value for 8-chloro-6-phenyl-4H-*s*-triazolo[4,3-*a*][1,4] benzodiazepine (estazolam) was reported to be 2.84 from the UV absorption spectral change (23). Considering the structural difference mentioned, the estimated pKa value for triazolam, 1.52, is reasonable.

The bioavailability or the pharmacological effect of a drug would greatly depend on the formation rate in the cyclization reaction from the opened form to the closed form because only the cyclized 1,4-benzodiazepines possess pharmacological CNS activity (24), which are discussed in reports on diazepam (8) and desmethyldiazepam (12). The half-time of the forward reaction of I at pH 7.4, which was calculated to be 80.6 min (Fig. 5), indicates that much time is required to convert I into the closed form II, only if the *in vivo* reaction proceeds chemically.

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Extended Hildebrand Solubility Approach: Testosterone and Testosterone Propionate in Binary Solvents

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Abstract □ Solubilities of testosterone and testosterone propionate in binary solvents composed of the inert solvent, cyclohexane, combined with the active solvents, chloroform, octanol, ethyl oleate, and isopropyl myristate, were investigated with the extended Hildebrand solubility approach. Using multiple linear regression, it was possible to obtain fits of the experimental curves for testosterone and testosterone propionate in the various binary solvents and to express these in the form of regression equations. Certain parameters, mainly K and $\log \alpha_2$, were employed to define the regions of self-association, nonspecific solvation, specific solvation, and strong solvation or complexation.

Keyphrases □ Testosterone—extended Hildebrand solubility approach, solubility in binary solvents □ Solubility—extended Hildebrand solubility approach, testosterone and testosterone propionate in binary solvents □ Binary solvents—solubility of testosterone and testosterone propionate, extended Hildebrand solubility approach

Solute–solvent complexes of testosterone and testosterone propionate in binary solvents composed of cyclohexane with ethyl oleate, isopropyl myristate, and octanol have been reported previously (1). These solvents are pharmaceutically important; the first two are useful as solvents for steroid injectable preparations.

The calculated complexation constants (1) between the steroids and solvents were based on a previous method (2). The solute-mixed solvent systems are analyzed here with the extended Hildebrand solubility approach (3), an extension of the Hildebrand regular solution theory (4) which was introduced to allow the calculation of solubility of nonpolar and semipolar drugs in mixed solvents having a wide range of solubility parameters.

THEORETICAL

Solubility on the mole fraction scale, X_2, may be represented by the expression:

$$-\log X_2 = -\log X_2^i + \log \alpha_2 \qquad \text{(Eq. 1)}$$

where X_2^i is the ideal solubility of the crystalline solid, and α_2 is the solute activity coefficient in mole fraction terms. Scatchard (5) and Hildebrand and Scott (4) formulated the solubility equation for regular solutions in the form:

$$\log \frac{a_2^s}{X_2} = \log \alpha_2 = \frac{V_2 \phi_1^2}{2.303RT}(a_{11} + a_{22} - 2a_{12}) \qquad \text{(Eq. 2)}$$

where

$$\phi_1 = \frac{V_1(1 - X_2)}{V_1(1 - X_2) + V_2 X_2} \qquad \text{(Eq. 3)}$$

The activity of the crystalline solid (a_2^s), taken as a supercooled liquid, is equal to X_2^i as defined in Eq. 1. Variable V_2 is the molar volume of the

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hypothetical supercooled liquid solute (subscript 2), ϕ_1 is the volume fraction of the solvent (subscript 1), R is the molar gas constant, and T is the absolute temperature of the experiment.

The terms a_{11} and a_{22} are the cohesive energy densities of solvent and solute, and a_{12}, referred to in other reports (3, 6) and elsewhere in this report as W, is expressed in regular solution theory as a geometric mean of the solvent and solute cohesive energy densities:

$$a_{12} = W = (a_{11}a_{22})^{1/2} \quad \text{(Eq. 4)}$$

The square roots of the cohesive energy densities of solute and solvent, called solubility parameters and given the symbol δ, are obtained for the solvent from the energy or heat of vaporization per cubic centimeter:

$$\delta_i = (a_{ii})^{1/2} = \left(\frac{\Delta E^v}{V_i}\right)^{1/2} \cong \left(\frac{\Delta H^v - RT}{V_i}\right)^{1/2} \quad \text{(Eq. 5)}$$

When the solubility parameters and the geometric mean are introduced into Eq. 2, the expression becomes:

$$\log \alpha_2 = A(\delta_1^2 + \delta_2^2 - 2\delta_1\delta_2) = A(\delta_1 - \delta_2)^2 \quad \text{(Eq. 6)}$$

where

$$A = \frac{V_2\phi_1^2}{2.303RT} \quad \text{(Eq. 7)}$$

By substituting Eq. 6 into Eq. 1, one obtains:

$$-\log X_2 = -\log X_2^i + A(\delta_1 - \delta_2)^2 \quad \text{(Eq. 8)}$$

which is the Hildebrand–Scatchard solubility equation (4) for a crystalline solid compound of solubility parameter δ_2 dissolved in a solvent of solubility parameter δ_1. Equation 8 may be referred to as the regular solution equation; the term regular solution will be defined. The ideal solubility term is ordinarily expressed in terms of the heat of fusion of the crystalline solute at its melting point:

$$-\log X_2^i \cong \frac{\Delta H_m^f}{2.303RT}\frac{T_m - T}{T_m} \quad \text{(Eq. 9)}$$

although this is an approximation that disregards the molar heat capacity difference ΔC_p of the liquid and solid forms of the solute. An approximation involving the entropy of fusion, ΔS_m^f, was introduced (7) as:

$$-\log X_2^i \cong \frac{\Delta S_m^f}{R}\log\frac{T_m}{T} \quad \text{(Eq. 10)}$$

to partially correct for the failure to include ΔC_p in Eq. 9, and this form of log ideal solubility is employed in the current report. Equations 9 and 10 are approximations, and currently it has not been determined which is more appropriate for use in solubility analysis.

