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# RESEARCH ARTICLES

# Structural Approach to Partitioning: Estimation of Steroid Partition Coefficients Based upon Molecular Constitution

**GORDON L. FLYNN**

**Abstract** ☐ Partition coefficients are directly related to the free energy of transfer of a substance between two immiscible phases and thus have a first principal character rarely ascribed to them. In addition, partition coefficients have been shown to be additive constituitive in character, allowing for their calculation from the individual contributions of the molecular components. These factors, taken together, make partitioning a meaningful and convenient physical phenomenon to match against biological activity. For these reasons, the structural relationships between a large group of steroids and their ether–water parition coefficients were explored. Hansch-like $\pi_e$ values were estimated for a number of functional groups. These data allow the calculation of partition coefficients of highly substituted steroids from stripped steroid skeletons. The implications of these results from both physical–chemical and biological activity standpoints are discussed.

**Keyphrases** ☐ Corticosteroids—partition coefficients ☐ Partition coefficients—ether–water partitioning, steroids ☐ Structural group contribution—steroid partition coefficients ☐ Blue tetrazolium, isonicotinic acid procedures—analysis

The fundamental work of Meyer (1) and Overton (2) at the turn of the century that introduced the lipid-partitioning hypothesis brought a significant new dimension to biopharmaceutical research. Since then a myriad of investigators have sought correlation of biological activity with some measurable physical–chemical

parameter of a drug family. It is obvious that no single property is capable of correlating all drug activities, because every type of bonding makes its contribution to the forces of action between the pharmacological agent and its environment and, in particular, its interaction with the "receptor." In the cases where one interaction factor predominates or is variant while all other factors are invariant, good correlations will be obtained with a closely related physical–chemical parameter. Good correlation is also possible with relatively meaningless parameters if the choice of compounds is limited to a homologous series or is restricted in some similar fashion.

Invariably, activities are compared with partition coefficients obtained in some seemingly arbitrary partitioning system. Hansch and coworkers (3–5) were extremely successful in correlating a spectrum of biological response data with octanol–water partition coefficients. Beckett and Moffat (6) found that *n*-heptane–water partitioning gives excellent rank correlation with buccal absorption for several series of compounds. Other investigators made similar contributions (7, 8).

From the practical standpoint, it is perhaps of equal importance that recent investigators (in particular, Hansch) have provided evidence that the partition co-

