Intrinsic Solubility Estimation and pH-Solubility Behavior of Cosalane (NSC 658586), an Extremely Hydrophobic Diprotic Acid

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Purpose. The selection of cosalane (NSC 658586) by the National Cancer Institute for further development as a potential drug candidate for the treatment of AIDS led to the exploration of the solubility behavior of this extremely hydrophobic drug, which has an intrinsic solubility (S_0) approaching 1 ng/ml. This study describes attempts to reliably measure the intrinsic solubility of cosalane and examine its pH-solubility behavior.

Methods. S_0 was estimated by 5 different strategies: (a) direct determination in an aqueous suspension; (b) facilitated dissolution; (c) estimation from the octanol/water partition coefficient and octanol solubility; (d) application of an empirical equation based on melting point and partition coefficient; and (e) estimation from the hydrocarbon solubility and functional group contributions for transfer from hydrocarbon to water.

Results. S_0 estimates using these five methods varied over a 5×10^9-fold range. Method (a) yielded the highest values, two-orders of magnitude greater than those obtained by method (b) (facilitated dissolution, 1.4 ± 0.5 ng/ml). Method (c) gave a value 20-fold higher while that from method (d) was in fair agreement with that from facilitated dissolution. Method (e) yielded a value several orders-of-magnitude lower than other methods. A molecular dynamics simulation suggests that folded conformations not accounted for by group contributions may reduce cosalane's effective hydrophobicity. Ionic equilibria calculations for this weak diprotic acid suggested a 100-fold increase in solubility per pH unit increase. The pH-solubility profile of cosalane at 25°C agreed closely with theory.

Conclusions. These studies highlight the difficulty in determining solubility of very poorly soluble compounds and the possible advantage of the facilitated dissolution method. The diprotic nature of cosalane enabled a solubility enhancement of $>10^7$-fold by simple pH adjustment.

KEY WORDS: cosalane; solubility; facilitated dissolution; partition coefficient; group contribution approach; pH-solubility behavior.

INTRODUCTION

Cosalane (NSC 658586) (Scheme I) is a potent inhibitor of HIV with a broad range of activity against a variety of HIV-1 isolates, HIV-2, Rauscher murine leukemia virus, HSV-1, HSV-2, and human cytomegalovirus (1–3). Its mechanism of anti-HIV activity appears to be mainly due to interference with gp120-CD4 binding, but cosalane also inhibits the enzymatic activities of HIV-1 reverse transcriptase, protease, and integrase enzymes (2,3). The hydrophobic steroid portion of this molecule is considered important in directing the compound to the cell membrane and viral envelope, thus enhancing the ability of cosalane to inhibit viral binding to cell membranes.

In response to the need for new agents with diverse antiviral mechanisms of action against HIV, the National Cancer Institute (NCI) recently selected cosalane for further development as a potential candidate for the treatment of AIDS. A parenteral formulation containing >10 mg/mL of drug was desired for the assessment of toxicity and also for the determination of the absolute bioavailability of an orally administered dosage form. As might be expected from its extremely hydrophobic nature, cosalane has an extremely low intrinsic solubility in water of ≈1 ng/mL, a factor of 10^7 below the required target concentration of 10 mg/mL. This report describes attempts to reliably measure the intrinsic aqueous solubility of cosalane by a variety of techniques and to obtain the desired $>10^7$-fold enhancement in solubility.

The determination of the intrinsic aqueous solubility of very poorly soluble organic compounds presents several practical difficulties that lead to wide variations in the reported equilibrium solubility values. The aqueous solubility of cholesterol reported in the literature, for example, ranges from 0.025 to 2600 μg/mL (4–6). Reliable determination of the true solubility may require comparisons between the values obtained by several methods. In this paper, five approaches were employed to estimate the intrinsic aqueous solubility of cosalane: (a) direct determination by centrifugation of aqueous suspensions; (b) the facilitated dissolution method of Higuchi *et al.* (7); (c) estimation from the octanol/water partition coefficient and octanol solubility; (d) calculation from the melting point and octanol/water partition coefficient (8,9); and (e) estimation from the hydrocarbon solubility combined with group contributions for the transfer from hydrocarbon to water (7,10).

An ionic equilibria treatment of the ionization behavior of cosalane based on the examination of its structure suggested that since it is a weak diprotic acid, the slope of the log (solubility) vs. pH profile should approach 2 at pH values above its second pKa, thereby resulting in a 100-fold increase in solubility per pH unit increase. Therefore it may be possible to employ a simple strategy of pH adjustment to achieve the desired 10^7 fold enhancement in aqueous solubility. To test this prediction, the solubility of cosalane was studied as a function of pH.