The Hildebrand–Scatchard equation (Eq. 8) may be used to estimate solubility only for relatively nonpolar drugs in nonpolar solvents which adhere to regular solution requirements. The molar volumes of the solute and solvent should be approximately the same, and the solution should not expand or contract when the components are mixed. Dipole–dipole and hydrogen bonding interactions are absent from regular solutions, with only physical forces being present. In such a system the mixing of solvent and solute results in a random arrangement of the molecules. The entropy in a regular solution is the same as that in an ideal solution, and therefore, the entropy of mixing is zero. Only the enthalpy of mixing has a finite value and it is always positive.

In most solutions encountered in pharmacy, interactions and selective ordering of molecules occur; these systems are referred to as irregular solutions. In pharmaceutical solutions, the geometric mean rule (Eq. 4) is too restrictive, and Eq. 6 or 8 ordinarily provides a poor fit to experimental data in irregular solutions. Instead, $\delta_1\delta_2$ is replaced in Eq. 6 by $W = a_{12}$, which is allowed to take on values as required to yield correct mole fraction solubilities:

$$-\log X_2 = -\log X_2^i + A(\delta_1^2 + \delta_2^2 - 2W) \quad \text{(Eq. 11)}$$

It is not possible at this time to determine W by recourse to fundamental physical chemical properties of solute and solvent. It has been found, however, for drugs in binary solvent mixtures (3, 6, 8) that W may be regressed in a power series on the solvent solubility parameter:

$$W_{calc} = C_0 + C_1\delta_1 + C_2\delta_1^2 + C_3\delta_1^3 + \ldots \quad \text{(Eq. 12)}$$

A reasonable estimate, W_{calc}, is obtained by this procedure, and when W_{calc} is substituted in Eq. 11 for W, mole fraction solubilities in polar binary solvents are obtained ordinarily within ≤20% of the experimental results. Log α_2/A may also be regressed directly on powers of δ_1, bypassing W and obviating the need for δ_2. The estimated solubility, X_2, with this method is identical to that obtained with W_{calc} except for rounding-off errors. The entire procedure, referred to as the extended Hildebrand solubility approach (3), may be conducted by using a polynomial regression program and carrying out the calculations on a computer. It is useful to include a statistical routine which provides R^2, Fisher's F ratio, and a scatter plot of the residuals. Terms of the polynomial (*i.e.*, powers of δ_1) are added sequentially and the values of R^2 and F, together with the appearance of the residual scatter plot, indicate when the proper degree of the polynomial has been reached. A well-known polynomial program using multiple regression analysis, SPSS (9), is convenient for this purpose.

Parameters for Solute–Solvent Interaction—The activity coefficient of the solute, α_2, may be partitioned into a term, α_V, for physical or van der Waals (dispersion and weak dipolar) forces and a second term, α_R, representing residual and presumably stronger solute–solvent interactions (Lewis acid-base type forces). In logarithmic form:

$$\log \alpha_2 = \log \alpha_V + \log \alpha_R \quad \text{(Eq. 13)}$$

According to this definition of log α_2, Eq. 11 may be written:

$$(\log \alpha_2)/A = (\delta_1 - \delta_2)^2 + 2(\delta_1\delta_2 - W) \quad \text{(Eq. 14)}$$

where

$$(\log \alpha_V)/A = (\delta_1 - \delta_2)^2 \quad \text{(Eq. 15)}$$

and

$$(\log \alpha_R)/A = 2(\delta_1\delta_2 - W) \quad \text{(Eq. 16)}$$

Hildebrand *et al.* (10) introduced a parameter, l_{12}, to account for deviations from the geometric mean. In terms of W, l_{12} may be written:

$$W = (1 - l_{12})\delta_1\delta_2 \quad \text{(Eq. 17)}$$

Therefore, the second right-hand term of Eq. 14, representing the residual activity coefficient, is:

$$(\log \alpha_R)/A = 2l_{12}\delta_1\delta_2 \quad \text{(Eq. 18)}$$

and the modified equation for solubility of a drug in binary polar solvents becomes:

$$-\log X_2 = -\log X_2^i + A(\delta_1 - \delta_2)^2 + 2A(l_{12})(\delta_1\delta_2) \quad \text{(Eq. 19)}$$

The variable W may be related to the geometric mean, $\delta_1\delta_2$, by the introduction of a proportionality constant, K (11), such that:

$$W = K(\delta_1\delta_2) \quad \text{(Eq. 20)}$$

From Eqs. 17 and 20:

$$(1 - l_{12}) = W/(\delta_1\delta_2) = K \quad \text{(Eq. 21)}$$

or

$$l_{12} = 1 - K \quad \text{(Eq. 22)}$$

The extended Hildebrand solubility expression (Eq. 11) may now be written:

$$-\log X_2 = -\log X_2^i + A(\delta_1 - \delta_2)^2 + 2A(1 - K)\delta_1\delta_2 \quad \text{(Eq. 23)}$$

By employing Eq. 20 to replace W of Eq. 11, another form of the extended Hildebrand equation is obtained:

$$-\log X_2 = -\log X_2^i + A(\delta_1^2 + \delta_2^2 - 2K\delta_1\delta_2) \quad \text{(Eq. 24)}$$

or, with Eq. 17:

$$-\log X_2 = -\log X_2^i + A[\delta_1^2 + \delta_2^2 - 2(1 - l_{12})\,\delta_1\delta_2] \quad \text{(Eq. 25)}$$

It was found (12) that a plot of l_{12} against a branching ratio, r, provided a good linear correlation for testosterone in a number of branched hydrocarbon solvents.

Variable K was employed (11) to describe the dissolving power of solvents for polyacrylonitrile, and it was concluded that the solvent action of organic solvents on the polymer solute was determined "by a very delicate balance between the various intermolecular forces involved." Solvent power could not be explained alone in terms of dipolar interaction and hydrogen bonding; it depended rather on whether dipolar and hydrogen bonding energies for the solvent–polymer contacts were a few percentage points less than, equal to, or greater than the sum of the sol-

vent–solvent and polymer–polymer interaction energies. The same conclusions can be reached for steroids in the various solvents in the present study and are elaborated.

The various extended solubility equations (Eqs. 11, 24, and 25) are equivalent, and the deviation of polar (or nonpolar) systems from regular solution behavior may be expressed in terms of $(\log \alpha_2)/A$, $(\log \alpha_R)/A$, W, l_{12}, or K. Any one of the parameters may be regressed on a polynomial in δ_1 to obtain values of solubility, X_2. These quantities may also be regressed against the volume fraction or percent of one of the solvents in the mixture or against the mean molar volume of the binary solvent mixture (3). Volume percents and mean molar volumes of chloroform in mixtures of cyclohexane and chloroform are given in Table I. The $X_{2(\text{calc})}$ values may be converted to molal solubility units and, if densities of the solutions are available, to molar or gram per milliliter concentration.