# Table I—Partition Coefficients in Ascending Order of Their Experimentally Obtained and Averaged Values

| Avg. $k_p$ | Compound | Weight, mg. | Volume Water, ml. | Calcd. Volume Ether, ml. | $F$ | $A_E$ | $A_W$ | $k_p$ Experimental | $k_p$ Calculated |
|---|---|---|---|---|---|---|---|---|---|
| 0.757 | [1]Triamcinolone | 1.0230 | 100 | 48.7 | 1 | 0.248[a] | 0.685[a] | 0.743 | 0.800 |
| | | 1.0620 | 50 | 48.6 | 1 | 1.532 | 2.043 | 0.771 | |
| 1.13 | [2]Prednisolone | 1.1325 | 100 | 48.9 | 1 | 0.447 | 0.801 | 1.14 | Std. |
| | | 1.6450 | 48.5 | 56.0 | 1 | 2.315 | 1.811 | 1.11 | (1.13) |
| 1.40 | [3]Cortisone | 0.5040 | 50 | 48.2 | 1 | 0.938 | 0.722 | 1.35 | 1.55 |
| | | 1.0245 | 48 | 53.7 | 1 | 1.792 | 1.110 | 1.44 | |
| 1.60 | [4]Hydrocortisone | 0.9850 | 100 | 100 | 2 | 0.778 | 0.850 | 1.83 | 1.72 |
| | | 1.5550 | 50 | 49.0 | 1 | 2.663 | 1.811 | 1.50 | |
| | | 1.0510 | 50 | 49.2 | 1.67 | 1.688 | 1.943 | 1.47 | |
| 1.93 | [5]6$\alpha$-Fluoro-prednisolone | 1.2795 | 100 | 53.3 | 1.67 | 1.692 | 2.740 | 1.93 | 2.00 |
| 2.32 | [6]9$\alpha$-Fluoro-hydrocortisone | 1.3305 | 50 | 47.8 | 2 | 1.238 | 1.117 | 2.32 | 2.46 |
| 3.44 | [7]6$\alpha$-Methyl-prednisolone | 0.9948 | 100 | 100 | 2 | 1.010 | 0.518 | 3.90 | 3.24 |
| | | 1.2800 | 100 | 54.9 | 1.67 | 2.210 | 2.123 | 3.16 | |
| | | 1.1155 | 100 | 49.6 | 1.67 | 1.864 | 1.923 | 3.25 | |
| 3.87 | [8]Dexamethasone | 1.3865 | 100 | 49.2 | 1.67 | 2.379 | 2.078 | 3.88 | 4.09 |
| | | 1.2950 | 100 | 48.5 | 1.67 | 2.228 | 1.982 | 3.86 | |
| 4.17 | [9]6$\alpha$-Methyl-9$\alpha$-fluoro-prednisolone | 2.7510 | 200 | 48.7 | 2 | 0.839 | 1.652 | 4.17 | 4.64 |
| 4.52 | [10]Corticosterone[b] | 1.8600 | 100 | 49.3 | 4 | 0.403 | 0.771 | 4.24 | 6.97 |
| | | 1.4775 | 100 | 50.1 | 4 | 0.910 | 1.515 | 4.79 | |
| 4.76 | [11]Betamethasone | 1.0910 | 100 | 49.0 | 1 | 3.115 | 1.332 | 4.78 | 5.03 |
| | | 1.3300 | 100 | 48.8 | 1.67 | 1.706 | 1.232 | 4.73 | |
| 7.50 | [12]6$\alpha$-Fluorodexa-methasone | 2.1150 | 200 | 45.5 | 5 | 0.841 | 2.480 | 7.36 | 7.24 |
| | | 1.1745 | 150 | 49.3 | 2.5 | 2.242 | 2.235 | 7.63 | |
| 14.6 | [13]Triamcinolone acetonide | 2.2150 | 100 | 47.9 | 20 | 1.047 | 3.375 | 13.0 | 15.5 |
| | | 1.7010 | 50 | 50.1 | 10 | 1.803 | 1.032 | 17.4 | |
| | | 1.1600 | 50 | 49.3 | 10 | 1.251 | 0.935 | 13.6 | |
| 21.1 | [14]Prednisolone-21-acetate | 1.6330 | 100 | 47.6 | 5 | 0.765 | 1.900 | 21.1 | 20.8 |
| 25.1 | [15]Cortisone-21-acetate | 1.0075 | 100 | 46.1 | 10 | 1.181 | 1.023 | 25.1 | 28.6 |
| 25.5 | [16]6$\alpha$-Fluoro-triamcinolone acetonide | ~1 | 100 | 47.6 | 10 | 0.975 | 0.670 | 30.6 | 27.1 |
| | | ~1 | 100 | 47.8 | 10 | 0.970 | 0.925 | 22.0 | |
| | | 0.9950 | 100 | 49.5 | 5 | 2.223 | 0.901 | 24.9 | |
| | | 1.2050 | 100 | 49.4 | 5 | 2.707 | 1.111 | 24.7 | |
| 26.0 | [17]Hydrocortisone-21-acetate | 0.9418 | 100 | 100 | 10 | 1.184 | 0.440 | 26.9 | 31.6 |
| | | ~1 | 100 | 100 | 10 | 1.102 | 0.735 | 15.0 | |
| | | 0.9639 | 100 | 100 | 10 | 1.180 | 0.462 | 25.3 | |
| | | 0.9935 | 100 | 100 | 10 | 1.230 | 0.462 | 26.6 | |
| | | 1.0000 | 100 | 21.4 | 10 | 0.940 | 1.823 | 24.1 | |
| | | 0.9690 | 100 | 47.0 | 10 | 1.150 | 0.933 | 26.2 | |
| | | 0.9450 | 100 | 97.3 | 10 | 1.160 | 0.315 | 37.8 | |
| 32.7 | [18]6$\alpha$-Methyl-9$\alpha$-fluoro-21-desoxy-prednisolone[b] | 1.957 | 100 | 48.7 | 10 | 1.150 | 0.725 | 32.6 | 36.4 |
| | | 2.435 | 100 | 48.9 | 10 | 1.367 | 0.874 | 32.0 | |
| | | 2.243 | 100 | 48.9 | 10 | 1.312 | 0.802 | 33.5 | |
| 34.4 | [19]6$\alpha$-Methyl-triamcinolone acetonide | 4.8855 | 750 | 38.6 | 40 | 0.723 | 1.942[c] | 34.9 | 44.3 |
| | | 0.9500 | 100 | 46.4 | 10 | 1.120 | 0.707 | 34.1 | |
| | | 1.1110 | 100 | 48.1 | 10 | 1.065 | 0.650 | 34.1 | |
| 36.7 | [20]6$\alpha$-Fluoro-prednisolone-21-acetate | 2.454 | 100 | 48.7 | 20 | 1.372 | 1.577 | 35.7 | 36.8 |
| 45.5 | [21]6$\alpha$,16$\alpha$-Difluoro-prednisolone-21-acetate | 1.200 | 100 | 46.2 | 10 | 0.765 | 0.408 | 40.6 | 44.6 |
| | | 2.229 | 100 | 47.2 | 20 | 1.245 | 1.103 | 47.8 | |
| | | 1.5090 | 100 | 49.6 | 10 | 1.743 | 0.729 | 48.2 | |
| 45.7 | [22]9$\alpha$-Fluorohydro-cortisone-21-acetate | 1.6605 | 100 | 46.2 | 10 | 1.986 | 0.940 | 45.7 | 45.2 |
| 51.5 | [23]6$\alpha$-Methyl-9$\alpha$-fluoro-21-desoxy-hydrocortisone[b] | 2.374 | 85 | 45.6 | 20 | 0.720 | 0.515 | 52.1 | 56.6 |
| | | 2.698 | 100 | 47.6 | 20 | 0.928 | 0.768 | 50.8 | |
| 52.0 | [24]Desoxycorticos-terone[b] (Cortexone) | 2.1590 | 100 | 49.0 | 10 | 1.355 | 0.533 | 50.0 | 80.2 |
| | | 1.9055 | 100 | 50.1 | 10 | 1.517 | 0.561 | 54.0 | |
| 53.2 | [25]6$\alpha$-Methyl-9$\alpha$-fluoro-16$\alpha$-hydroxy-hydrocortisone acetonide | ~1 | 100 | 46.6 | 10 | 1.180 | 0.475 | 53.3 | 67.4 |
| | | 1.9610 | 100 | 47.8 | 20 | 1.030 | 0.812 | 53.0 | |