MATERIALS AND METHODS

Chemicals

Cosalane [1,1-di(3′-carboxy-5′-chloro-4′-hydroxyphenyl)-4(3β)-cholestanyl-1-butene] (NSC 658586) was obtained from the National Cancer Institute (Bethesda, MD). Its purity was established by several different methods including DSC (mp, 262°C), HPLC and TLC. A determination of cosalane's equivalent weight by titration of a methanolic solution with 0.1 N NaOH gave an estimated purity of 99.1%. Soybean oil

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(Sigma, St Louis, MO), octanol (Baker, Phillipsburg, NJ) and 3-choloro-salicylic acid (Aldrich, Milwaukee, WI) were used as received. All other chemicals were HPLC grade (mobile phase solvents) or reagent grade (HCl, NaOH, buffers) obtained from commercial sources and used without further purification. Deionized water was used to prepare all aqueous solutions.

HPLC Analyses

Cosalane samples were analyzed by HPLC using a system comprised of a manual sample injector (Rheodyne Model 7125, Rainin Instrument Co., Woburn, MA), a solvent pump (Model 110A, Beckman Instruments Inc., Fullerton, CA), a variable wavelength UV absorbance detector (Model 1050, Hewlett Packard, Avondale, PA, or Waters Model 480, Millipore Corp., Milford, MA) operated at 230 nm, an integrator (Model 3396A, Hewlett Packard, or Waters 740, Millipore Corp., Milford, MA), and a reversed phase column packed with 5 μ Supelcosil LC-18-S (Supelco, 4.6 mm i.d. × 25 cm, Rainin) at room temperature. The mobile phase [methanol:tetrahydrofuran:phosphoric acid (74:25:1 % v/v)] was pumped at a flow rate of 2 mL/min. The retention time was ≈5 minutes. Cosalane concentrations in some aqueous samples in the acidic pH range were extremely low (≈1 ng/mL). Therefore it was necessary to concentrate these samples by evaporating at least 3 mL of aqueous sample under a stream of nitrogen, using a Reacti-Therm heating module (Pierce Chemical Co., Rockford, IL) to provide slight warming of the sample. The dry residue was dissolved in 300 μl methanol and 250 μl was taken for HPLC analysis (200 μl injection loop). The 3-chloro-salicylic acid (3Cl-SA) samples generated in partitioning experiments were analyzed using HPLC but without sample concentration. The mobile phase [methanol:choloroacetic acid buffer (I = 0.01, pH 2.2), 60:40% v/v] was pumped at a flow rate of 1.5 mL/min and UV detection was carried out at 245 nm.

Solubility of Cosalane in Organic Solvents

An accurately weighed amount of cosalane in excess of the amount expected to dissolve was placed in a clear 4 mL HPLC glass vial with an appropriate volume of solvent. The vial was sealed with a teflon-lined cap and placed on a laboratory rotator (Labquake, Berkeley, CA) at 25 ± 1°C. After equilibration (48 h) the sample was filtered through a suitable filter (Acrodisc PTFE, 0.45 μm, Gelman Sciences, Ann Arbor, MI), and the solution was appropriately diluted for HPLC analysis.

Methods for Determination of the Intrinsic Aqueous Solubility of Cosalane

Direct Determination in Aqueous Suspensions

An excess amount of cosalane was weighed into clear 8 mL glass centrifuge tubes to which was added 6 mL of aqueous buffer (pH 2–7, ionic strength ≈0.01). The vials were sealed with a teflon lined cap and the samples were placed on a laboratory rotator at 25 ± 1°C for 3–10 days. The samples were centrifuged (Sorvall, DuPont, Hoffman Estates, IL) at 3000 rpm for 15 min, and the clear aqueous solutions were carefully withdrawn using a microliter syringe. Appropriate volumes (0.5–1 mL) of the sample solutions were analyzed by HPLC. Samples for solubility determination at higher pH (>7.5) were prepared by addition of 0.1 N NaOH as required to obtain the desired final pH. These samples were filtered (Acrodisc PVDF, 0.45 μm, Gelman Sciences, Ann Arbor, MI) and diluted prior to analysis.

