Prostaglandin Prodrugs VI: Structure–Thermodynamic Activity and Structure–Aqueous Solubility Relationships

BRADLEY D. ANDERSON ˣ **and ROBERT A. CONRADI**

Received May 7, 1979, from *Pharmacy Research, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication November 6, 1979.

Abstract □ Solubilities in isooctane and water were determined for several C_1-phenolic esters of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 and acetates having the same phenol moiety. Linear free energy relationships for solubility among the series were observed with slopes of ~1. These results suggest that the contributions of the phenyl substituent to the free energies of these processes are similar in the three series, even though the structure of the acyl moiety is varied. In addition, aqueous solubility was separated into two thermodynamic components, reflecting transfer from the solid phase to an inert solvent and transfer from the inert solvent to water, to evaluate the relative effects of various substituents on the escaping tendency of the drug from the solid phase and on solution interactions. It was found that polar, hydrogen-bonding functional groups in many cases do not bring about increased water solubility because of a corresponding increase in intermolecular interaction in the solid phase.

Keyphrases □ Prostaglandins—prodrugs, structure–thermodynamic activity and structure–aqueous solubility relationships □ Prodrugs—prostaglandins, structure–thermodynamic activity and structure–aqueous solubility relationships □ Solubility—prostaglandin prodrugs, solubility in isooctane and water

The physicochemical properties of a drug affect every aspect of drug design, from initial purification to optimization of drug concentration in the receptor region. Consequently, relationships between molecular structure and physical properties are extremely useful to the pharmaceutical or medicinal chemist. Predictive relationships are particularly important in prodrug design since the primary goal often is the improvement of pharmaceutical or pharmacodynamic properties of already active compounds through modifications of physical properties.

A variety of prostaglandin prodrugs have been synthesized in an attempt to improve the pharmaceutical or biological properties of the parent compounds (1–5). Among the problems encountered with prostaglandins are solid-state instability and their frequent existence as liquids, which pose difficulties in handling and formulation. In the E_2 series, some crystalline high-melting C_1-phenolic esters with improved solid-state stability were reported (1). A rough correlation was observed between the melting points of the esters and the parent phenols, suggesting that strong intermolecular interactions existing in the crystalline phenols may be retained upon ester formation. Similar melting-point correlations were observed for C_1-phenolic esters in the prostaglandin $F_{2\alpha}$ series[1].

This report describes an attempt to gain a more quantitative understanding of the effect of various prodrug moieties on aqueous solubility and on the thermodynamic activity of drugs in the pure crystalline solid as reflected by alkane solubility. Alkane and aqueous solubilities have been determined for several phenolic C_1-esters of prostaglandins of the E_2 and $F_{2\alpha}$ series and for acetates having the same phenolic moieties. Linear free energy relationships between series with slopes of ~1 have been observed in the alkane and aqueous solubilities, suggesting that the effect of a given substituent on the free energies of these processes is similar in all three series. Such relationships may be useful in the prediction of solubilities of other prostaglandin derivatives.

[1] W. Morozowich, The Upjohn Co., Kalamazoo, Mich., personal communication.

EXPERIMENTAL

The compounds examined in this study and their melting points are listed in Table I[2]. The syntheses of the prostaglandin E_2 esters were reported previously (1). The C_1-phenolic esters of prostaglandin $F_{2\alpha}$ and the corresponding acetates were prepared by a similar procedure[3].

Solubilities were determined by various methods, depending on the particular difficulties encountered with each compound. For pure, relatively soluble compounds, 10–50-mg samples were shaken in vials containing 10–25 ml of solvent at 25° for at least 24 hr. These solutions were filtered or centrifuged to remove undissolved solid; after the appropriate dilutions or concentrations, they were analyzed for dissolved drug.

Solution concentrations were determined spectrophotometrically[4] only if solubilities were quite high. Thus, the isooctane and water solubilities of acetates having solubilities greater than 2×10^{-5} *M* were determined by UV analysis, as were the isooctane solubilities of the biphenyl, naphthyl, and *p*-acetylphenyl esters of prostaglandin E_2. In the spectrophotometric analyses, solubilities were determined as a function of the amount of solid added to the vials. A constant apparent solubility suggested that impurities did not contribute significantly to the UV absorbance reading.

All other analyses were performed by high-pressure liquid chromatography[5] (HPLC) using a reversed-phase column[6] and acetonitrile–water solvent systems. Sample detection was at 254 nm.

In many cases, especially for compounds having low aqueous solubility but much higher solubility in isooctane, the method proposed by Higuchi *et al.* (6) was utilized. Solid samples, 10–50 mg, were shaken in vials at 25° in both isooctane and water for not less than 24 hr. When possible, the saturation solubility in both phases was determined as described previously. Because of the low water solubility in isooctane and vice versa (7), it was assumed that solubilities in water-saturated isooctane or in isooctane-saturated water were equal within experimental error to the solubilities in the pure solvents.