Table I—*Continued*

| Avg. $k_p$ | Compound | Weight, mg. | Volume Water, ml. | Calcd. Volume Ether, ml. | $F$ | $A_E$ | $A_W$ | $k_p$ Experimental | $k_p$ Calculated |
|---|---|---|---|---|---|---|---|---|---|
| 67.1 | [26]6α-Methyl-prednisolone-21-acetate | ~1 | 100 | 23.6 | 10 | 1.150 | 0.684 | 71.2 | 59.7 |
| | | ~1 | 100 | 23.0 | 10 | 1.138 | 0.632 | 78.2 | |
| | | ~1 | 100 | 48.5 | 10 | 1.222 | 0.390 | 64.6 | |
| | | ~1 | 100 | 47.4 | 10 | 1.202 | 0.350 | 72.3 | |
| | | 2.060 | 100 | 22.3 | 10 | 2.342 | 1.775 | 59.3 | |
| | | 1.969 | 100 | 47.3 | 20 | 1.143 | 0.850 | 56.8 | |
| 80.8 | [27]6α,9α-Difluoro-21-desoxy-hydrocortisone-17-acetate[b] | 3.121 | 200 | 49.2 | 20 | 0.540 | 0.587 | 74.8 | 97.6 |
| | | 2.769 | 150 | 46.4 | 20 | 0.619 | 0.462 | 86.7 | |
| 82.4 | [28]6α-Methyl-9α-fluoro-prednisolone-21-acetate | 2.504 | 100 | 48.7 | 20 | 1.390 | 0.693 | 82.4 | 85.4 |
| 85.3 | [29]6α,9α,16α-Trifluoro-prednisolone-21-acetate | 1.100 | 100 | 47.1 | 10 | 1.347 | 0.365 | 78.3 | 78.9 |
| | | 1.581 | 200 | 46.6 | 10 | 1.868 | 0.883 | 90.7 | |
| | | 1.8085 | 200 | 48.8 | 10 | 2.230 | 1.051 | 86.9 | |
| 87.3 | [30]Testosterone | 6.133 | 200 | 48.3 | 10 | 1.231 | 0.584 | 87.3 | 53.9 |
| 93.7 | [31]6α-Methyl-9α-fluoro-21-desoxy-prednisolone-17-acetate | 4.750 | 500 | 48.2 | 16 | 1.152 | 2.092 | 91.4 | 104 |
| | | 3.356 | 150 | 47.5 | 20 | 1.811 | 1.192 | 95.9 | |
| 145 | [32]6α-Fluoro-dexamethasone-21-acetate | 3.702 | 200 | 45.7 | 20 | 2.121 | 1.250 | 149 | 133 |
| | | 2.5505 | 200 | 48.3 | 12.5 | 2.425 | 0.884 | 142 | |
| 222 | [33]Hydrocortisone-21-isobutyrate | 5.461 | 200 | 48.3 | 100 | 0.543 | 0.982 | 229 | 312 |
| | | 5,001 | 200 | 49.2 | 40 | 1.083 | 0.824 | 214 | |
| 242 | [34]Hydrocortisone-21-butyrate | 4.787 | 300 | 52.3 | 40 | 1.412 | 1.551 | 215 | 334 |
| | | 6.120 | 200 | 51.1 | 40 | 1.552 | 1.083 | 224 | |
| | | 8.888 | 200 | 50.8 | 40 | 2.272 | 1.245 | 288 | |
| 243 | [35]6α,9α-Difluoro-16α-chloro-prednisolone-21-acetate | 3.363 | 200 | 46.8 | 20 | 2.029 | 0.723 | 240 | 262 |
| | | 2.7825 | 500 | 46.5 | 12.5 | 2.630 | 1.436 | 246 | |
| 250 | [36]6α-Methyl-21-desoxy-prednisolone-17-propionate[b] | 4.850 | 750 | 45.1 | 40 | 1.259 | 3.245 | 258 | 237 |
| | | 4.876 | 200 | 45.6 | 40 | 1.342 | 0.978 | 241 | |
| 272 | [37]9α-Fluoro-11β-hydroxy-6α-methyl-4-pregnene-3,20-dione[b] | 4.950 | 750 | 40.7 | 40 | 0.977 | 2.535 | 284 | 225 |
| | | 4.907 | 200 | 46.6 | 40 | 1.028 | 0.680 | 259 | |
| 509 | [38]Betamethasone-17-valerate | 4.458 | 750 | 42.1 | 40 | 0.650 | 0.910 | 509 | 494 |
| 604 | [39]Progesterone[b] | 2.7960 | 150 | 47.6 | 20 | 1.108 | 0.120 | 582 | 630 |
| | | 9.788 | 250 | 46.9 | 50 | 1.875 | 0.798 | 626 | |
| 857 | [40]6α-Methyl-9α-fluoro-16α-hydroxy-prednisolone-16,17-acetonide-21-acetate | 6.811 | 750 | 25.4 | 40 | 1.680 | 2.320 | 857 | 815 |
| 960 | [41]6α-Methyl-9α-fluoro-16α-hydroxyhydro-cortisone-16,17-acetonide-21-acetate | 4.829 | 900 | 45.2 | 50 | 0.985 | 1.023 | 960 | 1240 |
| 1380 | [42]6α-Methyl-9α-fluoro-16α-hydroxy-hydrocortisone-16,17-acetonide-21-propionate | ~15 | 900 | 42.0 | 200 | 0.132 | 0.410 | 1380 | 3550 |
| 1530 | [43]6α-Fluorodexa-methasone-21-butyrate | 9.932 | 900 | 43.8 | 100 | 1.170 | 1.575 | 1530 | 1440 |
| 1690 | [44]6α-Methyltriam-cinolone acetonide-21-propionate | ~8.4 | 900 | 42.8 | 100 | 0.145 | 0.180 | 1690 | 2650 |
| 1730 | [45]6α-Fluoro-dexamethasone-21-isobutyrate | 10.132 | 900 | 44.3 | 100 | 1.083 | 1.275 | 1730 | 1320 |

*(continued)*

**Table I—***Continued*

| Avg. $k_p$ | Compound | Weight, mg. | Volume Water, ml. | Calcd. Volume Ether, ml. | $F$ | $A_E$ | $A_W$ | $k_p$ Experimental | $k_p$ Calculated |
|---|---|---|---|---|---|---|---|---|---|
| 3620 | [46]Hydrocortisone-21-caproate (Hexanoate) | 4.9695 | 500 | 48.8 | 40 | 1.345 | 0.153 | 3600 | 3530 |
| | | 11.01 | 1000 | 48.6 | 50 | 2.316 | 0.713 | 3340 | |
| | | 13.01 | 900 | 48.3 | 100 | 1.513 | 0.720 | 3910 | |
| | Blank—blue tetrazolium procedure | None | 100 | 47.4 | 20 | $0.012^d$ | $0.017^d$ | — | — |
| | Blank—isonicotinic acid hydrazide procedure | None | 100 | 50 | 20 | $0.051^e$ $0.000^f$ | $0.042^e$ $0.000^f$ | — — | — — |

[a] 1-cm. cells. [b] By isonicotinic acid hydrazide method. [c] Required a $1/10$ dilution with blank. [d] At 525 nm. [e] At 375 nm. [f] At 400 nm.

efficients are of additive-constituitive character (9, 10) and thus can be readily calculated once the functional group contributions are known for a given partitioning system. This has freed the investigator from the tedium of experimentally determining all the partition coefficients in a given drug series. The purpose of this report is to explore in detail the structural approach to partitioning. Cosaturated ether and water phases, henceforth referred to as the ether and the water phases, were used as a system for the determination of the partition coefficients of a large group of polyfunctional steroids, and the $\pi_e$ values for various groups with varied positioning were determined.

## EXPERIMENTAL

The selection of the ether–water partition system was based on partition coefficient data of Katz and Shaikh (11), which showed ether–water corticosteroid partition coefficients in this system to be in an experimentally accessible range. Initial experiments were with their procedure until its limitations became apparent. Shoulders on UV absorption peaks and double peaks were observed in the 240–250-nm. range. Partition coefficients appeared to be dependent on the phase ratio. Partition coefficients starting with a dilute solution of the drug did not remotely agree with those obtained with the filtrate from a saturated solution. Analysis of steroid-free ether-saturated water filtrates through several Whatman filter papers, including No. 41 used by Katz and Shaikh, (11) revealed that significant UV absorbing material is leached out of the filter (Fig. 1). The same problem is observed with other "inert" filters such as the solvent inert Gelman and Millipore types. Because of these problems and the necessity of conserving the compound with many steroids, the following procedures requiring no filtration and more specific assays were employed.

