations, as determined by X-ray crystallography (Baker, Chothia, Pauling & Petcher, 1971), as predicted from molecular oribital calculations (Kier, 1967, 1968: Pullman & Courrière, 1972), or as estimated from n.m.r. spectroscopy of solutions in deuterium oxide (Partington, Feeney & Burgen, 1972). Possible effects of ion size on activity and affinity for acetylcholine receptors have been discussed by Barlow, Lowe, Pearson, Rendall & Thompson (1971).

Because acetylcholine and compounds related to it are biologically active in low concentrations, it is preferable to use physical measurements with dilute solutions, rather than in high concentrations or in the solid state, in any attempted correlation with biological activity. The present paper describes measurements of the osmotic coefficients and enthalpies of dilution of acetylcholine and related compounds in order to assess some thermodynamic properties of their solutions. With present equipment for measuring the depression of the freezing point of water, osmotic coefficients can be measured with solutions of about

0.05 mol/kg upwards; accurate measurements of enthalpies of dilution are possible with solutions of 0.1 mol/kg upwards. Although these concentrations are still very high compared with those which are biologically active, they are considerably lower than those used in n.m.r. spectroscopy (Partington *et al.*, 1972, used solutions about 1 M in deuterium oxide). They are roughly in the same range as those used in density measurements made by Barlow *et al.* (1971) and Barlow & Franks (1973), though the estimates of apparent molal volume derived from these were extrapolated to infinite dilution.

The compounds studied included acetylcholine and analogues in which trimethylammonium was replaced by triethylammonium and the ester group was replaced by $-CH_2CH_2-$, $-CH_2O-$, and $-CH_2CO-$. All the compounds were iodides. Measurements were also made with tetraethylammonium iodide, whose osmotic coefficients and enthalpies of dilution have already been reported (Lindenbaum & Boyd, 1964; Lindenbaum, 1966).

Although the biological properties of these compounds appear to depend only on the cation, their thermodynamic properties depend also on the anion. All the compounds studied had the same anion (iodide) but it was not feasible to study series derived from other anions. Although, therefore, it may not be justifiable to attempt to correlate thermodynamic properties directly with biological properties, the effects of changes in structure on thermodynamic properties could indicate changes, perhaps due to effects on water structure, which might be related to changes in biological activity.

From the osmotic coefficients the excess free energies (G^{EX}) were calculated as described by Lindenbaum & Boyd (1964), the excess function being the difference between the value for the solution and that for an ideal solution of the same composition. The enthalpy of dilution of an ideal solution is zero, so the experimental values are estimates of minus the excess enthalpy (H^{EX}; if this is negative the solution will absorb heat when diluted and the enthalpy of dilution will be positive) and can be used with the excess free energies to calculate the excess entropies of the solutions (S^{EX}; Lindenbaum, 1966). Allowance for possible ion-association can be made by applying Debye-Hückel theory and the osmotic coefficients can be used to estimate the ion-association constants. Values for some of the compounds have already been obtained by Lowe, MacGilp & Pritchard (1973) from conductance measurements at 25°C. These could be used to calculate the degree of ion-association in the measurements of the enthalpies of dilution and hence to estimate the enthalpy of formation of ion-pairs. This can then be used in conjunction with the free energy of formation, obtained from the association constant, to calculate their entropy of formation.

However, Lindenbaum, Leifer, Boyd & Chase (1970) have suggested that the concept of ion-pairing is not valid when either cations or anions reduce the amount of local order in the solvent. It is arguable whether this applies to the compounds studied, so both the excess entropies and the entropies of formation of ion-pairs have been calculated and compared with the biological properties of the compounds in order to see how far the effects of changes in structure on biological activity might be related to changes in thermodynamic properties.

Although all the compounds are closely related to acetylcholine it was necessary to supplement existing information about their biological properties and some experiments were performed with the guinea-pig ileum and frog rectus preparations in order to estimate the affinity of compounds for acetylcholine receptors and/or their activity relative to acetylcholine.

Methods

Osmotic coefficients

These were obtained from the depression of the freezing-point of water, measured with an Advanced Instruments Model 3R Osmometer, calibrated with standard solutions of sodium chloride which had freezing-points of −0.1858 and −0.929°C.

Solutions of the compounds were made up by weight in at least six different concentrations ranging between 0.05 and 0.5 molal. Duplicate samples of approximately 2.5 cm^3 of each concentration were taken and their freezing-points measured in order of increasing concentration. They were then immediately covered with 'Parafilm', allowed to thaw, and the measurements repeated. The four estimates of the freezing-point for each concentration were consistent to within 0.0001°C and it is the errors in the calibration which limit the accuracy of the results.

The osmotic coefficient, ϕ, was calculated from the depression of freezing-point, ΔT, and the molality of the solution, m:

$$\phi = \frac{\Delta T}{1.858 \times 2\,m}$$

Enthalpies of dilution

These were measured with an LKB batch

microcalorimeter connected to a Keithley 150B amplifier and a Vitatron UR403 electronically integrating potentiometric recorder. The temperature was 25.0 ± 0.01° C. Solutions were made up by weight and measurements were made with at least three concentrations of each compound, about 0.10, 0.16 and 0.25 mol/kg. The dilution was usually 1 to 20, with samples of 200 ± 3 mg of solution diluted with 3.800 ± 0.008 g water. The final concentrations were therefore approximately 0.005, 0.008, and 0.0125 mol/kg. In many experiments with 0.10 mol/kg solutions, however, the dilution was 1 to 10, with 400 ± 4 mg of solution and 3.600 ± 0.008 g water, and the final concentration was approximately 0.010 mol/kg. It was necessary to use the larger amounts of solution when the heat change was small; in some experiments it was as low as 6 mJ (1.5 mcal). One of the compounds with a particularly high enthalpy of dilution, 4-ketopentyltriethylammonium iodide, was investigated in a concentration of 0.04 mol/kg, diluted 1 to 10.

Usually at least four measurements were made with each solution and the mean estimate of the enthalpy of dilution/mol, Q, was calculated with its standard error, which was usually about 1% of the mean. In experiments with 0.04 mol/kg solutions of 4-ketopentyltriethylammonium iodide it was 3% but in none of the others was it greater than 2%.