Water solubilities of extremely insoluble compounds or compounds that appeared to adsorb readily to glassware, filters, *etc.*, were determined by recycling the solvent through a cartridge containing a filter[7] and a 1–20-mg sample. The change in UV absorbance was monitored at 254 nm[8]. The solvent was pumped[9] through the filtration system at ~10–15 ml/min.

This system offered several advantages over conventional methods:

1. By monitoring absorbance with time, one can ascertain whether equilibrium is established within the system.
2. Withdrawal of filtered samples can be done easily and rapidly with a minimum temperature change.
3. Possible adsorption onto extraneous glassware or onto the filter no longer is a problem since all components become saturated with solute.

Equilibrium solubilities generally were approached within 1 day for

[2] Compounds used in this study were provided by W. Morozowich and S. L. Douglas, The Upjohn Co., Kalamazoo, Mich.

[3] W. Morozowich and S. L. Douglas, The Upjohn Co., Kalamazoo, Mich., personal communication.

[4] Zeiss DMR-21.

[5] DuPont model 840 liquid chromatograph.

[6] Lichrosorb RP-8, 10 μ, Merck, Darmstadt, West Germany.

[7] Swinnex-13 filter unit with 0.45-μm pore size filters (type HA), Millipore Corp., Bedford, MA 01730.

[8] Altex detector 153-00.

[9] FMI RP-58 solvent pump.

0022-3549/80/0400-0424$01.00/0

Table I—Melting Points of Investigated Compounds

Phenolic Moiety	Acyl Moiety: Prostaglandin $F_{2\alpha}$	Acyl Moiety: Prostaglandin E_2	Acyl Moiety: Acetic Acid
Phenyl	59–60.6°	Liquid	Liquid
p-Biphenyl	114.3–116.8°	91.8–92.8°	87.0–88.0°
β-Naphthyl	98.8–100°	79.3–80.3°	68.9–69.9°
p-Nitrophenyl	—	—	81–82°
p-Acetylphenyl	85.3–86.5°	76.8–77.8°	52.1–53.7°
p-Benzamidophenyl	139.8–143.8°	132.8–135.0°	172–173°
p-Acetamidophenyl	114–115.8°	102.3–103.3°	154–156°
p-Carbamoylphenyl	129.5–130.8°	106.3–108.3°	175–184.1°
p-(*p*-Acetamidobenzamido)phenyl	165.6–168.2°	173.2–176.2°	256–260°
p-Ureidophenyl	133.8–135°	105.3–108.3°	189.6–191.2°
N-Acetyl-L-tyrosinamide	109.8–113.8°	137.3–140.8°	222–225.7°
N-Benzoyl-L-tyrosinamide	142.5–144.3°	160.8–164.8°	221.7–228°
α-Semicarbazono-*p*-tolyl	110.8–113.3°	125.3–126.5°	194–198°
2-Oxo-5-indolinyl	109.8–112.0°	—	146–147°
p-(3-Phenylureido)phenyl	145.0–147.3°	—	198–199°

the major component, although impurities often leached out of the solid phase more slowly. Because impurities often contributed more to the UV absorbance than did the compound of interest, HPLC analysis of the saturated solution was necessary. All solubilities are reported in moles per liter.

RESULTS AND DISCUSSION

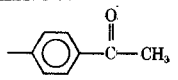
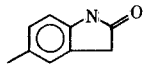
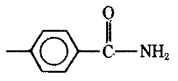
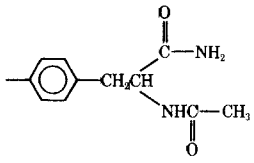
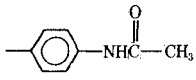
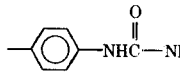
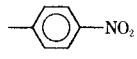
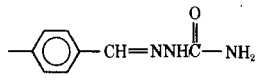
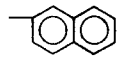
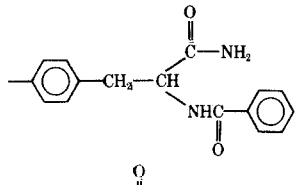
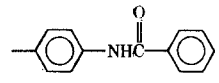
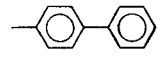
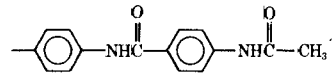
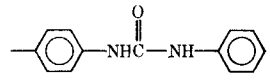
Molecular Structure–Aqueous Solubility Relationships—Aqueous solubilities of prostaglandin E_2 and $F_{2\alpha}$ derivatives and their corresponding acetates are listed in Table II. Plots of log solubility of the prostaglandin $F_{2\alpha}$ esters and the prostaglandin E_2 esters *versus* log solubility of the acetates are shown in Figs. 1 and 2. These quantities were obtained experimentally. Slopes of ~1 (Fig. 1: slope = 0.98, r = 0.9467; Fig. 2: slope = 1.03, r = 0.9468) are observed.

