Spectroscopic Studies of Surfactant Solubility. III. Side-chain Effects of Phosphatidyl Compounds in Chloroform Solutions

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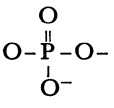
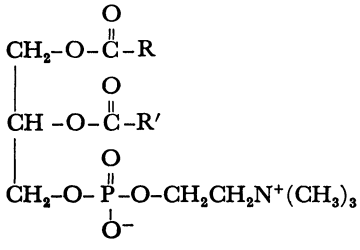
The infrared spectra of phosphatidyl and related compounds were studied with respect to their side-chain effects on solubility. It was clarified that the existence of a hydrogen-bonding between chloroform and a phosphate group is essential for these phosphatidyl compounds to form stable solutions in chloroform. The existence of double alkyl-chains and acyloxyl groups is clarified to be very useful for those compounds to have a strong solvent-phobic character without losing their solubility in solvents. The role of a choline moiety was also clarified. The details of the analysis of the infrared intensity were also described. It was emphasized that the intensity enhancement due to hydrogen-bond formation can be quantitatively related to the enthalpy change due to hydrogen-bond formation.

In our previous publications,[1-3] the infrared spectra of some water-soluble surfactants have been reported. On the basis of the quantitative analysis of the absorption intensity, it has been shown that molecules that dissolve in chloroform form a complex with several solvent-chloroform molecules. The bonding between a surfactant and the solvent chloroform has been shown to be of the hydrogen-bond type.

The present study concerns itself with a further study of the solubilities, in chloroform, of the molecules which have a phosphate group:



by the use of the infrared spectra and solubility data. Our special interest lies in the solubility of the phosphatidyl compounds, which have this structure:



with respect to:

1) the role of a phosphate group (a polar group),
2) the role of a choline moiety (an end group),
3) the role of an acyloxyl group, and
4) the role of double alkyl chains,

to the solubilities in chloroform. We focus our attention on the infrared-absorption band of chloroform-*d*, which is related to the hydrogen bonding between chloroform-*d* and a phosphate group. If we can get quantitative knowledge about the hydrogen bonding between chloroform-*d* and a phosphate group, we can relieve the effects of the other moieties on the solubilities.

Experimental

Meterials. The chloroform-*d* was purchased from Merck and Co., Ltd., and was dried over zeorite A-3. The chloroform was purchased from Wako Pure Chemical Industries, Ltd., and was dried over zeorite A-3 after eliminating the ethanol by column chromatography on alumina. The samples used in this study were both synthesized and natural products. The purities of these samples were checked by means of thin-layer chromatography. The samples are (with the abbreviations in parentheses): (**1**) propylphosphorylcholine (C_3PC), (**2**) butylphosphorylcholine (C_4PC), (**3**) pentylphosphorylcholine (C_5PC), (**4**) heptylphosphorylcholine (C_7PC), (**5**) decylphosphorylcholine (C_{10}PC), (**6**) tetradecylphosphorylcholine (C_{14}PC), (**7**) octadecylphosphorylcholine (C_{18}PC), (**8**) *rac*-1,2-dipalmitoylglycerol-3-phosphorylcholine (DL-di-$C_{16:0}$PC), (**9**) palmitoylglycerolphosphorylcholine ($C_{16:0}$-g-PC), (**10**) 1,2-dioctadecanoylglycerol-3-phosphorylcholine (L-di-$C_{8:0}$PC), (**11**) *rac*-1,2-dilauroylglycerol-3-phosphorylcholine (DL-di-$C_{12:0}$PC), (**12**) 1,2-dilinoleoylglycerol-3-phosphorylcholine (L-di-$C_{18:2}$PC), (**13**) 1,3-dipalmitoylglycerol-2-phosphorylcholine (di-$C_{16:0}$-β-PC), (**14**) *rac*-1-palmitoylglycerol-3-phosphorylcholine (DL-$C_{16:0}$-lyso-PC), (**15**) spingomyelin from beef brain (beef brain $C_{21:0,3}$, or spm.), (**16**) phosphatidylcholine from soy beans (soybean PC, or L-di-$C_{17.5:1.4}$PC), (**17**) *rac*-1,2-dipalmitoylglycerol-3-phosphoryl-*N*,*N*-dimethylethanolamine (DL-di-$C_{16:0}$diMePE), (**18**) *rac*-1,2-dipalmitoylglycerol-3-phosphoryl-*N*-methylethanolamine (DL-di-$C_{16:0}$MePE), (**19**) phosphatidylethanolamine from pig brain (pig PE, or L-di-$C_{19.5:2.1}$PE), and (**20**) phosphatidylserine from pig brain (pig PS, or L-di-$C_{18.9:1.6}$PS).

Absorption Measurements. The absorption spectra were recorded with a JASCO IR-G grating spectrometer at a resolution of 1 cm^{-1}. The spectra of chloroform-*d* solutions containing the acceptor in the concentration range from 0 to 0.5 M were measured with a KBr cell having a thickness of about 0.1 mm. The thickness of the sample cell was checked by the interference-fringe method.

Sample solutions containing various amounts of the solute molecule were prepared in a dry-box just before measurements by weighing the solute and chloroform-*d* in the sample flask. Since all of the solutes are very hygroscopic, they were dried over P_2O_5 under a reduced pressure at 60 °C for more than two days. The elimination of water from the sample solution was confirmed by observing the infrared spectra in the region of 2000—4000 cm^{-1}.

All the measurements were done at 20±2 °C, and the absorption spectra of the C-D stretcing vibration for the frequency region of 2000—2500 cm^{-1} were measured with a resolution of 1 cm^{-1} and a scanning speed of 33.3 cm^{-1}/min. The absorption due to chloroform-*d* was eliminated, when

necessary, by the use of a variable-pathlength cell on the reference side. The thickness of the variable cell was adjusted so that the absorption band of chloroform-*d* at 2254 cm^{-1} disappeared completely.