To obtain the enthalpy of dilution, ϕL, it is necessary to allow for the dilution of the residual solutions. Lindenbaum (1966) assumed this was the same as for solutions of sodium chloride but in the range of the final concentrations used in this work (0.005 to 0.0125 mol/kg) the dilution of sodium chloride is exothermic. The dilution of the quaternary iodides in this range, however, appeared still to be endothermic, though the heat was too small to be measured accurately, usually being less than 15 cal/mol (less than 25 µcal in the conditions of the experiment). For the calculation of H^{EX} an allowance was made for the heat of dilution of the residual solutions by extrapolating the results obtained with higher concentrations to the value zero for infinite dilution. In most experiments this involved the deduction of about 10 cal/mol (range, 5 to 25 cal/mol). For the calculations of the enthalpy of formation of ion-pairs, however, there was no need to estimate ϕL because it was possible to work with the enthalpy of dilution/mol, Q, between the two concentrations used.

Biological activity

Experiments were made on the guinea-pig isolated ileum and frog rectus preparations with the compounds which had not already been tested and also to check published values for some of the others (Welsh & Taub, 1951: Ing, Kordik & Tudor Williams, 1952; Barlow *et al.*, 1971). The ileum was suspended in aerated Tyrode solution (containing hexamethonium 2.76×10^{-4} M) at 37° C and the rectus abdominis *(Rana temporaria)* in frog-Ringer solution at room temperature (Edinburgh Staff, 1970). The time-cycle in the experiments with the rectus, however, was lengthened and agonist drugs were allowed to act for 2 min and applied once every 8 minutes.

The activities of agonists were expressed as equipotent molar ratios relative to acetylcholine by comparing the concentrations producing comparable responses. As was pointed out by Ing *et al.* (1952) the graph of log dose against response with the frog rectus is much flatter for acetylcholine than for the other compounds and only a rough comparison can be made.

With compounds which were antagonists the affinity constant was measured if possible (Abramson, Barlow, Mustafa & Stephenson, 1969).

Compounds

Acetylcholine iodide, chromatographically pure, was obtained from B.D.H. Ltd. Acetoxyethyltriethylammonium iodide had m.p. 119.5-119.8° C; found I^-, 40.13; theory, 40.26%. 4-Keto-*n*-pentyltrimethylammonium iodide had m.p. 97.6-98.6° C; found I^-, 46.65: theory 46.79%. Ing *et al.* (1952) recorded m.p. 100-102° C; Welsh & Taub (1951) recorded m.p. 87-90° C. 4-Keto-*n*-pentyltriethylammonium iodide had m.p. 90.0-90.8° C; found I^-, 40.42; theory, 40.52%. For details of the other compounds see Abramson *et al.* (1969).

Results

Results obtained with tetraethylammonium iodide provide a check on the estimates of both the osmotic coefficients and the enthalpies of dilution. Values of ϕ obtained by interpolation on the graph of osmotic coefficient against (molality)$^{1/2}$ agreed well those obtained by Lindenbaum & Boyd (1964) from isopiestic measurements at 25° C (shown in parentheses): $m = 0.10$, $\phi = 0.855$ (0.865); $m = 0.15$, $\phi = 0.831$ (0.832); $m = 0.20$, $\phi = 0.806$ (0.804); $m = 0.25$, $\phi = 0.783$ (0.782). Lindenbaum & Boyd had noted that values they obtained for tetra-alkylammonium salts at 25° C did not differ much from those reported by earlier workers from the depression of the freezing-point of water and although Lindenbaum *et al.* (1970) observed that the osmotic coefficients at 25° C and

65°C differed appreciably for quaternary halides with small anions, such as chloride, those for iodides did not.

Lindenbaum (1966) obtained values for the enthalpy of dilution of tetraethylammonium iodide from 0.298 to 0.005 mol/kg and from 0.222 to 0.003 mol/kg of 292 and 227 cal/mol respectively. When the results obtained in this present study were used to calculate, by the method of least squares, the best fit for a linear relation between enthalpy of dilution and (molality)$^{1/2}$ the interpolated values for these concentrations were 286 and 226 cal/mol respectively. This indicates reasonable agreement between the two sets of results, even allowing for the differences in the concentrations of the diluted solutions.

Calculations of excess free energies, enthalpies, and entropies (G^{EX}, H^{EX}, and S^{EX})

The procedure followed was the same as that used by Boyd, Schwarz & Lindenbaum (1966) and Lindenbaum (1966), the only difference being that the osmotic coefficients were obtained from freezing-point depression and that it was assumed from the results of Lindenbaum *et al.* (1970) that these would not be much different from values at 25°C. The osmotic coefficients (Table 1a) were plotted against (molality)$^{1/2}$ and also against the expression $(1-\phi)/m^{1/2}$, whose limiting value ($m \rightarrow 0$) was taken as 0.3903 (Boyd *et al.*, 1966). From the graphs, drawn by eye, values of $(1-\phi)$ and of

$$\int_0^{m^{1/2}} \frac{(1-\phi)}{m^{1/2}}\, d(m)^{1/2}$$

were calculated for selected values of $m^{1/2}$. The integration was made graphically. The activity coefficients were then calculated from the relation:

$$-\ln\gamma = 1-\phi+2\int_0^{m^{1/2}} \frac{(1-\phi)}{m^{1/2}}\, d(m)^{1/2}$$

and the excess free energies from the relation:

$$G^{EX} = 2RT\,(1-\phi+\ln\gamma).$$

Values of H^{EX}, obtained from the calorimetry experiments by making an allowance for the dilution of the residual solutions (see above) are shown in Table 2. The graphs of H^{EX} against $m^{1/2}$ (Fig. 1) were used to calculate values of H^{EX} for $m^{1/2} = 0.3$, 0.4, and 0.5 and an estimate of the value for $m^{1/2} = 0.2$ was made by taking an average of the value obtained by joining the origin to the result for $m^{1/2} = 0.3$ and the value obtained by extrapolating the results for $m^{1/2} = 0.3$, 0.4, and 0.5. It was possible to check this estimated value for $m^{1/2} = 0.2$ with 4-ketopentyltriethylammonium iodide, for which the enthalpy of dilution was large enough to be measured reasonably accurately, and the experimental value was 101 cal/mol, compared with the calculated value of 108 cal/mol.