Since log solubility is proportional to the solution free energy, the plots in Figs. 1 and 2 are indicative of approximate linear free energy relationships for solubility; the effect of a substituent on the solution free energy in one series is linearly related to its effect in another series. Slopes of 1, as observed in the series of compounds examined, suggest that not only is there a proportionality in free energy but that the substituent effects on solubility are nearly the same for both prostaglandin series and the acetates. This effect was not known to be observed previously for polar crystalline materials. These observations should not be extrapolated to other series since the constancy in substituent group contributions probably depends on similar crystal lattice arrangements of molecules in all compounds examined.

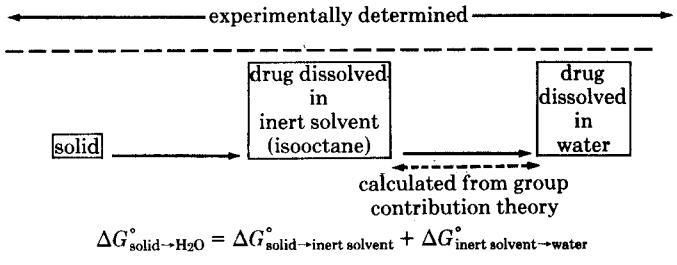
The aqueous solubilities in Table II are listed from highest to lowest. There appears to be no correlation between the apparent polarity of the phenol moiety and aqueous solubility. This lack of correlation illustrates the inadequacy of simple maxims such as "like dissolves like" for predicting relative solubilities of solids in water. A more quantitative understanding of the relationship between molecular structure and aqueous solubility can be gained by separating aqueous solubility into two thermodynamic components.

Separation of Aqueous Solubility into Two Thermodynamic Components—As shown in Scheme I, the solution free energy of a drug in water can be expressed as a sum of the energies from the escape of the drug from the crystal into an inert solvent and the transfer of the drug from an inert solvent to water.

Table II—Water Solubilities of C_1-Phenolic Esters of Prostaglandin $F_{2\alpha}$ and Prostaglandin E_2 and Acetates Having the Same Phenol Moiety

Phenol Moiety	Water Solubility, *M*		
	Acetate	Prostaglandin E_2	Prostaglandin $F_{2\alpha}$
	3.0×10^{-2}	1.6×10^{-5}	5.0×10^{-5}
	2.9×10^{-2}	—	6.0×10^{-5}
	2.3×10^{-2}	4.4×10^{-5}	8.3×10^{-5}
	1.3×10^{-2}	1.7×10^{-4}	1.4×10^{-4}
	1.1×10^{-2}	5.4×10^{-5}	9.5×10^{-5}
	Liquid	Liquid	9.1×10^{-5}
	3.2×10^{-3}	2.8×10^{-5}	6.9×10^{-5}
	2.9×10^{-3}	1.1×10^{-5}	2.1×10^{-5}
	1.4×10^{-3}	2.5×10^{-6}	1.6×10^{-5}
	3.6×10^{-4}	3.9×10^{-7}	5.7×10^{-7}
	1.3×10^{-4}	4.7×10^{-7}	1.8×10^{-6}
	7.2×10^{-5}	2.3×10^{-7}	3.0×10^{-7}
	3.9×10^{-5}	6.6×10^{-8}	9.6×10^{-8}
	3.9×10^{-5}	~9.8×10^{-8}	2.8×10^{-7}
	3.6×10^{-5}	—	—

Component 1: Drug Escape from Crystal into Inert Solvent—The excaping tendency of a compound from its solid phase obviously is dependent on the strength of the intermolecular forces in the crystal. *A priori* attempts to predict the magnitude of these forces from molecular structural considerations still are largely undeveloped. Ideally, partial



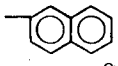
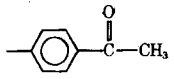
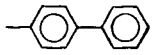
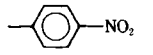
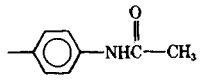
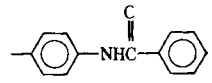
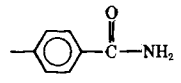
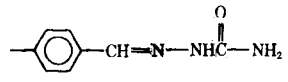
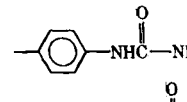
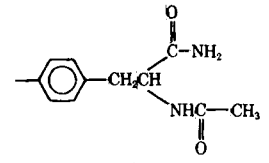
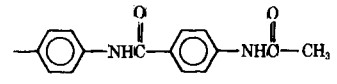
Scheme I—Separation of aqueous solubility into two thermodynamic components

vapor pressures of the drug above the solid phase give relative escaping tendencies directly (8), but these quantities are not easily measured for most compounds. Consequently, it has been suggested (9, 10) that solubility in a relatively inert solvent such as an aliphatic hydrocarbon should be employed to compare escaping tendencies of drugs from the solid phase.

