Quantitative Analysis of Partition Behavior of Substituted Phenols from Aqueous Phase into Liposomes Made of Lecithin and Various Lipids

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The partition coefficient of a series of substituted phenols in lecithin-liposome-water systems, P(L/W), was quantitatively analyzed by use of physicochemical molecular and substituent parameters. The effects on the P(L/W) value of the incorporation of cholesterol and charged lipids such as cardiolipin or stearylamine into the liposomal membranes were examined with the perturbation of the bilayered membrane structure induced by these lipids being taken into account. The partition behavior of phenols was primarily decided by the hydrophobicity, represented in terms of the 1-octanol-water partition coefficient, P(O/W). The partitioned phenols seemed to be in membrane close to the interface. The steric and electronic effects of substituents on the partitioning into liposomes varied significantly depending upon the type of lipids incorporated. The variations in these effects reflect differences in the structure of the interfacial region of the lecithin bilayer modified by the incorporation of various lipids.

The partition behavior of a variety of medicinal and agricultural chemicals has been studied extensively with the use of artificial lipid membranes as models of biomembranes.[1-5] In most of these studies, the effects of lipid composition,[1,2] temperature,[3,4] or the pH of the bulk aqueous phase[5] on the partition coefficient have been examined for a limited series of compounds. Only a few investigations have dealt with effects of physicochemical characteristics on the partition behavior of a series of systematically selected chemicals.[6,7]

We have quantitatively analyzed various physicochemical factors that decide the partition coefficient, P(L/W), of a series of substituted phenols in the nonionized form in a lecithin-liposome-water system.[8] The partition was governed most strongly by molecular hydrophobicity represented in terms of the partition coefficient in the 1-octanol-water system, P(O/W), as a reference. It was also affected by the acidity of phenols: The higher the electron-withdrawing ability of the substituent, the easier was the partitioning into the liposomal membrane. The basicity of the hydrogen-bond acceptor in the liposomal membrane seemed to be greater than that of the oxygen of 1-octanol used as the reference phase for the partitioning. The steric bulkiness of substituents was more unfavorable to partitioning into the liposomes than into 1-octanol. The 2,6-disubstitution showed the most unfavorable steric effect. The steric bulkiness of substituents at the meta or para position was less unfavorable. That of monoortho substitution was negligible. From these results, we assumed that the partitioned phenol molecules would be oriented with the benzene ring in the apolar interior and the phenolic OH group in the polar interfacial region of the membrane.

In this study, we measured the P(L/W) values for a number of substituted phenols including newly synthesized compounds to continue examinations of the partition behavior of phenols in artificial membranes. The substitution pattern was varied so that the steric effect of substituents specific for their positions could be analyzed in more detail than in our previous study. The effects of the incorporation of various kinds of lipids, such as cardiolipin (negatively charged), stearylamine (positively charged) and cholesterol, into bilayered lecithin liposomes on the partition behavior of substituted phenols were analyzed by comparison of quantitative structure-partitioning relationships derived with these partitioning systems.

Here, we report details of these analyses and discuss differences in physicochemical factors that govern the partitioning behavior of substituted phenols in various kinds of membranes.

Experimental

Materials. Lecithin was prepared from fresh egg yolk and purified by the method of Singleton et al.[9] Most of the phenols were the same as those studied before.[8,10] Additional phenols were purchased from Nakarai Chemicals, Ltd., except for 2-alkyl-4,6-dinitrophenols, which were synthesized by the nitration of 2-alkylphenols.[11] The structures of newly synthesized phenols were confirmed by elementary analysis and spectra. Phenols were purified by either distillation or silica-gel column chromatography before use. Reagent-grade stearylamine, cardiolipin, *N*-dansylhexadecylamine (DSHA), and anthroylstearic acid (AS) were obtained from the Sigma Chemical Co. Cholesterol was of reagent grade and was further purified by recrystallization from ethanol.

