

PO₂⁻ Symmetric-Stretching Raman Line and Molecular Aggregation States of Barium Dialkyl Phosphates

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Abstract: The Raman spectra of barium dialkyl phosphates having various chain lengths were investigated. Dipentyl phosphate (DPP) was found to form a liquid crystal that is stable at room temperature. Calorimetric measurements of the DPP liquid crystal exhibited a phase transition at 60 °C with $\Delta H = 1.7$ kcal/mol. The PO₂⁻ symmetric-stretching Raman line of DPP was observed at 1106 cm⁻¹ for the crystal and at 1075 cm⁻¹ for an aqueous solution. For the liquid crystal, two lines were observed at 1096 and 1075 cm⁻¹. The relative intensities of these lines changed sharply at the phase-transition temperature but were essentially constant below and above the transition point. On the other hand, the intensity of the diester OPO symmetric-stretching Raman line as well as that of the accordion vibration was hardly altered by the phase transition. This suggested little structural change in the phosphoalkyl group upon phase transition and the coexistence of two types of aggregation structures with different Ba²⁺...PO₂⁻ interaction modes in the liquid crystal. The PO₂⁻ symmetric-stretching Raman lines of dibutyl phosphate were observed at 1090 and 1068 cm⁻¹, and their relative intensities changed significantly upon micellization. The all-trans form of the alkyl group was favored in the micelle state of dibutyl phosphate. It was concluded that the cation-phosphate interaction mode is important for investigation of the aggregation structure of dialkylphosphates and is characterized by the PO₂⁻ symmetric-stretching Raman lines. The frequencies of the accordion vibrations for mono- and dialkyl phosphates fell on identical straight lines when they were plotted against the reciprocal of the carbon number.

Surfactant molecules with both hydrophobic and hydrophilic groups form a variety of aggregation structures in an aqueous solution that include liquid crystals, micelles, bilayers, and monolayers, and they occasionally serve as a model of biomembranes. Synthesized amphiphiles such as didecyl, didodecyl, and dihexadecyl phosphates have been shown to form vesicles and lamellars that possess physicochemical characteristics similar to those of biomembranes.¹⁻³

Conformational studies on dialkyl phosphates involved in an aggregated structure are important for understanding physical properties of phospholipid bilayers. So far, the conformation of simple dialkyl phosphate anions in an aqueous solution has been investigated mainly by infrared and Raman spectroscopic methods.⁴ The X-ray crystallographic analysis was performed for barium diethyl phosphate, for which the two C-O bonds were found to occupy the gauche positions with regard to the P-O bonds,⁵ in agreement with the conclusion of spectroscopic investigations. However, much less is known about the conformation of dialkylphosphates with longer hydrocarbon chains. We report here the Raman spectra of barium dipentyl phosphate in the liquid crystal and barium dibutyl phosphate in micelles, as well as various mono- and dialkyl phosphates in aqueous solutions, and discuss the Raman intensity changes upon alteration of the aggregated structures.

Experimental Section

Materials. Barium dimethyl phosphate (DMP), barium diethyl phosphate (DEP), barium dibutyl phosphate (DBP), and barium dipentyl phosphate (DPP) were synthesized by dealkylation of the corresponding phosphoric acid triester in anhydrous barium iodide-acetone solution⁶ and were purified by recrystallization in methanol-water. Didecyl phosphate was obtained from phosphoryl chloride and decyl alcohol in benzene^{7,8} and was recrystallized from benzene. The samples of disodium monoalkyl phosphates (carbon number = 6, 8, 10, and 12) were synthesized from pyrophosphoric acid and the corresponding alcohols^{9,10} and were purified by recrystallization.

Raman Scattering. The measurements of Raman spectra of the samples in the crystalline state and in an aqueous solution were carried out at room temperature on a JASCO R-800 or R-500 Raman spectrometer with the 514.5-nm line of an Argon-ion laser. All spectra are simple averages of several scans accumulated without smoothing. The frequencies of Raman lines were calibrated with indene as a standard. The intensity of a Raman line was evaluated as a peak height from a straight base line drawn between bases around 1150 and 650 cm⁻¹.