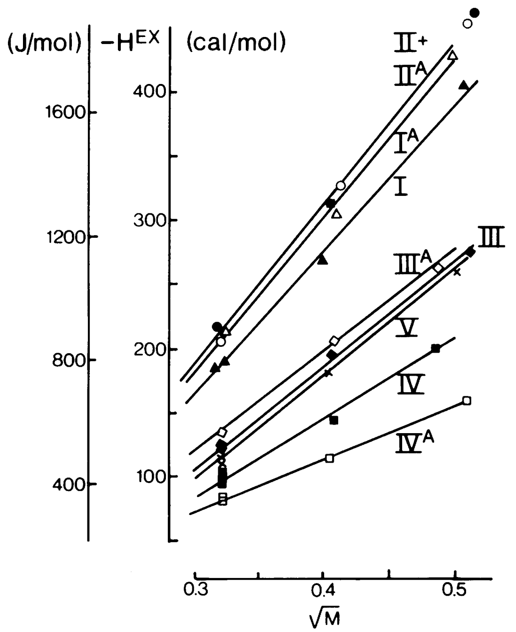

Fig. 1 Values of $-H^{EX}$ plotted against (molality)$^{1/2}$ for acetylcholine iodide (I), acetoxyethyltriethylammonium iodide (I^A), the 4-ketopentyl compounds (II and II^A), the ethoxyethyl compounds (III and III^A), the *n*-pentyl compounds (IV and IV^A) and tetraethylammonium iodide (V). The trimethylammonium compounds are indicated by the solid symbols. The estimates have been derived from the calorimetry experiments by making an allowance for the dilution of the residual solutions (see text).

Values of TS^{EX}, calculated from the relation:

$$TS^{EX} = H^{EX} - G^{EX}$$

are shown in Table 3, together with the values of G^{EX} and H^{EX}, and are illustrated in Figure 2.

Calculation of association constants (K), enthalpy of formation (ΔH) and entropy of formation (ΔS) of ion-pairs

If the results are interpreted in terms of ion-pair formation, departure from the predictions of Debye-Hückel theory should be accounted for by

Table 1a Osmotic coefficients (ϕ) calculated from the depression of the freezing-point of water (ΔT); each entry is the average of duplicate estimates with duplicate samples

Molality	ΔT	ϕ	*DHLL*	α
$Et_4\overset{+}{N}I^-$; a = 5.61; K = 3.8				
0.0515	0.1735	0.907 (0.902)	0.914	0.890
0.1038	0.3336	0.865 (0.865)	0.878	0.825
0.1509	0.4654	0.830 (0.843)	0.853	0.782
0.1944	0.5808	0.804	0.834	
0.3002	0.8436	0.756	0.793	
0.3874	1.0406	0.723	0.765	
n-PentNMe$_3$ I$^-$; a = 5.64; K = 4.5				
0.0478	0.1602	0.902 (0.898)	0.917	0.881
0.0988	0.3127	0.852 (0.859)	0.881	0.809
0.1493	0.4517	0.814 (0.833)	0.854	0.759
0.1877	0.5512	0.780	0.836	
0.3142	0.8567	0.734	0.788	
0.4009	1.0428	0.700	0.761	
0.4896	1.2212	0.671	0.736	
n-Pent$\overset{+}{N}$Et$_3$ I$^-$; a = 5.99; K = 6.0				
0.0506	0.1671	0.889 (0.883)	0.915	0.845
0.0964	0.3002	0.838 (0.844)	0.883	0.771
0.1500	0.4428	0.795 (0.814)	0.854	0.711
0.1932	0.5490	0.765	0.834	
0.3000	0.7852	0.704	0.793	
0.3675	0.9182	0.672	0.771	
0.4069	0.9929	0.657	0.759	
0.5020	1.1602	0.622	0.733	
$EtOCH_2CH_2\overset{+}{N}Me_3\,I^-$; a = 5.55; K = 4.0				
0.0498	0.1675	0.904 (0.901)	0.916	0.889
0.0980	0.3151	0.865 (0.866)	0.882	0.825
0.1492	0.4608	0.831 (0.840)	0.854	0.777
0.1945	0.5845	0.809	0.834	
0.2971	0.8503	0.770	0.794	
0.3890	1.0748	0.743	0.765	
$EtOCH_2CH_2\overset{+}{N}Et_3\,I^-$; a = 5.91; K = 5.0				
0.0510	0.1690	0.892 (0.891)	0.915	0.864
0.1013	0.3180	0.845 (0.880)	0.880	0.791
0.1448	0.4346	0.808 (0.829)	0.856	0.745
0.2019	0.5797	0.773	0.830	
0.2963	0.8036	0.730	0.795	
0.4025	1.0334	0.691	0.760	
$CH_3COCH_2CH_2CH_2\overset{+}{N}Me_3\,I^-$; a = 5.60; K = 6.0				
0.0501	0.1646	0.884 (0.883)	0.915	0.847
0.0992	0.3102	0.842 (0.841)	0.881	0.768
0.1505	0.4502	0.805 (0.813)	0.853	0.712
0.1972	0.5705	0.778	0.832	
0.2923	0.7992	0.736	0.796	
0.4172	1.0783	0.696	0.756	
$CH_3COCH_2CH_2CH_2\overset{+}{N}Et_3\,I^-$; a = 5.98; K = 7.0				
0.0513	0.1666	0.875 (0.874)	0.914	0.825
0.1013	0.3101	0.823 (0.830)	0.880	0.741
0.1534	0.4441	0.779 (0.801)	0.852	0.682
0.2016	0.5592	0.747	0.831	
0.2934	0.7595	0.697	0.796	
0.3996	0.9697	0.653	0.761	
Acetylcholine iodide; a = 5.51; K = 4.30				
0.0495	0.1661	0.902 (0.898)	0.916	0.882
0.0980	0.3122	0.858 (0.862)	0.882	0.816
0.1478	0.4533	0.825 (0.836)	0.855	0.767
0.1930	0.5730	0.799	0.834	
0.2968	0.8305	0.753	0.794	
0.3869	1.0400	0.723	0.765	
0.4882	1.2580	0.693	0.736	