Preparation of Liposome. Lecithin bilayered liposome was prepared by a procedure slightly different from that reported previously.[8] Dry lecithin (100 mg) was suspended in 20 ml of an aspartate buffer (0.04 M† sodium aspartate, 0.25 M Na_2SO_4, and 0.2 mM EDTA), adjusted to pH 6.0 by the addition of NaOH, and sonicated in an ice-cooled bath under Ar gas. Bilayered liposome was separated from the undispersed lecithin by centrifugation and from multilayered liposome by gel filtration on Sepharose 4B at 4 °C. Cholesterol-lecithin liposome (1 : 10, m/m), cardiolipin-lecithin liposome (1 : 5, m/m), and stearylamine-lecithin

† 1 M=1 mol dm^{-3}.

liposome (1 : 5, m/m) were prepared from the corresponding dry lipid mixture in a similar way. The amount of lecithin in the liposome suspension was estimated from that of phosphorus by a modification of the Bartlett method.[12]

Measurement of Partition Coefficient. The partition coefficient, P(L/W), of each substituted phenol between the liposomal membrane and the external aqueous phase was measured by equilibrium dialysis at pH 6.0.[8] A solution of phenols, the concentration range of which is from 10^{-5} to 10^{-4} M, was prepared in the same pH 6.0 aspartate buffer as for the preparation of liposome. At this pH, all of these compounds except for 2,4,6-trichlorophenol and 2,4-dinitro derivatives exist almost completely as the nonionized form. In an equilibrium dialysis cell (ten 1-ml chambers, Sanko Plastic Co.), the liposome suspension (1 ml) was equilibrated with the phenol solution (1 ml) through a cellophane dialysis membrane at 25 °C for 12 h. The partition coefficient, P(L/W), was calculated by Eq. 1,

$$P(\mathrm{L/W}) = \frac{2(C_0 - C)}{C \times C_L} \quad \left[\frac{\text{mol phenols/kg lipids}}{\text{mol phenols/l buffer}}\right] \qquad (1)$$

where C is the concentration at equilibrium of the phenol in the liposome-free chamber, C_0 is that of the phenol in a control experiment without liposome, and C_L is the concentration of lecithin (kg l^{-1}) in the liposome suspension. The total amount of lecithin in 1 ml of the liposome suspension was about 1 mg. The concentration of the phenol was measured spectrophotometrically on a Shimadzu UV-360 spectrophotometer. For the 2,4-dinitro derivatives, the P(L/W) value was measured at pH 3.0. Since the dinitro derivatives exist as an equilibrated mixture of ionized and nonionized species even at this pH (3—8% ionized), this gave the apparent value. Using lecithin-liposome, we examined the effect of variations in the pH of the bulk aqueous phase on the apparent P(L/W) value for 2,4,6-trichloro- and 2,4-dinitrophenols.

Measurement of Polarity of Liposomal Membrane. Variations in the polarity of interfacial and interior regions of the liposome membrane were estimated from the change in λ_{max} of the emission spectrum of DSHA and AS, respectively, incorporated in liposomes.[13] DSHA or AS was mixed with lecithin at the molar ratio of 1 : 50 in chloroform. After removal of the chloroform, liposomes containing the fluorescent probe were prepared in a similar way. All fluorescent measurements were made on a Shimadzu RF-503A spectrophotometer equipped with a thermoregulated cell compartment at 25 °C. The emission λ_{max} of DSHA- and AS-liposome was observed at around 520 and 450 nm, respectively, with excitation at 330 nm and with the slit width of 5 nm for both excitation and emission sides. As an index of polarity, the emission λ_{max} value of DSHA and AS in various organic solvents was also measured.