Facilitated Dissolution Method

Since the intrinsic solubility of cosalane is extremely low (<1 μg/mL), a modification of the facilitated dissolution approach as described earlier by Higuchi *et al.* (7) was explored. The facilitated dissolution method employs a water-immiscible organic solvent to solubilize the drug and thereby facilitate its equilibration with water. The equilibrium solubilities of cosalane in octanol, soybean oil, and isooctane were determined to be 244 mg/mL, 4.2 mg/mL, and 0.0097 mg/mL respectively. Soybean oil was chosen as the water-immiscible solvent in these studies. An excess amount of cosalane was placed in 240 μL of soybean oil and 6 mL of aqueous solution in 8 mL centrifuge tubes. When the required aqueous sample volume exceeded 6 mL (e.g., ultrafiltration studies at low pH), multiple samples were prepared at the same pH. Dilute solutions of HCl were used to adjust pH in the low pH range while buffers of 0.01 ionic strength were employed above pH 3. Equilibration was achieved by rotating the samples at 25 ± 1°C for over 48 hours.

Samples at low pH were analyzed after either centrifugation or ultrafiltration to obtain a clear aqueous phase. Samples at pH > 3 emulsified, necessitating separation of the aqueous phase by ultrafiltration. Ultrafiltration of samples was performed using Ultrafree-PFL, 300,000 MWCO membranes (Millipore Corp., Bedford, MA). Samples equilibrated at pH > 5 were filtered directly without any pretreatment of the filter membrane. However, to minimize the effects of adsorption by the ultrafiltration membrane, which was a severe problem in the ultrafiltration of extremely dilute samples, the filter membrane was saturated by filtering 10 mL of a 3×10^{-4} mg/mL aqueous solution of cosalane at pH 8.5 before filtration of samples at pH < 5. The filter unit was then rinsed with deionized water three times to remove excess solution within the housing and then successive 1-mL aliquots of sample were ultrafiltered and analyzed by HPLC. Typically, a plateau in the plot of the cosalane concentration versus filtration volume was achieved after the ultrafiltration of 10–30 mL of sample at low pH and 2–10 ml of sample at higher pH. The equilibrium solubility was determined by fitting the ultrafiltrate concentration versus volume profiles to an equation assuming an exponential (i.e., first-order) approach to the plateau values. Drug suspensions containing soybean oil and water equilibrated at pH < 3 phase separated readily. For these samples, a clear aliquot of the aqueous phase could be obtained after centrifugation. Sample was transferred to a clean 15-mL glass centrifuge tube and centrifuged at 3000 rpm for 15 minutes. The aqueous solution was carefully withdrawn and placed into a second 15-mL centrifuge tube, which was centrifuged at 3000 rpm for another 15 minutes. An aliquot was then carefully withdrawn for HPLC analysis.

Estimation from Octanol Solubility and Octanol/Water Partition Coefficient

The octanol/water partition coefficients for cosalane and 3-chloro-salicylic acid were measured as a function of solute

concentration using the shake-flask method. An appropriate volume of the solution of the solute in octanol was placed in contact with the aqueous phase. After vortexing (5 min) the sample was rotated overnight in a temperature controlled chamber at 25 ± 1°C. The two phases were then separated and allowed to stand in the chamber for several hours. Both phases were analyzed after appropriate dilution. The intrinsic aqueous solubility of cosalane was estimated from its solubility in octanol and its octanol/water partition coefficient.

Calculation Based on Melting Point and Octanol/Water Partition Coefficient

The intrinsic aqueous solubility of cosalane was also calculated using an empirical equation (Eq. 1) developed by Yalkowsky and Valvani (8). Considering the transfer of a solute from the solid state to octanol, followed by its transfer to the aqueous phase, they fitted the solubility data of 167 compounds to obtain an equation for solubility as a function of octanol/water partition coefficient and melting point of the solute:

$$\log S_{aq} = 0.54 - 8.16 \times 10^{-4}\Delta S_f(\theta_m - 25) - \log P_{o/w} \quad (1)$$

where ΔS_f is the entropy of fusion and θ_m is the melting point. A ΔS_f of 13.5 cal.mol^{-1}K^{-1} was assumed in these estimates.

Estimation Based on Functional Group Contributions

In this method the intrinsic aqueous solubility is calculated from the hydrocarbon (isooctane) solubility and the hydrocarbon/water partition coefficient, where the latter is estimated by the group contribution approach. The solute molecule is assumed to be comprised of a set of fundamental fragments whose individual contributions to the partition coefficient are summed with appropriate weighting factors consisting of the number of times each fragment appears in the molecule. This approach can be mathematically expressed as follows:

$$\log P = \sum a_i f_i + \sum c_j \quad (2)$$

where f_i and a_i are the fragment constants and the number of occurrences of the ith fragment, respectively (7,10,11). The group contribution of 3-chloro-salicylic acid (3Cl-SA) to the partition coefficient value is not available in the literature. Therefore the isooctane/water partition coefficient for 3Cl-SA was determined using the shake-flask method as described above for octanol/water partition coefficients.

