These errors have been carried over into standard texts on chemical bonding (2, 4). It is, therefore, in order to call attention to this matter; the use of the non-orthogonal set of d^2sp^3 hybrids in molecular orbital and ligand field calculations will result in errors in off-diagonal terms of matrixes used to calculate values of physical variables. A correct set of equivalent, normalized orthogonal d^2sp^3 hybrids is:

$$\psi_1 = \frac{1}{\sqrt{6}}s + \frac{1}{\sqrt{2}}p_z + \frac{1}{\sqrt{3}}d_z \qquad \text{(Eq. 7)}$$

$$\psi_2 = \frac{1}{\sqrt{6}}s - \frac{1}{\sqrt{2}}p_z + \frac{1}{\sqrt{3}}d_z \qquad \text{(Eq. 8)}$$

$$\psi_3 = \frac{1}{\sqrt{6}}s - \frac{1}{\sqrt{12}}d_z + \frac{1}{2}d_x + \frac{1}{\sqrt{2}}p_x \qquad \text{(Eq. 9)}$$

$$\psi_4 = \frac{1}{\sqrt{6}}s - \frac{1}{\sqrt{12}}d_z + \frac{1}{2}d_x - \frac{1}{\sqrt{2}}p_x \qquad \text{(Eq. 10)}$$

$$\psi_5 = \frac{1}{\sqrt{6}}s - \frac{1}{\sqrt{12}}d_z - \frac{1}{2}d_x + \frac{1}{\sqrt{2}}p_y \qquad \text{(Eq. 11)}$$

$$\psi_6 = \frac{1}{\sqrt{6}}s - \frac{1}{\sqrt{12}}d_z - \frac{1}{2}d_x - \frac{1}{\sqrt{2}}p_y \qquad \text{(Eq. 12)}$$

(1) A. Pullman and B. Pullman, "Quantum Biochemistry," Wiley, New York, N. Y., 1963.

(2) L. Pauling, "The Chemical Bond," Cornell University Press, Ithaca, N. Y., 1967.

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(4) Y. K. Syrkin and M. E. Dyatkina, "Structure of Molecules and the Chemical Bond," Dover, New York, N. Y., 1964.

S. G. SCHULMAN

Department of Pharmaceutical Chemistry
College of Pharmacy
University of Florida
Gainesville, FL 32601

Hydrophilic–Lipophilic Balance and Distribution Coefficients of Nonionic Surfactants

Sir:

The hydrophilic–lipophilic balance (HLB) of nonionic surfactants has been correlated with many of their physical properties such as dielectric constants, heats of hydration, critical micelle concentrations, cloud points, phase inversion temperatures of emulsions, spreading coefficients of oil on water or of water on oil, and interfacial tensions at oil–water interfaces. In general, good correlations were found between the
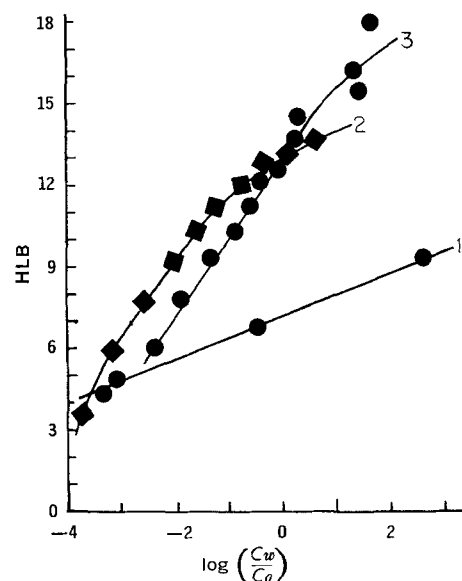

Figure 1—*Relationship between correct HLB values and the logarithms of the water–oil distribution coefficients. Key: Curve 1, sorbitan monoesters; Curve 2, homogeneous polyoxyethylated octylphenols; and Curve 3, normally distributed polyoxyethylated octylphenols.*

HLB and these properties within a given homologous series of surfactants. However, as soon as the chemical characteristics of the hydrophilic or lipophilic moieties were changed, e.g., by going from a polyoxyethylated surfactant to a sorbitan derivative or from a n-alkanol base to an alkylphenol base, different correlations were obtained. So far, the search for a universal correlation between the HLB and another property of the surfactant, which could be determined more readily than the HLB, has not been successful.

Intuitively, the distribution coefficient of the surfactants between water and oil, C_w/C_o, would seem to be well suited for correlation with the balance between their hydrophilic and lipophilic tendencies. Davies and Rideal (1) combined the kinetic treatment of emulsion coalescence with the free energy change of transferring a surfactant molecule from water to oil and arrived at the following correlation:

$$HLB = 7 + 0.829 \log (C_w/C_o) \qquad \text{(Eq. 1)}$$

Table I—Comparison between Experimental HLB Values and Values Derived from Distribution Coefficients

Surfactant	Experimental HLB[a]	$\left(\frac{C_w}{C_o}\right)^b$	HLB from Eq. 1
Sorbitan monooleate[c]	4.3	3.70×10^{-4}	4.2
Sorbitan monostearate[d]	4.7	6.19×10^{-4}	4.3
Sorbitan monopalmitate[e]	6.7	0.314	6.6
Polyoxyethylene sorbitol oleate[f]	9.2	367	9.1

Surfactant	HLB from Eq. 2a	$\left(\frac{C_w}{C_o}\right)^g$	HLB from Eq. 1
Octylphenol(EO)₁	3.5	1.84×10^{-4}	3.9
Octylphenol(EO)₃	7.8	3.13×10^{-3}	4.9
Octylphenol(EO)₆	11.2	5.92×10^{-2}	6.0
Octylphenol(EO)₁₀	13.6	3.85	7.5

[a] From *Reference 2.* [b] From *Reference 1.* [c] Span 80. [d] Span 60. [e] Span 40. [f] Atlox 1087. [g] From *Reference 3.*

The agreement between the experimental HLB values of a series of sorbitan derivatives and the values calculated by Eq. 1 was good (Table I).