Results

pH Dependence of Log P(L/W). For 2,4,6-trichloro- and 2,4-dinitrophenols, the effect of variations in pH on the apparent log P(L/W) was examined (Fig. 1). These phenols were partitioned into the liposome membrane even from the bulk aqueous phase, where they are invariably in the ionized form.[8]

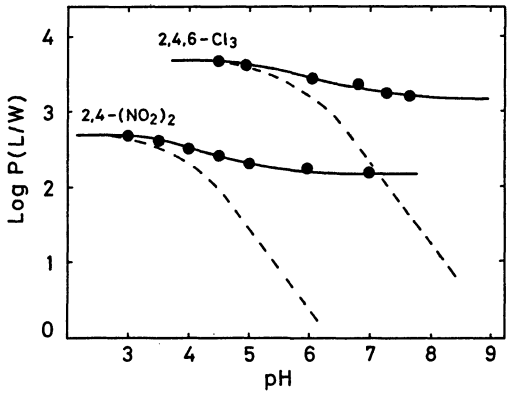

Fig. 1. Dependence on pH of the apparent log P(L/W) value for 2,4,6-trichloro- and 2,4-dinitrophenols. The dotted line is for the dependence of the log P(L/W) value according to the pH-partition model.[14]

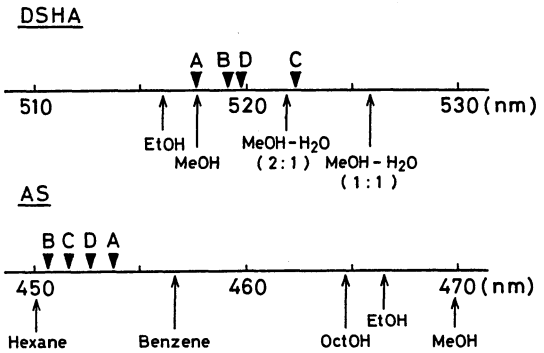

Fig. 2. Positions of the fluorescence emission maximum of DSHA and AS in lecithin liposome (A), cholesterol-lecithin liposome (B), cardiolipin-lecithin liposome (C), and stearyamine-lecithin liposome (D), together with those of various solvents.

Judging from the differences in the apparent log P(L/W) value in regions of lower to higher pH, the partition of the phenolate anions was lower than that of the neutral phenols, although the difference was not large. The partition behavior of 2,4,6-trichloro- and 2,4-dinitrophenols did not obey the pH-partition model,[14] in which the partition of ionized species from aqueous to organic phases such as 1-octanol can be neglected.

Polarity of Liposome Membrane. The emission λ_{max} values of DSHA and AS incorporated in various kinds of liposomes are shown in Fig. 2 together with those measured in various organic solvents. The fluorescent moieties of DSHA and AS have been said to be in the aqueous interface and the hydrophobic fattyacid regions of artificial membranes, respectively.[13] The λ_{max} of DSHA in the genuine lecithin bilayer was 518 nm, which corresponds to that in methanol. That of AS was between those in hexane and benzene. The λ_{max} of DSHA increased after the incorporation of cholesterol, stearylamine, or cardiolipin toward the λ_{max} in

Table 1. Partition Coefficient of Substituted Phenols and Physicochemical Parameters