Table 1a—*continued*

Molality	ΔT	ϕ	*DHLL*	α
$CH_3COOCH_2CH_2\overset{+}{N}Et_3I^-$; a = 5.89; K = 6.4				
0.0498	0.1631	0.882 (0.880)	0.916	0.839
0.0992	0.3071	0.833 (0.838)	0.881	0.757
0.1416	0.4209	0.800 (0.813)	0.858	0.709
0.1985	0.5630	0.763	0.832	
0.2936	0.7836	0.718	0.795	
0.3869	0.9814	0.683	0.765	

The values in parentheses are calculated from the Debye-Hückel theory with the values of the size parameter, *a*, and the association constant, K, shown; α is the degree of dissociation of the ion-pairs. The limiting values (α = 1, a = 0) are indicated by DHLL.

Table 1b Stoichiometric activity coefficients calculated from Debye-Hückel theory compared with values (shown in parentheses) obtained the expression

$$-\ln\gamma = 1-\phi+2\int_{0}^{m^{1/2}} \frac{(1-\phi)}{m^{1/2}}\, d(m)^{1/2}$$

	Molality			
	0.04	0.09	0.16	0.25
$Et_4\overset{+}{N}I^-$	0.774	0.683	0.608	0.546
	(0.782)	(0.687)	(0.605)	(0.531)
n-Pent$\overset{+}{N}Me_3I^-$	0.763	0.667	0.590	0.527
	(0.774)	(0.671)	(0.581)	(0.503)
n-Pent$\overset{+}{N}Et_3I^-$	0.743	0.639	0.558	0.493
	(0.760)	(0.652)	(0.555)	(0.470)
$EtOCH_2CH_2\overset{+}{N}Me_3I^-$	0.771	0.678	0.602	0.540
	(0.773)	(0.674)	(0.583)	(0.507)
$EtOCH_2CH_2\overset{+}{N}Et_3I^-$	0.757	0.658	0.579	0.515
	(0.773)	(0.674)	(0.587)	(0.513)
$CH_3COCH_2CH_2CH_2\overset{+}{N}Me_3I^-$	0.741	0.638	0.556	0.491
	(0.760)	(0.652)	(0.561)	(0.484)
$CH_3COCH_2CH_2CH_2\overset{+}{N}Et_3I^-$	0.730	0.622	0.538	0.473
	(0.743)	(0.629)	(0.532)	(0.450)
Acetylcholine iodide	0.766	0.671	0.594	0.531
	(0.780)	(0.679)	(0.590)	(0.515)
$CH_3COOCH_2CH_2\overset{+}{N}Et_3I^-$	0.737	0.632	0.549	0.484
	(0.748)	(0.640)	(0.547)	(0.468)

The values of *a* and K used in the Debye-Hückel calculations are given in part (a) of this Table.

Table 2 Enthalpies of dilution at 25°C

		Q				
Molality	α	*J/mole*	*cal/mole*	$-H^{EX}$	$-\phi L_{\pm}$ *(cal/mole)*	$-\Delta H$ *(cal/mole)*
$Et_4\overset{+}{N}I^-$; a = 5.61; K = 2.9						
0.44460	0.684	1654 ± 7 (4)	396	416	99.5	1628
0.02223	0.955				53.3	
0.25017	0.762	1039 ± 12 (4)	249	261	96.2	1436
0.01251	0.972				42.8	
0.15811	0.816	706 ± 9 (5)	169	178	90.7	1357
0.00790	0.981				35.4	
0.10262	0.860	436 ± 7 (5)	104	113	83.6	1269
0.01026	0.977				39.5	
0.10253	0.860	444 ± 3 (5)	106	115	83.6	1286
0.01025	0.977				39.5	
						1337 (Av.)

Table 2—*continued*

Molality	α	*Q*		$-H^{EX}$	$-\phi L_{\pm}$ *(cal/mole)*	$-\Delta H$ *(cal/mole)*
		J/mole	*cal/mole*			
n-Pent$\overset{+}{\mathrm{N}}\mathrm{Me_3I^-}$; a = 5.64; K = 3.3						
0.23359	0.751	793 ± 7 (4)	190	200	92.1	1095
0.01168	0.971				41.4	
0.16460	0.794	584 ± 5 (4)	140	146	88.4	1046
0.00823	0.978				35.9	
0.10259	0.845	370 ± 6 (5)	88	96	81.6	1017
0.01026	0.974				39.3	
0.10245	0.846	384 ± 1 (6)	92	100	81.6	1049
0.01025	0.974				39.3	
0.10140	0.847	354 ± 5 (4)	85	93	81.4	1000
0.01014	0.974				39.1	
						1041 (Av.)
n-Pent$\overset{+}{\mathrm{N}}\mathrm{Et_3I^-}$; a = 5.99; K = 3.7						
0.25885	0.716	630 ± 3 (4)	151	160	87.5	790
0.01294	0.964				42.5	
0.16203	0.778	462 ± 4 (4)	110	115	83.9	801
0.00810	0.976				35.3	
0.10293	0.830	309 ± 2 (6)	74	80	78.3	810
0.01029	0.971				38.8	
0.10288	0.830	304 ± 5 (4)	73	79	78.3	803
0.01029	0.971				38.8	
						801 (Av.)
$\mathrm{EtOCH_2CH_2}\overset{+}{\mathrm{N}}\mathrm{Me_3I^-}$; a = 5.55; K = 2.9						
0.26036	0.757	1109 ± 12 (4)	265	278	97.0	1486
0.01302	0.971				43.5	
0.16384	0.813	780 ± 8 (4)	187	195	91.5	1441
0.00819	0.981				36.0	
0.10237	0.860	457 ± 7 (4)	109	119	83.9	1317
0.01024	0.977				39.5	
0.10237	0.860	474 ± 5 (6)	113	123	83.9	1352
0.01024	0.977				39.5	
						1399 (Av.)
$\mathrm{EtOCH_2CH_2}\overset{+}{\mathrm{N}}\mathrm{Et_3I^-}$; a = 5.91; K = 3.30						
0.23474	0.748	1052 ± 16 (4)	252	266	90.3	1356
0.01174	0.970				41.2	
0.16465	0.793	826 ± 8 (4)	198	207	86.9	1344
0.00823	0.978				35.7	
0.10295	0.844	512 ± 2 (6)	122	134	80.4	1263
0.01029	0.974				39.1	
						1321 (Av.)
$\mathrm{CH_3COCH_2CH_2CH_2NMe_3I^-}$; a = 5.60; K = 3.70						
0.26232	0.717	1824 ± 26 (4)	436	461	90.2	1958
0.01312	0.964				43.1	
0.16216	0.780	1251 ± 3 (4)	299	315	86.1	1782
0.00811	0.976				35.5	
0.10188	0.834	865 ± 14 (3)	207	217	79.9	1701
0.00509	0.984				29.2	
						1814 (Av.)
$\mathrm{CH_3COCH_2CH_2CH_2}\overset{+}{\mathrm{N}}\mathrm{Et_3I^-}$; a = 5.98; K = 4.0						
0.25681	0.704	1809 ± 11 (4)	433	457	85.5	1852
0.01284	0.962				42.2	
0.16855	0.762	1310 ± 5 (4)	313	328	82.7	1700
0.00843	0.973				35.7	
0.10135	0.822	824 ± 26 (4)	197	206	76.9	1525
0.00507	0.983				28.9	
0.04034	0.905	390 ± 12 (3)	93	101	62.1	1546
0.00202	0.993				19.1	
						1656 (Av.)