Measurement of Physical Constants. The refractive index and the specific gravity of the solution were measured, with an Abbe refractometer and a picnometer respectively, at the same time as the absorption measurements.

The solubility was determined by a weight method. A sample was weighed in an NMR tube with a radius of 5 mm after having been dried over P_2O_5 under reduced pressure for 2 d. After dried chloroform-*d* has been added and the tube had beed sealed, the solubility was determined by weighing the sealed tube.

Results and Discussion

Infrared Spectra of Chloroform Solution. All the molecules studied in the present work have a phosphate group and show high solubilities in chloroform. As these substances are hardly soluble at all in nonpolar solvents and only slightly soluble in non-hydrogen-bonding polar solvents, the solubilities in chloroform suggest the existence of strong hydrogen-bonding between the solute and solvent molecules. Actually, the infrared spectra of chloroform-*d* show remarkable changes in the fundamental bands of chloroform-*d*. The ν_1 vibration (the C-D stretching) changes its frequency about -20 to -50 cm^{-1} from that of pure liquid chloroform-*d*, that is, 2254 cm^{-1}. We will hereafter designate the ν_1 band occurring at 2254 cm^{-1} as an F-band.

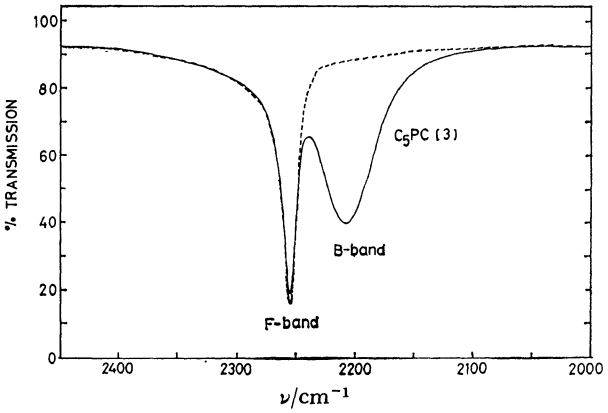


Fig. 1. Infrared spectra of C-D stretching vibration of chloroform-*d*: - - -pure liquid chloroform-*d*, ——C_5PC solution.

Figure 1 shows a typical example of the C-D stretching vibration spectra of the chloroform-*d* solution. The band at 2207 cm^{-1} is related with the chloroform-*d* molecule, which forms a hydrogen bonding with a phosphate group. We will hereafter designate this band as a B-band. For the molecules which have hydrogen-bonding sites other than a phosphate group, on the other hand, additional bands whose frequencies are higher than that of the B-band are observed. Figure 2 shows the spectra corresponding to those molecules which have an acyloxyl group in addition to a phosphate group. The reference spectra for a chloroform-*d*–methyl palmitate system is also shown in Fig. 2 in order to clarify the location of the absorption band due to the hydrogen-bond formation between chloroform and an acyloxyl group. Figure 3 shows the spectra corresponding to a group of molecules which have an acyloxyl group and a double bond in addition to a phosphate group. The spectra for a chloroform-*d* solution of methyl oleate and methyl linoleate are also shown as reference spectra in order to show the location of the additional bands which are related with the hydrogen bonding between chloroform and an acyloxyl group or between chloroform and a double bond. Figure 4 shows the spectra corresponding to a group of molecules which have a hydroxyl group in addition to phosphate and acyloxyl groups and a double bond. The spectrum for a chloroform-*d* solution of 1-pentanol is also included

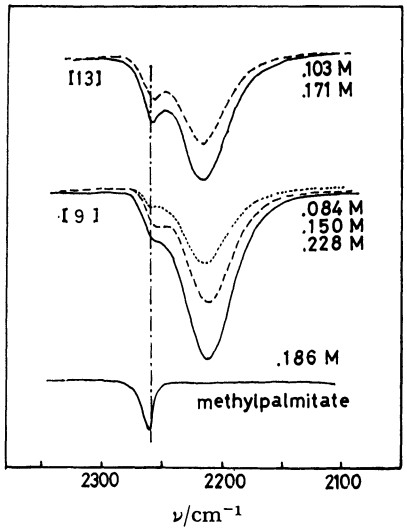


Fig. 2. Infrared spectra of C-D stretching vibration of chlrorform-*d* (solutions of these molecules having an acyloxyl group, (**9**) and (**13**)).

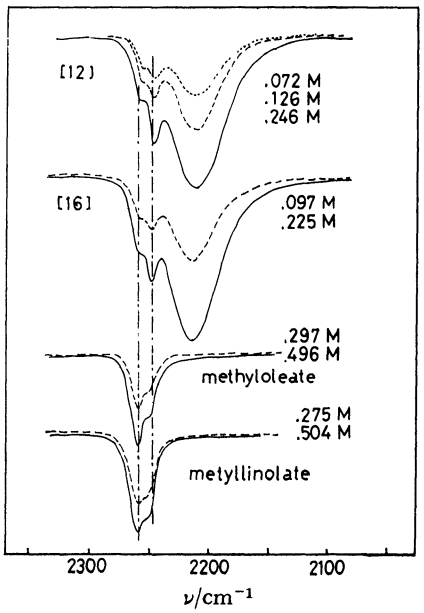


Fig. 3. Infrared spectra of C-D stretching vibration of chloroform-*d* (solutions of these molecules having a double bond and an acyloxyl group, (**12**) and (**16**)).

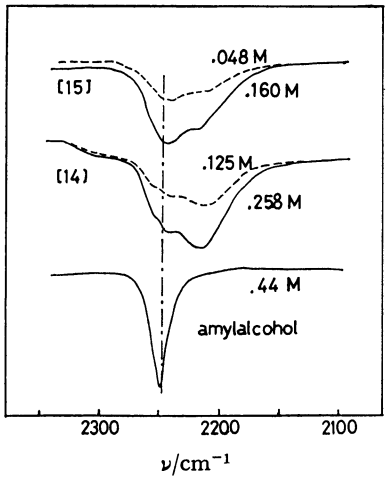


Fig. 4. Infrared spectra of C-D stretching vibration of chloroform-*d* (solutions of these molecules having a double bond, an acyloxyl group, and a hydroxyl group).

