Relationships Between the Surface Activity and Cholinesterase Inhibition of Mono- and bis[3-(*N*,*N*-Diethylcarbamoyl)piperidino]alkanes

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The static surface tension of equimolar aqueous solutions of a series of mono- and bis[3-(*N*,*N*-diethylcarbamoyl)piperidino]alkanes and of two mono[3-(*N*,*N*-diethylcarbamoyl)pyridinium]alkanes has been determined. The extent of relationships existing between the surface activity of these compounds and their inhibitory effect upon human plasma pseudo-cholinesterase is reported.

During the course of synthetic work on carbamoylpiperidine derivatives (1), it became apparent that some compounds among the derivatives possessed surface-active properties. This observation, coupled with (*a*) an awareness of the work of Coleman and Eley (2) and that of Hoffmann and co-workers (3), from which one may expect a possible correlation between enzyme inhibition and surface activity, and (*b*) the suggestions of Long and Schueler (4) and Thomas and Marlow (5) that cholinesterase inhibition may in part be due to the surface activity of inhibitors, prompted us to explore relationships between the surface-active and inhibitory properties of our series of cholinesterase inhibitors.

While the literature records studies of relationships between surface activity and effect upon enzymes (*e.g.*, 6–10), we believe that this is the first published expression of relationships between observed and actually measured surface activity, and inhibition of an isolated cholinesterase system.

EXPERIMENTAL

Materials.—The chemistry and properties of the mono- and bis[3-(*N*,*N*-diethylcarbamoyl)piperidino]-alkanes and of the mono[3-(*N*,*N*-diethylcarbamoyl)-pyridinium]alkanes utilized in this investigation were described elsewhere (11, 12). All of the compounds employed were of analytically pure grade.

Solutions.—Measurements of surface tension were made on 0.01 and 0.005 *M* solutions in redistilled water obtained by distillation through a 45-cm. Vigreaux column from aqueous permanganate. The solvent water obtained by this method had a corrected surface tension of 70.26 to 70.87 dynes/cm.

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Instrumentation.—We employed the model TE03 du Nouy interfacial tensiometer (Kahlsico, Calif.) equipped with an insulated water-jacketed holder accommodating a 70-mm. diameter sample dish. The use of a Thermoboy model KTS5 circulating bath (Kahlsico, Calif.) in conjunction with the water-jacketed holder permitted rigid control of sample temperature. Measurements were made at 25.00 ± 0.02°.

Platinum-iridium rings having the following characteristics were employed: ring No. 1, mean circumference 5.950 cm., $R/r = 51.5$; ring No. 2, mean circumference 5.978 cm., $R/r = 51.5$.

The operation of the calibrated tensiometer was evaluated by determining the surface tension of spectro grade benzene. The corrected surface tension obtained (28.21 dynes/cm.) was in excellent agreement with the reported value (28.22 dynes/cm.) (13).

Methods.—Measurement of surface tension was performed by the ring method, in accordance with those directives described in the "Standard Methods of Test for Surface and Interfacial Tension of Solutions of Surface-Active Agents" (ASTM designation: D 1331-56) (14) and the "Standard Method of Test for Interfacial Tension of Oil against Water by the Ring Method" (ASTM designation: D 971-50) (15).

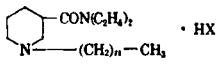
Measurements were made on 50-ml. samples at each concentration; in each case at least five determinations were employed in calculating an average value for the static surface tension (16). To determine the static value, the surface tension of each solution was checked periodically; in some instances measurements were made on solutions approximately 1.5 to 12.5 hours old. With each determination providing the static value, an equivalent determination was made on a control sample of redistilled water. Appropriate correction factors (13) were applied to the values obtained, and the surface pressure was then determined by subtracting the value for the surface tension of the solution from that of the redistilled water control.

Measurements of pH were made with a Beckman model 72 pH meter. Specific gravities, required in calculating correction factors, were determined by use of a Westphal balance (Fisher Scientific Co.).