Solubility Parameters for Crystalline Solids—It is not possible to obtain solubility parameters of crystalline drugs by vaporization using Eq. 5, because many organic compounds decompose above their melting points. Instead, it has been shown (13) that the solubility parameter of solid drugs can be estimated from the point of maximum solubility in a binary solvent such as ethyl acetate and ethyl alcohol. The solubility parameter of the solute must lie between the δ values of the two solvents for this technique to be successful. In a regular solution, when:

$$\log X_2 = \log X_2^i \qquad \text{(Eq. 26)}$$

the system represents an ideal solution, and the maximum solubility is obtained, excluding specific solvation effects. When a pure solvent or solvent mixture is found that yields a peak in the solubility profile for a regular solution, δ_1 is assumed to equal δ_2, and the final term of Eq. 8 becomes zero, then Eq. 26 holds.

In an irregular solution, these relations do not hold exactly as in a regular solution. Equation 24 may be written as:

$$\frac{1}{A}(\log X_2^i - \log X_2) = \frac{\log \alpha_2}{A} = \delta_1^2 + \delta_2^2 - 2K\delta_1\delta_2 \qquad \text{(Eq. 27)}$$

The partial derivative of $(\log \alpha_2)/A$ then is taken with respect to δ_1 and the result set equal to zero to obtain the value of δ_2 at the peak in the solubility profile:

$$\left[\frac{\partial(\log \alpha_2/A)}{\partial(\delta_1)}\right]_{\delta_2} = 2\delta_1 - 2K\delta_2 = 0 \qquad \text{(Eq. 28)}$$

$$\delta_1 = K\delta_2 \qquad \text{(Eq. 29}a\text{)}$$

or, from Eq. 5 and the corresponding equation for the solute:

$$a_{11} = K^2 a_{22} \qquad \text{(Eq. 29}b\text{)}$$

Thus, $\delta_1 \neq \delta_2$ at the maximum in the solubility curve, but rather is equal to $K\delta_1$ (11). In irregular solutions, K is slightly greater than unity (~1.01) when solvation occurs between the solute and solvent; K is slightly less than unity (~0.98) when the species of the solution self-associate; and $K = 1.00$ when the solution is regular. As pointed out (11), a very small change in K can bring about large changes in solvent action; this phenomenon is considered in another report (8). Since K is nearly unity, even for highly solvated solutions, δ_2 is almost equal to δ_1 at the point of peak solubility in the system. This gives the researcher a good method for estimating solubility parameters of crystalline drugs. A differentiation method was introduced to obtain this value more precisely (12). Methods for calculating the solubility parameters of solid drugs, involving a regression of $(\log \alpha_2)/A$ on δ_1 in a second degree power series, have been introduced (14, 15). Satisfactory values of δ_2^2 and K are obtained[1] by use of the coefficients of the polynomial in moderately polar systems, but the technique is inadequate for highly irregular solutions. Another approach was introduced (16) to calculate δ_2 of solid compounds. Solubility parameters for solutes may also be obtained by a group contribution method (17).

EXPERIMENTAL

The solubility analyses of testosterone and testosterone propionate in solvent mixtures (*i.e.*, cyclohexane–chloroform, cyclohexane–octanol, cyclohexane–isopropyl myristate, and cyclohexane–ethyl oleate) were reported earlier (1), and the reported values were used in this study.

[1] The K value reported in Ref. 15 is constant over the range of solvent solubility parameters used. It differs from K introduced in the extended Hildebrand solubility approach which has a different value for each solvent used. The term in Ref. 15 should properly be differentiated from K by use of another symbol, such as κ, kappa.

Table I—Testosterone in Chloroform–Cyclohexane at 25° [a]

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Volume, % $CHCl_3$	δ_1	V_1	A	$\log \alpha_2$	$\frac{\log \alpha_2}{A}$	$\frac{\log \alpha_{2(\text{calc})}}{A}$ [b]	W	W_{calc} [c]	K	l_{12}	$\frac{\log \alpha_R}{A}$	X_2	$X_{2(\text{calc})}$	Percent Error
0	8.19	108.8	0.1863	2.458	13.194	12.714	86.347	86.586	0.9672	0.0328	5.849	0.000253	0.000311	−22.9
2	8.21	108.3	0.1862	2.232	11.987	11.916	87.113	87.149	0.9734	0.0266	4.572	0.000426	0.000439	−3.1
4	8.23	107.7	0.1860	2.070	11.129	11.138	87.706	87.703	0.9777	0.0223	4.002	0.000618	0.000616	0.3
6	8.25	107.1	0.1857	1.880	10.124	10.378	88.373	88.247	0.9827	0.0173	3.103	0.000957	0.000859	10.2
8	8.27	106.6	0.1853	1.712	9.239	9.638	88.982	88.783	0.9871	0.0129	2.323	0.00141	0.00119	15.6
10	8.29	106.0	0.1849	1.611	8.713	8.916	89.412	89.309	0.9895	0.0105	1.897	0.00178	0.00163	8.4
20	8.38	103.2	0.1807	1.049	5.805	5.884	91.614	91.575	1.0030	−0.0030	−0.545	0.00649	0.00628	3.2
30	8.48	100.4	0.1677	0.537	3.202	2.899	93.760	93.911	1.0143	−0.0143	−2.655	0.0211	0.0237	−12.3
40	8.57	97.6	0.1387	0.100	0.721	0.516	95.767	95.870	1.0252	−0.0252	−4.708	0.0577	0.0616	−6.8
50	8.67	94.8	0.1096	−0.147	−1.341	−1.842	97.662	97.910	1.0334	−0.0334	−6.319	0.102	0.116	−13.7
60	8.76	92.0	0.0788	−0.351	−4.454	−3.743	100.001	99.645	1.0473	−0.0473	−9.034	0.163	0.143	12.3
70	8.86	89.2	0.0705	−0.394	−5.589	−5.657	101.448	101.483	1.0505	−0.0505	−9.748	0.180	0.182	−1.1
80	8.95	86.4	0.0606	−0.448	−7.393	−7.242	103.157	103.077	1.0574	−0.0574	−11.204	0.204	0.200	2.0
90	9.05	83.6	0.0549	−0.475	−8.652	−8.898	104.685	104.805	1.0612	−0.0612	−12.080	0.217	0.224	−3.2
100	9.14	80.8	0.0487	−0.506	−10.390	−10.334	106.368	106.342	1.0677	−0.0677	−13.484	0.233	0.232	0.4

[a] Parameters: $\Delta H_m{}^f$ = 6190 cal/mole; T_m = 427.2°K, δ_2 = 10.9 $(\text{cal/cm}^3)^{1/2}$; V_2 = 254.5 cm^3/mole; $X_2{}^i$ = 0.07264; $-\log X_2{}^i$ = 1.1388. [b] Equation 33. [c] Equation 32.