Table 1. Spectroscopic information on B-bands andsolubilities

Substance	$\nu^\circ-\nu$ / cm^{-1}	$\Delta\nu_{1/2}$ / cm^{-1}	Solubility(20 °C) / mol l^{-1}
(1) C_3PC	47—47.5	45	0.06*[a]
(2) C_4PC	47	45	0.15*[a]
(3) C_5PC	47	45	2.0
(4) C_7PC	46.5	45	1.6
(5) C_{10}PC	46	45	1.5
(6) C_{14}PC	46	44.5	1.2
(7) C_{18}PC	45.5	44—45	0.9
(8) DL-Di-$C_{16:0}$PC	39.5	42	0.7
(9) $C_{16:0}$-g-PC	43	42.5	1.0
(10) L-Di-$C_{8:0}$PC	42	45	1.0
(11) DL-Di-$C_{12:0}$PC	41.5	41.5	0.9
(12) L-Di-$C_{18:2}$PC	42.5	45	0.8
(13) Di-$C_{16:0}$-β-PC	39.5	42	0.6
(14) DL-$C_{16:0}$lyso-PC	39	48	0.4
(15) Beef brain $C_{21:0,3}$(spm)	37—40	≈45	0.15
(16) L-Di-$C_{17.5\cdot11.4}$PC	42.5	41	>0.6
(17) DL-Di-$C_{16:0}$diMePE	30	42.5	0.21
(18) DL-Di-$C_{16:0}$MePE	23	40	0.20
(19) Pig PE	≈20	30	>0.16
(20) Pig PS	≈10	Not observed	>0.16

a) * Phase separation (see the text).

in Fig. 4 as a reference. Attention must be paid for the fact that, in the spectra of Figs. 2—4, the F-bands are completely cancelled out when pure liquid chloroform-*d* is put in the reference side of the spectrometer.

The half-band width, $\Delta\nu_{1/2}$, and the frequency shift, $\nu^\circ-\nu$, for a B-band are listed in Table 1, where ν° is the maximum absorption frequency of the F-band. For the sake of simplicity, we do not describe in detail the spectral information for any of these hydrogen-bonding bands other than the B-bands.

Determination of Absolute Intensities. In order to determine the absolute intensities, the absorption spectra for F- and B-bands of chloroform-*d* solutions containing various amounts of the solute molecule were measured in each system. We will here begin by considering the spectra which are composed of one F-band and one B-band only, that is, the spectra for those molecules which have only one phosphate group.

The relative intensities of the F- and B-bands are defined as

$$I_f = \frac{1}{l}\int_{\text{band (F)}} \ln(I_0/I)\mathrm{d}(\ln\nu) \quad (1)$$

and

$$I_b = \frac{1}{l}\int_{\text{band (B)}} \ln(I_0/I)\mathrm{d}(\ln\nu)$$

where the subscripts b and f refer to the bonded and free states (or to the B- and F-bands) respectively. The integration covers the entire band area.

The molar concentration of chloroform-*d* in the solution

$$C_{CDCl_3} = C_f + C_b \quad (2)$$

was determined from the observed weight concentration and the density of the solution. After being corrected for the local-field effect,[4] the relative intensities, I_f and I_b, are reduced to the values at the molar concentration of pure liquid chloroform-*d*, $C^\circ_{CDCl_3}=12.30$ M, thus:

$$I_f^* = f_d f_c I_f$$
$$I_b^* = f_d f_c I_b \quad (3)$$

and

$$f_c = 12.30/C_{CDCl_3}$$
$$f_d = 9n_D/(n_D^2+2)^2, \quad (4)$$

where n_D is the refractive index of the solution.

Now the absolute intensities, Γ_f and Γ_b, are defined as:

$$C_b^*\Gamma_b = I_b^*$$
$$C_f^*\Gamma_f = I_f^* \quad (5)$$

and

$$(C_f^*+C_b^*) = f_c(C_f+C_b) = C^\circ_{CDCl_3}. \quad (6)$$

Equations 5 and 6 give the relation:

$$\frac{I_f^*}{\Gamma_f} + \frac{I_b^*}{\Gamma_b} = C^\circ_{CDCl_3} \quad (7)$$

or

$$I_b^* = -(\Gamma_b/\Gamma_f)I_f^* + \Gamma_b C^\circ_{CDCl_3}.$$

By plotting I_b^* against I_f^* at a series of concentrations, a straight line with a slope of $-(\Gamma_f/\Gamma_b)$ is generated as long as the absolute intensities, Γ_f and Γ_b, are constant over the concentration range employed. The extrapolated I_f^* value at $I_b^*=0$ corresponds to the Γ_f value for pure liquid chloroform-*d*, and may be compared with the Γ_f^* value directly observed for pure liquid chloroform-*d*.

The above procedure can be expanded for the case where a solute molecule has many hydrogen-bonding sites. If we focus our attention especially on the B-band, *i. e.*, the hydrogen-bonded absorption band which is related with a phosphate group, Eqs. 6 and 7 are modified to:

$$C_f^* + C_b^* + \sum_n \frac{I_{bn}^*}{\Gamma_{bn}} = C^\circ_{CDCl_3} \quad (8)$$

or

$$\frac{I_f^*}{\Gamma_f} + \frac{I_b^*}{\Gamma_b} + \sum_n \frac{I_{bn}^*}{\Gamma_{bn}} = C^\circ_{CDCl_3}, \quad (9)$$

where the subscript bn refers to the bonded states which are related with these sites other than a phosphate group. If we assume a complete association of the