Substituent	log P (O/W)[a]	Δlog K_A[a]	ΔV_w (m, p)[b]	ΔV_w (di-ortho)[b]	log P(L/W)		log P(L/W)[c]		log P(L/W)$^-$		log P(L/W)$^+$	
			$10\,cm^3\,mol^{-1}$	$10\,cm^3\,mol^{-1}$	Obsd	Calcd[c]	Obsd	Calcd[d]	Obsd	Calcd[e]	Obsd	Calcd[f]
H	1.46	0	0	0	1.97	2.04	1.93	1.95	2.10	2.06	1.96	1.96
2-Me	1.97	−0.30	0	0	2.45	2.42	2.27	2.31	2.48	2.41	2.38	2.34
3-Me	2.02	−0.10	1.12	0	2.34	2.43	2.21	2.36	2.32	2.45	2.25	2.38
4-Me	1.94	−0.16	1.12	0	2.42	2.36	2.33	2.30	2.43	2.39	2.40	2.32
2-Et	2.47[g]	−0.22[h]	0	0	2.81	2.82	2.69	2.69	2.69	2.77	2.72	2.72
4-Et	2.58	−0.23	2.14	0	2.88	2.82	2.78	2.77	2.73	2.85	2.83	2.80
2-*n*-Pr	2.93[g]	−0.36[h]	0	0	3.13	3.19	3.03	3.04	3.04	3.10	3.06	3.07
4-*n*-Pr	3.06	−0.23	3.07	0	3.09	3.17	3.18	3.13	3.09	3.19	3.13	3.17
2-*s*-Bu	3.27	−0.36	0	0	3.47	3.45	3.34	3.28	3.28	3.33	3.35	3.32
4-*s*-Bu	3.27[i]	−0.25[i]	4.09	0	3.43	3.29	3.45	3.29	3.32	3.34	3.43	3.32
2-*t*-Bu	3.31	−1.18	0	0	3.51	3.42	3.46	3.26	3.47	3.32	3.53	3.33
3-*t*-Bu	3.31	−0.14	4.09	0	3.25	3.33	3.18	3.33	3.27	3.37	3.26	3.36
4-*t*-Bu	3.31	−0.25	4.09	0	3.43	3.32	3.31	3.32	3.40	3.37	3.34	3.35
2-Ph	3.09	0.01	0	0	3.40	3.33	3.37	3.17	3.40	3.22	3.40	3.20
4-Ph	3.20	0.43	4.33	0	3.24	3.28	3.25	3.28	3.43	3.32	3.40	3.29
4-*t*-Pent	3.87	−0.25	5.20	0	3.64	3.72	3.55	3.74	3.66	3.76	3.57	3.78
2,6-$(Me)_2$	2.30	−0.64	0	2.24	2.47	2.39	2.37	2.26	2.39	2.45	2.38	2.45
2,6-$(Et)_2$	3.03	−0.59	0	4.28	2.73	2.73	2.60	2.57	3.02	2.82	2.91	2.89
2-Cl	2.15	1.50	0	0	2.73	2.70	2.43	2.56	2.51	2.63	2.47	2.53
3-Cl	2.50	0.96	0.95	0	2.78	2.90	2.81	2.79	2.80	2.85	2.75	2.78
4-Cl	2.39	0.60	0.95	0	2.89	2.79	2.73	2.68	2.88	2.75	2.76	2.68
3-Me-4-Cl	3.10	0.43	2.07	0	3.29	3.29	3.31	3.21	3.21	3.25	3.19	3.22
2,4-Cl_2	3.06	2.09	0.95	0	3.54	3.43	3.22	3.28	3.45	3.31	3.21	3.24
2,6-Cl_2	2.75	3.19	0	1.90	2.84	3.08	2.78	2.89	2.80	3.00	2.75	2.93
2,4,6-Cl_3	3.69	3.99	0.95	1.90	3.61	3.85	3.50	3.64	3.70	3.72	3.67	3.66
4-SO_2Me	0.58	2.15	3.80	0	1.27	1.35	1.30	1.43	1.41	1.54	1.32	1.36
4-CN	1.60[g]	2.03[k]	1.20	0	2.11	2.26	2.01	2.18	2.35	2.26	2.08	2.13
3-CF_3	2.95[g]	1.03[l]	1.90	0	3.25	3.22	3.05	3.13	3.17	3.18	3.00	3.12
3-NO_2	2.00	1.58	1.43	0	2.56	2.53	2.49	2.45	2.61	2.53	2.41	2.42
2,4-$(NO_2)_2$	1.54	5.89	1.43	0	2.70	2.50	2.63	2.39	2.60	2.42	2.24	2.21
2-Me-4,6-$(NO_2)_2$	2.13[m]	5.54[m]	1.43	2.60	2.66	2.63	2.60	2.49	2.59	2.63	2.67	2.49
2-Et-4,6-$(NO_2)_2$	2.67[m]	5.55[m]	1.43	3.50	3.02	2.95	2.90	2.78	2.91	2.95	2.85	2.85
2-*i*-Pr-4,6-$(NO_2)_2$	3.10[m]	5.51[m]	1.43	4.50	3.14	3.17	3.09	3.20	3.27	3.18	3.36	3.40
2-*s*-Bu-4,6-$(NO_2)_2$	3.56	5.47	1.43	5.52	3.50	3.41	2.98	2.98	3.34	3.43	3.15	3.12