RESULTS

Testosterone in Cyclohexane–Chloroform—The solubilities of testosterone at 25° in mixtures of cyclohexane and chloroform are found in Table I. The $\Delta H_m{}^f$ value for testosterone is 6190 cal/mole and T_m is 427.2°K. The $-\log X_2{}^i$ value is 1.1388 ($X_2{}^i$ = 0.07264), and δ_2 is 10.90 $(\text{cal/cm}^3)^{1/2}$. The solubility parameter for cyclohexane is 8.19 and for

chloroform is 9.14. The molar volume of testosterone is 254.5 cm^3/mole (12). The log activity coefficients are calculated using the expression:

$$\log \alpha_2 = \log X_2{}^i - \log X_2 \qquad \text{(Eq. 30)}$$

The values of W for the various mixtures are obtained directly from the solubility data, using a rearranged form of Eq. 11:

$$W = \tfrac{1}{2}\left[\delta_1{}^2 + \delta_2{}^2 - \frac{\log (X_2{}^i/X_2)}{A}\right]$$

$$= \tfrac{1}{2}\left[\delta_1{}^2 + \delta_2{}^2 - (\log \alpha_2)/A\right] \qquad \text{(Eq. 31)}$$

Also included in Table I are the calculated values of $(\log \alpha_2)/A$ and W obtained by regressing $(\log \alpha_2)/A$ and W on δ_1 in a third degree polynomial:

$$W = -3298.82 + 1084.56\delta_1 - 116.904\delta_1{}^2 + 4.26742\delta_1{}^3 \qquad \text{(Eq. 32)}$$

$$n = 15, R^2 = 0.999, F = 6702, F(3, 11, 0.01)^2 = 6.22$$

and:

$$\frac{\log \alpha_2}{A} = 6716.38 - 2169.12\delta_1 + 234.809\delta_1{}^2 - 8.53485\delta_1{}^3 \qquad \text{(Eq. 33)}$$

$$n = 15, R^2 = 0.998, F = 2352, F(3, 11, 0.01) = 6.22$$

The observed mole fraction solubilities, and the calculated values (obtained with Eqs. 30 and 33), together with percent differences between calculated and observed solubilities, are given in Table I. Variables K, l_{12}, and $(\log \alpha_R)/A$ were also regressed on δ_1 and the equations are:

$$K = -41.1320 + 13.7685\delta_1 - 1.50351\delta_1{}^2 + 0.0549512\delta_1{}^3 \qquad \text{(Eq. 34)}$$

$$n = 15, R^2 = 0.997, F = 1476, F(3, 11, 0.01) = 6.22$$

$$l_{12} = 42.1320 - 13.7685\delta_1 + 1.50351\delta_1{}^2 - 0.0549512\delta_1{}^3 \qquad \text{(Eq. 35)}$$

$$n = 15, R^2 = 0.997, F = 1476, F(3, 11, 0.01) = 6.22$$

and:

$$\frac{\log \alpha_R}{A} = 918.478\delta_1 - 300.154\delta_1{}^2 + 32.7765\delta_1{}^3 - 1.19794\delta_1{}^4 \qquad \text{(Eq. 36)}$$

$$n = 15, R^2 = 0.997, F = 1476, F(4, 10, 0.01) = 5.99$$

Since $K = 1 - l_{12}$ from Eq. 21 and $(\log \alpha_R)/A = 2l_{12}\delta_1\delta_2$ from Eq. 18, any one of the regression equations for K, l_{12}, and $(\log \alpha_R)/A$ can be obtained from the others. For example, replacing K in Eq. 34 by $(1 - l_{12})$ yields Eq. 35 for l_{12}. It is seen that the only differences are in the constant terms, −41.1320 in Eq. 34 and +42.1320 in Eq. 35, and the change in sign of each coefficient. Equation 36 for $(\log \alpha_R)/A$ is observed to take on an interesting form: no constant term exists and the polynomial is carried to the fourth rather than the third power.

Once the calculated value for one of these parameters is obtained from the regression equation, it may be substituted in the appropriate expression given earlier to obtain $X_{2(calc)}$. For example, $l_{12(calc)}$ for testosterone solubility in 50% chloroform–50% cyclohexane (v/v) ($\delta_1 = 8.67$) is obtained with Eq. 35:

$$l_{12(calc)} = 42.1320 - 13.7685(8.67) + 1.5035(8.67)^2$$

$$-0.0549512(8.67)^3 = -0.0362$$

Then, from the second right hand term of Eq. 25:

$$\frac{\log \alpha_2}{A} = \delta_1{}^2 + \delta_2{}^2 - 2(1 - l_{12})\delta_1\delta_2 = (8.67)^2 + (10.9)^2$$

$$- 2(1 + 0.0362)\,(8.67)\,(10.9) = -1.8691$$

[2] $F(3, 11, 0.01)$ is the tabulated F value with p degrees of freedom in the numerator and n-p-1 degrees of freedom in the denominator, where $p = 3$ is the number of independent variables and $n = 15$ is the total number of samples. The value 0.01 signifies that the F ratio is compared with the tabular value obtained at the 99% level of confidence.