(1 : 1) type for the hydrogen-bonding between chloroform and these sites,[5] the third term on the left-hand side of Eq. 8 or 9 can be expressed as:

$$\sum_n C^*_{bn} = nC_s f_c, \tag{10}$$

where n is the number of hydrogen-bonding sites other than a phosphate group, and C_s, the concentration of the solute. Thus, Eq. 9 is reduced to:

$$\frac{I^*_f}{\Gamma_f} + \frac{I^*_b}{\Gamma_b} = C^\circ_{CDCl_3} - nC_s f_c = C^\circ_{CDCl_3}\left(\frac{C_{CDCl_3} - nC_s}{C_{CDCl_3}}\right). \tag{11}$$

Eq. 11 indicates that this relation holds:

$$\frac{I^{**}_f}{\Gamma_f} + \frac{I^{**}_b}{\Gamma_b} = C^\circ_{CDCl_3} \tag{12}$$

if we define I^{**}_f and I^{**}_b as:

$$I^{**}_f = f'_c I^*_f = f'_c f_c f_d I_f$$
$$I^{**}_b = f'_c I^*_b = f'_c f_c f_d I_b \tag{13}$$

and:

$$f'_c = \frac{C_{CDCl_3}}{C_{CDCl_3} - nC_s}. \tag{14}$$

In Fig. 5, the plots of I^*_b *vs.* I^*_f (or I^{**}_b *vs.* I^{**}_f) for C_5PC (or $C_{16:0}$-g-PC) are shown. It can be seen from the figures that a linear relationship exists between I^*_b and I^*_f (or I^{**}_b and I^{**}_f). Consequently, the validity of Lambert-Beer's law was verified in these concentration ranges. It can also be seen from the figures that the extrapolated I^*_f (or I^{**}_f) values at $I^*_b=0$ (or $I^{**}_b=0$) are almost the same for these systems. For the chloroform-*d*–C_3PC system, for example, Γ_f and Γ_b were 106 and 2268 cm²/mol respectively. The absolute intensities observed by this method are summarized in Table 2. The values of Γ_f are fairly identical for all the systems. It must be added here that the beautiful linear relationship between I^{**}_b and I^{**}_f of Fig. 5b (for $C_{16:0}$-g-PC) confirms the validity of the assumption used in the derivation of

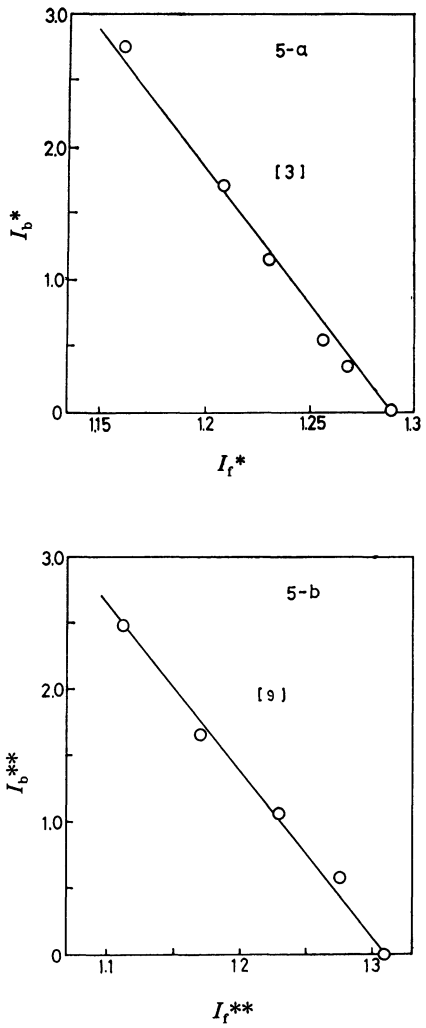


Fig. 5. (a) I_b^* *vs.* I_f^* plot for the chloroform-*d*–C_5PC system. (b) I_b^{**} *vs.* I_f^{**} plot for the chloroform-*d*–(**9**) system.

TABLE 2. CALCULATED PARAMETERS AND ABSOLUTE INTENSITIES

	Γ_f/cm² mol⁻¹	Γ_b/cm² mol⁻¹	n_s	$\sqrt{\Gamma_b} - \sqrt{\Gamma_f}$	E_s
(**1**) C_3PC	106	2268	2.8—3.1	37.3	110
(**2**) C_4PC	106	2226	3.1—3.3	36.9	118
(**3**) C_5PC	105	2205	2.7—3.4	36.7	112
(**4**) C_7PC	105	2006	3.0—3.4	34.5	110
(**5**) C_{10}PC	108	1944	3.0—3.6	33.4	111
(**6**) C_{14}PC	106	1688	3.7—4.0	30.8	118
(**7**) C_{18}PC	104	1460	3.6—4.2	29.3	109
(**8**) DL-Di-$C_{16:0}$PC	107	920	5.8—6.7	20.0	125
(**9**) $C_{16:0}$-g-PC	107	1305	4.8—5.1	25.8	127
(**10**) L-Di-$C_{8:0}$PC	105	1050	5.8—6.3	22.2	134
(**11**) DL-Di-$C_{12:0}$PC	105	1008	6.4—6.6	21.5	139
(**12**) L-Di-$C_{18:2}$PC	106	977	6.1—6.7	21.0	134
(**13**) Di-$C_{16:0}$-β-CP	105	966	6.0—6.2	20.8	127
(**14**) DL-$C_{16:0}$lyso-PC	105	861	3.8—4.2	19.1	76
(**15**) Beef brain $C_{21:0,3}$(spm)	107	578	7.4—7.8	13.7	104
(**16**) L-Di-$C_{17,5:1,4}$PC	107	984	6.1—6.6	21.0	133
(**17**) DL-Di-$C_{16:0}$diMePE	106	837	6.5—7.1	18.6	126
(**18**) DL-Di-$C_{16:0}$MePE	106	742	5.1—5.8	17.0	92
(**19**) Pig PE	105	420	1.5	10.3	15
(**20**) Pig PS	106	350	1.5	8.4	13

Eq. 10.

