Partition Coefficients and the Structure–Activity Relationship of the Anesthetic Gases†

Corwin Hansch,* Antonio Vittoria,‡ Carlo Silipo,§ and Priscilla Y. C. Jow

Department of Chemistry, Pomona College, Claremont, California 91711. *Received December 4, 1974*

Partition coefficients of 32 gaseous anesthetics in the octanol–water system have been determined. It is shown that relative anesthetic potency depends on hydrophobicity of the anesthetic (as defined by log P) and on a polar factor. The presence of a polar hydrogen in the anesthetic greatly increases potency. A quantitative structure–activity relationship is formulated based on these two factors.

Since the work of Meyer and Overton at the turn of the century, it has been generally held that the potency of gaseous anesthetics roughly increases with increasing oil–water partition coefficients. An exception to this view is Mullins' emphasis on the importance of solubility parameters.[1] The Meyer–Overton view is that the anesthetics bring about their action in a fatty phase of nerve tissue. A dramatic departure from this view was advanced by Pauling[2] and, independently, Miller.[3] They suggested that the critical action occurs in an aqueous phase and is better correlated by the tendency of anesthetics to form hydrates. In a thoughtful analysis of the facts, Miller et al.[4,5] conclude that the phase for anesthetic action is nonaqueous. Moreover, they showed that there is a very high correlation between anesthetic pressure and olive oil–gas partition coefficients.[5]

One of the critical elements missing in our current discussion is a complete set of partition coefficients of the important gases in a single solvent–water system. Although many investigators have measured partition coefficients of anesthetics in many different systems, we have lacked a large enough set in a single system to make a general assessment of the Meyer–Overton hypothesis. To correct this problem we have measured the octanol–water[6] partition coefficient (P) of 32 volatile compounds whose anesthetic action has been studied. We have formulated eq 1–3 which correlate anesthetic potency with partition coefficients and the polar character of the compounds from the log P values and the anesthetic pressure (p) in Table I.

Experimental Section

Instrumentation. Two types of gas chromatography were used for analysis of the aqueous and octanol phases. A Loenco Model 70 Hi-Flex instrument with a thermal conductivity detector was employed for inert gases which could not be detected by a flame ionization detector. A special injection system was utilized for gas dissolved in fluid (obtained from Carlo Erba, Milan, Italy). This device consists of a Plexiglas stripping chamber (of about 10-ml capacity) a stainless steel carrier gas two-way valve, a stirrer, and a column set in a cooling bath (Dry Ice–acetone) to trap solvents. By switching the valve, carrier gas can be allowed to bypass the chamber and directly enter into the column, or it can be entered after bubbling through the stripping chamber. For the chromatography, one-third of a column of copper tubing (3 m by 4 mm i.d.) was packed with 0.2–0.5 mm silica gel (E. Merck, Darmstadt, Germany) and the remaining two-thirds was packed with 60–80 mesh molecular sieve 5A (Matheson Coleman and Bell). During analysis, carrier gas flowed through the column passing over the silica gel first. For the analysis of N_2O, a copper column (1.80 m by 4 mm) packed with 100–120 mesh Chromosorb 102 was used (Johns-Mansville, Denver, Colo.). He, N_2, and argon were used for carrier gases, depending on the type of gas being analyzed. The technique for stripping the gases dissolved in octanol or water by carrier gas is similar to that employed by McAuliffe.[8]

Variable amounts of the partitioned phases (0.5–4.0 ml) were injected into the stripping chamber by means of a Pressure-Lok syringe (Precision Sampling Corp., Baton Rouge, La.). After 3 min of stirring, carrier gas was allowed to bubble directly through the fluid in the chamber and then passed through the cooling coil (−80°) and into the chromatographic column. The value of the partition coefficient (P) was obtained by taking the ratio of the areas under the peaks for octanol and water phases. All determinations were made at room temperature (24 ± 3°). Partition coefficients for three gases (H_2, N_2O, and Ar) were determined at 0°.

A Varian Model 2740 chromatograph with a Vidar (6300) digital integrator was employed for the carbon compounds (except CF_4 and C_2F_6). The column (6 ft) was packed with Se-30 (5%) on 80–100 mesh Chromosorb W AW-DMCS. Since injecting water into the column gave poor results, an idea of McAuliffe[9] was used. A U tube of 4.5 in., one-third packed with 60–80 mesh firebrick and two-thirds packed with 8–20 mesh ascarite, 0.2–0.5 silica gel, or 8–12 mesh $CaCl_2$, was placed in the oven before the column. This trap removed the water. It is necessary to replace the drying agent after three or four injections. To be consistent, the trap was used for analysis of both octanol and water phases. The temperatures employed were in the 60–90° range. The halogen compounds such as CH_3I and $ClCH_2CH_2Cl$ gave good results when ascarite was used; however, silica gel or $CaCl_2$ is recommended for reactive compounds. All of the gases studied were research grade material of 99+% purity.