Calorimetry. The temperature of the thermotropic transition of the DPP liquid crystal was determined with a differential scanning calorimeter (Rigaku-Denki, TG-DTA-M8076). For the DPP liquid crystal, the calorimeter was scanned with a rate of 10 °C/min with α -Al₂O₃ in the reference pan.

Preparation of Liquid Crystal of DPP. After a 7:3 DPP-H₂O mixture was heated to 100 °C and then cooled to room temperature, a white turbid and very viscous liquid crystal was formed. In a sealed glass tube, the liquid crystal was stable in a temperature range of 15-60 °C, but it changed into a crystalline state below 14 °C or into a clear solution above 60 °C.

Critical Micelle Concentration (cmc). DBP is easily solubilized in water and forms a micelle. The cmc of DBP, 0.35 M (121.1 mg/mL), was determined from a inflection point in the plot of the ³¹P NMR chemical shift vs. the inverse of the concentration.^{11,18} DPP was only

- (1) Kunitake, T.; Okahata, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3860.
- (2) Kunitake, T.; Okahata, Y.; Tamaki, K.; Kumamaru, F.; Takayanagi, M. *Chem. Lett.* **1977**, 387.
- (3) Kunitake, T.; Okahata, Y. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1877.
- (4) Shlmanouchi, T.; Tsuboi, M.; Kyogoku, Y. *Adv. Chem. Phys.* **1964**, *7*, 435.
- (5) Kyogoku, Y.; Iitaka, Y. *Acta Crystallogr.* **1966**, *21*, 49.
- (6) Zervas, L.; Dilaris, I. *J. Am. Chem. Soc.* **1955**, *77*, 5354.
- (7) Kunitake, T.; Okahata, Y. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1877.
- (8) De Pont, Co. U.S. Patent 2005619, 1935.
- (9) Nelson, K. *Inorg. Chem.* **1963**, *2*, 775.
- (10) Nakagaki, M.; Handa, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 630.
- (11) Matsushita, K.; Okabayashi, H., to be published.
- (12) Harada, K.; Sugeta, H.; Kyogoku, Y. "Abstracts of Papers", Annual Meeting of the Chemical Society of Japan, 1978; p 537.
- (13) Thomas, G. J.; Kyogoku, Y. *Pract. Spectrosc.* **1977**, *1C*, 717.
- (14) Lippert, J. L.; Peticolas, W. L. *Proc. Natl. Acad. Sci.* **1971**, *68*, 1570.
- (15) Gaber, B. P.; Peticolas, W. L. *Biochim. Biophys. Acta* **1977**, *465*, 260.

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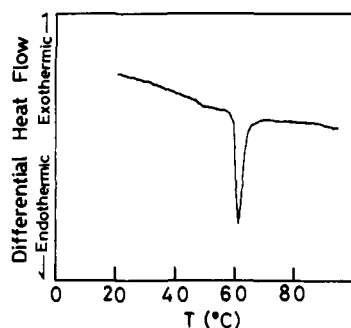


Figure 1. Differential scanning calorimetry of the DPP liquid crystal. The concentration of DPP is 70 wt %.

Table I. $\nu_s(\text{PO}_2^-)$ Frequencies of Simple Dialkyl Phosphates in a Crystal and an Aqueous Solution

	crystal	solution	$\Delta\nu^a$	ref
DPP	1106	1075 (monomer)	31	this work
DBP	1108	1090 (micelle)	18	this work
		1068 (monomer)	40	this work
DMP	1098	1086	12	this work
		1063	35	this work
DEP	1093	1082	11	this work
		1060	33	this work
DIP ^b	1093	1073	20	12
MIP ^c	1109	1074	35	12

^a $\Delta\nu = \nu_{\text{crystal}} - \nu_{\text{soln}}$. ^b Barium diisopropyl phosphate.

^c Barium methylisopropyl phosphate.

slightly soluble in water (ca. 30 mg/mL at room temperature). As no cmc was found for DPP in the concentration range of 5–30 mg/mL, the DPP molecules seem to exist as a monomolecular dispersion state in this concentration range.

Results and Discussion

Thermotropic Properties of DPP–H₂O. Figure 1 shows the results of differential scanning calorimetry of the DPP liquid crystal in the sealed tube. An endothermic peak was observed at 60 °C, and ΔH of the phase transition was calculated to be 1.7 kcal/mol from the peak area. The transition was reversible only in a sealed tube. DBP never formed a liquid crystal at any mixing ratio of DBP/H₂O. We conclude therefore that DPP is the shortest dialkyl phosphate that forms a liquid crystal.