RESULTS AND DISCUSSION

Values for the corrected, static surface tension of 0.01 and 0.005 *M* solutions of the compounds investigated, together with the corresponding surface

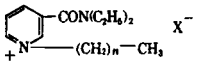
TABLE I.—INHIBITION OF ISOLATED HUMAN PLASMA PSEUDO-CHOLINESTERASE AND SURFACE-ACTIVE PROPERTIES OF MONO[3-(*N,N*-DIETHYLCARBAMOYL)PIPERIDINO]ALKANES



Compd.	*n*	Acid Component	Human Plasma Cholinesterase Inhibition (I_{50} ± S.E.)[a]	Surface Pressure,[b] dynes/cm. at 0.01 *M*	Surface Pressure,[b] dynes/cm. at 0.005 *M*	Surface Tension,[c] dynes/cm. ± S.E.[d] at 0.01 *M*	Surface Tension,[c] dynes/cm. ± S.E.[d] at 0.005 *M*
I	0	HCl	$(63.5 \pm 1.0) \times 10^{-5}$ *M*	1.98	0.58	68.59 ± 0.08	69.99 ± 0.04
II	1	HBr	$(118.5 \pm 0.5) \times 10^{-5}$ *M*	1.23	0.50	69.34 ± 0.05	70.07 ± 0.08
III	2	HBr	$(101.0 \pm 1.0) \times 10^{-5}$ *M*	3.12	2.41	67.36 ± 0.03	68.07 ± 0.04
IV	3	HBr	$(78.0 \pm 2.0) \times 10^{-5}$ *M*	5.60	2.75	65.05 ± 0.11	67.90 ± 0.09
V	4	HBr	$(26.1 \pm 1.1) \times 10^{-5}$ *M*	4.43	3.69	66.10 ± 0.02	66.84 ± 0.07
VI	5	HBr	$(8.13 \pm 0.33) \times 10^{-5}$ *M*	5.82	3.65	64.64 ± 0.05	66.81 ± 0.06
VII	9	HBr	$(0.527 \pm 0.011) \times 10^{-5}$ *M*	26.15	20.68	44.49 ± 0.06	49.96 ± 0.04

[a] Summarized from a paper by Lasslo and co-workers (17). [b] The surface pressure is the corrected surface tension of a redistilled water control minus the corrected surface tension of the solution. [c] The static surface tension is reported. [d] Standard error (18).

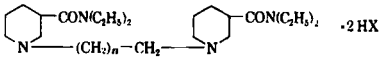
TABLE II.—INHIBITION OF ISOLATED HUMAN PLASMA PSEUDO-CHOLINESTERASE AND SURFACE-ACTIVE PROPERTIES OF MONO[3-(*N,N*-DIETHYLCARBAMOYL)PYRIDINIUM]ALKANES



Compd.	*n*	Halide	Human Plasma Cholinesterase Inhibition (I_{50} ± S.E.)[a]	Surface Pressure,[b] dynes/cm. at 0.01 *M*	Surface Pressure,[b] dynes/cm. at 0.005 *M*	Surface Tension,[c] dynes/cm. ± S.E.[d] at 0.01 *M*	Surface Tension,[c] dynes/cm. ± S.E.[d] at 0.005 *M*
VIII	0	I	Insignificant inhibition at at 100 × 10^{-5} *M* concn.	0.53	0.18	69.98 ± 0.08[e]	70.33 ± 0.05
IX	9	Br	$(0.365 \pm 0.000) \times 10^{-5}$ *M*	17.95	12.29	52.87 ± 0.05[e]	58.53 ± 0.05

[a] Summarized from a paper by Lasslo and co-workers (17). [b] The surface pressure is the corrected surface tension of a redistilled water control minus the corrected surface tension of the solution. [c] The static surface tension is reported. [d] Standard error (18). [e] Ciusa (19) has reported the surface tension of the corresponding chlorides.

TABLE III.—INHIBITION OF ISOLATED HUMAN PLASMA PSEUDO-CHOLINESTERASE AND SURFACE-ACTIVE PROPERTIES OF BIS[3-(*N,N*-DIETHYLCARBAMOYL)PIPERIDINO]ALKANES