**Materials**—All steroids tested were used as received[1]. Deionized water and analytical reagent ether were used to prepare the cosaturated phases.

**Partitioning Procedure**—Water and ether were added to a 2-l. separator and shaken to obtain cosaturation of the phases. The separator was set aside for 24 hr. to allow for temperature and further phase equilibrium. All remaining steps were carried out at room temperature (23 ± 1°). An appropriate amount of steroid (from ~1 to ~10 mg., depending on the steroid) was accurately weighed and placed in a 50-ml. conical flask. This was dissolved in the ether phase, which was maintained at approximately 50 ml. An appropriate amount of water phase (from 50 to 1000 ml., depending on the compound) was placed in an appropriately sized separator (from 125 to 1000 ml.). The steroid solution in the ether phase was added with no attempt to rinse any residual steroid from the flask or funnel used. The phases were shaken intermittently, allowing sufficient time between agitations for the phases to reform.

[1] From The Upjohn Company's Biological Screening Office.

The system was allowed to stand for 20–30 min. to assure complete phase separation. Then the aqueous phase was transferred to a clean, appropriately sized separator, with great care taken to assure that no ether phase contaminated it at this point. The ether phase was immediately transferred to a 250-ml. round-bottom flask, the phase weight was recorded, and the phase was brought to dryness on a Buchi evaporator. The aqueous phase was extracted with two 50-ml. portions of methylene chloride, and the extracts were collected in a 250-ml. round-bottom flask. The extractions were followed by an additional 10–15 ml. methylene chloride rinse. The combined extracts and rinse were then brought to dryness on a Buchi evaporator. The dried phase residues were reconstituted with 95% ethanol USP (usually 20 ml. of ethanol for the aqueous phase residue and 200 ml. of ethanol for the ether phase residue), appropriately diluted if necessary, and assayed by one of the following procedures.
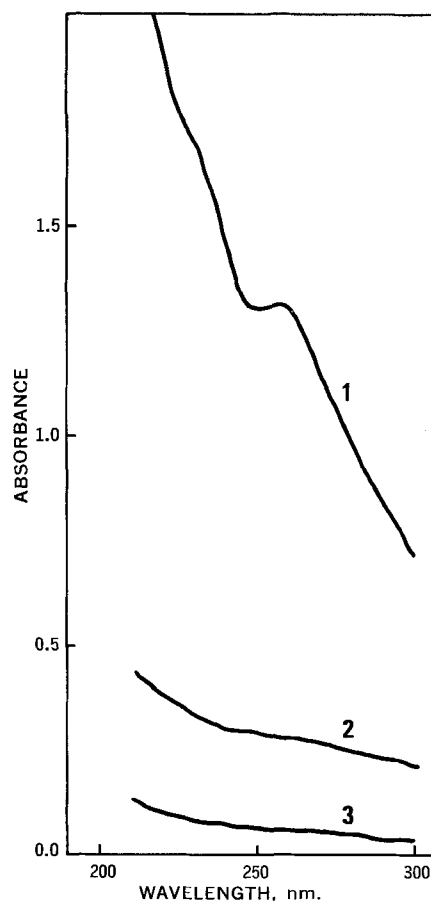


**Figure 1**—*Absorbance of repetitive 50-ml. water (ether-saturated) filtrates passed through Whatman No. 41 filter paper of approximately 5-cm. diameter. Key: 1, first extract; 2, second extract; and 3, third extract.*

**Blue Tetrazolium Procedure (12)**—This procedure was used on all steroids for which it was applicable. Twenty milliliters of 95% ethanol USP reconstituted ether phase, diluted to contain approximately 0.1 mg., was transferred to a clean 50-ml. glass-stoppered conical flask. Two milliliters of blue tetrazolium reagent (500 mg. of blue tetrazolium in 100 ml. of 95% ethanol USP) and 2 ml. of tetramethylammonium hydroxide reagent (10 ml. of 10% tetramethylammonium hydroxide made to 100 ml. with 95% ethanol USP) were added to this flask and the 250-ml. round-bottom flask containing the aqueous phase residue in 20 ml. of ethanol. A third flask, containing 40 ml. of ethanol to be used as a blank, was similarly and simultaneously prepared. After a lapsed time of 90 min., the solutions were analyzed in the visible range (525 nm.). The Cary 11 used was zeroed at 525 nm. with the blank in both cells. The spectrum was scanned from 650 to 475 nm. Usually, 5-cm. cells were employed, but on occasion other pathlength cells were chosen for analytical convenience.

**Isonicotinic Acid Hydrazide Procedure (13)**—This procedure was used on compounds for which it was applicable when the blue tetrazolium procedure was not. The procedures are of comparable sensitivity, allowing exact duplication of the blue tetrazolium procedure up to the point of bringing both the ether phase and the methylene chloride extracted aqueous phase residues to dryness. Two hundred milliliters of reagent grade methanol was added to the dried ether extract, the steroid was dissolved, and an appropriately sized aliquot was transferred to a 50-ml. glass-stoppered conical flask. This aliquot ranged from 1 to 20 ml., depending on the original sample size. The methanol aliquot was brought to dryness on a steam bath. The dried aqueous extract, still in its 250-ml. round-bottom flask, was similarly heated to remove any traces of water. Twenty milliliters of isonicotinic acid hydrazide reagent (25 mg. of isonicotinic acid hydrazide and 0.31 ml. of concentrated HCl in 500 ml. of absolute reagent grade methanol) was added to the residues from both the ether and the water phases. After swirling to assure solution, the flasks were tightly sealed and placed in a 47° oven for 1 hr. The flasks were removed from the oven and cooled to room temperature; then the peak absorbance was read between 375 and 405 nm., using cells of appropriate pathlength (usually 5 cm.). A reagent solution that had received identical treatment was used as the blank.