Raman Spectra of DPP. Figure 2 shows the Raman spectra of DPP in an aqueous solution, a liquid crystal, and a crystal. On the basis of the Raman spectral analysis of various dialkyl phosphates,^{4,11} a very intense line of crystalline DPP at 1106 cm^{-1} is assigned to the PO_2^- symmetric-stretching vibration [$\nu_s(\text{PO}_2^-)$]. The $\nu_s(\text{PO}_2^-)$ line of DPP is shifted to 1075 cm^{-1} in the monomolecular aqueous solution. The similar size of frequency shift between a crystal and an aqueous solution has also been observed for other simple barium dialkylphosphates, as summarized in Table I.

In the spectrum of the DPP liquid crystal, two prominent Raman lines were observed at 1075 and 1096 cm^{-1} . On normalizing the Raman intensities of the aqueous solution and the liquid crystal with the use of the CH_2 scissoring line at 1450 cm^{-1} , we find that the sum of intensities of the 1096- and 1075- cm^{-1} lines for the liquid crystal corresponds well to the intensity of the single 1075- cm^{-1} line of the aqueous solution. Hence, the 1096- and 1075- cm^{-1} lines are likely to be associated with the $\nu_s(\text{PO}_2^-)$ mode and to indicate the presence of two kinds of the Ba^{2+} and PO_2^- interaction in the DPP liquid crystal. The $\text{Ba}^{2+} \cdots \text{PO}_2^-$ interaction of the 1075- cm^{-1} species of the liquid crystal is presumably similar to that of the monomeric solution. As the intensity ratio of the two lines, I_{1096}/I_{1075} , qualitatively reflects the relative

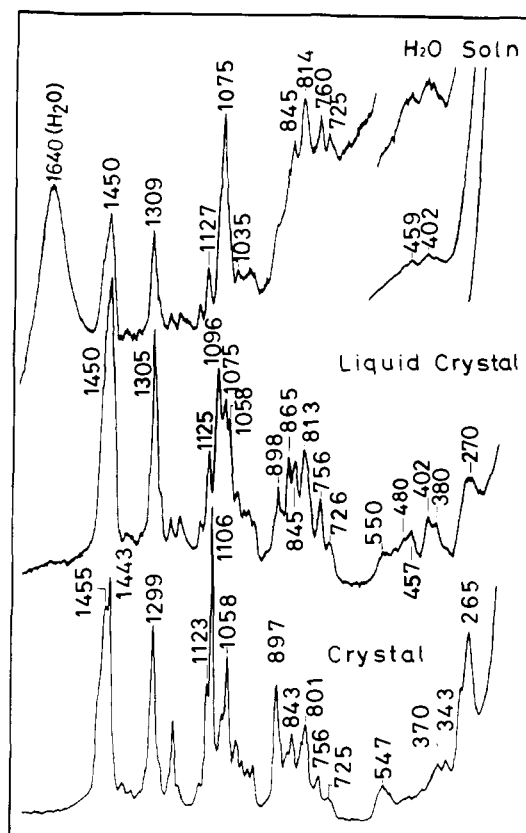


Figure 2. Raman spectra of DPP in an aqueous solution (top), liquid crystal (middle), and crystal (bottom). Slit width: 190 μm ; laser power at sample: 300 mW.

populations of the two species, the 1075- cm^{-1} species would be dominant in the dilute aqueous solution, while the 1096- cm^{-1} species would be a major component in the liquid crystal.

In order to examine the above interpretation, we measured the temperature dependence of Raman spectra for the DPP liquid crystal in the 20–80 °C range. The Raman spectra in higher and lower frequency regions are shown in Figure 3 a and b, respectively. The Raman spectra changed significantly at 60 °C with regard to relative intensities but without frequency shifts of Raman lines. This feature strongly suggests that in the liquid crystal there are two molecular species and the relative populations change sharply on the phase transition. On the basis of the relative intensity, I_{1096}/I_{1075} , the population ratio of the 1096 to the 1075- cm^{-1} species is 1.2 at 15–60 °C and 1.6 at 80 °C, and accordingly, the relative population seems to be important to produce a liquid crystal.