a) Unless otherwise noted, from Ref. 8. b) From A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964). c) By Eq. 4. d) By Eq. 5. e) By Eq. 6. f) By Eq. 7. g) From log P data bank compiled by Pomona College Medicinal Project, Claremont, California, U. S. A., 1983. h) From J. Epstein, *J. Am. Chem. Soc.*, **86**, 3075 (1964). i) Taken as the same as that of 2-*s*-butylphenol. j) Taken as the same as that of 4-*t*-butylphenol. k) From A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London (1962), p. 130. l) Estimated according to a procedure published by T. Fujita, *Prog. Phys. Org. Chem.*, **12**, 1 (1976). m) Newly measured.

aqueous alcohol, indicating that the polarity of the membrane surface region increased. The incorporation of cardiolipin had the strongest effect. The λ_{max} of AS decreased after the incorporation of one of these lipids, suggesting that the polarity was lowered in the membrane interior.

Analysis of Log *P*(L/W). The log P(L/W) values in four different liposome-water systems are listed in Table 1, where P(L/W), P(L/W)^C, P(L/W)$^+$, and P(L/W)$^-$ represent the partition coefficients measured with liposomes of genuine lecithin, cholesterol-lecithin, stearylamine-lecithin, and cardiolipin-lecithin, respectively. As shown in Fig. 1, the log P(L/W) of 2,4-dinitrophenol ionized by 7.5% at pH 3.0 did not seem to differ much from that of the nonionized form. Along with previous data on 2-*s*-butyl-4,6-dinitrophenol,[8] the log P(L/W) values measured at pH 3.0 for 2,4-dinitrophenol and its 6-substituted derivatives were used without further correction for the following analyses, as approximate values for their nonionized form.

We previously measured the log P(L/W) values of nonionized phenols in the lecithin-liposome-water system using Tris-HCl buffer (10 mM Tris, 50 mM NaCl, 0.2 mM EDTA).[8] The previous relationship between log P(L/W) and log P(O/W) was analyzed by regression analysis with the steric and electronic effects of substituents taken into account, as shown in Eq. 2:

$$\log P(\mathrm{L/W}) = \underset{(0.070)}{0.870} \log P(\mathrm{O/W}) + \underset{(0.029)}{0.064}\,\Delta \log K_A$$
$$- \underset{(0.042)}{0.111}\,\Delta V\mathrm{w(di\text{-}ortho)} - \underset{(0.033)}{0.074}\,\Delta V\mathrm{w(m,p)} + \underset{(0.182)}{0.629} \quad (2)$$
$$(n=26,\ s=0.117,\ r=0.986)$$

In this and the following equations, n is the number of phenols, s is the standard deviation, and r is the correlation coefficient. The figures in parentheses are the 95% confidence interval. As a steric parameter, the relative van der Waals volume of substituents, ΔVw, was used. The ΔVw is the Vw value of substituents relative to that of H and multiplied by 0.1 to make the magnitude similar to other parameters according to Eq. 3.[15]

$$\Delta V\mathrm{w} = [V\mathrm{w(X)} - V\mathrm{w(H)}] \times 0.1 \quad (3)$$

ΔVw(di-ortho) is the sum of the parameters of both ortho substituents for di-ortho substituted phenols. ΔVw(m,p) is such a sum for meta and para substituents. The $\Delta \log K_A$ value [$\log K_A$(substituted phenols)$-\log K_A$(unsubstituted phenol)] is used as the electron-withdrawing index of substituents. Here, the possible relationship between the newly measured $\log P$(L/W) and $\log P$(O/W) values was also analyzed in a similar way. Equations 4—7 gave the best correlation for the various liposome-water systems.