**Calculations**—In both procedures, the following calculations were used:

$$k_p = F \times \frac{C_E}{C_W} = F \times \frac{W_E/V_E}{W_W/V_W} \quad \text{(Eqs. 1 and 2)}$$

However, in the analytical procedure employed, weight is directly proportional to the absorbance; therefore:

$$k_p = F \times \frac{A_E}{A_W} \times \frac{V_W}{V_E} \quad \text{(Eq. 3)}$$

where:

$k_p$ = ether–water partition coefficient between cosaturated phases
$F$ = dilution factor
$C_E$ = concentration in ether phase
$C_W$ = concentration in water phase
$W_E$ = weight in ether phase
$W_W$ = weight in water phase
$V_E$ = volume of ether phase
$V_W$ = volume of water phase
$A_E$ = absorbance of ether phase
$A_W$ = absorbance of water phase

$V_E$ was determined by dividing the weight of the ether phase by its density, which was independently determined to be 0.712 g./ml. The dilution factor was calculated for each run from

$$F = \frac{V_s}{A_s} \times \frac{L_W}{L_E} \quad \text{(Eq. 4)}$$

where:

$V_s$ = volume of reconstituted ether phase extract (usually 200 ml.)
$A_s$ = aliquot in milliliters taken from $V_s$
$L_E$ = cell pathlength ether phase
$L_W$ = cell pathlength water phase

Relatively small amounts of compound were required for the partition coefficient determinations. The amount required for a given compound depended upon the phase volume used and the partition coefficient itself and was calculated from the following equation which assumes that 0.1 mg. must be retained in the water phase:

$$W_T \simeq W_E = 0.1 \text{ mg.} \times \frac{V_E}{V_W} \times k_p \quad \text{(Eq. 5)}$$

The total weight, $W_T$, is approximately equal to the weight of the ether phase, $W_E$, for compounds with large partition coefficients (>10). Thus, for a compound with a partition coefficient of 500 and a phase ratio of 0.1 (50 ml. of ether phase to 500 ml. of water phase), one must use approximately 5 mg. to get desired results. Initially, selecting suitable conditions for determining a particular partition coefficient was a matter of trial and error. As facility in their estimation grew, phase ratios and weights of compound were satisfactorily determined in advance with the aid of Eq. 5.

## RESULTS

Partition coefficient data for close to 50 steroids are presented in Table I. The compounds are identified according to either their common name (for example, progesterone) or their structural relationship to either hydrocortisone, prednisolone, triamcinolone, dexamethasone, or betamethasone. A knowledge of these few structures will facilitate understanding of the structural dependencies presented. The data in this table include the weight of compound used, the phase volumes, and the dilution factor for each run. In some cases, the weight of compound was approximated and these weights are so indicated.

Hydrocortisone-21-acetate and 6α-methylprednisolone-21-acetate were the compounds used in perfecting the experimental procedure. It was expected that the partition coefficient would be invariant with changing phase volume ratio, and this was the case over a fourfold range for the hydrocortisone ester. The average of the seven hydrocortisone-21-acetate values is 26.0, with a standard deviation of 6.95. As experience was gained with the procedure, experimental conditions were sharpened and points of probable error were eliminated, leading to the likelihood that most of the later data are of better precision and accuracy. In particular, additional care was taken in separating the respective phases. Approximately 0.5 ml. of the aqueous phase was left in the separator to prevent contamination by the ether phase. This was then discarded prior to weighing of the ether phase. This introduced a small error in the value of the water phase (1% or less, depending on volume) but guarded against the far larger error introduced by chance phase contamination.

## CALCULATION OF $\pi_e$ VALUES FOR VARIOUS FUNCTIONAL GROUPS

The estimation of group contribution factors, $\delta$, or their respective log values ($\pi$ values) is a simple, straightforward process. For example, the group contribution to the partition coefficient for the making of an acetate ester at position 21 would be obtained from

$$\text{group contribution factor} = \delta = \frac{k_p \, (21\text{-acetate})}{k_p \, (\text{corresponding alcohol})}$$

$$\text{(Eq. 6)}$$

and the $\pi$ value is then

$$\pi = \log \delta = \log [k_p \, (21 \text{ acetate})] - \log [k_p$$

$$(\text{corresponding alcohol})] \quad \text{(Eq. 7)}$$

In the case of the ether–water system, $\pi_e$ will be used. This will affiliate the ether–water values with the $\pi$ values of Hansch and at the same time differentiate them with respect to the choice of organic phase.

The partitioning influence of the many functional substitutions for the steroids in Table I in terms of $\delta$ and $\pi_e$ values are listed in Table II. In this table the data are grouped in pairs of compounds,
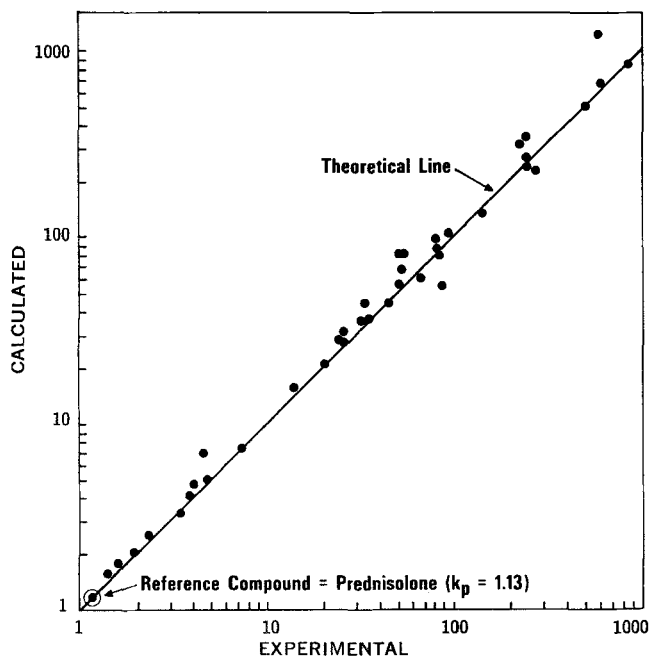
**Figure 2**—*Log–log plot of $\pi_e$ value estimated partition coefficients against those determined experimentally. Analysis of the statistical line for all compounds reported in Table I has a correlation coefficient of 0.999.*

one having a particlar substitution relative to the other. For convenience, numbers referring to the order of listing in Table I instead of full compound names are used in Table II. As many as 10 pairs and as few as one pair of compounds have been used to estimate the $\pi_e$ values. These are given as the log of the averaged group contribution factor for a particular substitution.