Determination of the Solvation Number for a Phosphate Group. At a low concentration of the proton acceptor, the phosphate groups of all the acceptor molecules are considered to be bonding to chloroform-*d* molecules.[6] Therefore, the ratio of the concentration, C_b, of the bonded chloroform-*d* to the concentration, C_s, of the solute, C_b/C_s, corresponds to the number of the chloroform-*d* molecule attached to one phosphate group. Using the observed Γ_b value, C_b is determined as:

$$C_b = f_d I_b/\Gamma_b. \quad (15)$$

As C_s is known, the C_b/C_s ratio can easily be determined. We call this ratio the solvation number, n_s, for a phosphate group. The calculated results for n_s are summarized in Table 2. As an example, the detailed data for the C_3PC system are shown in Table 3. The solvation number obtained in this case is 2.8—3.1 (see n_s of Table 2). Therefore, it is concluded that about three chloroform-*d* molecules and one C_3PC (or one phosphate group in C_3PC) form a complex molecule in solution.

Table 3. Relative intensities of the C-D stretching vibrations of the chloroform-*d*–C_3PC system (n_D is the refractive index, and *d* is the specific gravity of this solution)

Sample No.	Wt% of C_3PC	n_D	*d*	I_f^*	I_b^*
1	0	1.4470	1.48	1.300	0
2	0.337	1.4476	1.478	1.293	0.142
3	0.504	1.4474	1.476	1.287	0.215
4	0.676	1.4475	1.475	1.282	0.274
5	0.808	1.4476	1.474	1.284	0.340
6	0.990	1.4477	1.473	1.281	0.392

Spectroscopic Informations and Solubility Parameter. It has been established that there exists a considerable correlation between the frequency shift, $\nu^\circ-\nu$, and the intensity enhancement, Γ_b/Γ_f, due to hydrogen-bond formation, so long as the type of hydrogen-bonding is not very different.[7] We prefer, for the reason described in the Appendix, a $(\sqrt{\Gamma_b}-\sqrt{\Gamma_f})$ value as an appropriate intensity enhancement parameter to a (Γ_b/Γ_f) value. In Fig. 6, the observed $(\sqrt{\Gamma_b}-\sqrt{\Gamma_f})$ values are plotted against the $(\nu^\circ-\nu)$ values. Obviously, the observed data points fall on two different lines, indicating that the (**1**)—(**16**) group is related with a similar type of hydrogen bonding. Actually, the (**1**)—(**16**) molecules all have a choline moiety, while the (**17**)—(**20**) molecules have either a 2-(dimethylamino)ethanol, 2-(methylamino)ethanol, a 2-aminoethanol, or a serine moiety instead. Thus, the nature of the hydrogen bonding between chloroform-*d* and a phosphate group is dependent on the number of methyl groups attached to an amino group. The (**18**), (**17**), and (**8**) molecules are typical examples of those molecules having mono-, di-, and trimethylamino groups respectively.

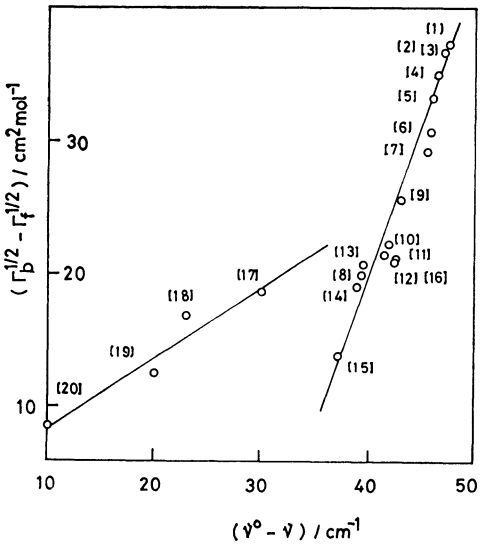


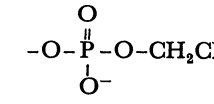
Fig. 6. $(\Gamma_b-\Gamma_f)$ *vs.* $(\nu^\circ-\nu)$ plot for the chloroform-*d* solutions of the molecules (**1**)—(**20**).

Table 2 shows the absolute intensities and the solvation numbers observed for various acceptor molecules. It may be seen from Table 2 that several solvent molecules attach to a phosphate group by forming a strong hydrogen bond. For a series of alkyl phosphorylcholines ((**1**)—(**7**)), for example, the absolute intensity of the B-band, Γ_b, is largest for C_3PC. As the intensity enhancement originates from the hydrogen-bond formation, the intensity difference, $(\sqrt{\Gamma_b}-\sqrt{\Gamma_f})$, can represent the strength of the hydrogen bonding between chloroform-*d* and a phosphate group. Therefore, the product:

$$E_s = \bar{n}_s \cdot (\sqrt{\Gamma_b} - \sqrt{\Gamma_f}), \quad (16)$$

where $\bar{n}_s$ is a mean solvation number, is an appropriate estimate of the stabilization energy of one acceptor molecule through hydrogen-bond formation with a chloroform-*d* molecule in solution. We call this product, E_s, a spectroscopic solubility parameter.

The last column of Table 2 shows the calculated E_s values. The results indicate that the E_s value takes almost identical values for those molecules which have this group:

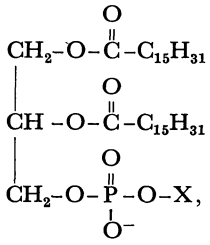


Hereafter, we will designate this group (PC) and call it the PC-group. For all the molecules which have a PC-group, the stabilization energies due to the hydrogen bonding between chloroform-*d* and a phosphate group are almost the same. In case a weak hydrogen bond is formed (*i. e.*, a small Γ_b), a larger number of solvent molecules are attached to a phosphate group. In case a strong hydrogen bond is formed, on the other hand, only a few solvent molecules are attached to a phosphate group. As is shown in the Appendix, $E_s=100$ is equivalent to an enthalpy change of about 2.5 kcal/mol. Therefore, the existence of a phosphate group is essential for these molecules to give a solubility in chloroform.