Partitioning. The gases were allowed to bubble through octanol and water placed in a vacutainer (Becton-Dickinson Co., Rutherford, N.J.) (100 × 16 mm) with a rubber serum stopper. The gases were introduced via a needle and withdrawn via a syringe. In the process of withdrawing a sample, the system was kept at atmospheric pressure by a second needle connected to a reservoir of gas at atmospheric pressure.

Table I contains both log P values and the anesthetic pressure of the various compounds. Both phases were analyzed and the values are the ratios of the peak areas. The peak area was found to be linear with respect to concentration of solute over the ranges studied. Each value listed in Table I is the average of four to five analyses made with different concentrations of solutes.

Results and Discussion

Equations 1–3 have been formulated from the data in Table I. In these equations p in log $1/p$ is the effective anesthetic pressure (ATA), n is the number of data points

		n	r	s	
log $1/p$ =	1.193 (±0.59) log P − 1.327 (±0.87)	30	0.613	1.056	(1)
log $1/p$ =	1.913 (±0.69) I − 0.596 (±0.45)	30	0.734	0.909	(2)
log $1/p$ =	1.166 (±0.25) log P + 1.881 (±0.33) I − 2.106 (±0.39)	30	0.947	0.438	(3)

used in deriving the equations, r is the correlation coefficient, and s is the standard deviation. The figures in parentheses are the 95% confidence intervals. I is an indicator variable assigned a value of 1 to all compounds containing a "polar hydrogen atom". All other compounds are assigned a value of zero for I.

†This investigation was supported by Public Health Service Research Grant No. CA-11110 from the National Cancer Institute.

‡Visiting Scientist from the Institute of Pharmaceutical and Toxicological Chemistry, University of Naples, Italy.

§NATO Fellow and Visiting Professor of Chemistry from the Institute of Pharmaceutical and Toxicological Chemistry, University of Naples, Italy.

Table I. Data Used in the Formulation of Eq 1–3 Correlating Righting Reflex in Mice with Anesthetic Pressure (p)

No.	Gas	Log $1/p$ Obsd[a]	Log $1/p$ Calcd[b]	$\lvert\Delta \log 1/p\rvert$	Log P[c]	I[c]	μ[d]
1	He	−2.28	−1.779	0.50	0.28	0.0	0.00
2[e]	H_2	−2.14	−1.581	0.56	0.45	0.0	0.00
3	Ne	−1.94	−1.779	0.16	0.28	0.0	0.00
4	N_2	−1.52	−1.325	0.20	0.67	0.0	0.00
5	CF_4	−1.24	−0.730	0.51	1.18	0.0	0.00
6[f]	C_2F_6	−1.19	0.226	1.42	2.00	0.0	0.00
7[e]	Ar	−1.18	−1.243	0.06	0.74	0.0	0.00
8	SF_6	−0.75	−0.147	0.60	1.68	0.0	0.00
9	CH_4	−0.66	−0.835	0.18	1.09	0.0	0.00
10	Kr	−0.65	−1.068	0.42	0.89	0.0	0.00
11[e]	N_2O	−0.18	0.276	0.46	0.43	1.0	0.17
12	$CH_2{=}CH_2$	−0.15	−0.789	0.64	1.13	0.0	0.00
13	C_2H_6	−0.11	0.004	0.11	1.81	0.0	0.00
14	Xe	0.02	−0.614	0.63	1.28	0.0	0.00
15	C_3H_8	0.05	0.645	0.60	2.36	0.0	0.00
16	C_2H_2	0.15	0.206	0.06	0.37	1.0	0.00
17	CF_2Cl_2	0.40	0.412	0.01	2.16	0.0	0.55
18	$CH_3CH{=}CH_2$	0.40	−0.043	0.44	1.77	0.0	0.35
19	c-C_3H_6	0.80	−0.101	0.90	1.72	0.0	0.00
20	$CFCl_3$	0.82	0.843	0.02	2.53	0.0	0.45
21	CH_3F	0.85	0.369	0.48	0.51	1.0	1.81
22	CH_3Cl	0.85	0.836	0.01	0.91	1.0	1.87
23	CH_3I	1.15	1.535	0.39	1.51	1.0	1.62
24	C_2H_5Cl	1.40	1.442	0.04	1.43	1.0	2.06
25	C_2H_5Br	1.40	1.652	0.25	1.61	1.0	2.03
26	EtOEt	1.52	0.812	0.71	0.89	1.0	1.27
27	CH_2Cl_2	1.52	1.232	0.29	1.25	1.0	1.54
28	CH_3CHCl_2	1.59	1.861	0.27	1.79	1.0	2.06
29	$CHCl_3$	2.08	2.071	0.01	1.97	1.0	1.02
30	$CF_3CHClBr$	2.11	2.456	0.35	2.30[g]	1.0	
31	$Cl_2CHCF_2OCH_3$	2.66	2.351	0.31	2.21	1.0	
32	$ClFCHCF_2OCF_2H$				2.10		