Some of the group contributions are themselves based upon calculated partition coefficients. This procedure was employed where no direct comparison and estimation were possible. The value for $9\alpha$-fluoroprednisolone used in these estimations was determined by three methods as follows:

| prednisolone 1.13 | $\times$ | $9\alpha$-fluoro 1.40 | $=$ | $9\alpha$-fluoroprednisolone 1.58 | (Eq. 8) |
|---|---|---|---|---|---|
| $9\alpha$-fluorohydrocortisone 2.32 | $\div$ | $\Delta 4$ to $\Delta 1,4$ 1.52 | $=$ | 1.55 | (Eq. 9) |
| $6\alpha$-methyl-$9\alpha$-fluoro-prednisolone 4.17 | $\div$ | $6\alpha$-methyl 2.87 | $=$ | 1.45 | (Eq. 10) |
| | | | | Average $\overline{1.53}$ | |

This value (1.53) was then used to calculate the value of $6\alpha,9\alpha$-difluoroprednisolone acetate in the following manner:

| log 1.53 | 0.1847 |
|---|---|
| $\pi$ ($6\alpha$-fluoro) | 0.2480 |
| | $\overline{0.4327}$ |
| $\pi$ (21-acetate) | 1.2648 |
| | $\overline{1.6975}$ |

$$\text{antilog } 1.6975 = 49.9 = k_p \qquad \text{(Eq. 11)}$$

These two calculations illustrate the use of both the factor values, $\delta$, or their logs or $\pi$ values. The agreement between the individual values for $9\alpha$-fluoroprednisolone is noteworthy in that they are obtained from the experimentally determined partition coefficients of three different compounds.

A column of calculated partition coefficients using these $\pi_e$ values appears in Table I. The partition coefficient of prednisolone was arbitrarily chosen as the standard (baseline value) for this series, and all calculated values are based on differences with respect to it. Many of these estimations have built-in redundancies due to the fact that the compounds to which they pertain were

also used in $\pi_e$ value estimation. Numerous compounds in the table were not used in $\pi_e$ value estimation, however, and these serve to validate the $\pi$ value system. The agreement between the calculated and the experimental values is good and within experimental error in most cases. The values for the $\Delta 4$ compounds are consistently high, and this may indicate that the $\Delta 1,4$ to $\Delta 4$ $\pi$ value is slightly larger than it should be. This procedure accurately predicts the value of progesterone (calculated $= 630$; experimental $= 604$), a very long extrapolation. Even more interesting is the prediction of 53.9 for testosterone (found 87.3), because it involves the removal of two methylene groups representing $C_{20}$ and $C_{21}$ from progesterone and the correction of a ketone ($C_{20}$) to a hydroxyl ($C_{17}$).

The good agreement between experimentally determined and calculated partition coefficients is graphically shown in Fig. 2. For the $C_4$ and longer esters, the agreement between theory and experiment breaks down some and can only be said to be fair. It can be shown, however, that as little as a 0.1% contamination of a hypothetical long-chain ester ($k_p = 3000$), with its corresponding alcohol ($k_p = 10$), will result in a 10% error in the experimental partition coefficient. Since the compounds were not rigorously purified, this represents a potentially substantive source of error. The statistical line generated by all 46 estimations on a log calculated *versus* log experimental basis has an intercept not significantly different from zero, a slope of 1.007, and a correlation coefficient of 0.999.

## DISCUSSION

**Theoretical Significance of Partition Coefficients**—Since partitioning is a process involving molecular equilibria, a partition coefficient is an equilibrium constant of fundamental significance in terms of free energies. It can be seen from Eq. 12:

$$\Delta G = -RT \ln K_{e.g.} = RT \ln k_p \qquad \text{(Eq. 12)}$$

that the partition coefficient is directly relatable to the free energy change in the system when a mole of the partitioning agent is transferred between the phases. A mounting body of evidence indicates that when two structurally closely related molecules—such as a corticosteroid free alcohol and its corresponding acetate—are partitioned between the same two immiscible phases, the differences in the free energies of transfer calculated by Eq. 12 are directly relatable to the specific structural modification (7, 9, 10). This generality is at least applicable to solutes that form regular solutions in both phases or for which entropy of mixing is maximized. Thus,

$$\Delta G_{\text{alcohol}} = -RT \ln k_{p\,\text{alcohol}} \qquad \text{(Eq. 13)}$$

$$\Delta G_{\text{ester}} = -RT \ln k_{p\,\text{ester}} \qquad \text{(Eq. 14)}$$

and

$$\Delta G_{\text{acetate moiety}} = \Delta G_{\text{alcohol}} - \Delta G_{\text{ester}} = RT \ln \frac{k_{p\,\text{ester}}}{k_{p\,\text{alcohol}}} = \text{constant} \qquad \text{(Eq. 15)}$$

or in general:

$$\Delta G_{\text{functional group free energy of partitioning}} = \Delta G_{GFE} =$$
$$-RT \ln \frac{k_{p\,\text{derivative}}}{k_{p\,\text{parent molecule}}} = \text{constant} \qquad \text{(Eq. 16)}$$

If these relationships are valid, it is apparent that by working from the reverse direction one can generate a system whereby partition coefficients can be calculated from a knowledge of the functional