By choosing the infinitely dilute solution of the solute in an alkane solvent as the reference state and setting the thermodynamic activity of a hypothetical 1 *M* solution equal to 1, the thermodynamic activity of a drug in the solid phase may be defined as its alkane solubility (providing that the solubility is low). Differences in the free energies thus obtained reflect differences in such interactions as hydrogen bonding and Debye forces and the entropic content between the two states (10). Whereas specific interactions may be quite significant in the solid phase, solution interactions between the drug substance and alkane solvent are expected to be nonspecific and not highly sensitive to minor changes in molecular structure.

Alkane Solubilities—The alkane solubilities of acetates reported here were determined by direct measurement as described under *Experi-*

Table III—Isooctane Solubilities of C_1-Phenolic Esters of Prostaglandin $F_{2\alpha}$ and Prostaglandin E_2 and Acetates Having the Same Phenol Moiety

Phenol Moiety	Isooctane Solubility, *M*		
	Acetate	Prostaglandin $F_{2\alpha}$	Prostaglandin E_2
	—	5.4×10^{-5}	—
	9.6×10^{-2}	3.3×10^{-6}	1.9×10^{-5}
	6.2×10^{-2}	1.7×10^{-6}	1×10^{-5}
	4.4×10^{-2}	2.8×10^{-6a}	2.7×10^{-5}
	1.6×10^{-2}	4.1×10^{-6}	6.7×10^{-6}
	1.8×10^{-5}	3.9×10^{-9a}	1.4×10^{-8a}
	1.7×10^{-5}	1.8×10^{-9a}	8.7×10^{-9a}
	5.8×10^{-6}	5.4×10^{-10a}	1.8×10^{-9a}
	1.9×10^{-7}	5.5×10^{-11a}	5.5×10^{-11a}
	1.8×10^{-8}	1.1×10^{-11a}	2.8×10^{-11a}
	1.7×10^{-8}	6.2×10^{-12a}	1.0×10^{-11a}
	$\sim 1.3 \times 10^{-9}$	2.4×10^{-13a}	5.5×10^{-13a}

[a] Estimated from water solubility data (Table II) and alkane–water partition coefficients (Table V).

mental. Prostaglandin alkane solubilities were in many cases too low to measure experimentally. These values were estimated from their water solubilities and alkane–water partition coefficients estimated from group contribution theory (the method employed for calculating alkane–water partition coefficients of highly insoluble prostaglandin esters will be described).

Table III gives the alkane solubilities of the C_1-esters of prostaglandins E_2 and $F_{2\alpha}$ and the corresponding acetates. Plots of log alkane solubility in the prostaglandin series *versus* log alkane solubilities of the corresponding esters in the acetate series are shown in Figs. 3 and 4. The plots

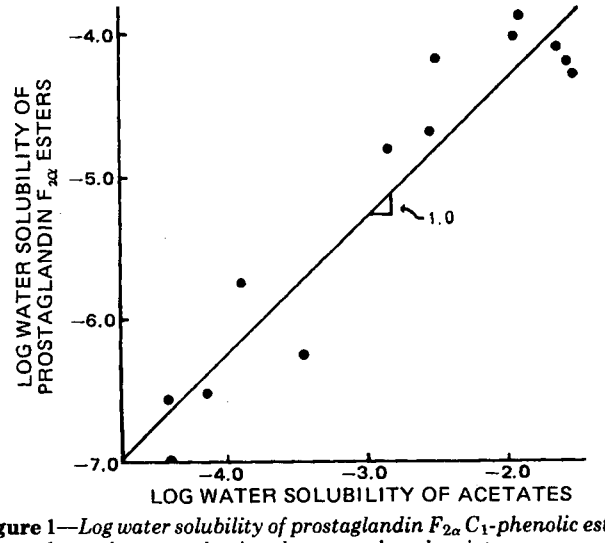


Figure 1—*Log water solubility of prostaglandin $F_{2\alpha}$ C_1-phenolic esters* versus *those of acetates having the same phenol moiety.*

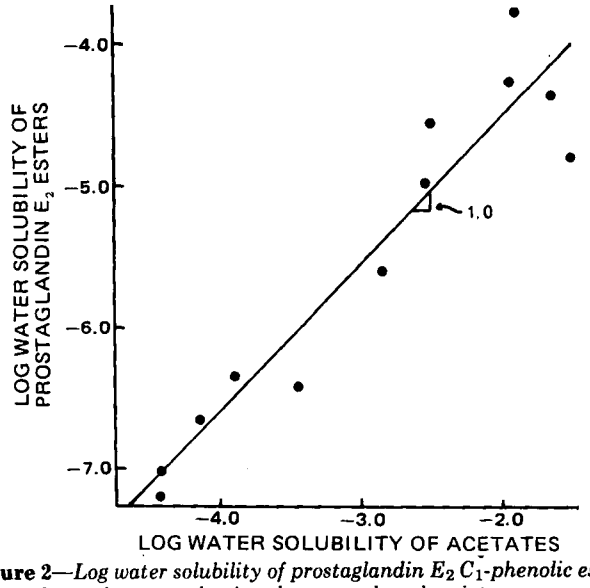
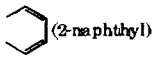
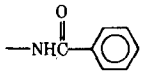
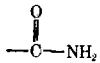
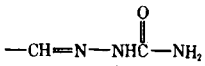
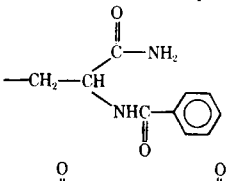
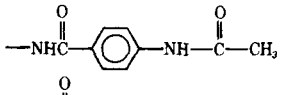