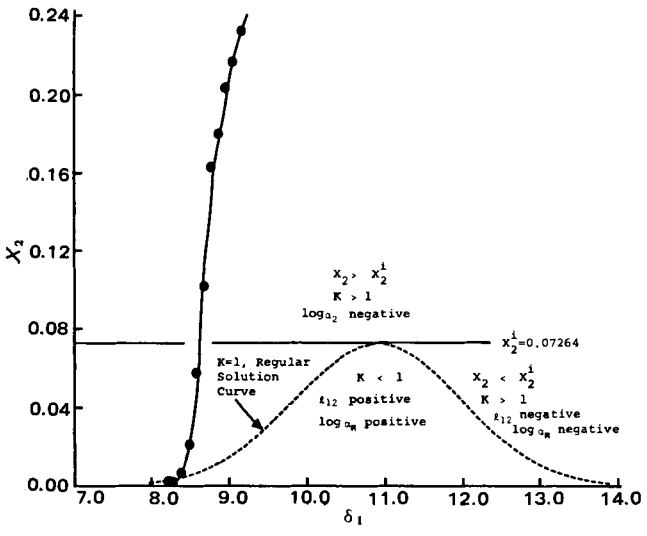

Figure 1—*Mole fraction solubility of testosterone ($\delta_2 = 10.9$) at 25° in cyclohexane and chloroform. Key: (●) experimental points; (—) solubility calculated by extended Hildebrand solubility approach; (- - - -) solubility curve calculated using regular solution theory.*

where the solubility parameter of testosterone is 10.9 $(cal/cm^3)^{1/2}$. Log $X_2{}^i$ is equal to −1.1388 for testosterone at 25°, and A from Table I is 0.1096 at 50% by volume chloroform. Continuing with Eq. 25, one obtains:

$$-\log X_2 = 1.1388 + (0.1096)(-1.8691) = 0.9340$$

$$X_{2(obs)} = 0.102$$

$$X_{2(calc)} = 0.116\ (-13.7\%\ \text{error})$$

Variables K, l_{12}, and $\log \alpha_R$ are three different means of expressing deviation from regular solution behavior. Log α_R (Column 12, Table I) is a measure of the residual activity coefficient due to dipolar interactions between solvent and solute, inductive effects, and hydrogen bonding. Variables K and l_{12} are also used to represent solution irregularities. When log α_R is negative, l_{12} (Column 11) becomes negative and K (Column 10) becomes greater than unity, indicating that X_2 is greater than the mole fraction solubility in a regular solution. As observed in Table I, this effect occurs at 20% chloroform in cyclohexane. Above this concentration of chloroform, it may be assumed that the predominant factor promoting the solubility of testosterone is solvation of the drug by chloroform, most probably in this case through hydrogen bonding. At 50% chloroform in cyclohexane, the interaction between testosterone and chloroform has increased sufficiently to elevate the drug solubility above the ideal mole fraction solubility, $X_2{}^i = 0.0726$. At this point the total logarithmic activity coefficient, log α_2, as well as log α_R, is negative, indicating the beginning of strong solvation. It is suggested that the term complexation is appropriate for interactions between solute and solvent when $X_2 \gg X_2{}^i$, observed in Table I for testosterone in pure chloroform.

The various parameters, and the manner in which they may be used to express self-association ($K < 1$), nonspecific solvent effects or regular solution ($K \cong 1$), weak solubilization ($K > 1$ and $X_2 < X_2{}^i$), and complexation or strong solubilization ($K > 1$ and $X_2 > X_2{}^i$), are depicted in Fig. 1 for testosterone in a mixture of chloroform and cyclohexane. As the real or irregular solubility line crosses the regular solution line at the lower left side of Fig. 1, K changes from <1.0 to >1.0. Then, as the irregular solution line crosses the ideal solubility line, K remains >1.0, X_2 becomes greater than $X_2{}^i$, and log α_2 becomes negative. At 100% chloroform, log $\alpha_2 = -0.506$, which means that the ratio of X_2 to $X_2{}^i$ is ~3:1. The curve for testosterone propionate in chloroform–cyclohexane (not shown) is similar to Fig. 1 for testosterone, demonstrating complexation between the steroid ester and chloroform >30% by volume chloroform in the chloroform–cyclohexane mixture.

Testosterone Propionate in Mixed Solvents—The solubilities of the steroidal ester, testosterone propionate, at 25° in octanol–cyclohexane, ethyl oleate–cyclohexane, and isopropyl myristate–cyclohexane are plotted in Figs. 2–4 as a function of the solubility parameter of the mixed solvent. The logarithmic ideal solubility of testosterone propionate, log $X_2{}^i$, is −0.81356 at 25°; $X_2{}^i = 0.15362$. The solubility parameter, δ_2, and

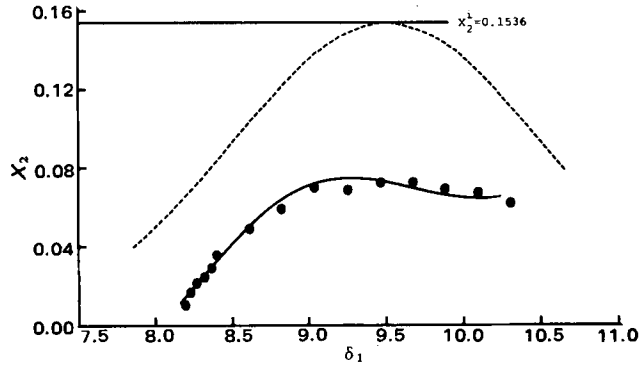

Figure 2—*Mole fraction solubility of testosterone propionate (δ_2 = 9.5) at 25° in cyclohexane and octanol. Key: (●) experimental points; (—) solubility calculated by extended Hildebrand solubility approach; (- - -) solubility curve calculated using regular solution theory.*

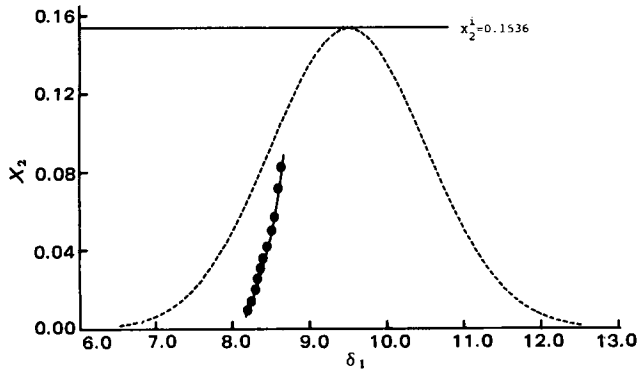

Figure 3—*Mole fraction solubility of testosterone propionate (δ_2 = 9.5) at 25° in cyclohexane and ethyl oleate. Key: (●) experimental points; (—) solubility calculated by extended Hildebrand solubility approach; (- - -) solubility curve calculated using regular solution theory.*