**Table II—Effect of Various Functional Groups on Ether–Water Partition Coefficients of Corticosteroids**

| Compound | $k_p$ | Corresponding | $k_p$ | δ | $\pi_e$ |
|---|---|---|---|---|---|
| **6α-Methyl** | | | | | |
| 1. 6α-Methyl group | | | | | |
| 13 | 14.6 | 19 | 34.4 | 2.35 | |
| 2 | 1.13 | 7 | 3.44 | 3.04 | |
| 14 | 21.1 | 26 | 67.1 | 3.18 | |
| | | | | $\overline{2.87}$ | 0.4579 |
| **6α-Fluoro** | | | | | |
| 2. 6α-Fluoro group | | | | | |
| 13 | 14.6 | 16 | 25.5 | 1.73 | |
| 8 | 3.87 | 12 | 7.50 | 1.94 | |
| 2 | 1.13 | 5 | 1.93 | 1.71 | |
| 14 | 21.1 | 20 | 35.7 | 1.69 | |
| | | | | $\overline{1.77}$ | 0.2480 |
| **9α-Fluoro** | | | | | |
| 3. 9α-Fluoro group | | | | | |
| 4 | 1.60 | 6 | 2.32 | 1.45 | |
| 7 | 3.44 | 9 | 4.17 | 1.21 | |
| 17 | 26.0 | 22 | 45.7 | 1.76 | |
| 26 | 67.1 | 28 | 82.4 | 1.21 | |
| | | | | $\overline{1.43}$ | 0.1553 |
| **16α-Methyl** | | | | | |
| 4. 16α-Methyl group | | | | | |
| 9α-Fluoroprednisolone | 1.53[a] | 8 | 3.87 | 2.53 | 0.4031 |
| **16β-Methyl** | | | | | |
| 5. 16β-Methyl group | | | | | |
| 9α-Fluoroprednisolone | 1.53[a] | 11 | 4.76 | 3.11 | 0.4928 |
| **16α-Hydroxy** | | | | | |
| 6. 16α-Hydroxy group | | | | | |
| 9α-Fluoroprednisolone | 1.53[a] | 1 | 0.757 | 0.495 | −0.3054 |
| **16α-Fluoro** | | | | | |
| 7. 16α-Fluoro group | | | | | |
| 20 | 35.7 | 21 | 45.5 | 1.29 | |
| 6α,9α-Difluoroprednisolone acetate | 49.9[a] | 29 | 85.3 | 1.71 | |
| | | | | $\overline{1.50}$ | 0.1761 |
| **16α-Chloro** | | | | | |
| 8. 16α-Chloro group | | | | | |
| 6α,9α-Difluoroprednisolone acetate | 49.9[a] | 35 | 243 | 4.97 | 0.6964 |
| **16,17-Acetonide** | | | | | |
| 9. 16,17-Diol versus the 16,17-acetonide | | | | | |
| 1 | 0.757 | 13 | 14.6 | 19.3 | 1.2856 |
| **17-Acetate** | | | | | |
| 10. $C_{17}$ Acetate group | | | | | |
| 18 | 32.7 | 31 | 93.7 | 2.86 | 0.4564 |
| **21-Acetate** | | | | | |
| 12. $C_{21}$ Acetate group | | | | | |
| 19 | 34.4 | 41 | 857 | 25.3[b] | |
| 4 | 1.60 | 17 | 26.0 | 16.3 | |
| 25 | 53.2 | 42 | 960 | 18.0 | |
| 7 | 3.44 | 26 | 67.1 | 19.8 | |
| 9 | 4.17 | 28 | 82.4 | 19.8 | |
| 12 | 7.50 | 32 | 145 | 19.4 | |
| 3 | 1.40 | 15 | 25.1 | 17.9 | |
| 5 | 1.93 | 20 | 35.7 | 18.5 | |
| 2 | 1.13 | 14 | 21.1 | 18.7 | |
| 6 | 2.32 | 22 | 45.7 | 19.7 | |
| | | | | $\overline{18.7}$ | 1.2648[c] |
| **$C_{11}$ Desoxy** | | | | | |
| 13. Desoxy at $C_{11}$ | | | | | |
| 10 | 4.52 | 24 | 52.0 | 11.5 | 1.0607 |
| **$C_{17}$ Desoxy** | | | | | |
| 14. Desoxy at $C_{17}$ | | | | | |
| 23 | 51.5 | 37 | 272 | 5.28 | |
| 4 | 1.60 | 10 | 4.52 | 2.83 | |
| | | | | $\overline{4.06}$ | 0.6085 |

*(continued)*

**Table II**—*Continued*

| Compound | $k_p$ | Corresponding | $k_p$ | $\delta$ | $\pi_e$ |
|---|---|---|---|---|---|
| **C21 Desoxy** | | | | | |
| 15. Desoxy at C21 | | | | | |
| 9 | 4.17 | 18 | 32.7 | 7.85 | 0.8949 |
| **C21 Desoxy** | | | | | |
| 16. C21 Acetate *versus* C21 desoxy | | | | | |
| 28 | 82.4 | 18 | 32.7 | 0.397 | −0.4012 |
| **Hydroxy** | | | | | |
| 17. C11 Keto *versus* C11 hydroxy | | | | | |
| 15 | 25.1 | 17 | 26.0 | 1.07 | |
| 3 | 1.40 | 4 | 1.60 | 1.14 | |
| | | | | 1.105 | 0.0434 |
| **Δ4** | | | | | |
| 18. Δ1,4 *versus* Δ4 | | | | | |
| 2 | 1.13 | 4 | 1.60 | 1.42 | |
| 18 | 32.7 | 23 | 51.5 | 1.57 | |
| 19 | 34.4 | 25 | 53.2 | 1.57 | |
| | | | | 1.52 | 0.1818 |
| **β** | | | | | |
| 19. 16α-Methyl *versus* 16β-methyl | | | | | |
| 8 | 3.87 | 11 | 4.96 | 1.23 | 0.899 |
| **Branched** | | | | | |
| 20. Branching | | | | | |
| 34 | 242 | 33 | 222 | 0.917 | −0.0376 |

21. Methylene contribution (calculated):

    (a) From hydrocortisone butyrate

        Log ($k_p$ hydrocortisone butyrate)      2.3838

        − Log ($k_p$ hydrocortisone acetate)    1.4150

                                       0.9688

        ÷ 2 (—$CH_2$—$CH_2$—) 

        Antilog = 3.05 = $\delta$

    (b) From hydrocortisone hexanoate

        Log ($k_p$ hydrocortisone hexanoate)    3.5587

        − Log ($k_p$ hydrocortisone acetate)    1.4150

                                           2.1437

        ÷ 4 (—$CH_2$—$CH_2$—$CH_2$—$CH_2$—)    0.5359

        Antilog = 0.5359 = 3.44 = $\delta$

    (c) $\sum\limits_{n} \delta/n$

$$\frac{\delta_i + \delta_2}{2} = 3.25$$

$$\pi_e = \log 3.25 = 0.5119$$

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$^a$ Calculated (see text).   $^b$ Not included in average.   $^c$ Corresponds to a previous average with fewer comparisons of 18.4; used in all the calculations.

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group free energies of partitioning. This is exactly what Hansch and Steward (3) accomplished with the octanol–water system. The $\pi$ values obtained from Eq. 7 are directly related to the group free energy change (Eq. 17):

$$\pi = \frac{\Delta G_{GFE}}{2.303\,RT} \qquad \text{(Eq. 17)}$$

Under isothermal conditions, the group free energy of partitioning can be generated by simply multiplying the $\pi$ value times the constant factor of 2.303 $RT$.