Figure 2—*Log water solubility of prostaglandin E_2 C_1-phenolic esters* versus *those of acetates having the same phenol moiety.*

Table IV—Log Isooctane–Water Partition Coefficient and Group Contribution to the Free Energy of Transfer of Phenyl Substituents in Various Acetates

X	Log Partition Coefficient of $CH_3CO-C_6H_4-X$	π_{alk}[a]	$\Delta(\Delta G^{\circ}_{H_2O\rightarrow alkane})_X$, kcal/mole
	3.05	1.92	−2.62
 (2-naphthyl)	2.43	1.30	−1.77
—H	1.13	0.0	0.0
$-NO_2$	0.73	−0.39	0.53
	0.30	−0.83	1.13
	−0.64	−1.77	2.41
	−2.80	−3.92	5.3
	−2.60	−4.72	6.4
	−3.87	−5.0	6.8
	−3.78	−5.0	6.8
	−4.5	−5.6	7.6
	−5.3	−6.4	8.7

[a] π_{alk} = log partition coefficient of $RCO-C_6H_4-X$ minus log partition coefficient of $RC-O-C_6H_4-H$ in isooctane–water systems.

are linear with slopes of ~1 (Fig. 3: slope = 0.89, r = 0.994; Fig. 4: slope = 0.96, r = 0.9967). The linear free energy correlations observed in the alkane solubilities are a direct consequence of the observed correlations in the aqueous solubilities since group contribution theory was employed in estimating the alkane–water partition coefficients used in calculating many of the alkane solubilities. Nevertheless, the exercise is useful because it enables one to separate structural effects on thermodynamic activity in the solid phase from structural effects on interactions in solution.

Some interesting observations can be made from Table III on the effect

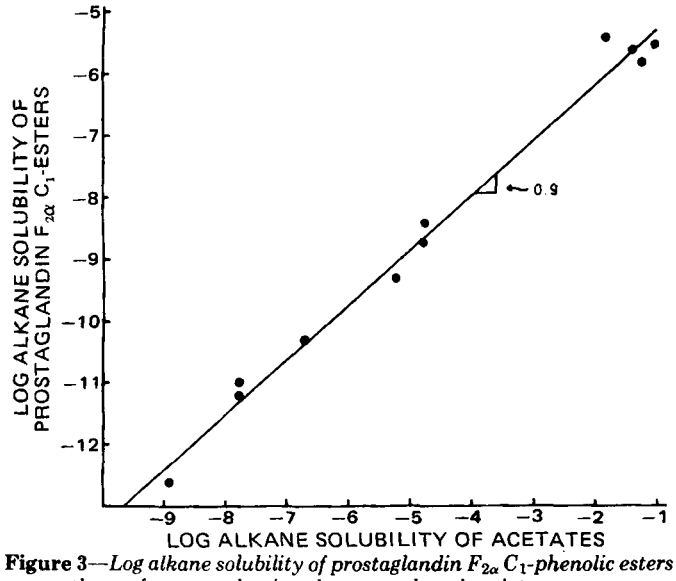


Figure 3—*Log alkane solubility of prostaglandin $F_{2\alpha}$ C_1-phenolic esters* versus *those of acetates having the same phenol moiety.*