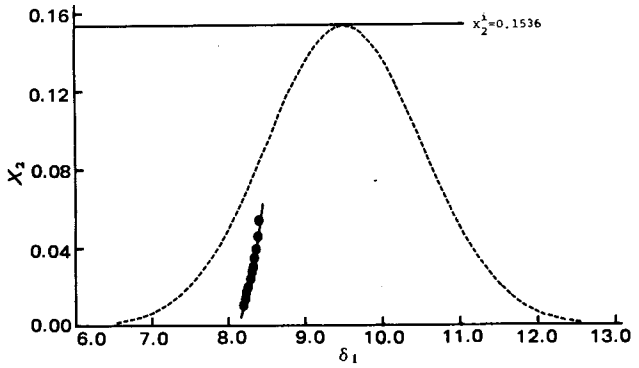

Figure 4—*Mole fraction solubility of testosterone propionate (δ_2 = 9.5) at 25° in cyclohexane and isopropyl myristate. Key: (●) experimental solubility; (—) solubility calculated by extended Hildebrand solubility approach; (- - -) solubility curve calculated using regular solution theory.*

the molar volume, V_2, of testosterone propionate are, respectively, 9.5 $(cal/cm^3)^{1/2}$ and 294.0 cm^3/mole. The solubility parameter is 8.19 for cyclohexane, 10.30 for octanol, 8.63 for ethyl oleate, and 8.85 for isopropyl myristate.

Use of the extended Hildebrand solubility approach to calculate solubilities yields good results for these systems as observed by the fit of the calculated line to the points in Figs. 2–4.

As seen by comparing the regular solution curve (calculated using Eq. 8) with the extended Hildebrand solubility line (calculated using Eq. 11, 24, or 25), the observed solubilities are smaller than those predicted for a regular solution over most of the range of δ_1 values of the mixed solvents, as contrasted to the chloroform–cyclohexane mixture. At no composition of mixed solvent do the solubilities exceed the ideal solubility, as observed earlier in chloroform–cyclohexane (Fig. 1). The regression equations used to calculate solubilities in these systems are:

Octanol–Cyclohexane Mixtures (Fig. 2):

$$\frac{\log \alpha_2}{A} = 1142.47 - 356.237\delta_1 + 37.0357\delta_1^2 - 1.28137\delta_1^3 \quad \text{(Eq. 37)}$$

$$n = 15, R^2 = 0.965, F = 101, F(3, 11, 0.01) = 6.22$$

Ethyl Oleate–Cyclohexane Mixtures (Fig. 3):

$$\frac{\log \alpha_2}{A} = 27888.99 - 9867.80\delta_1 + 1164.77\delta_1^2 - 45.8617\delta_1^3 \quad \text{(Eq. 38)}$$

$$n = 11, R^2 = 0.999, F = 2589, F(3, 7, 0.01) = 8.45$$

Isopropyl Myristate–Cyclohexane Mixtures (Fig. 4):

$$\frac{\log \alpha_2}{A} = 157348.62 - 56738.3\delta_1 + 6821.48\delta_1^2 - 273.439\delta_1^3 \quad \text{(Eq. 39)}$$

$$n = 11, R^2 = 0.999, F = 3970, F(3, 7, 0.01) = 8.45$$

Nonlinear Regression—The solubility of testosterone in octanol–cyclohexane and in ethyl oleate–cyclohexane are plotted in Figs. 5 and 6. The extended Hildebrand solubility approach with polynomial regression, used with success for the other systems, failed to provide a satisfactory fit of the data, as shown by the dotted lines in Figs. 5 and 6.

The polynomial regression method contains potential numerical difficulties which show themselves only in certain applications. The source of these difficulties may be seen by recognizing that to date the extended

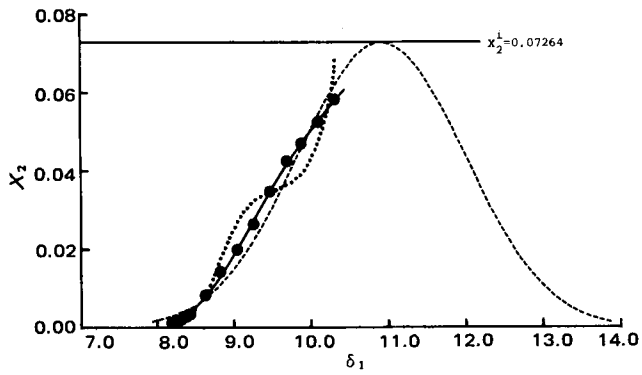

Figure 5—*Mole fraction solubility of testosterone (δ_2 = 10.9) at 25° in cyclohexane and octanol. Key: (●) experimental points; (—) extended Hildebrand solubility curve based on NONLIN polynomial regression; (. . . .) extended Hildebrand solubility curve based on ordinary polynomial regression; (- - - -) regular solution curve.*

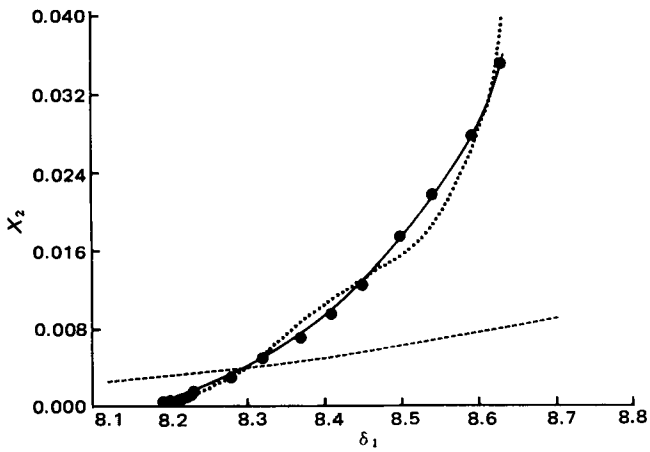

Figure 6—*Mole fraction solubility of testosterone (δ_2 = 10.9) at 25° in cyclohexane and ethyl oleate. Key: (●) experimental points; (—) extended Hildebrand solubility curve based on NONLIN polynomial regression; (·····) extended Hildebrand solubility curve based on ordinary polynomial regression; (- - -) regular solution curve.*