[a] From ref 4 and 5. [b] Using eq 3. [c] See Experimental Section for sources of these constants. [d] From the Chemical Rubber Company Handbook of Chemistry and Physics and A. L. McClellan, "Tables of Experimental Dipole Moments", W. H. Freeman, San Francisco, Calif., 1963. [e] The partition coefficients for these three gases were determined at 0° to obtain log P_{H_2} = 0.38, log P_{Ar} = 0.58, and log P_{N_2O} = 0.36. [f] This point not used in deriving eq 1–3. [g] Improved value over that reported.[7]

The definition of a polar hydrogen atom is simply a phenomenological one. Those compounds which contain an electronegative element (O, halogen, etc.) attached directly to a carbon atom holding a hydrogen atom are observed to be more potent anesthetics than compounds lacking such a hydrogen atom. I is given a value of 1 for such compounds and zero for all others except HC≡CH and N_2O. We have assumed that the C≡C function is electronegative enough to confer polar character on its H atoms. In the case of N_2O, the hydrated form may be the active species.

Equation 1 accounts for only 38% (r^2) of the variance in log $1/p$ while eq 3 accounts for 90%. The coefficient of 1.9 with I in eq 3 indicates that on the average, other factors being equal, molecules having a "polar" hydrogen (CH_3Cl, HC≡CH, $HCCl_3$, EtOEt, etc.) are about 80 times as potent anesthetics as are compounds lacking such a function (CH_4, CF_4, $CFCl_3$, CF_2Cl_2, etc.). Carbon tetrachloride (log P 2.83) with log P not far from halothane (2.30) has very poor anesthetic activity. The coefficients with the log P terms in eq 1 and 3 are characteristic of those we have found for correlation equations for the narcotic action of organic compounds on membranes and nerve processes.

Adding a term in $(\log P)^2$ to eq 3 makes a slight improvement in the correlation (r = 0.956). However, confidence limits cannot be placed on log P_0; hence, this higher order equation is of little value. Although the log P_0 of 2.7 is not very reliable, it does suggest a parabolic dependence of anesthetic potency on log P which is in accord with our earlier findings with a series of 28 anesthetic ethers.[7] This could be interpreted to mean that highly lipophilic gaseous anesthetics do not reach equilibrium between gas phase and receptor sites. Other interpretations are also possible.[12]

One approach to interpreting eq 1 and 3 is that anesthetic action is brought about by two properties of organic compounds: (1) hydrophobic and (2) polar. This conclusion was reached some time ago for the hypnotic action of alcohols, amides, and barbiturates.[13] Thus, in eq 3 we see the normal increase in narcotic activity as log P increases. In effect, there are two such curves; one for the polar and one for the nonpolar anesthetics, separated by about 1.9 log units. Our inclination is to rationalize the I term by postulating that perturbation of the lipid space is also brought about by a polar interaction of the anesthetic. It occurred to us that this might be related to the dipole moment of the anesthetic; however, using μ in eq 3 instead of I does not yield as good a correlation (r = 0.858 for 28 data points; see Table I). The addition of $\mu + \mu^2$ to eq 3 in place of I also falls far short of the correlation of eq 3 (r = 0.893). Thus it appears that μ is not nearly as effective a parameter as I to model polar character. This does not rule out a role for the dipole

moment, however, since there is considerable collinearity between I and μ as shown in the following correlation matrix.

	log P	μ	I
log P	1.00	0.03	0.01
μ		1.00	0.67
I			1.00

The values in the above matrix are for r^2; hence, the correlation between I and μ is high although log P is orthogonal to these two vectors. Our model definitely brings out a polar component in anesthetic action, the nature of which is somewhat ambivalent because of the collinearity between I and μ. It is interesting to note that the most potent anesthetics, chloroform, halothane, methoxyflurane, and ethrane, all contain a "polar" hydrogen atom.

A fact which must be considered is that Miller et al.[5] found an excellent correlation between anesthetic pressure and olive oil–gas partition coefficients. This partition coefficient, obtained from a *nonaqueous* system, correlates anesthetic potency without the additional term I needed with octanol–water partition coefficients (r = 0.994 for 16 molecules from Table I for which olive oil–gas log P values are available). Assuming an approximate equilibrium between anesthetic in the gas phase and anesthetic on receptor sites causing anesthesia, it would seem that olive oil–gas partitioning models the lipid receptor site quite well. This single partition coefficient contains both the hydrophobic and polar information contained in the two terms of eq 3.