Compd.	*n*	Acid Component	Human Plasma Cholinesterase Inhibition (I_{50} ± S.E.)[a]	Surface Pressure,[b] dynes/cm. at 0.01 *M*	Surface Pressure,[b] dynes/cm. at 0.005 *M*	Surface Tension,[c] dynes/cm. ± S.E.[d] at 0.01 *M*	Surface Tension,[c] dynes/cm. ± S.E.[d] at 0.005 *M*
X	1	HBr	$(17.5 \pm 1.0) \times 10^{-5}$ *M*	3.34	1.67	67.39 ± 0.02	69.06 ± 0.02
XI	2	HCl	$(99.3 \pm 1.8) \times 10^{-5}$ *M*	3.38	2.22	66.99 ± 0.03	68.11 ± 0.04
XII	3	HBr	$(42.0 \pm 1.7) \times 10^{-5}$ *M*	2.00	0.94	68.87 ± 0.04	69.93 ± 0.04
XIII	4	HCl	$(27.1 \pm 1.3) \times 10^{-5}$ *M*	5.15	4.72	65.18 ± 0.05	65.54 ± 0.05
XIV	5	HBr	$(15.0 \pm 0.3) \times 10^{-5}$ *M*	3.58	1.90	66.95 ± 0.04	68.63 ± 0.05
XV	9	HBr	$(2.59 \pm 0.06) \times 10^{-5}$ *M*	4.78	4.98	65.63 ± 0.04	65.43 ± 0.03

[a] Summarized from a paper by Lasslo and co-workers (17). [b] The surface pressure is the corrected surface tension of a redistilled water control minus the corrected surface tension of the solution. [c] The static surface tension is reported. [d] Standard error (18).

pressure at these concentrations and the inhibitory characteristics, previously reported by Lasslo and co-workers (17), are given in Tables I, II, and III.

The graphic interpretation of surface pressure values (at 0.01 *M* concentration) is illustrated along with the corresponding plot of the values determined by Lasslo and co-workers (17). Whereas in Fig. 1 the correlation between surface pressure and inhibitory effect is striking, no such relationship is apparent in Fig. 2. (In Fig. 2 the straight line representing surface pressure was computed by the method of least squares.)

It is indeed interesting to note the parallel between the cholinesterase inhibition and surface pressure of the mono[3-(*N,N*-diethylcarbamoyl)-piperidino]alkanes (Fig. 1, Table I) and the lack of it in the corresponding bis[3-(*N,N*-diethylcarbamoyl)piperidino]alkanes (Fig. 2, Table III).

In addition, it is instructive to compare the parallel between the surface activity and cholinesterase inhibition elicited by certain specific members of our groups of compounds. Considering the carbamoylpiperidinodecanes, for example, it can be seen that the *monosubstituted* alkane is the more potent one, both in terms of enzyme inhibition as well as in its ability to lower surface tension. In the case of the corresponding ethanes, the *bis-substituted* derivative more strongly inhibits the

enzyme system and is, at the same time, more powerful in lowering surface tension.

The determined surface tension values seem to be consistent with Ciusa's (19) results, reported for some corresponding pyridinium derivatives, and with Gadebusch and Cavallito's (20) figures for their α,ω-bis(2,2′-dipyridylamino)alkane bisquaternary salts.

In the course of determining the static surface tension, it was observed that, for a few compounds, values of dynamic surface tension varied considerably from the static value (*e.g.*, III, 5.6 dynes/cm.; IV, 5.3 dynes/cm.; XI, 9.4 dynes/cm.; XIII, 4.6 dynes/cm.). In most cases, however, variation from the static value was small, *i.e.*, 1 to 2 dynes/cm.

The pH of all solutions of our mono[3-(*N,N*-diethylcarbamoyl)piperidino]alkanes lay between 5.40 and 6.05 and between 5.65 and 6.50 for the corresponding pyridinium compounds. In the series of bis[3-(*N,N*-diethylcarbamoyl)piperidino]-alkanes, the pH of all solutions fell between 5.10 and 6.15, except for compounds X and XI (Table III). The values for 0.01 and 0.005 *M* solutions of X were 3.65 and 3.80, respectively; those for XI were 4.55 and 4.70, respectively.

SUMMARY

The static surface tension of 0.01 and 0.005 *M* aqueous solutions of a series of mono- and bis[3-(*N,N*-diethylcarbamoyl)piperidino]alkanes and some pyridinium analogs has been measured utilizing the ring method.

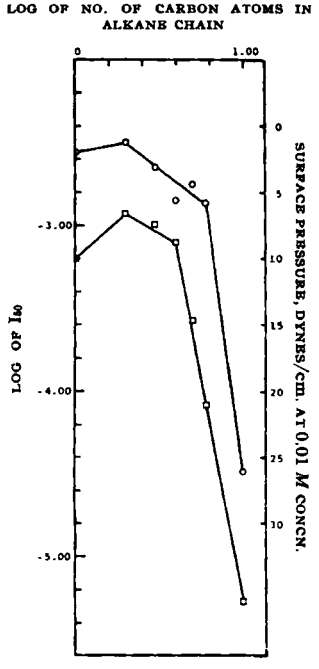


Fig. 1.—Graphic comparison of inhibitory (□—□) and surface-active (○—○) characteristics of mono[3-(*N,N*-diethylcarbamoyl) piperidino] alkanes.