Hildebrand solubility approach has fitted observed values of X_2 to a model that defines X_2 as a function of δ and other variables and constants. That is, the relations expressed by Eq. 11 may be written as:

$$\log (X_2^i/X_2) - \frac{V_2}{2.303RT}\left[\frac{V_1(1-X_2)}{V_1(1-X_2)+V_2X_2}\right]^2 f(\delta) = 0 \quad \text{(Eq. 40)}$$

where $f(\delta)$ is a polynomial in δ. Thus, Eq. 40 defines the dependent variable X_2 as an implicit function of the independent variables δ and V_1. The terms X_2^i, R, T, and V_2 are known constants, and the parameters to be estimated are the coefficients of $f(\delta)$.

The polynomial regression method contains a circular element in that it uses the observed values of X_2 in W or $(\log \alpha_2)/A$ to estimate the coefficients of $f(\delta)$, and then uses these values of $f(\delta)$ to obtain calculated values of X_2. This circular process can be thought of as the first step in an iteration; the conditions necessary for this process to converge are not known. In many applications the iteration gives acceptable results; in some cases, as shown in Figs. 5 and 6, the results are poor.

Another potential source of difficulty is that the values of W or $(\log \alpha_2)/A$ are fit by least squares to the polynomials of δ. Thus, the coefficients are estimated by minimizing the squared deviations between observed and predicted (model) values of functions of X_2 [W or $(\log \alpha_2)/A$]. When X_2 then is calculated, this is equivalent to weighted least squares, with the weights being complicated functions of the constants in W or in $(\log \alpha_2)/A$.

To use Eq. 40 as a model for predicting X_2 as a function of δ and V_1, it must be determined that there is a unique value of X_2 that satisfies the equality. Writing the expression in Eq. 40 as $F(X_2)$, it can be verified that $\lim F(X_2)_{X_2 \to 0} = \infty$ and $F(X_2)_{X_2 \to 0} = \log X_2^i < 0$. Thus, $F(X_2)$ has a root between 0 and 1. It can also be shown that if $f(\delta) > 0$ then $F^1(X_2) < 0$ for $0 \le X_2 \le 1$. Thus, $F(X_2)$ is monotonic decreasing on [0,1] and has one, and only one, root.

Equation 40 can be used in any nonlinear regression program that accepts the model defined as an implicit function. In this application good inital estimates are important; they can be obtained as the coefficients of the polynomial in δ used in the polynomial regression method.

The regression equations for testosterone in octanol–cyclohexane and in ethyl oleate–cyclohexane were obtained by fitting Eq. 40 with the nonlinear regression program NONLIN (18). The results are:

Testosterone in Octanol–Cyclohexane (Fig. 5):

$$\frac{\log \alpha_2}{A} = 895.34 - 264.088\delta_1 + 26.1217\delta_1^2 - 0.865608\delta_1^3 \quad \text{(Eq. 41)}$$

and

Testosterone in Ethyl Oleate–Cyclohexane (Fig. 6):

$$\frac{\log \alpha_2}{A} = 50518.74 - 17713.3\delta_1 + 2071.82\delta_1^2 - 80.8331\delta_1^3 \quad \text{(Eq. 42)}$$

To solve Eq. 40 for X_2, the rootfinder subroutine ZBRENT (19) was called from DFUNC of NONLIN. Figures 5 and 6 show that fitting observed values of X_2 directly to values of X_2 predicted by Eqs. 41 and 42, respectively, greatly improves the fit. NONLIN was also used to fit the data shown in Figs. 1–4. In these applications the improvement in fit was so small as to be of little importance.

CONCLUSIONS

In another report (1), the interaction of testosterone and testosterone propionate was shown in mixed solvents of cyclohexane combined with ethyl oleate, isopropyl myristate, and octanol. The interaction was analyzed using association constants (2) with limited success. The present study shows that the interaction of testosterone and testosterone propionate with mixed solvents may be represented accurately by use of the extended Hildebrand solubility approach, a method that employs a polynomial on δ_1 rather than association constants.

The solubility is plotted in Figs. 1–6 in reference to the regular solution curve and the ideal solubility, X_2^i of the drug. This method of plotting the results delineates systems in which self-association or solvation predominates, and it differentiates these from regular and ideal solutions. It is suggested that although the drug and active (solvating) solvent (cyclohexane is considered to be the inactive solvent of the binary solvent mixture) interact to a lesser or greater extent, strong interaction or specific solvation, such as that resulting from hydrogen bonding effects, occurs when the solubility rises well above the ideal solubility line, X_2^i. Some self-association of solute or solvent may exist above X_2^i, leading to reduced solubility, but the overriding effect is strong solvation (complexation between solute and solvent).

A combination of X_2^i and K may be used to define various classes of interaction between solute and solvent. As observed in Fig. 1, when a solubility point falls on or near the regular solution line, it is defined as a regular solution (4). Referring to Eq. 24, when $K \cong 1$, the geometric mean obtains, and the solution may be considered to be a regular system. However, it is conceivable that W equals $\delta_1\delta_2$ (*i.e.*, $K \cong 1$) in polar systems by cancellation of solvating and self-associating effects, rather than because of the criteria layed down (4) for regular solution behavior.

When $K < 1$, the solubility points in a graph such as Fig. 2 fall below the regular solution line. The solute, solvent, or both are ordinarily considered to be self-associated when $K < 1$, resulting in decreased solubility.

When $K > 1$ and $X_2 > X_2^i$, association of a specific nature (*i.e.*, hydrogen bonding, dipolar interaction, or charge transfer complexation) is considered to exist between solute and solvent.

Finally, when $K > 1$ but $X_2 < X_2^i$, an intermediate situation exists. Some self-association may be present, but association results in solubilities above those found on the regular solution line. Various classes of solvation, self-association, and regular solution behavior have been defined (4), but the case where $X_2 < X_2^i$ and $K > 1$ was not addressed. This is neither a regular, self-associated, or strongly solvated solution, but rather an intermediate or weakly solvated system. The system treated in the present study are interesting because most of them are of the self-associated–weakly solvated class (*i.e.*, except for the chloroform systems, $X_2 < X_2^i$ and, depending on the composition of the solvent, K is greater or less than unity).