$$\log P(\mathrm{L/W}) = \underset{(0.064)}{0.820} \log P(\mathrm{O/W}) + \underset{(0.025)}{0.079}\,\Delta \log K_A$$
$$- \underset{(0.037)}{0.124}\,\Delta V\mathrm{w(di\text{-}ortho)} - \underset{(0.029)}{0.050}\,\Delta V\mathrm{w(m,p)} + \underset{(0.168)}{0.821} \quad (4)$$
$$(n=34,\ s=0.114,\ r=0.981)$$

$$\log P(\mathrm{L/W})^{c} = \underset{(0.063)}{0.750} \log P(\mathrm{O/W}) + \underset{(0.026)}{0.064}\,\Delta \log K_A$$
$$- \underset{(0.037)}{0.122}\,\Delta V\mathrm{w(di\text{-}ortho)} + \underset{(0.174)}{0.854} \quad (5)$$
$$(n=34,\ s=0.119,\ r=0.976)$$

$$\log P(\mathrm{L/W})^{-} = \underset{(0.059)}{0.714} \log P(\mathrm{O/W}) + \underset{(0.024)}{0.053}\,\Delta \log K_A$$
$$- \underset{(0.034)}{0.076}\,\Delta V\mathrm{w(di\text{-}ortho)} + \underset{(0.162)}{1.015} \quad (6)$$
$$(n=34,\ s=0.111,\ r=0.978)$$

$$\log P(\mathrm{L/W})^{+} = \underset{(0.052)}{0.756} \log P(\mathrm{O/W}) + \underset{(0.021)}{0.031}\,\Delta \log K_A$$
$$- \underset{(0.030)}{0.057}\,\Delta V\mathrm{w(di\text{-}ortho)} + \underset{(0.144)}{0.859} \quad (7)$$
$$(n=34,\ s=0.099,\ r=0.985)$$

Each of the terms is significant at a level higher than 99.5% except for the $\Delta \log K_A$ in Eq. 7, which is significant at the 99% level. The addition of the ΔVw(m,p) term to Eqs. 5—7 was not justified over the 95% level. In each correlation, the regression coefficients of the $\Delta \log K_A$ and ΔVw terms were much lower than that for the $\log P$(O/W) value. This does not mean that the steric and electronic effects of substituents were not important. In general, the variations in $\log P$(O/W) values were much lower than those in $\Delta \log K_A$ and ΔVw values throughout the series of compounds. The lower regression coefficients for the $\Delta \log K_A$ and ΔVw terms were attributable to a range of variations much higher than that for the $\log P$(O/W) term.

Discussion

Yeagle et al.[16] showed that the choline moiety of the lecithin head groups is oriented almost parallel to the surface plane of bilayered liposomes and that the positively charged trimethylammonio groups are associated electrostatically with the negatively charged phosphates of neighboring lipids. Here, we examined the polarity of the interfacial region of various kinds of liposomes in terms of the shift in the emission maximum of DSHA. The polarity increased with the incorporation of negatively as well as positively charged lipid, suggesting that the inter-head group interactions of the kind studied by Yeagle et al. were weakened and that the interfacial structure was loosened so that the binding of water molecules with the charged head groups on the membrane surface was enhanced. The effects of the cardiolipin molecule, with a large polar head group, were particularly strong. This molecule could perturb the membrane surface structure markedly, increasing the surface polarity very much.