This temperature dependence of Raman spectrum of the DPP liquid crystal clearly differs from that of phospholipids. In the case of phospholipids, a new Raman line appears around 1090 cm^{-1} upon the phase transition, and its intensity increases as temperature is raised,¹⁴ whereas the intensities of the Raman lines at 1064 and 1130 cm^{-1} decrease. The 1090- cm^{-1} line is assigned to the C–C stretching mode of the alkyl groups with the gauche conformation, whereas the 1064- and 1130- cm^{-1} lines are assigned to the same mode of the all-trans alkyl group. Accordingly, the temperature dependence of the Raman spectra of phospholipids has been attributed to the intra- and intermolecular disordering of the alkyl groups.¹⁵

The Raman lines of the DPP liquid crystal at 1125 and 1058 cm^{-1} are assigned to the C–C stretching modes of the all-trans alkyl group. The 1096- cm^{-1} band of the liquid crystal may have some contribution from the C–C stretching mode of the gauche forms, but this line seems to be too intense and too narrow to be assigned to the gauche line. Furthermore, the relative intensity of the accordion vibration at 270 cm^{-1} did not significantly decrease at 80 °C compared with those at 20–80 °C (Figure 3b). This

(16) Okabayashi, H.; Okuyama, M.; Kitagawa, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2264.

(17) Okabayashi, H.; Abe, M. *J. Phys. Chem.* **1980**, *84*, 999.

(18) Okabayashi, H.; Matsushita, K., to be published.

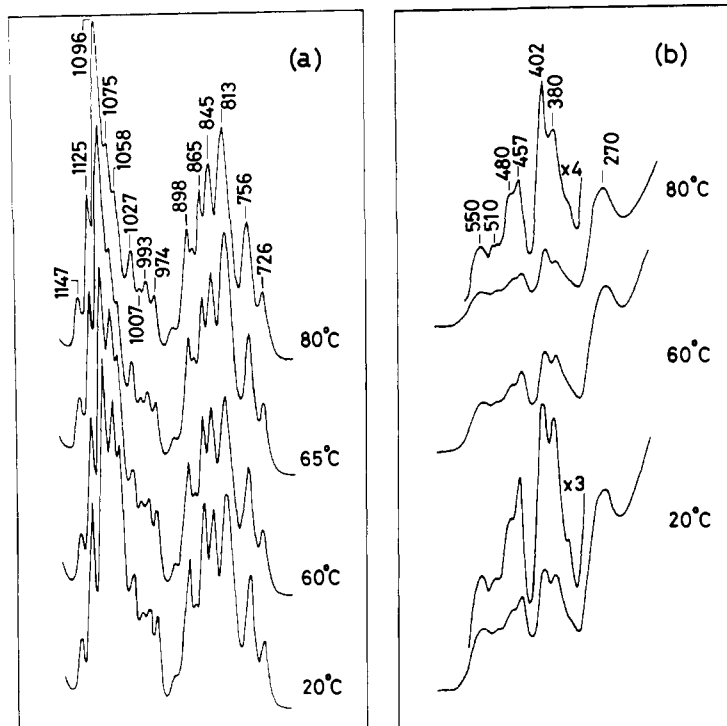


Figure 3. Temperature dependence of the Raman spectra in the (a) 700–1200-cm⁻¹ region and (b) 200–600-cm⁻¹ region for the DPP liquid crystal. Slit width: 180 μm; laser power at sample: 350 mW.

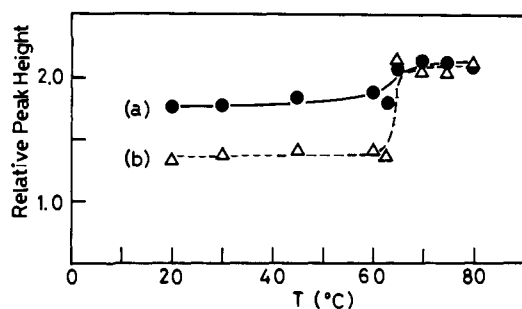


Figure 4. Temperature dependence of relative peak heights (●, I_{1096}/I_{1125} ; Δ, I_{1096}/I_{1058}) of the DPP liquid crystal.

shows that a large amount of molecules still remains in the all-trans form even at this temperature. Consequently, the contribution of the C–C stretching mode of the gauche isomer to the 1096-cm⁻¹ band would not be significant for the DPP liquid crystal.