Table 2—*continued*

Acetylcholine iodide; a = 5.5; K = 3.1						
0.25646	0.749	1618 ± 11 (4)	387	407	95.4	1989
0.01282	0.970				43.2	
0.15756	0.808	1077 ± 19 (4)	258	271	89.9	1817
0.00788	0.980				35.4	
0.10307	0.853	729 ± 3 (4)	174	190	83.2	1776
0.01031	0.975				39.5	
0.09994	0.855	745 ± 13 (3)	178	186	82.7	1761
0.00499	0.987				29.0	
						1826 (Av.)
$CH_3COOCH_2CH_2\overset{+}{N}Et_3I^-$; a = 5.89; K = 3.80						
0.24622	0.719	1716 ± 30 (4)	410	430	87.2	1857
0.01233	0.965				41.8	
0.16605	0.772	1224 ± 20 (4)	293	306	84.1	1679
0.00830	0.975				35.6	
0.10304	0.827	871 ± 10 (3)	208	216	78.4	1645
0.00515	0.984				29.1	
						1727 (Av.)

Results for tetramethylammonium iodide with a = 4.80 and K = 2.02 (Fernandez-Prini, 1968); the first three values of Q are from Lindenbaum (1966) and the last was obtained in this work.

		Q				
Molality	α	*J/mole*	*cal/mole*	$-H^{EX}$	$-\phi L_{\pm}$ *(cal/mole)*	$-\Delta H$ *(cal/mole)*
0.23136	0.826	961	230	232	110.2	1899
0.00118	0.998				15.1	
0.23136	0.826	890	213	215	110.2	1799
0.00121	0.998				15.3	
0.11357	0.890	539	129	134	94.8	1916
0.00212	0.996				19.9	
0.10200	0.897	363 ± 2 (5)	87	103	92.2	1610
0.01020	0.984				40.5	
						1806 (Av.)

The mean value of the heat of dilution/mole, Q, is shown with the standard error and with the number of results in parentheses. The excess enthalpy, H^{EX}, has been calculated from Q by allowing for the heat of dilution of the residual solution. From the initial and final molalities, m_1 and m_2, and the association constant, K, and size-parameter, a, the degree of dissociation, α, of the ion-pairs has been calculated, together with the calculated enthalpy of dilution of the ions, $\phi L_{\pm}$, and the enthalpy of formation of the ion-pairs, ΔH. The average of ΔH shown (marked Av.) does not include the result with 0.44 molal tetraethylammonium iodide. Note that the dilution of the solutions is endothermic; dilution of the ions is exothermic, but break-up of the ion-pairs is endothermic and their enthalpy of formation is negative (the process is exothermic).

ion-association. The endothermic nature of the dilution of the solutions, which should be exothermic according to Debye-Hückel theory, suggests that the formation of ion-pairs is an exothermic process and this is supported by the observations of Fernandez-Prini (1968) that ion-association is increased by reducing the temperature. Estimates of the association constants at 25°C have been obtained for tetraethylammonium iodide (Fernandez-Prini, 1968; Lowe & Rendall, 1972) and for the *n*-pentyl- and ethoxyethyl-compounds (Lowe *et al.*, 1973); the values at 0°C will be somewhat higher.

If the association constant is known, the degree of dissociation of ion-pairs, α, and the ionic activity coefficient, $\gamma_{\pm}$, can be calculated for a particular molality, m, from the expression:

$$K = \frac{(1-\alpha)}{m\alpha^2\gamma_{\pm}^2}$$

and the Debye-Hückel relation:

$$-\log_{10}\gamma_{\pm} = \frac{A(\alpha m)^{1/2}}{1 + Ba(\alpha m)^{1/2}}$$

where the constants A and B have the values 0.4918 and 0.3248, respectively, at 0°C (0.5115 and 0.3291 at 25°C; Robinson & Stokes, 1965) and the size parameter, a, can be estimated from the apparent molal volume at infinite dilution, ϕ_v^o, to which it was assumed to be linearly related