According to Sunamoto et al.[17] the addition of cholesterol molecules to the lecithin bilayer allows closer molecular packing and reduces the mobility of the fatty acid chain of the lecithin above the phase transition temperature of lipids (that of the egg yolk lecithin is approx. -7 to -15 °C[18]). The emission maximum of AS was shifted toward a shorter wavelength. The polarity of the interior region of the liposome was decreased by the incorporation of cholesterol, consistent with the highly packed structure (Fig. 2). However, the addition of cholesterol caused an increase in the polarity around the fluorescent moiety of DSHA. This suggests that the inter-head group interactions were also weak in the cholesterol-lecithin liposome. Our result is consistent with the ^{31}P NMR studies[19] demonstrating that the inter-polar head interactions of phospholipid bilayered membranes are slightly weakened by the addition of cholesterol molecules.

Phenolate anions were partitioned into the liposome membrane, although the extent of partitioning is lower than that of neutral phenols.[8] Generally, the partitioning of charged species into the hydrophobic interior of a lipid membrane is energetically unfavorable.[3] Thus, the phenol molecules, regardless of their dissociation conditions, would be in a region close to the surface of the membrane holding the polar OH group toward the external aqueous phase. Colly and Metcalfe[20] studied the partition behavior of benzyl alcohol in liposomes using NMR spectroscopy and showed that the hydroxyl group projects toward the

aqueous phase. Their observation would be consistent with the model stated above for the orientation of phenols.

The coefficient of the log P(O/W) term in Eqs. 4—7 was from 0.71 to 0.82, which suggests that the partitioning of phenols occurs mainly into a phase less hydrophobic than 1-octanol.[3] Although the variations in the slope were not great, they seemed to correspond to those in the polarity of the membrane surface region; the slope decreased as the polarity increased. This would also suggest that the partitioned phenol molecules are mainly in the region of the membrane surface rather than the more hydrophobic interior.

The significance of the $\Delta \log K_A$ term would be that the basicity of the hydrogen-bond acceptor in the membrane surface region is higher than that of the oxygen of 1-octanol. We have suggested that the phenolic OH group interacts with the phosphate group of the neighboring lecithin.[8] This suggestion may need revision because this type of the interaction seems to be energetically unfavorable from the ^{31}P NMR studies of the lecithin-cholesterol interaction by Yeagle et al.[16] They have shown that the hydrogen bonding of the OH group of the incorporated cholesterol with the carbonyl group of the ester moiety of the lecithin molecule is energetically more favorable than that with the phosphate. If the hydrogen bonding occurs with the phosphate group, a part of nonpolar steroid ring should be exposed in the polar aqueous phase that is energetically less favorable. Thus, the interaction of the OH group of phenols with the carbonyl moiety of lecithin may also be more favorable although the acidity of the OH group in phenols is higher than that in cholesterol (Fig. 3-A). Incorporation of stearylamine (a positively charged lipid) to lecithin liposome reduced the coefficient of $\Delta \log K_A$ term, as shown by Eq. 7, suggesting a reduction in the hydrogen-bonding effect. It is probable that the positively charged amino groups compete with the phenols for the above kind of hydrogen-bonding interaction with hydrogen acceptors.

As shown in Eqs. 2 and 4, the bulkiness of meta and para substituents but not of the mono-ortho substituent was unfavorable to partition into the liposome. We have suggested[8] that the substituted phenols are incorporated from the side of the benzene ring, with the polar OH group projecting toward the external aqueous phase. In this situation, meta and para substituents would have an unfavorable effect on packing in the lecithin molecules, the effect depending upon their volume (Fig. 3-B). For ortho substituted derivatives, both the OH group and the single ortho substituent would be close to the outside of the membrane, with little steric effect on the packing structure of the membrane.[8] Such positional specificity in the steric effect was, however, not observed in Eqs. 5—7. Steric demands for the meta and para substituents were apparently made less strict in the partitioning into the mixed lipid liposomes. These findings are in accord with the partitioning model where the partitioned phenol molecules are close to the surface region of the membrane. With incorporation of lipids other than lecithin, the structure of the membrane surface was loosened even in regions corresponding to the meta and para positions of phenols.