Molecular Dynamics (MD) Simulation

MD simulations of cosalane were conducted in both the gas phase and in an aqueous environment using a Silicon Graphics IRIS 47/70GT system (San Jose, CA) supported by Insight II and Discover (Biosym Technologies, San Jose, CA) software programs. Unionized cosalane and the aqueous environment around the molecule were constructed using Insight II and Discover was used to perform energy minimization at 25°C to find the conformation with the global minimum free energy.

Determination of pKa of 3-Chloro-Salicylic Acid by Titration

An accurately weighed amount of 3Cl-SA was transferred into a 10 mL volumetric flask and dissolved in water (final concentration was 0.00448 M). A 3 ml aliquot of this solution was titrated with dilute sodium hydroxide (0.455 M) and the pH of the solution was measured after each addition. The pKa was determined by computer fitting the data for pH as a function of volume of NaOH added.

RESULTS AND DISCUSSION

Cosalane is one of several anti-HIV analogues recently synthesized with increased potency possibly resulting from incorporation of large, hydrophobic moieties such as steroid, adamantyl, or fullerene into the molecule (2,12,13). From the standpoint of formulation, such analogues are likely to present severe challenges because of their hydrophobic nature. Beyond the problem of solubilization is the added difficulty of obtaining precise measurements of solubility under conditions in which these compounds are in their unionized form. This study addresses both the determination of solubility and the solubilization of cosalane, which serves as a useful model compound for drugs of this type.

Comparison of Various Approaches to Intrinsic Solubility Determination

The intrinsic solubility of cosalane was determined by a variety of approaches, the results of which are summarized in Table I. The apparent intrinsic solubility of cosalane at 25°C obtained by the classical method, direct determination in aqueous suspensions, was found to be 1.6×10^{-4} mg/mL but the large standard deviations in the individual determinations at low pH (Table III) raised concerns as to the reliability of this method. Usually the direct determination of solubility of moderately (>1 mg/mL) or highly soluble compounds poses few problems. However, in the case of compounds that are very poorly soluble (<1 μg/mL), several issues need to be considered. From the Noyes-Whitney relationship (14), it is apparent that the rate of attainment of equilibrium increases with an increase in the surface area available for dissolution. Therefore, a larger excess of solid would be required for very poorly soluble compounds as compared to the case for moderately soluble solutes to maintain equivalent surface areas. For example, in the case of norethindrone (aqueous solubility ≈6 μg/mL) a ≈500 fold excess of solid would be required to achieve the same terminal rate of approach to equilibrium as in the case of benzoic acid (aqueous solubility = 3.4 mg/mL) wherein a

Table I. Intrinsic Aqueous Solubility of Cosalane Determined by Five Different Approaches

Approach taken to obtain solubility estimate	Solubility (mg/mL) mean (CV%)
Direct determination from aqueous suspensions	1.6×10^{-4} (47%)
Facilitated dissolution (from pH-solubility profile)	1.4×10^{-6} (39%)
Octanol solubility and octanol/water partition coefficient measurement	2.8×10^{-5} (46%)
Empirical equation based on melting point and octanol/water partition coefficient	7.3×10^{-7}
Group contribution method	3.1×10^{-14}

two fold excess of solid is present (7). However, this strategy of increasing the effective surface area available may lead to discrepancies in the determined solubility value as a result of two different independent effects (7,15). Firstly, when an extremely large amount of solid is present, the effect of impurities on the apparent solubility value may be magnified several-fold (this should not be a factor when a specific method of analysis is used, as in the present study). Secondly, during dissolution, the energetic heterogeneity (e.g., crystal defects, amorphous regions, etc.) within the otherwise chemically pure crystals will result in the early dissolution of the higher energy regions thereby leading to a supersaturated system temporarily reflecting the thermodynamic activity of the higher energy component. In other words, the initial apparent solubility will depend on the heterogeneity within the crystals, and may be erroneously high since the apparent solubility increases with the energy content of the crystals. Furthermore since the solubility is very low, the rate of recrystallization will be extremely slow, thereby prolonging this effect.