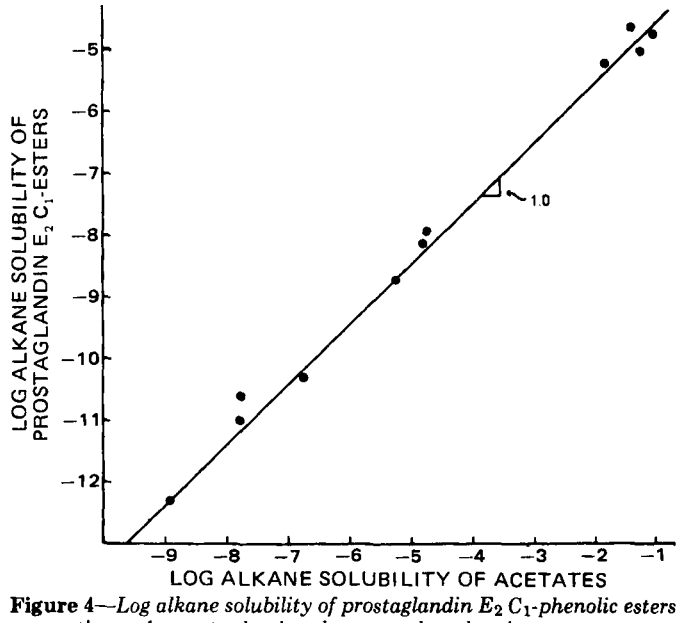
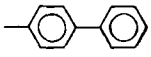
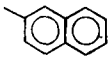
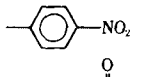
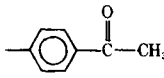
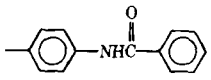
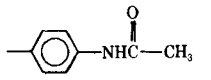
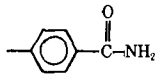
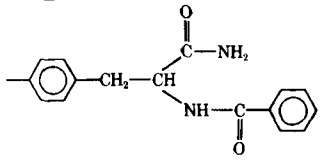
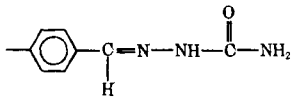
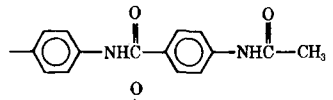
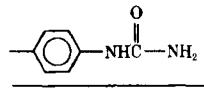
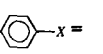


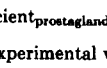
Figure 4—*Log alkane solubility of prostaglandin E_2 C_1-phenolic esters* versus *those of acetates having the same phenol moiety.*

Table V—Calculated [a] Log Isooctane–Water Partition Coefficients of Prostaglandin $F_{2\alpha}$ and Prostaglandin E_2 *para*-Substituted Phenyl Esters [b]

Phenol Moiety	Log Partition Coefficient: Prostaglandin $F_{2\alpha}$	Log Partition Coefficient: Prostaglandin E_2
	1.46	2.26 (2.61)
	0.84 (0.77)	1.64 (1.69)
	(−0.46)	(0.34)
	−0.85 (−0.71)	−0.05 (−0.20)
	−1.28 (−1.47)	−0.48 (−0.22)
	−2.2	−1.42
	−4.4	−3.6
	−5.2	−4.4
	−5.4	−4.6
	−5.4	−4.6
	−6.1	−5.3
	−6.8	−6.0

[a] Calculated values were obtained from:

log partition coefficient$_{\text{prostaglandin}}$  =

log partition coefficient$_{\text{prostaglandin}}$  + $(\pi_{alk})_X$

where $(\pi_{alk})_X$ values are listed in Table IV. [b] Experimental values are given in parentheses.

of molecular structure, particularly the incorporation of hydrogen-bonding substituents into the molecule, on the escaping tendency of drug from the solid phase. For example, a difference of over 3000-fold in the alkane solubility of *p*-acetylphenyl acetate compared to *p*-acetamidophenyl acetate shows the dramatic influence of a single hydrogen-bonding moiety on crystalline energy. This factor represents a difference of ~4.8 kcal in free energy, a difference that can be rationalized in terms of hydrogen-bonding differences in the crystals. Replacement of a terminal methyl group with a phenyl group (*p*-acetamidophenyl acetate *versus* *p*-benzamidophenyl acetate) has a small influence on alkane solubility, while addition of another acetamido substituent again lowers the solubility by the equivalent of 5.6 kcal in free energy.

Clustering of points is evident in Figs. 3 and 4. Points at high alkane solubilities represent compounds containing no hydrogen-bonding phenyl substituents, while addition of a single hydrogen-bonding group results in a second cluster of points 3–4 log units lower in solubility.

Component 2: Transfer of Drug from Inert Solvent to Water—Prediction of organic solvent–water partition coefficients from molecular structures is complicated by the present lack of understanding of solute–solvent interactions at the molecular level. Consequently, a semiempirical approach often is employed based on the group contribution concept (11).

Briefly, it is assumed that the free energy of the partitioning process

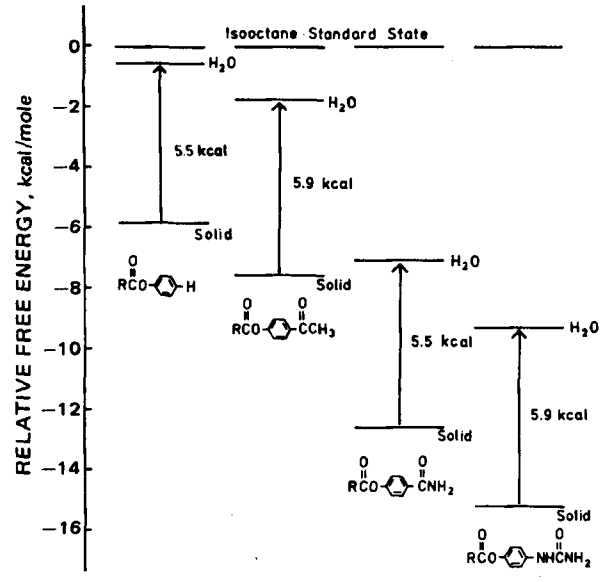


Figure 5—*Relative free energy diagram illustrating the effect of polar substituents on the thermodynamic activity of drug in the solid, on the free energy of transfer from alkane to water, and on aqueous solubility in which RCOO represents prostaglandin $F_{2\alpha}$.*

can be divided into independent contributions from the constituent groups in a molecule.