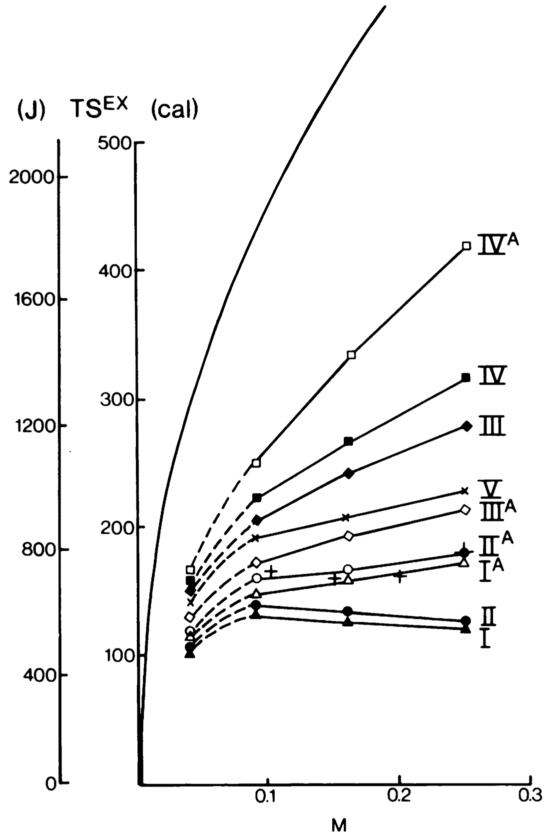

Fig. 2 Values of TS^{EX} (cal/mol) plotted against molality for acetylcholine iodide (I), acetoxyethyltriethylammonium iodide (I^A), the 4-ketopentyl compounds (II and II^A), the ethoxyethyl compounds (III and III^A), the pentyl compounds (IV and IV^A) and tetraethylammonium iodide (V). Estimates for tetramethylammonium iodide are indicated by + and the continuous curve indicates the predictions of the Debye-Hückel limiting law.

(Lowe *et al.*, 1973). Values of ϕ_v^0 obtained by MacGilp (personal communication) were used to obtain *a* by interpolation where necessary. An iterative procedure was used to calculate α for particular values of m and it was found that a cycle of five calculations was sufficient to give estimates of α which had become consistent to within one part in 10,000 (these, and the following, calculations were made with the Edinburgh Regional Computing Centre's IBM 370/155 computer and a Fortran computer programme).

The ionic osmotic coefficiant, $\phi_\pm$, was then calculated from the Debye-Hückel expression:

$$1 - \phi_\pm = \frac{2.3\ A}{3}\ (\alpha m)^{1/2}$$

$$\times \left[\frac{3}{u^3}\left(1 + u - \frac{1}{(1 + u)} - 2 \ln (1 + u)\right)\right]$$

where $u = Ba(\alpha m)^{1/2}$. This was used to derive the stoichiometric osmotic coefficient (Pitzer, 1972), $\phi_{sto} = \alpha\phi_\pm + 1/2\ (1 - \alpha)$, which was compared with the experimental value.

The Debye-Hückel expressions used are only likely to be valid for concentrations up to about 0.1M and it was not expected that it would be possible to obtain close agreement between the calculated and experimental osmotic coefficients over the whole range of concentrations tested. The values of K were altered, however, starting from a guess based on the values of K at 25° C, until the calculated values of ϕ_{sto} agreed reasonably with the experimental values for the two lowest concentrations (approximately 0.05 and 0.10 mol/kg). At the lowest concentration the calculated value was less than the observed value but at the next concentration it was higher by roughly the same amount (Table 1a). The calculated values were clearly sensitive to changes in K and it seemed probable that the error in the estimate of K obtained in this way was about 0.5 units. Table 1a also includes the values of the osmotic coefficient predicted by Debye-Hückel theory in limiting conditions ($a = 0$, $\alpha = 1$, $1 - \phi = (2.3A/3)m^{1/2}$) and these are all bigger than the experimental values.

The values of $\gamma_\pm$ for the particular values of K were used to calculate the stoichiometric activity coefficient, γ_{sto} ($= \alpha\gamma_\pm$; Pitzer, 1972) and these agree well with the activity coefficients calculated from the experimental results by the method of Boyd *et al.* (1966, see above and Table 1b).

The effects of ion-pair formation on enthalpies of dilution have been considered by Pitzer (1972) and it has only been necessary to adapt the calculations for 1 : 1 electrolytes diluted between two concentrations. The observed enthalpy of dilution, $\phi L = \phi L_\pm - (1 - \alpha)\Delta H$, where $\phi L_\pm$, the molar enthalpy of dilution (for ions), can be calculated from the expression:

$$\phi L_\pm = -\alpha\ \frac{A_H}{A_\gamma}\ (\ln \gamma_\pm + 1 - \phi_\pm),$$

and at 25° C A_H is 688 cal/mol and A_γ has the value 1.1766. The observed enthalpy of dilution, Q, from m_1 to m_2 is ($\phi L_1 - \phi L_2$) and if the association constant, K, and the ion size parameter, *a*, are known, α and $\phi L_\pm$ can be calculated for the two molalities and ΔH can therefore be estimated (Table 2).

The ketones and esters were not included in the conductance studies of Lowe *et al.* (1973), however, so it has been necessary to estimate the values of K. This can be done by a rough extrapolation from the values at 0° C, assuming that the effects of temperature are similar to those observed for the other compounds. The temperature dependence of K, however, is related to:

$$\Delta H = \frac{-R\Delta \ln K}{\Delta 1/T}$$

so an alternative procedure is to alter K until the value of ΔH in the dilution experiment is similar to that obtained from the difference in the values of ln K at 0° and at 25° C. For tetraethylammonium iodide the value of K at 25° C obtained in this way is intermediate between the two estimates from conductance data but the values for the *n*-pentyl and ethoxyethyl compounds are appreciably higher than the values from conductance (Table 3). In spite of the uncertainty about their exact values, it is possible to see the effects of changes in structure on K, on ΔH, and hence on ΔS, the entropy of formation of ion-pairs (Table 4b).