It is shown in this communication that for a different category of nonionic surfactants, namely, polyoxyethylated octylphenols, $C_8H_{17}C_6H_4O(C_2H_4O)_pH$, Eq. 1 does not apply. These are homogeneous or single-species surfactants; each compound has a single value of p (3). Because experimental HLB determinations of these surfactants have not been made, their HLB values were calculated by

$$HLB = \frac{E}{5} \qquad (Eq.\ 2)$$

where E is the percentage of polyoxyethylene in the surfactant molecule. For polyoxyethylated octylphenols, Eq. 2 can be rewritten as

$$HLB = \frac{4405p}{5(206.32 + 44.05p)} \qquad (Eq.\ 2a)$$

where 206.32 is the molecular weight of octylphenol. Equation 2 was derived by Griffin from experimental measurements and is reported to be applicable to all polyoxyethylated nonionic surfactants (2); this is the basis of the HLB system for rating these surfactants. The HLB values calculated according to Eq. 2 are those listed in a recent compilation (2) and are considered to be correct.

These HLB values are compared in Table I with the HLB values calculated from the distribution coefficients (3) according to Eq. 1 for several surfactants of the homologous series. The correct HLB values (experimental or calculated by Eq. 2) for the entire series, as well as for the sorbitan-based surfactants, are plotted in Fig. 1 against the logarithms of the distribution coefficients. The curve for the polyoxyethylated octylphenols diverges considerably from the straight line of the sorbitan-based surfactants. A universal correlation between the distribution coefficients and the HLB, applicable to all nonionic surfactants, requires that the two curves coincide. An alternative, although less likely, explanation for the divergence is that Eq. 2 is not applicable to all nonionic surfactants; specifically, that it breaks down for homogeneous surfactants.

The distribution coefficients of normally distributed polyoxyethylated octylphenols (3, 4), where each surfactant has a range of p values, are also plotted against the HLB in Fig. 1. A broadened molecular weight distribution of the nonionic surfactants affects the relationship between HLB and distribution coefficient far less than a change in their chemical characteristics.

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(2) P. Becher, in "Nonionic Surfactants," M. J. Schick, Ed., Marcel Dekker, New York, N. Y., 1967, chap. 18.

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HANS SCHOTT

Temple University School of Pharmacy
Philadelphia, PA 19140

Isolation and Characterization of Cytotoxic Principles from *Hyptis verticillata* Jacq.

Sir:

A recent observation (1) that aqueous extracts of *Hyptis verticillata* Jacq. (Labiatae) inhibited development of sea urchin eggs led to a systematic study of extractable substances from this source. As a result of this study, two antimitotic agents, 4'-demethyldesoxypodophyllotoxin (I) and β-peltatin (II) were isolated and identified by comparison with authentic materials.

The ubiquity of lignans related to the 2,3-dimethylphenylnaphthalenes (2–10) is well documented, and the breadth of occurrence of cytotoxic substances related to podophyllotoxin is continually expanding (11–12). Isolation of these materials from *H. verticillata* represents, to our knowledge, the first report of their occurrence in the Labiatae family and attests to their widespread occurrence.

I   II
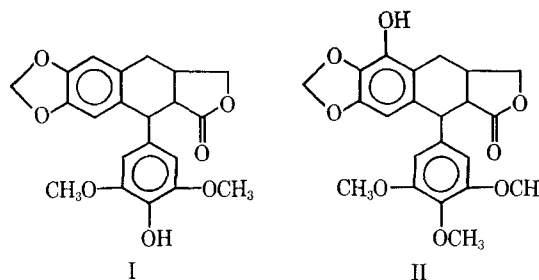
Experimental[1]—Melting points were determined on a Hoover-Thomas capillary melting-point apparatus. IR spectra were determined in chloroform solution on a Beckman IR-8. NMR spectra were determined on a Varian A-60 spectrometer. Mass spectra were determined on a Hitachi RMU-6H mass spectrometer. Antimitotic activity of the fractions and materials obtained as a consequence of purification was determined[2] using Earle's L929 mouse fibroblast cell line as the test system.

Antimitotic Assay—Earle's L929 mouse fibroblast cells (1.5 × 10⁵ cells/tube) were incubated 10 hr. at 36° in culture tubes, each tube containing 1 ml. of Eagle's minimum essential medium supplemented with 10% calf serum. To pairs of tubes containing the incubated cells was added 0.1 ml. of the test solution containing the suspected antimitotic agent. The cells were incubated an additional 18–24 hr. Generally, three to four dilutions varying by factors of 10 were