One approach to overcome these problems is the facilitated dissolution method developed by Higuchi *et al.* (7). In this method, the equilibration time is reduced by including a water-immiscible solvent (soybean oil) in which the compound is soluble. The advantage provided by the facilitated dissolution method is that it overcomes the influence of crystal heterogeneity on equilibrium solubility determination by allowing the use of less excess solid and by facilitating recrystallization. Thus, drug which dissolves from high energy sites will recrystallize on lower energy surfaces more rapidly when the equilibrium concentration of the drug in solution is high. The intrinsic solubility of cosalane at 25°C using this approach was determined to be 1.4×10^{-6} mg/mL (Table I), approximately 2 orders of magnitude lower than the value determined by the direct method. The inherent assumptions made in this method are as follows: (i) the solid drug particles effectively remain in the oil phase thereby enhancing dissolution surface area, and (ii) soybean oil is water insoluble and therefore has no effect on the aqueous phase (15). Visual observations confirmed that cosalane particles essentially remained in the oil phase. In addition, the octanol/water partition coefficient of soybean oil was estimated by the group contribution approach using fragment values obtained from the literature (15) to be on the order of 10^{22} thus establishing that soybean oil is insoluble in water. It is possible, of course, that impurities in reagent grade soybean oil (e.g., free fatty acids) have substantially smaller partition coefficients such that their concentration in the aqueous phase might be higher than that of soybean oil itself. Such impurities would be expected to increase the aqueous solubility of cosalane. Thus, it is difficult to envision contributions to error in the facilitated dissolution method as employed in this study which might account for the 2 orders-of-magnitude lower apparent intrinsic solubility value obtained in comparison to the direct method. It seems more likely that the values obtained by the direct method are in error due to crystal heterogeneity.

A third method of solubility estimation which closely resembles the facilitated dissolution method for lipophilic compounds which are sparingly soluble in water, utilizes the experimentally obtained solubility in a water immiscible organic solvent and the partition coefficient (15). For example, in this case, the solubility of cosalane was determined in octanol (S_{oct}). Following this measurement, the octanol-water partition coefficient for cosalane was measured ($K_{o/w}$). From these two values the aqueous solubility (S_{aq}) was calculated by the following relationship:

$$S_{aq} = S_{oct}/K_{o/w} \tag{3}$$

where S_{oct} = 244 mg/mL, $K_{o/w} = 8.8 \times 10^6$, and thus, $S_{aq} = 2.8 \times 10^{-5}$ mg/mL.

The calculated value is 20-fold higher than the intrinsic solubility value (1.4×10^{-6} mg/mL) obtained from the facilitated dissolution approach. The above relationship between the solubility values and partition coefficient is based on the thermodynamic cycle for predicting the standard molar free energy of solution of the solute in water. The route for the solute is considered to be as follows: *pure solid solute* → *solution in octanol* → *solution in water.* The above approach requires that the solute concentration in the two phases is low, a condition which is violated in the octanol phase, thus perhaps accounting for a portion of the discrepancy. Perhaps more importantly, this solubility estimate is based on the partitioning of drug between octanol and a saturated solution of octanol in water, a more favorable solvent for a hydrophobic drug than water alone.

Yalkowsky and Valvani have described an empirical equation (Eq. 1) to predict the solubility of organic compounds from their octanol/water partition coefficients and melting points (8). The intrinsic aqueous solubility is calculated using this approach to be 7.3×10^{-7} mg/mL, a value within a factor of two of that obtained by the facilitated dissolution method. Given the errors associated with estimates obtained by these two methods, these values are not significantly different.

A final alternative method to predict the aqueous solubility of very sparingly soluble, lipophilic organic compounds makes use of measured solubility in a hydrocarbon solvent and the group contribution approach to estimate the hydrocarbon/water partition coefficient. In this approach, the constituent groups comprising the molecule are assumed to contribute independently to the overall partition coefficient of the solute. This procedure may be particularly attractive, and has been used previously (16) for extremely water insoluble solutes having aqueous solubilities that cannot be measured directly. Indeed, Higuchi *et al.* (7) observed that this approach may be the only feasible method of obtaining an accurate solubility estimate for extremely water insoluble compounds.