Table 3 Entropy changes for the dilution of compounds related to acetylcholine at 25°C

	$m^{1/2}$			
	0.2	*0.3*	*0.4*	*0.5*
$Et_4\overset{+}{N}I^-$				
G^{EX}	−189	−290	−387	−491
H^{EX}	(−45)	−97	−180	−262
TS^{EX}	(144)	193	207	229
K	0°	25°	ΔH	
			(i)	(ii)
	3.8	2.9; 3.5	1750; 535	1335; 1125
		3.1	*1320*	*1270*
$n\text{-Pent}\overset{+}{N}Me_3I^-$				
G^{EX}	−196	−304	−413	−526
H^{EX}	(−40)	−82	−146	−209
TS^{EX}	(156)	222	267	317
K	4.5	3.3	2005	1040
		3.9	*920*	*920*
$n\text{-Pent}\overset{+}{N}Et_3I^-$				
G^{EX}	−205	−321	−446	−574
H^{EX}	(−38)	−71	−114	−156
TS^{EX}	(167)	250	332	418
K	6.0	3.7	3115	800
		5.4	*680*	*610*
$EtOCH_2CH_2\overset{+}{N}Me_3I^-$				
G^{EX}	−203	−312	−430	−548
H^{EX}	(−50)	−107	−188	−270
TS^{EX}	(153)	205	242	278
K	4.0	2.9	2075	1400
		3.3	*1245*	*1270*
$EtOCH_2CH_2\overset{+}{N}Et_3I^-$				
G^{EX}	−191	−290	−392	−493
H^{EX}	(−60)	−118	−199	−280
TS^{EX}	(131)	172	193	213
K	5.0	3.3	2685	1320
		4.2	*1130*	*1120*
$CH_3CO(CH_2)_3\overset{+}{N}Me_3I^-$				
G^{EX}	−210	−330	−448	−566
H^{EX}	(−107)	−190	−316	−441
TS^{EX}	(103)	140	132	125
K	6.0	3.7	3115	1815
		4.7	*1575*	*1555*

Table 3—*continued*

$CH_3CO(CH_2)_3\overset{+}{N}Et_3I^-$				
G^{EX}	−217	−347	−479	−611
H^{EX}	−101	−186	−313	−441
TS^{EX}	116	161	166	170
K	7.0	4.0	3605	1655
		5.7	*1320*	*1310*
Acetylcholine iodide				
G^{EX}	−194	−297	−406	−515
H^{EX}	(−80)	−165	−279	−393
TS^{EX}	(114)	132	127	122
K	4.3	3.1	2110	1835
		3.3	*1705*	*1754*
$CH_3COOCH_2CH_2\overset{+}{N}Et_3I^-$				
G^{EX}	−212	−338	−463	−588
H^{EX}	(−109)	−190	−305	−414
TS^{XE}	(103)	148	158	174
K	6.4	3.8	3355	1725
		5.1	*1455*	*1435*
		$m^{1/2}$		
	0.317	*0.388*	*0.447*	*0.500*
$Me_4\overset{+}{N}I^-$				
G^{EX}	−272	−318	−363	−420
H^{EX}	−110	−160	−200	−240
TS^{EX}	162	158	163	180
K	2.47*	2.02*	1295	1806

Values of G^{EX}, from the activity and osmotic coefficients (Table 1), and of H^{EX}, from the calorimetry experiments (Table 2), are shown with the corresponding values of TS^{EX} (= $H^{EX} - G^{EX}$) for various values of $m^{1/2}$. Results for $m^{1/2} = 0.2$, in parentheses, are based on an extrapolated estimate of H^{EX} (see text). Values for tetramethylammonium iodide have been calculated from osmotic and activity coefficients published by Lindenbaum & Boyd (1964) and enthalpies of dilution listed in Table 2.

Values of the association constants, K, for ion-pair formation at 0° C are taken from Table 1; those at 25° C are either published values from conductance measurements (Fernandez-Prini, 1968, and Lowe & Rendall, 1973, give estimates for tetraethylammonium iodide; Lowe *et al.*, 1973, give estimates for the *n*-pentyl and ethoxyethyl compounds) or are extrapolations based on these. The estimates of the enthalpy of formation of ion-pairs, ΔH, have been calculated (i) from the effect of temperature on K, and (ii) from the calorimetry experiments. The value of K at 25° C in italics is that which gives roughly the same value of ΔH calculated by either method. Values of K for tetramethylammonium iodide at 0° and 25° C, marked with an asterisk, are taken from Fernandez-Prini (1968).

Biological activity

Values of log equipotent molar ratios relative to acetylcholine, shown in Table 4a, are the average of two estimates. The equipotent molar ratios of the agonists on the ileum differed by up to a factor of two from those previously reported (Holton & Ing, 1949; Ing, *et al.*, 1952). The biggest difference was with 4-ketopentyltrimethylammonium, and probably arises from its ganglion-stimulant activity. In the present work the tests were performed in the presence of hexamethonium (2.8×10^{-4} M). The mean estimate of log affinity constant for 4-ketopentyltriethylammonium at the receptors in the ileum was 3.91 (± 0.09, s.e.; 7 estimates); values in the table for the other compounds on this preparation are those of Abramson *et al.* (1969). The mean estimate of log affinity constant for *n*-pentyltriethylammonium at the receptors in the rectus was 3.35 (± 0.08, s.e.; 3 estimates) which is not very different from the value for diethylpiperidinium, to which it is closely related (3.49; Barlow, Scott & Stephenson, 1967).

The main effects of changes in structure on activity are that replacement of ester by $-CH_2CO-$ markedly lowers activity at postganglionic receptors in the ileum but not at those in the rectus. Replacement of ester by $-CH_2CH_2-$ reduces activity at both but the reduction is less at

Table 4a Biological properties of acetylcholine and related compounds

	Ileum		*Rectus*	
	log epmr	*log K*	*log epmr*	*log K*
$CH_3COOCH_2CH_2\overset{+}{N}Me_3$	0		0	
$CH_3COOCH_2CH_2\overset{+}{N}Et_3$	3		>3	
$CH_3CH_2OCH_2CH_2\overset{+}{N}Me_3$	0.83	*4.07*	1.23	
$CH_3CH_2OCH_2CH_2\overset{+}{N}Et_3$		*3.97*	⩾3 or	<2.5
$CH_3COCH_2CH_2CH_2\overset{+}{N}Me_3$	2.12		$\bar{1}.89$	
$CH_3COCH_2CH_2CH_2\overset{+}{N}Et_3$		*3.91*	>3	
$CH_3(CH_2)_4\overset{+}{N}Me_3$	1.30	*3.73*	0.78	
$CH_3(CH_2)_4\overset{+}{N}Et_3$		*4.59*		*3.35*
$Et_4\overset{+}{N}$				*2.87*
$Me_4\overset{+}{N}$	3		1	

The numbers in italics show the log affinity constant (log K); the other values are the log equipotent molar ratio (log epmr) relative to acetylcholine (note that this is 3 for a compound with one-thousandth of the activity of acetylcholine; $\bar{1}.89$ indicates 1.3 times as active as acetylcholine). Acetylcholine itself is much less active on the frog rectus than on the ileum, requiring about 100 times the concentration to produce effects. All the compounds were iodides but the biological activity is not affected by the nature of the anion.