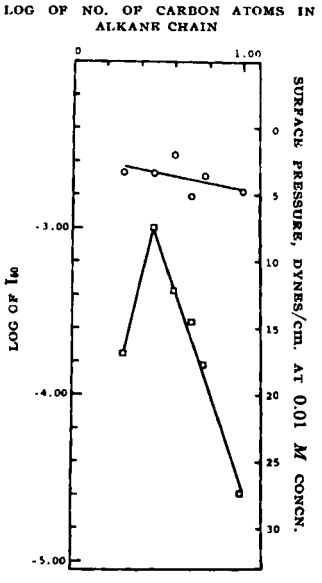


Fig. 2.—Graphic comparison of inhibitory (□—□) and surface-active (○—○) characteristics of bis[3-(*N,N*-diethylcarbamoyl) piperidino] alkanes.

A parallel was observed between the ability of mono[3-(*N,N*-diethylcarbamoyl)piperidino]alkanes to lower surface tension and their inhibition of human plasma pseudo-cholinesterase. No such relationship was noted in the case of the corresponding bis-substituted alkanes.

REFERENCES

(1) Quintana, R. P., and Shrader, W. A., This Journal, **52**, 1186(1963).
(2) Coleman, M. H., and Eley, D. D., *Biochim. Biophys. Acta*, **58**, 231(1962).
(3) Hoffmann, F. W., King, J. W., and Michel, H. O., *J. Am. Chem. Soc.*, **83**, 706(1961).
(4) Long, J. P., and Schueler, F. W., This Journal, **43**, 79(1954).
(5) Thomas, J., and Marlow, W., *J. Med. Chem.*, **6**, 107 (1963); *cf.* Thomas, J., *J. Med. Pharm. Chem.*, **3**, 309(1961).
(6) Fredrick, J. F., *Physiol. Plantarum*, **10**, 844(1957); through *Chem. Abstr.*, **52**, 20279*i*(1958).
(7) Kalman, S. M., *Nature*, **171**, 568(1953).
(8) Katagiri, H., and Ikemiya, M., *Koso Kagaku Shimposiumu*, **12**, 5(1957); through *Chem. Abstr.*, **51**, 16605*b* (1957).
(9) Marron, T. U., and Moreland, F. B., *Enzymologia*, **6**, 225(1939).
(10) Sugishima, W., *Osaka Daigaku Igaku Zasshi*, **8**, 901 (1956); through *Chem. Abstr.*, **51**, 7477*f*(1957).
(11) Lasslo, A., Marine, W. M., and Waller, P. D., *J. Org. Chem.*, **21**, 958(1956).
(12) Lasslo, A., and Waller, P. D., *ibid.*, **22**, 837(1957).
(13) Harkins, W. D., and Jordan, H. F., *J. Am. Chem. Soc.*, **52**, 1751(1930).
(14) "1961 Book of ASTM Standards (Including Tentatives)," Part 10, American Society for Testing and Materials, Philadelphia, Pa., 1961, pp. 1088–1092.
(15) "1961 Book of ASTM Standards (Including Tentatives)," Part 7, American Society for Testing and Materials, Philadelphia, Pa., 1961, pp. 421–424.
(16) Harkins, W. D., and Alexander, A. E., in Weissberger, A., ed., "Technique of Organic Chemistry: Physical Methods of Organic Chemistry," Vol. I, Part 1, 3rd ed., Interscience Publishers, Inc., New York, N. Y., 1959, p. 766.
(17) Lasslo, A., Beasley, J. G., Nelms, G. G., and Epperson, G. J., *J. Med. Chem.*, **6**, 811(1963).
(18) Snedecor, G. W., and Cochran, W. G., "Statistical Methods," 5th ed., Iowa State College Press, Ames, 1956, pp. 42–45.
(19) Ciusa, W., *Gass. Chim. Ital.*, **86**, 667(1956); *cf.* Ciusa, W., and Lipparini, L., *ibid.*, **90**, 147(1960).
(20) Gadebusch, H. H., and Cavallito, C. J., *Antibiot. Chemotherapy*, **7**, 549(1957).