Alkane–Water Partitioning Behavior—Logs of the partition coefficients at 25° for acetates containing various substituted phenyl moieties are listed in Table IV. In most cases, these values were determined from solubility ratios, which are assumed to be reliable estimates of partition coefficients because isooctane and water are almost completely immiscible and because the solubilities usually were sufficiently low so that solute–solute interactions in solution were considered negligible.

Phenyl acetate was chosen as a reference compound for determining group contributions of various substituents to the free energy of transfer from water to isooctane. The standard free energy of transfer of a drug containing a substituent, X, from water to isooctane can be expressed as:

$$\Delta G^{\circ RX}_{H_2O\rightarrow alk} = -RT \ln PC_{RX} \qquad \text{(Eq. 1)}$$

where:

$$PC_{RX} = \frac{C_{alk}}{C_{H_2O}} \qquad \text{(Eq. 2)}$$

in which C is expressed in moles per liter.

The change in transfer free energy brought about by substitution is:

$$\Delta(\Delta G^{\circ}_{H_2O\rightarrow alk})_X = \Delta G^{\circ RX} - \Delta G^{\circ RH} = -RT \ln \frac{PC_{RX}}{PC_{RH}} \qquad \text{(Eq. 3)}$$

Values of $\Delta(\Delta G^{\circ}_{H_2O\rightarrow alk})_X$ for various substituents on the phenyl ring, derived from the acetate partition coefficients, also are listed in Table IV along with π_{alk} values that represent the quantity log PC_{RX}/PC_{RH}.

Partition coefficient data for the prostaglandin esters were generated from solubility ratios when possible or were estimated from group contributions. This calculation was done by adding the π_{alk} value for a phenyl substituent obtained from acetate partitioning data (Table IV) to the measured log of the partition coefficient of the unsubstituted phenyl ester of either prostaglandin E_2 or $F_{2\alpha}$ to obtain the log of the partition coefficient for the substituted phenyl prostaglandin ester. Calculated values of log partition coefficient for the substituted phenyl esters of prostaglandins $F_{2\alpha}$ and E_2 are shown in Table V (experimental values in parentheses).

Influence of Polar, Hydrogen-Bonding Substituents on Aqueous Solubility—Because of the large effect of polar groups on the partitioning behavior of a molecule, it occasionally is suggested that the introduction of polar groups into a molecule should increase aqueous solubility. The underlying assumption is that log solubility is inversely related to log partition coefficient, as has been shown for liquid solutes. However, the effect of polar groups capable of hydrogen bonding on the crystal lattice energies also must be considered for solids.

A diagram of relative free energy levels of solute in the solid phase, in

the aqueous solution standard state, and in the isooctane standard state (Fig. 5) illustrates the effect of polar groups in a series of prostaglandin $F_{2\alpha}$ esters. An alkane solution of drug at infinite dilution was chosen as the reference state according to the convention proposed previously (9, 10). Thus, the free energies of various derivatives in isooctane arbitrarily were set equal to zero so that relative energies in the solid phase and in water could be compared. Polar substituents dramatically lowered the energy level of a drug in the solid phase and in water relative to an alkane solution; but the free energy difference between solid and aqueous solution, which is reflected by aqueous solubility, remained similar upon addition of increasingly polar substituents.

The conclusion is that polar, hydrogen-bonding substituents may not result in increased aqueous solubility. Even though lipophilicity as measured by partition coefficients may be decreased drastically, the effect of polar groups on the thermodynamic activity of a drug in the solid may tend to offset the change in lipophilicity (Fig. 5). Both thermodynamic components of aqueous solubility must be considered in predicting aqueous solubility.

REFERENCES

(1) W. Morozowich, T. O. Oesterling, W. L. Miller, C. F. Lawson, J. R. Weeks, R. G. Stehle, and S. L. Douglas, *J. Pharm. Sci.*, **68**, 833 (1979).

(2) W. Morozowich, T. O. Oesterling, W. L. Miller, and S. L. Douglas, *ibid.*, **68**, 836 (1979).

(3) W. Morozowich, T. O. Oesterling, W. L. Miller, C. F. Lawson, J. C. Cornette, J. R. Weeks, and S. L. Douglas, *ibid.*, **68**, 949 (1979).