Table 4b Physical properties of solutions of acetylcholine iodide and related compounds

	ϕ_V^0	TS^{EX} ($m = 0.09$)	K	$-T\Delta S$	*Conform.*
$CH_3COOCH_2CH_2\overset{+}{N}Me_3I^-$	174.5	132	3.3	2.4	(g)
$CH_3COOCH_2CH_2\overset{+}{N}Et_3I^-$	220.1	148	5.1	2.4	(g)
$CH_3CH_2OCH_2CH_2\overset{+}{N}Me_3I^-$	179.2	205	3.3	2.0	(g)
$CH_3CH_2OCH_2CH_2\overset{+}{N}Et_3I^-$	222.7	172	4.2	1.9	(g*)
$CH_3COCH_2CH_2CH_2\overset{+}{N}Me_3I^-$	185.4	140	4.7	2.5	
$CH_3COCH_2CH_2CH_2\overset{+}{N}Et_3I^-$	230.8	161	5.7	2.3	
$CH_3(CH_2)_4\overset{+}{N}Me_3I^-$	189.7	222	3.9	1.7	
$CH_3(CH_2)_4\overset{+}{N}Et_3I^-$	232.9	250	5.4	1.6	
$Et_4\overset{+}{N}I^-$	185.0	193	3.1	2.0	
$Me_4\overset{+}{N}I^-$	126.0	158	2.0	2.2	
Limiting value from Debye-Hückel theory		420			

Values shown are TS^{EX} for 0.09 mol/kg solutions (in cal/mole; from Table 3), and K (in litres/mole) and $T\Delta S$ (in kcal/mole) assuming ion-pair formation. Values of the apparent molal volume at infinite dilution (ϕ_V^0 in cm^3/mole) are taken from results of Barlow *et al.* (1971), Lowe *et al.* (1973) and results of MacGilp (personal communication). n.m.r. spectra of the compounds in D_2O (about 0.5 M) were studied by Dr R.R. Ison and the symbol (g) indicates a predominantly gauche conformation; (g*) indicates a ratio of *gauche* to *trans* of about 4 : 1; all the other compounds should have the *trans* conformation about the α-β carbon-carbon bond, though there are too many methylene protons for this to be inferred from the spectra.

the receptors in the rectus. Replacement of ester by $-CH_2O-$, however, reduces activity at receptors in the rectus more than at those in the ileum. Much higher concentrations are needed to produce effects on the rectus, however, and the much lower affinity of pentyltriethylammonium for these receptors suggests that high concentrations are needed because the compounds have low affinity. Some compounds did not produce effects, either agonist or antagonist, in concentrations as high as 3×10^{-3} M. Increasing the size of the onium group reduces efficacy at receptors in the ileum, as it does with simple salts on the frog rectus (Barlow *et al.*, 1971) and with the pentyl compounds studied in this work.

Discussion

The values of the excess entropies are all positive but the increase in entropy is less than that predicted from the Debye-Hückel limiting law. In these solutions, the ester and keto groups in particular must be regarded as increasing order and methylene groups as tending to decrease it, whether they are part of the chain or part of the onium group. The ethoxyethyl compounds are intermediate though the value of TS^{EX} for ethoxyethyltriethylammonium iodide is less than that for its trimethylammonium analogue. Possibly this is because the triethylammonium compound contains about 20% of the *trans* conformer, instead of being predominantly *gauche*. The change in size produced by replacing trimethylammonium by triethylammonium can be assessed from the increase in ϕ_v^o, which is 45.6 and 45.4 cm^3/mol for the esters and ketones, respectively, and only 43.5 and 43.2 cm^3/mol in the ethers and *n*-pentyl compounds. This seems to be consistent with the idea that the esters and ketones have similar effects on water and the effects of the ethers are closer to those of the methylene compounds.

It is arguable whether the decrease in order is big enough to invalidate the interpretation of the results in terms of ion-pair formation, as suggested by Lindenbaum *et al.* (1970). When the values of entropy of formation of such pairs are calculated, however, they lead to much the same conclusions as the excess entropies (Table 4b). The values of the ion-association constant, K, however, indicate extensive ion-pair formation by the keto compounds, with least association by the ethers. The extent to which ion-association may occur is important because it is likely to complicate most n.m.r. studies of preferred conformation. These have usually been made with concentrations between 0.5 and 1 mol/kg in D_2O and may well indicate preferred conformation in the ion-pair, which may not be the same as in the simple cation. As it is not often possible to obtain spectra over a range of concentrations, it would seem advisable to study salts with at least two different anions.

It is difficult to see any connexion between the solution properties of the compounds and their activity or affinity at muscarine-sensitive receptors in the ileum but there is some support for the idea that ability to activate nicotine-sensitive receptors may be associated with the presence of groups which increase order in the system. Barlow, Thompson & Scott (1969) noted that in substituted phenylalkyl onium salts nicotine-like activity was only enhanced by substituents, such as amino or hydroxyl, which could interact with water. It is likely that these groups have effects on water similar to those of ester or carbonyl, and these may be associated with conformational changes in the receptor. Because the triethylammonium compounds have properties similar to those of their trimethylammonium analogues but lack agonist activity, it is also necessary to suppose that ability to activate receptors is lowered by increasing the size of the onium group and some evidence for this view has already been discussed (Barlow, *et al.*, 1971).

Although work involving drug solutions studied apart from receptors can only lead to suggestions, rather than conclusions, about how drugs act, the information obtained in this present work is important because it is a preliminary to the study of the thermodynamics of drug-receptor interactions. The free energy of adsorption of very many compounds for receptors is known from affinity constant measurements and once purified receptor material becomes available, it may be possible to measure enthalpies of adsorption and hence to calculate the entropy changes involved. If the entropies of dilution are already known, the entropies of adsorption can then be estimated.

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