The isooctane-water system was used in this calculation since the group contribution values for many of the constituent fragments are available. The contributions of the 17-androstyl, methyl and methylene groups to the standard molar free energy of transfer of cosalane from water to isooctane (−10,700, −2000 and −850 cal/mol, respectively) were obtained from the literature (7,10). The group contribution of 3-chloro-salicylic acid (3Cl-SA) was obtained experimentally. In these experiments, the partition coefficient of 3Cl-SA was found to vary with concentration. Since carboxylic acids are known to self-associate in nonpolar solvents, the isooctane/water partition coefficients (K_{app}) were determined as a function of solute concentration at 25°C. The intrinsic partition coefficient at infinite dilution (0.04) was obtained by plotting K_{app} as a function of the aqueous concentration of the solute and extrapolating to zero. The log ($K^c_{iso/w}$) of cosalane in a isooctane/water system was estimated using the group contribution approach to be 11.7 (Table II, Scheme I). The solubility of cosalane in isooctane was determined experimentally to be $0.97 \times 10^{-2} \pm 6 \times 10^{-4}$ mg/mL. The aqueous solubility of cosalane was then calculated

Table II. Estimation of the Free Energy of Transfer from Water to Isooctane and Isooctane/Water Partition Coefficient for Cosalane Using the Group Contribution Method. The Group Contributions and Correction Factors Were Obtained from Davis *et al.* (10). Also see Scheme I

Constituent Group	ΔΔG (cal/mol)	Comments
3-chlorosalicylic acid (I & II)	+610 each	experimentally determined (see text for details)
(III)	−2470	4 $-CH_2$ groups[a] *corrections*[b] double bond: 1 branching: 1
17-androstyl (IV)	−10,700	literature value[c]
(V)	−9890	3 $-CH_3$ groups[a] 5 $-CH_2$ groups[a] *corrections*[b] branching: 2
	+1150 each	4 H atom correction[b]
Cosalane	−17240 $K^x = 4.4 \times 10^{12}$[d] $K_C = 4.9 \times 10^{11}$[d] log ($K^C_{iso/wat}$) = 11.7	

[a] $\Delta\Delta G^\circ$ ($-CH_3$) = −2000 cal/mol; $\Delta\Delta G^\circ$ ($-CH_2$) = −850 cal/mol.
[b] *Corrections*: H atom, $\Delta\Delta G_o$ = −1150 cal/mol; double bond, $\Delta\Delta G^\circ$ = 750 cal/mol; branching, $\Delta\Delta G^\circ$ = 180 cal/mol.
[c] Ref. (7).
[d] $\Delta\Delta G^\circ = -RT \ln K^x$. The conversion between mole fraction and molar concentration scale based partition coefficients, K_x and K_c, respectively, based on molar volume differences is shown by the following equation:

$$K^x = K^c \frac{\bar{V}_{isooctane}}{V_w}$$

to be 2×10^{-14} mg/mL, approximately 8 orders of magnitude below the value obtained by facilitated dissolution. Thus, the group contribution approach completely failed in estimating the aqueous solubility of cosalane.

In an attempt to rationalize this discrepancy, a molecular dynamics simulation was performed to determine the minimum free energy conformation of unionized cosalane in an aqueous environment. The results, shown in Fig. 1, indicate that in aqueous solution one of the substituted phenyl rings participates in a hydrophobic interaction with the steroid portion of the molecule, such that a much smaller and more compact molecule is presented to the aqueous environment. This intramolecular folding to minimize hydrophobic surface area, which leads to decreases in the effective hydrophobic group contributions, has been observed previously in studies of the solubility of n-alkanes (17). The discrepancy in the present study between the observed aqueous solubility and the group contribution estimated value corresponds to a molecule which would have ~14 fewer $-CH_2$ groups. It is not clear whether the intramolecular folding evident in the molecular dynamics simulation is sufficient to fully account for this sizeable reduction in overall hydrophobicity. Nevertheless, the effects of intramolecular folding, which are not generally taken into account in the group contribution method, may represent a serious potential error in using the group contribution approach for estimating the aqueous solubility of extremely hydrophobic molecules.

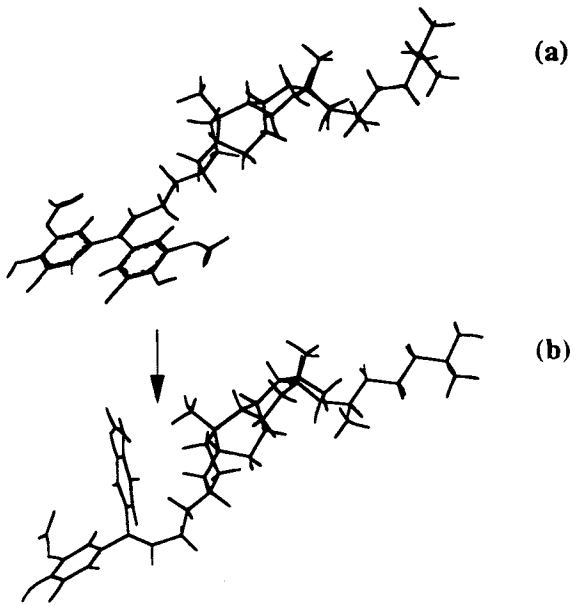
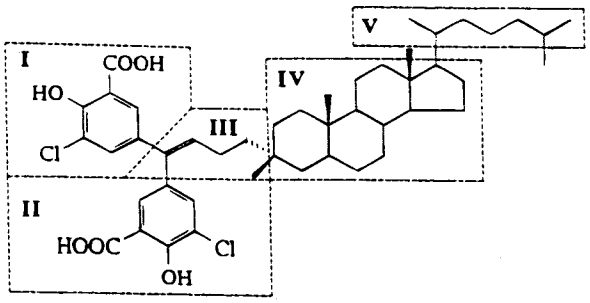