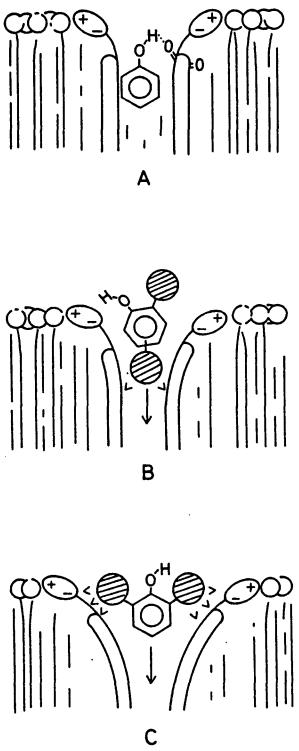

Fig. 3. Partition model of substituted phenols in liposome membrane. 3-A) The OH group of unsubstituted phenol interacts with the carbonyl moiety of lecithin. 3-B) Insertion of meta and para substituents into packed fatty acid chains is unfavorable, whereas that of the ortho substitution into phenols shows negligible effect. 3-C) Di-ortho substituents act as a wedge against the parallel arrangement of lecithin molecules and hinder the hydrogen bond of the OH group.

The steric bulkiness of di-ortho substituents lowered the partitioning into liposome in each of the liposome-water systems. Di-ortho substituents probably act as a wedge against the parallel arrangement of lecithin molecules and hinder the interaction of OH groups with hydrogen-acceptor groups (Fig. 3-C).[8] This kind of steric hindrance is somewhat reduced by the addition of charged lipids to liposome (Eqs. 6 and 7). The conformation of the head groups was more perturbed by the charged lipids more than by cholesterol, as observed by fluorescent probe studies, which is consistent with the above difference in the steric effect of di-ortho substituents.

Equation 4 for log P(L/W) measured in the genuine lecithin liposome was essentially equivalent to Eq. 2 published previously[8] except for the intercept. The higher intercept value for Eq. 4 is probably due to the

Table 2. Log P(L/W) of 2,6-Dialkyl-4-(2,2-dicyanovinyl)phenols

Alkyl	log P(O/W)[a]	Δlog K_A[b]	ΔV_w(m, p)[c]	ΔV_w(di-ortho)[c]	log P(L/W)	
			10 cm^3 mol^{-1}	10 cm^3 mol^{-1}	Obsd	Calcd[d]
H	2.23	2.94	4.04	0	2.80	2.68
Me	3.08	3.07	4.04	2.24	3.20	3.11
Et	4.12	3.00	4.04	4.28	3.61	3.70

a) Newly measured. b) From Ref. 10. c) From A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964). d) By Eq. 4.

higher ionic strength in the aqueous phase used here. The higher the ionic strength, the higher would be the partitioning into the lipid phase. Although the ionic strength would affect the membrane structure, the perturbation may be not so significant since the other terms, which decide the partitioning behavior of phenols, in two equations are almost equal.

To confirm the validity of Eq. 4 for the partition coefficient in the lecithin-liposome-water system, we measured the log P(L/W) value for structurally complex 2,6-dialkyl-4-(2,2-dicyanovinyl)phenols, and compared the observed values with the ones calculated by Eq. 4 (Table 2). The fact that observed values for the three dicyanovinyl phenols agreed with those calculated by Eq. 4 indicates the applicability of the equation to a wide range of phenols to predict their partition coefficient in the lecithin-liposome-water system.

The accumulation of this kind of knowledge on partition behavior of series of systematically selected compounds into various artificial membrane systems from aqueous media should provide reliable means for explanation of their mode of interactions with various biomembranes.

The calculations were done with a FACOM M382 computer at the Data Processing Center of this university. We thank Dr. Takaaki Nishioka, Institute for Chemical Research, Kyoto University for invaluable discussion.

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