There is a very high correlation between oil–water partition coefficients and octanol–water partition coefficients. This is illustrated[6] by eq 4 which correlates solutes that do

$$\log P_{\text{oil-water}} = 1.10 \log P_{\text{octanol-water}} - 1.30 \quad (4)$$

n = 65, r = 0.981, s = 0.271

not contain a strong hydrogen bond donor such as OH or NH_2 and is therefore applicable to the compounds of Table I. Since it is the oil–gas partition coefficient which rationalizes anesthetic potency and not the oil–water constant, the conclusion is that solubility of the gases in olive oil must be determined by dispersion and polar forces (including hydrogen bonding) in such a way that olive oil models the effects of these forces in the critical lipophilic sites of action.

Equation 3 factors these two properties modeled by olive oil. The role of polar forces and especially hydrogen bonding is deemphasized in the octanol–water or olive oil–water partition coefficient. The polar interactions of the solute in water are still operative in the octanol, especially in the octanol–water system where about 4% water is present in the octanol phase, although to a lower degree. In effect, octanol–water or olive oil–water constants do not contain much polar information and this must be introduced using the variable I.

A number of comments about specific cases in Table I should be made. The congener C_2F_6 is very poorly fit and has not even been used to derive eq 1 and 3. It is about 25 times less active than expected. Perfluoromethane is reasonably well fit; however, SF_6 is off by 1.5 standard deviations. All of these fluoro compounds are less active than one would expect from their high partition coefficients. The fluoro compounds are better fit by olive oil–gas partition coefficients. Our results suggest that they partition into octanol more readily than into nerve membrane.

Although our log P values for the rare gases are, to our knowledge, the first to be published, partition coefficients for these gases have been calculated by taking the ratio of their oil and water solubilities.[14] The values obtained by this method are: He, 0.23; H_2, 0.37; O_2, 0.56; N_2, 0.55; Ar, 0.60; Kr, 0.88; Xe, 1.16; Rn, 2.04. The values we have found by direct measurement of both phases of these gases partitioned between octanol–water are very close indeed to the calculated values obtained using different oils by different

$$\log P_{\text{octanol}} = 1.018\ (\pm 0.17) \log P_{\text{oil}} + 0.076\ (\pm 0.11) \quad (5)$$

n = 7, r = 0.990, s = 0.049

investigators. Equation 5 expresses the correlation between the two sets of values. This equation is quite significant ($F_{1,5}$ = 247) even though there is only a small difference between the two sets of values. We did not measure Rn in octanol–water; however, a value can be calculated for it from eq 5.

In conclusion, we would agree with Meyer and Overton, Mullins, and Miller, Paton, and Smith that the results of this study, taken with our earlier results,[7,10,11] strongly suggest that the critical phase in which anesthetic action occurs is lipophilic in character. However, our results clearly establish that there is an important polar component which plays a major role in the disruption of nerve function.

References and Notes

(1) L. J. Mullins, *Chem. Rev.*, **54**, 289 (1954).
(2) L. Pauling, *Science*, **134**, 15 (1961).
(3) S. L. Miller, *Proc. Natl. Acad. Sci. U.S.A.*, **47**, 1515 (1961).
(4) K. W. Miller, W. D. M. Paton, and E. B. Smith, *Nature (London)*, **206**, 574 (1965).
(5) K. W. Miller, W. D. M. Paton, E. B. Smith, and R. S. Smith, *Anesthesiology*, **36**, 339 (1972).
(6) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
(7) W. R. Glave and C. Hansch, *J. Pharm. Sci.*, **61**, 589 (1972).
(8) C. McAuliffe, *Chem. Technol.*, 1, 46 (1971).
(9) C. McAuliffe, *J. Phys. Chem.*, **70**, 1267 (1966).
(10) C. Hansch and W. J. Dunn III, *J. Pharm. Sci.*, **61**, 1 (1972).
(11) C. Hansch and W. R. Glave, *Mol. Pharmacol.*, **7**, 337 (1971).
(12) C. Hansch and J. M. Clayton, *J. Pharm. Sci.*, **62**, 1 (1973).
(13) C. Hansch, A. R. Steward, S. M. Anderson, and D. Bentley, *J. Med. Chem.*, 11, 1 (1968).
(14) J. H. Lawrence, W. F. Loomis, C. A. Tobias, and F. H. Turbin, *J. Physiol. (London)*, **105**, 197 (1946).