Side-chain Effects on Solubilities. By comparing the solubility values given in Table 1 and the E_s values of Table 2, a few important conclusions can be drawn as to the effects of the side chains and the molecular

structure on the solubilities.

First, let us compare the solubility data for the (**8**), (**17**), (**18**), (**19**), and (**20**) molecules, whose molecular structures are:



where X is:

(**8**) $-(CH_2)_2N^+(CH_3)_3$ (2-(trimethylammonio)ethyl),

(**17**) $-(CH_2)_2N^+H(CH_3)_2$ (2-(dimethylammonio)ethyl),

(**18**) $-(CH_2)_2N^+H_2(CH_3)$ (2-(methylammonio)ethyl),

(**19**) $-(CH_2)_2N^+H_3$ (2-ammonioethyl),

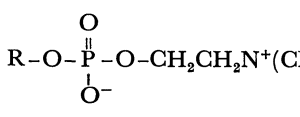
or (**20**) $-(CH_2)-CH(COO^-)-N^+H_3$ (serine).

As has been described in the preceding paragraph, the stregth and the nature of the hydrogen bonding between chloroform and a phosphate group are quite different for these molecules (see Fig. 6). The observed solubility decreases in magnitude in this order;

$$(8) > (17) > (18) > (19),\ (20).$$

The spectroscopic information shows that both the solvation number and the strength of the hydrogen bonding between chloroform and a phosphate group decrease in this order, resulting in a rapid decrease in the spectroscopic solubility parameter. This rapid decrease in the E_s value may arise from the intramolecular hydrogen-bond formation between a N–H group and a phosphate group.[8] If the hydrogen atoms of the NH_3 group are replaced by methyl radicals, this type of hydrogen-bond cannot be formed (see the structures of (**19**) and (**20**)). It must be added that the slight solubility of the (**19**) and (**20**) molecules arises from the existence of the unsaturated bonds. The corresponding synthesized sample (*rac*-1,2-dipalmitoylglycerol-3-phosphorylethanolamine or *rac*-1,2-dipalmitoylglycerol-3-phosphorylethanolserine) is insoluble in chloroform, so the spectroscopic study could not be performed. Anyway, the end group, X, must be a 2-(trimethylammonio)ethyl group in order to show a high solubility in chloroform. The (**8**) molecule will be used as the reference sample in the following discussion.

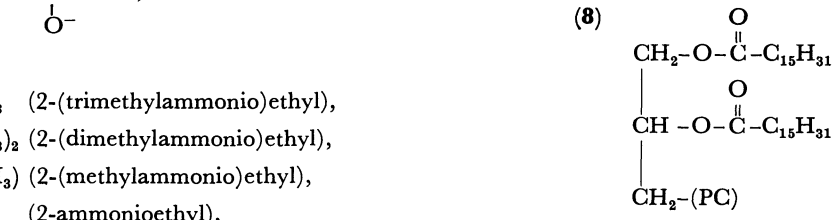
Secondly, let us compare the (**1**)—(**7**) molecules, whose molecular structures are:



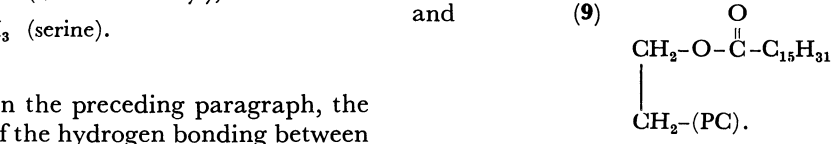
where R is an alkyl chain. The solubility decreases with the increase in chain-length ((**3**)—(**7**)). Although the strength of hydrogen bonding between chloroform and a phosphate group decreases with the increase in the chain length, the solvation number increases at the same time, thus keeping the spectroscopic solubility parameter almost constant for all the molecules. Therefore, the gradual increase in solubility with the increase in the chain-length may be ascribed to the solvent-phobic character of the alkyl chains. In fact, the solubilities of *n*-$C_{20}H_{41}$PC and *n*-$C_{22}H_{45}$PC are observed to be about 0.002 mol/l at room temperature. The abnormal solubility values for the (**1**) and (**2**) molecules will be referred to in a later paragraph in view of the mixing state of these alkylphosphorylcholines and chloroform.

Thirdly, let us compare the (**8**) and (**9**) molecules, whose molecular structures are:

(**8**)

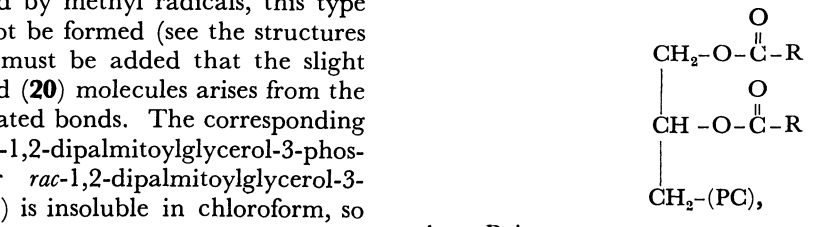


and (**9**)



On passing from a single alkyl-chain to double chains, the solubility decreases from 1.0 to 0.7 mol/l. This change is not so much as is expected for a long-chain alkylphosphorylcholine, probably because the effect of the alkyl chains is compensated for by the stabilization energy due to hydrogen bonding between the chloroform and an acyloxyl group. Again, the solubility parameters, E_s, are almost the same for these two molecules. An acyloxyl group plays a role in the solubility in chloroform second in importance only to a phosphate group.

Fourthly, let us compare the (**10**), (**11**), and (**12**) molecules with the (**8**) molecule. The molecular structures of these molecules are;



where R is:

(**8**) $-C_{15}H_{31}$,

(**10**) $-C_7H_5$,

(**11**) $-C_{11}H_{23}$,

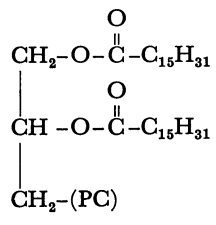
or: (**12**) $-(CH_2)_3-(CH_2-CH=CH)_2-(CH_2)_7CH_3$.