Fig. 1. Molecular dynamics simulation of minimum free energy conformation of unionized cosalane using a Silicon Graphics computer: gas state (a); aqueous environment (b). The arrow shows the tri-substituted phenyl ring folded inward in the aqueous environment.



Scheme I. Structure of cosalane showing the individual segments utilized in group contribution calculations (see Table II).

pH-Solubility Behavior of Cosalane

Cosalane, H_2A, is a diprotic acid which is capable of donating two protons. When the solid phase is the unionized drug, the solubility (S) is given by:

$$S = S_0 \left[1 + \frac{10^{(pH-pK_1)}}{\gamma_{HA^-}} + \frac{10^{(2pH-pK_1-pK_2)}}{\gamma_{A^{2-}}}\right] \quad (4)$$

where S_0 is the intrinsic solubility and the ionization constants are defined as:

$$K_1 = \frac{a_{H^+}\gamma_{HA^-}[HA^-]}{[H_2A]} \quad (5)$$

$$K_2 = \frac{a_{H^+}\gamma_{A^{2-}}[A^{2-}]}{\gamma_{HA^-}[HA^-]} \quad (6)$$

Table III. Solubility of Cosalane Determined by the Direct and Facilitated Dissolution Methods at 25°C

Direct Method					Facilitated Dissolution[a]					
					Ultrafiltration			Centrifugation		
pH	Eq. Time (Days)	Sol'ty (mg/ml)	S.D.[b]	n[c]	pH	Eq. Time (Days)	Sol'ty (mg/ml)	pH	Eq. Time (Days)	Sol'ty (mg/ml)
2.08	7–10	1.4×10^{-4}	8×10^{-5}	3	1.61	2	2.7×10^{-6}	1.60	2	1.4×10^{-6}
3.14	7–10	1.9×10^{-4}	8×10^{-5}	3	2.05	2.5	1.6×10^{-6}	2.37	2	1.4×10^{-6}
4.26	4	1.1×10^{-4}	1×10^{-5}	2	2.05	2.5	1.5×10^{-6}	2.99	10	2.0×10^{-6}
	8–10	2.7×10^{-4}	1.9×10^{-4}	3	2.99	10	1.2×10^{-6}			
4.93	7	1.8×10^{-4}	-	1	4.51	2	4.2×10^{-5}			
5.34	7–10	4.2×10^{-4}	2.7×10^{-4}	3	4.51	3	4.3×10^{-5}			
5.81	10	1.2×10^{-3}	-	1	5.33	2.5	3.2×10^{-4}			
6.85	3	0.75	-	1	5.33	2.5	2.1×10^{-4}			
	7–8	0.88	0.01	2	6.74	2.5	0.072			
7.19	8	0.52	-	1	6.91	2.5	0.119			
7.30	8	1.13	-	1						
7.73	10	58.6	-	1						

[a] Single determinations.
[b] S.D.: Standard deviation.
[c] n: Number of determinations.

From Eq. (4) it is clear that the solubility of cosalane increases with increasing pH, with a slope in the log S vs. pH line approaching 2 at pH values exceeding pK_2. Ionic activity coefficients were estimated from the Davies correction(18).

The solubility of cosalane was determined as a function of pH in the pH range of 1.5–8 (Table III and Figure 2). Under the pH conditions and over the time frame of these studies cosalane was shown to be completely stable, as evidenced by the absence of growth of extra peaks in the chromatograms with increasing time and more extensive studies of the stability of solutions. At pH 2–4, direct determination provided solubility values about 100-fold higher than those determined by the facilitated dissolution method, and, as shown in Table III, neither set of results appeared to exhibit significant changes with equilibration time. Non-linear regression analysis of the data generated by the direct method according to Eq. (4) yielded a satisfactory fit, but the values of pK_1 and pK_2 were unreasonable, with the value of pK_1 exceeding that of pK_2. This observation, combined with the concerns raised previously regarding the suitability of the direct method for poorly soluble compounds, led us to assume that the facilitated dissolution method provided a more accurate estimate of the true intrinsic solubility at low pH.