The solvents consisting of chloroform in cyclohexane exhibit weak to strong solvating effects on testosterone, depending on the concentration of chloroform in the solvent mixture (Fig. 1).

When the ester, testosterone propionate, is dissolved in the binary solvent, octanol in cyclohexane, a self-associating system results. As observed in Fig. 2, $K < 1$ across the range of solvent composition. This system exhibits a peak in the solubility because the solubility parameter of testosterone propionate, 9.5, lies between the solubility parameters of cyclohexane, $\delta = 8.2$, and octanol, $\delta = 10.3$. The methyl xanthines in dioxane–water mixtures have been reported (3, 6, 8) to show this kind of solubility profile. The calculated curve (solid line) of Fig. 2 should exhibit a parabolic shape somewhat like the dashed regular solution curve above it and should attain a maximum X_2 value at $\delta_1 = \sim 9.5$. The scatter of the experimental points caused the regression line (solid line) to take on an irregular shape and to rise slightly rather than fall on the right hand side of the figure. Testosterone propionate in ethyl oleate–cyclohexane (Fig. 3) yields solutions that appear to be self-associating rather than weakly solvating (*i.e.*, $K < 1$). Testosterone propionate in isopropyl myristate–cyclohexane (Fig. 4) follows a pattern similar to the drug in ethyl oleate–cyclohexane. The solubilities fall under the bell-shaped regular solution curve and may be classified as predominately self-associating ($K < 1$).

As observed in Fig. 5, testosterone in octanol–cyclohexane appears to follow regular solution behavior rather closely over the composition from 0 to 100% octanol. However solutions of a polar solvent, octanol, and a multifunctional solute, testosterone, do not meet the requirements of a regular solution (*e.g.*, molecules of approximately the same size, no change in entropy on mixing, no specific interaction of either species). The fact that such solutions follow the regular solution line are probably due to cancellation of self-association and solvation effects in these polar systems rather than an absence of specific interactions. Such solutions should not be called regular, although $K \cong 1$.

Testosterone in ethyl oleate–cyclohexane (Fig. 6) forms solutions that are characterized for the most part as weakly solvating (*i.e.*, $K > 1$ and $X_2 < X_2^i$).

The solubility parameters for testosterone and testosterone propionate have not been determined unequivocally. Testosterone is assigned a tentative value of 10.8 and testosterone propionate, 9.5. The latter value has more validity than the former at this time. If the solubility parameter of testosterone later is found to be essentially that of testosterone propionate, as some results appear to indicate, the position of the regular solution line will need to be moved relative to the solubility data points, and the above interpretations will change. For example, if it is found that δ for testosterone is 9.5 rather than 10.8, both testosterone and testosterone propionate will be observed to form weakly self-associated rather than weakly solvated solutions in ethyl oleate–cyclohexane.

However, the findings of chloroform as strongly solvating and the other solvents—octanol, ethyl oleate, and isopropyl myristate—as weakly solvating or self-associating is evident in these results, regardless of the exact solubility parameters of testosterone and testosterone propionate.

In earlier work (3, 4, 6, 8) the extended Hilidebrand solubility approach employed a polynomial regression routine for calculating quantities such as W and $(\log \alpha_2)/A$, and this statistical method has proved successful in most instances. It is demonstrated in the current study that direct polynomial regression sometimes may produce an unsatisfactory fit of solubility data. A nonlinear regression program, NONLIN (17), has been shown to improve the fit when ordinary polynomial regression fails.

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Antitumor Agents LVI: The Protein Synthesis Inhibition by Genkwadaphnin and Yuanhuacine of P-388 Lymphocytic Leukemia Cells

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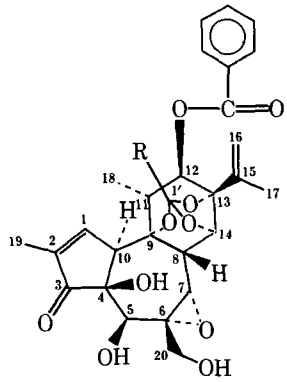
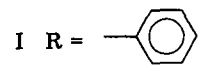
Abstract □ Two natural products isolated from the plant *Daphne genkwa* have been shown to possess antileukemic activity in mice. Genkwadaphnin and yuanhuacine were observed to inhibit DNA and protein synthesis in P-388 leukemic cells. A detailed study of the effects of these two diterpene esters on protein synthesis of leukemic cells was undertaken. The major effects of genkwadaphnin and yuanhuacine on protein synthesis were blockage of the elongation process and interference with the peptidyl transferase reaction. The latter reaction was suppressed at concentrations of the diterpene esters which were commensurate with concentrations that inhibited whole cell *in vitro* protein synthesis in P-388 cells.

Keyphrases □ Antitumor agents—inhibition of DNA and protein synthesis by genkwadaphnin and yuanhuacine in P-388 lymphocytic leukemia cells, daphnane deterpene esters □ Genkwadaphnin—antitumor agents, inhibition of DNA and protein synthesis in P-388 lymphocytic leukemia cells, daphnane diterpene esters □ Yuanhuacine—antitumor agents, inhibition of DNA and protein synthesis in P-388 lymphocytic leukemia cells, daphnane diterpene esters



I R = 

II R = —CH=CH—CH=CH—$(CH_2)_4$—CH_3

Daphnane diterpene esters which possess an isopropylene side chain at C_{13} have previously been reported to have antileukemic activity (1). Genkwadaphnin and yuanhuacine (I and II) are two such esters which have been isolated from *Daphne genkwa* and chemically characterized (2). Genkwadaphnin (I) at 0.8 mg/kg/day was shown to produce a T/C% value of 173, whereas yuanhuacine (II) afforded a value of 151% against P-388 lymphocytic leukemia growth (2). These T/C% values were comparable to 5-fluorouracil at 12.5 mg/kg/day in the P-388 screen. Therefore, it was concluded that daphnane diterpene esters may have potential as antineoplastic therapeutic agents and that their modes of action on cellular metabolism were of interest, particularly since these agents resemble, structurally, phorbol esters which are tumor pro-

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