The solubilities of these molecules do not change so much with the change in the alkyl chain length as is seen for a series of alkylphosphorylcholines. Here again, the solubility-parameter values are almost constant. It must be emphasized that these molecules are distinguished from the long-chain alkylphosphorylcholines by having two long chains. That is to say, this type of molecule can have long alkyl chains without losing their solubilities in chloroform. The existence of the long alkyl chains might increase the solvent-phobic character of these molecules. The variety of alkyl chains might produce the variety of the characters of

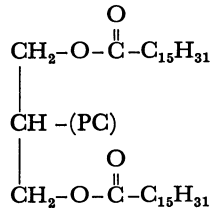
these molecules in solutions.

Lastly, let us compare the (**13**), (**14**), and (**15**) molecules with the (**8**) molecule. The structures of these molecules are:

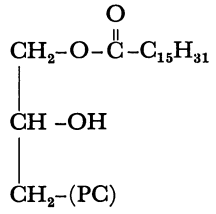
(**8**)



(**13**)

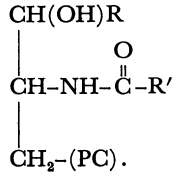


(**14**)



and

(**15**)



The (**14**) and (**15**) molecules are specified as having a hydroxyl group and/or an imino group. In these molecules, the hydrogen-bonding sites are expected to be deactivated because of the intramolecular hydrogen-bond formation between a phosphate group and the hydroxyl or imino group. If the intramolecular hydrogen-bond is formed, the solvation number, n_s, can be expected to decrease. In fact, the observed solvation number is quite small for the (**14**) molecule in comparison with the (**8**) molecule. Probably, the phosphate group and hydroxyl group from an intramolecular hydrogen bond. This situation is reflected in the low solubility value observed for the (**14**) molecule. In the case of the (**15**) molecule, on the other hand, the n_s value is observed to be slightly larger than that of the (**8**) molecule. This suggests that the intramolecular hydrogen bond is not formed in this molecule. As the strength of the hydrogen bonding between a phosphate group and solvent molecules is relatively small (see the $(\sqrt{\Gamma_b}-\sqrt{\Gamma_f})$ value), the resultant E_s value is relatively small, although the solvation number is relatively large. This situation might be responsible for the observed low solubility value.

The (**13**) molecule is an isomer of the (**8**) molecule. As can naturally be expected, the spectroscopic and physical parameters are quite similar in these two molecules, because the polar group and the side-chain groups are essentially the same. The positional difference of a PC-group does not change the solubility and the spectroscopic solubility parameter very much.

Mixing States of Alkylphosphorylcholines. In the preceding, we have left C_3PC and C_4PC out of our discussion in considering the effects of the alkyl chains on the solubilities for a series of alkylphosphorylcholines, although C_3PC and C_4PC have very low solubilities in chloroform (see Table 1). The (**3**)—(**7**) molecules dissolve into chloroform homogeneously so long as the solute concentration is lower than the solubilities. In the cases of C_3PC and C_4PC, however, their chloroform solutions separate into two solution phases if the solute concentrations exceed the solubility values of Table 1. The upper layers are relatively solute-rich solutions, while the lower layers are chloroform-rich solutions.

The concentration of the lower layers are 0.06 and 0.15 mol/l for C_3PC and C_4PC respectively. The spectroscopic information in Tables 1 and 2 was obtained for these lower layers, because the upper layers were so viscous and seemed unstable.

Taking the observed Γ_b and n_s values into account, we may explain those phenomena as follows. The C_3PC (or C_4PC) molecule forms a very stable complex molecule which is composed of one C_3PC (or C_4PC) and three chloroform molecules. The hydrogen bonding between chloroform molecules and a phosphate group is so strong that the complex molecule is precipitated as it is when the solute concentration exceeds 0.06 (or 0.15) mol/l. The sudden increase in solubility on passing from C_4PC to C_5PC may, therefore, suggest some kind of aggregate formation in the chloroform solutions of the (**3**)—(**7**) molecules. This aggregate formation may not correspond to micelle formation in the usual sense, because the critical solution temperature (CST) could not be observed for these solutions. Perhaps this aggregate has a structure analogous to that which has been clarified to be composed of a few solute molecules doubly solvated by chloroform molecules.[9] It must be emphasized that CST was observed for the alkylphosphorylcholines with longer chains, like *n*-$C_{20}H_{41}PC$ or *n*-$C_{22}H_{45}PC$ at about 40 °C. The mixing states of alkylphosphorylcholines, especially those of short alkyl chains, suggest ideas for future work into the problem.

Concluding Discussion. The main conclusions drawn from the above discussion are:

1) The hydrogen-bonding between chloroform and a phosphate group is the main factor in determining the solubility. The stabilization energy due to this type of hydrogen bonding is almost the same for all the molecules studied. When a weak hydrogen-bond is formed, a large number of solvent molecules are attached to the phosphate group, and *vice versa*. This situation may be helpful for the stabilization of those solutions in which the number of solvated molecules changes from time to time.

2) The hydrogen bonding between chloroform and an acyloxyl group may contribute to the stabilization of a long-chain molecule in a chloroform solution. With the help of the acyloxyl group, these molecules can exhibit various solvent-phobic characters when the long alkyl chains are changed without losing their solubility

in the solvent.

3) The existence of the double chains is also helpful in enabling these molecules to have a strong solvent-phobicity without losing their solubility.

4) The existence of any groups which can form intramolecular hydrogen bonding with a phosphate group can lower the solubilities of these solute molecules. A hydroxyl group is a good example of this.

Appendix. Derivation of Intensity Enhancement Parameter.

We will describe the relation between the intensity-enhancement parameter, $(\sqrt{\Gamma_b}-\sqrt{\Gamma_f})$, and the excess enthalpy, $-\Delta H$, originating from hydrogen-bond formation.