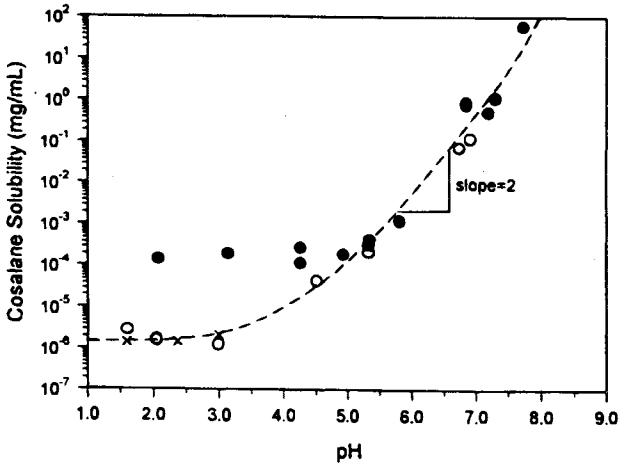


Fig. 2. pH-solubility profile of cosalane at 25°C. The data were fitted to Eq. (4) to obtain the dashed theoretical line shown. The slope approaches 2 at pH values exceeding the second pK_a. Key: (●), direct method; (○), facilitated dissolution method with ultrafiltration; (x) facilitated dissolution method with centrifugation.

At low pH, similar results were obtained using the facilitated dissolution method with either ultrafiltration or centrifugation to obtain a clear aqueous phase. At higher pH, the equilibrated samples containing soybean oil were emulsified (excess suspended solid was also present) and thus ultrafiltration was necessary to obtain a clear sample of the aqueous phase. Above pH 5, the results of the direct method and the facilitated dissolution method (with ultrafiltration) were the same.

As is evident in Eq. (4), the log S vs pH profile should have a slope of 2 at pH values exceeding pK_2. The pH-solubility data shown in Fig. 2 (lower curve) were fitted to Eq. (4) using a nonlinear least squares routine (SCIENTIST, Micromath, Salt Lake City, UT). The intrinsic solubility of cosalane was estimated to be 1.4 ± 0.5 ng/mL. The pK_1 and pK_2 values were estimated to be 3.3 ± 0.3 and 5.4 ± 0.3, respectively, and as predicted a slope of 2 is observed after the pH exceeds pK_2. Due to the extremely low intrinsic solubility of cosalane neither titrimetry nor UV spectrophotometry could be used to independently determine the dissociation constants.

It may be possible to estimate the ionization behavior of cosalane in aqueous conditions by examining the chemical structure of its acidic moieties, which closely resemble 3-chlorosalicylic acid. The dissociation behavior of 3-chlorosalicylic acid was determined independently by titration with dilute sodium hydroxide, from which a pK_a of 2.7 ± 0.1 was obtained.

The pK_1 value for cosalane determined from the solubility-pH profile is in reasonable agreement with the pK_a of 3-chlorosalicylic acid while pK_2 is much larger than would be expected from statistical effects alone. These discrepancies may reflect (a) the effects of conformational folding of cosalane which would tend to increase pKa values by providing a lower effective dielectric constant in the vicinity of the ionizable site; (b) electronic interactions between the two chlorosalicylic acid moieties; or (c) errors in the experimental measurements, particularly in the estimate of intrinsic solubility, which is particularly difficult to obtain for a compound having an apparent S_0 value of ≈ 1 ng/ml.

Dramatic Enhancement in Aqueous Solubility by pH Adjustment

The strategy of pH-adjustment is routinely employed to increase the solubility of ionizable drugs. In the case of cosalane, the influence of pH adjustment on solubility is amplified by the diprotic nature of this compound. From the pH-solubility profile it is clear that an aqueous solubility approaching 100 mg/mL for cosalane may be achieved by pH adjustment to a pH > 8 (Fig. 2), demonstrating a $>10^7$ fold increase in solubility of the compound by a pH adjustment of less than 4 units. This is attainable for cosalane because the slope of the log *S* vs. pH profile is 2 over a wide range of pH, resulting in a 100-fold increase in solubility per pH unit increase after the second pKa. Based on these results, an aqueous lyophilized parenteral formulation containing, after reconstitution, 20 mg/mL of cosalane at pH 8.5 was successfully prepared.

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