(4) M. J. Cho and W. Morozowich, *ibid.*, **68**, 1069 (1979).

(5) M. J. Cho, G. L. Bundy, and J. J. Biermacher, *J. Med. Chem.*, **20**, 15 (1977).

(6) T. Higuchi, F.-M. L. Shih, T. Kimura, and J. H. Rytting, *J. Pharm. Sci.*, **68**, 1267 (1979).

(7) S. S. Davis, *Sep. Sci.*, **10**, 1 (1975).

(8) G. N. Lewis and M. Randall, "Thermodynamics," 2nd ed., McGraw-Hill, New York, N.Y., 1961, pp. 145–156.

(9) J. H. Rytting, S. S. David, and T. Higuchi, *J. Pharm. Sci.*, **61**, 816 (1972).

(10) T. Higuchi, in "Design of Biopharmaceutical Properties through Prodrugs and Analogs," E. B. Roche, Ed., American Pharmaceutical Association, Washington, D.C., 1977, pp. 409–421.

(11) S. S. Davis, T. Higuchi, and J. H. Rytting, in "Advances in Pharmaceutical Sciences," vol. 4, Academic, London, England, 1974, pp. 73–261.

ACKNOWLEDGMENTS

The authors thank W. Morozowich and S. L. Douglas for providing the compounds used in this study and for enlightening discussions.

Preliminary Examination of Rabbit Conjunctival Mucins

VINCENT HON-LEUNG LEE * and JOSEPH R. ROBINSON ˣ

Received September 19, 1979, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706.* Accepted for publication November 16, 1979. *Present address: School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Abstract □ Conjunctival mucins from albino rabbits were fractionated using gel filtration and anion-exchange chromatography. Charge homogeneity of the various conjunctival mucin fractions was confirmed by polyacrylamide gel electrophoresis. The molecular weight characteristics of the isolated fractions varied with the analytical scheme employed. Two schemes yielded mucins with molecular weights of 10^4, 10^5, and 10^6. However, when anion-exchange chromatography was the first step in the fractionation scheme, the dominant mucin had a molecular weight of 1.7×10^5. In contrast, when gel filtration chromatography was the first step, the dominant mucin had a molecular weight of 5.7×10^4. It was postulated that during migration through the anion-exchange matrix, the low molecular weight conjunctival mucin underwent trimer formation. Comparison with the mucin fractions isolated from tear mucoid threads revealed that the scheme beginning with anion-exchange chromatography preserved the fractionation pattern seen in tear mucoid threads. This result implies that conjunctival mucins undergo an association prior to or after their entry into the tear film. The molecular event of interest in this process is self-association of the species with a molecular weight of 5.7×10^4, resulting in a trimer with a molecular weight of 1.7×10^5. This trimer appears to resist deaggregation on exposure to a medium of lesser ionic strength. Several explanations are offered for its formation as well as for its stability. The implication of multiple conjunctival (tear) mucins for tear film stability also is discussed.

Keyphrases □ Mucins—isolation from rabbit conjunctiva, fractionation by gel filtration and anion-exchange chromatography, determination of molecular weight, role in tear film stability □ Conjunctival mucins—isolation from rabbits, fractionation by gel filtration and anion-exchange chromatography, determination of molecular weight, role in tear film stability □ Ocular mucins—isolation from rabbit conjunctiva, fractionation by gel filtration and anion-exchange chromatography, determination of molecular weight, role in tear film stability

The strategic role assumed by conjunctival mucin in tear film stability has been recognized, but not understood, for many years. Alteration in either its chemical nature or quantity (probably both) has been implicated in keratoconjunctivitis sicca as well as other dry-eye syndromes (1–5), all of which are characterized by poor wetting of the corneal epithelium. It has been proposed that this fascinating substance derives its role from both the surface activity it is expected to exhibit at the air–tear and tear–corneal epithelium interfaces and the molecular events following its absorption at these two interfaces.

Principally because conjunctival mucins have never been isolated or identified, statements about their surface activity are based on experiments using bovine submaxillary mucin (6–9). In contrast, three tear mucins have been isolated (10), but the interrelationship between tear and conjunctival mucins is unknown. The tear mucins were shown to possess an amino acid and carbohydrate composition not shared by typical epithelial mucins (10, 11). Since the function it serves depends on its structure, considerable caution must be exercised in extrapolating the findings on surface activity of bovine submaxillary mucin to conjunctival and tear mucins. Successful isolation of conjunctival mucins, in reasonable quantity and purity, is important in understanding the mechanisms by which they confer stability to the tear film. This study is a first attempt in this direction; specifically, it concerns identifying conjunctival mucins present in a crude conjunctival extract.

The three tear mucins isolated by Iwata and Kabasawa (10) from tear mucoid threads on the conjunctival surface of albino rabbits possessed molecular weights of 4×10^5,

0022-3549/80/0400-0430$01.00/0