Tsubomura[10] proposes that, in a hydrogen-bonded system like

$$\text{A}\text{——}\text{H}\cdots\text{B},$$

where A and B refer, respectively, to an acid and a base, the intensity change of the A–H stretching vibration is to be attributed to charge-transfer forms in the wave function. It is assumed that the wave function of the ground state of the complex may be expressed by this formula:

$$\Phi = c_1\Phi_1 + c_2\Phi_2, \tag{A1}$$

where c_1 and c_2 are constants normalized to unity, $c_1^2+c_2^2=1$, and Φ_1 and Φ_2 are the wave functions associated with the following resonance structure:

Structure 1: A——H B covalent A–H bond

Structure 2: A^- H——B^+ covalent H–B bond. (with charge transfer)

Therefore, the dipole moment, p, of the hydrogen-bonded system is:

$$p = \langle\Phi|p|\Phi\rangle \simeq p_1 + c_2^2 p_2, \tag{A2}$$

where p_1 and p_2 refer to the dipole moments corresponding to Structures 1 and 2 respectively. The derivative of p with respect to the normal coordinate, Q, which corresponds to the A–H stretching vibration, is:

$$\left(\frac{\partial p}{\partial Q}\right) = \left(\frac{\partial p_1}{\partial Q}\right) + 2c_2p_2\left(\frac{\partial c_2}{\partial Q}\right) \tag{A3}$$

with these definition of the absolute intensities, Γ_f and Γ_b, of the A–H stretching vibration:

$$\Gamma_f = \left(\frac{N_0\pi}{3c^2\omega}\right)\left(\frac{\partial p_1}{\partial Q}\right)^2,$$

$$\Gamma_b = \left(\frac{N_0\pi}{3c^2\omega}\right)\left(\frac{\partial p_2}{\partial Q}\right)^2. \tag{A4}$$

Eq. A3 is reduced to:

$$\sqrt{\Gamma_b} - \sqrt{\Gamma_f} = \sqrt{\frac{N_0\pi}{3c^2\omega}}\,2c_2p_2\left(\frac{\partial c_2}{\partial Q}\right). \tag{A5}$$

Based upon the same model and the same order of approximation, the stabilization energy due to hydrogen-bond formation is calculated to be:[11]

$$-\Delta H = c_2W_{12} - \Delta H_0, \tag{A6}$$

where W_{12} is a resonance energy term, $\langle\Phi_1|\tilde{H}|\Phi_2\rangle$ ($\tilde{H}$ being a Hamilton operator), and $-\Delta H_0$ is a stabilization energy due to an interaction other than hydrogen bonding. If $-\Delta H_0$ is ralatively small, as is usually the case for ordinaty intermolecular interactions, Eq. A6 becomes:

$$-\Delta H = c_2W_{12} \tag{A7}$$

combination of Eqs. A5 and A7 leads to this relation:

$$\sqrt{\Gamma_b} - \sqrt{\Gamma_f} = -\left(\frac{2N_0\pi}{3c^2\omega}\right)^{1/2}\frac{p_2}{W_{12}}\left(\frac{\partial c_2}{\partial Q}\right)\Delta H, \tag{A8}$$

where N_0 is the Avogadro constant; c, the velocity of light, and ω the frequency of light.

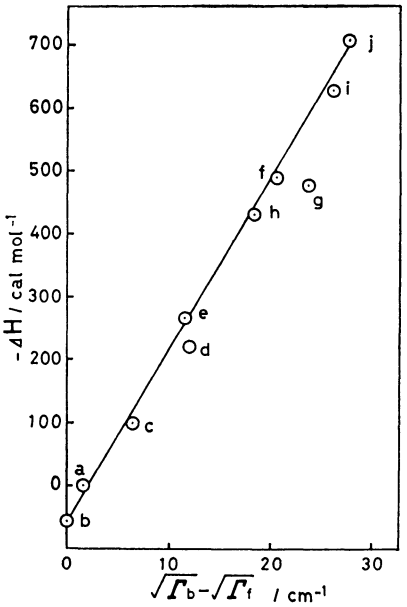


Fig. A1. $-\Delta H$ *vs.* $(\sqrt{\Gamma_b}-\sqrt{\Gamma_f})$ plot for the chloroform-*d* solutions of: (a) chloroform-*d*, (b) carbon tetrachloride, (c) benzene, (d) *p*-xylene, (e) 1,2,4-trichlorobenzene, (f) ethyl acetate, (g) acetone, (h) dibutyl ether, (i) diethyl ether, (j) diisopropyl ether.

In Fig. Al, the observed excess enthalpy values for chloroform solutions of various acceptors are plotted against the observed intensity difference values, $\sqrt{\Gamma_b}-\sqrt{\Gamma_f}$. Attention should be paid to the fact that the absolute intensity of the C–D stretching vibration of a carbon tetrachloride solution was chosen as Γ_f in this case. Obviously, a fine linear relation holds between the $-\Delta H$ and $(\sqrt{\Gamma_b}-\sqrt{\Gamma_f})$ values. Thus, we can safely conclude that the coefficient of the right-hand side of Eq. A8 is almost constant for a hydrogen bonding of a similar type. Conversely, it is very desirable to use the $(\sqrt{\Gamma_b}-\sqrt{\Gamma_f})$ value as a good estimate of the stabilization energy due to hydrogen-bond formation.

Incidentally, the E_s value defined by Eq. 16 can be converted into the excess enthalpy value. It may be seen from Fig. Al that $E_s=100/\text{cm}^{-1}$ is equivalent to $-\Delta H=2.5$ kcal/mol.

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5) Actually the complete association may not be realized in solution studies. The errors due to this assumption, however, do not spoil the conclusions drawn in this report.

6) It is ascertained that the C-D stretching band corresponding to the free state completely disappears at saturation.
7) See for example, G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freemen and Company, New York (1960).
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