In this study, linear free energy relationships were developed for an as yet uncharacterized group of steroids in a different solvent system; they were found valid within experimental error. The constancy of $\pi$ or $\delta$ values for the conversion of a 21-alcohol to a 21-acetate (Table II) is particularly notable. These values are for corticosteroid alcohols differing by as much as a factor of 50 in partition coefficient. The averaged group free energy of transfer for the 21-acetate at 25° is approximately 1.73 kcal./mole in the ether–water system. A perusal of Table II discloses that not all the $\pi$ values have been as sharply defined. For instance, $\delta$ for the 9α-fluoro group varies up to 19% about the mean of 1.43. Regardless, the consistently close approximation of experimentally determined $k_p$'s by $\pi_e$ value calculation gives good indication that the group contribution values are within reason.

An interesting conclusion readily drawn from Table II is that there is an inequality of effect for a given functional group located at different positions. The most notable difference is for esters at the 17- and 21-positions, although even the differences in partition coefficient brought about by positioning a methyl group in either the 6α-, 16α-, or 16β-positions are believed to be real. These differences are acceptable, considering the nonsymmetrical environment of the group locations. The marked variance in effect of derivitization at positions 17 and 21 is attributable to the marked difference between a tertiary alcohol enveloped by the huge steroid nucleus and a relatively unhindered, mobile primary alcohol.

The concept of summation of constituent properties to obtain or approximate the properties of the whole molecule is reasonably well established. The work of Hansch and coworkers has already been mentioned (3–5, 10). Cratin (14) recently published on the estimation of HLB values of surfactants using a similar approach. Small (15) and Rheineck and Lin (16) compiled evidence on the additive constitutive character of solubility parameters and related molar attraction constants; these approximations were used in turn by Ostrenga and Steinmetz (17) to characterize successfully the solubility of fluocinolone acetonide and its 21-acetate. These represent but a few examples of the diverse usefulness of this approach.

**Interrelationship of Several Common Structure–Activity Parameters**–In Fig. 3 the logs of the *experimentally determined* partition coefficients of several selected corticosteroid families are plotted against their respective molecular weights. These families are arbitrarily labeled prednisolone, monofluoroprednisolone, difluoroprednisolone, and triamcinolone. The family membership for
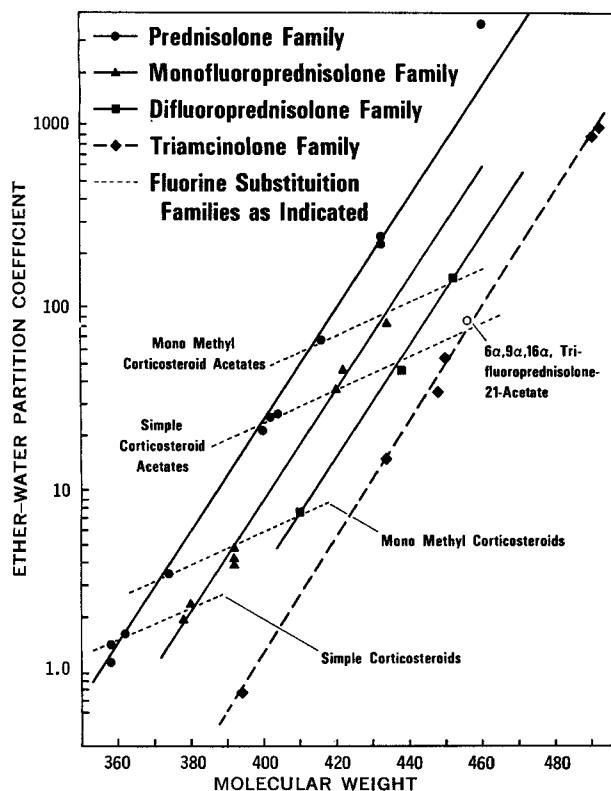
**Figure 3**—*Plot showing the linear relationship of molecular weight to log partition coefficient for selected families of compounds.*

prednisolone includes hydrocortisone, cortisone, methylprednisolone, and their respective 21-esters. The other families are similarly constituted. The positions of fluoronation are not differentiated from one another. The first point gleaned from this plot is that the very closely related analogs and their 21-acyl derivatives give a linear relationship with log $(k_p)$ (Eq. 18):

$$\log k_p = a(MW) + b \qquad \text{(Eq. 18)}$$

Similar relationships for simple homologous series only were pointed out by several other investigators (18). The significance of this is that good structure–activity correlations with molecular weight are possible for analogs by fortunate (or unfortunate, depending on one's perspective) choice of compounds. Such correlations are by themselves meaningless in terms of any fundamental property of the active species. It is noteworthy that a 17-ester, unlike a 21-ester, would be significantly displaced from the line containing its respective free alcohol. Thus, decreases in relative polarity by acylation are not necessarily directly proportional to molecular weight changes.

The monofluoro and difluoro lines are successively displaced along the molecular weight axis to the right of the unsubstituted series. These lines are drawn parallel, as is the dashed triamcinolone line. This is acceptable within experimental error and a necessary consequence of a working $\pi$ value system. The dotted intersecting lines represent another group of families, in which the fluorine substitution is varied while the remainder of the steroid nucleus is held relatively constant. The behavior on repetitive substitution of fluorine atoms is thus analogous to the behavior of a homologous series where methylene units are successively added. In an additive-constitutive system, the repetitive addition of any functional unit to positions of comparable molecular environment would be expected to show such correlation. In this case, the correlation is

reasonable even for nonsymmetrical substitution. Recently, Pinney and Walters (8) published data correlating the bactericidal activities of certain mono-, di-, and polysubstituted fluorophenols with oleyl alcohol–water partition coefficients, number of fluorine atoms, molar solubility, molecular weight, and surface-tension lowering. Although these authors proposed a nonspecific physical mode of action based on these multiple correlations, they actually better demonstrated the interdependence of various physical–chemical parameters.

In the same paper, it was observed that cyclohexane–water partition coefficients were virtually invariant for the fluorophenols and could not be used to establish a rank-order correlation of bactericidal activity. This leads to the question of how does one choose a partitioning system that will lead to a meaningful relationship with biological activity. The answer lies in closely approximating the biophases involved in transport to the receptor environment or interaction with the receptor site, whichever is activity determining. The choice actually narrows to selecting a lipid phase because water is the obvious choice for the polar phase. Based on knowledge of biological membranes and lipids, it seems reasonable that biophase simulation would be best accomplished with a semipolar solvent such as $n$-octanol or diethyl ether rather than a completely apolar solvent such as cyclohexane. When biophase simulation is good and the activity of the series of compounds is reflected in the free energy of transport into the lipid phase, significant structuring of data can be drawn from distribution coefficients obtained *in vitro*.

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