Since the Raman lines of the DPP liquid crystal at 402 and 480 cm⁻¹ are missing in the crystal, they should be assigned to conformers that are absent in the crystal. The relative intensities of these Raman lines, namely I_{402}/I_{380} and I_{480}/I_{457} , increase sharply upon the phase transition; I_{402}/I_{380} is 1.14–1.20 in the 20–60-°C and 1.30–1.40 in the 65–80-°C range. This fact also indicates the presence of two rotational isomers in the liquid crystal and the change of their populations at the phase transition.

In Figure 4, the relative peak intensities of the Raman lines, I_{1096}/I_{1058} and I_{1096}/I_{1125} , are plotted against temperature. The relative intensities are unexpectedly constant below and above the phase-transition temperature. The feature differs from the intensity vs. temperature curve observed for the same mode of lecithin multilayers,¹⁴ for which gradual intensity changes have been observed below and above the phase transition. If the intensity increase of the 1096-cm⁻¹ line reflected the conformational change from the all-trans form to the gauche forms, the population of the gauche forms above the phase-transition temperature should increase in proportion to the Boltzmann factor. This was, in fact, true for the lecithin multilayers but is not compatible with the intensity change observed in the present work. Furthermore, the intensity of the ester OPO symmetric-stretching line around 750

cm⁻¹, which has been found to be conformation sensitive,^{4,13} did not show any systematic intensity change in a temperature range between 20 and 80 °C. Therefore, the temperature dependence of the Raman spectrum should not be ascribed to a conformational change of the alkyl groups. Since the 1096-cm⁻¹ line is assignable to $\nu_s(\text{PO}_2^-)$, it is likely that the intensity change of the 1096-cm⁻¹ line upon the phase transition results from a cooperative change in the Ba²⁺...PO₂⁻ interaction and thus in the molecular aggregation state of dialkyl phosphates.

Raman Spectral Change of DBP on Micellization. The DBP aqueous solution exhibited weak Raman lines at 295, 376, 432, 469, 507, and 547 cm⁻¹ in the 250–600-cm⁻¹ region. In the measurements of the concentration dependence of relative Raman intensities, the I_{295}/I_{376} ratio increased with an increase in the concentration. As the 295-cm⁻¹ line arises from the accordion vibration of the butyl group with the all-trans form, the intensity increase of the 295-cm⁻¹ line implies the increased population of the all-trans conformation due to micelle formation. Similar observations have already been reported for aqueous solutions of potassium *n*-pentanoate, *n*-hexanoate,¹⁶ and *trans*-2-hexenoate.¹⁷

In the 1000–1150-cm⁻¹ region, the relative intensity of two prominent Raman lines at 1090 and 1068 cm⁻¹ evidently depended upon the concentration. The relative intensity, I_{1090}/I_{1068} , remained fairly constant (1.1–1.2) in the range of 1.5–100 mg/mL, but it drastically increased around the cmc (121 mg/mL)¹⁸ and finally amounted to 2.2 at the concentration of 600 mg/mL. The marked intensity change should be attributed to the micellization of DBP. However, this ratio was not sensitive to temperature (1.97 at 5 °C and 1.93 at 75 °C) and therefore does not reflect the population ratio of the gauche/trans isomers in the case of DBP.

We assume that the 1090- and 1068-cm⁻¹ lines of DBP correspond to the 1096- and 1075-cm⁻¹ lines of the DPP liquid crystal and accordingly are assigned to $\nu_s(\text{PO}_2^-)$. The 1090- and 1068-cm⁻¹ lines would be associated with the micellar and monomolecular DBP anions, respectively. The frequency difference in the $\nu_s(\text{PO}_2^-)$ mode between micellar and monomolecular DBP anions may be ascribed to different pairing modes of Ba²⁺ and DBP anions. Presumably, the ion-pair-like interaction as in a crystal is dominant in a micelle, and the number of such ion pairs increases with increasing concentration above the cmc, but the DBP molecules exist overwhelmingly as free ions below the cmc.

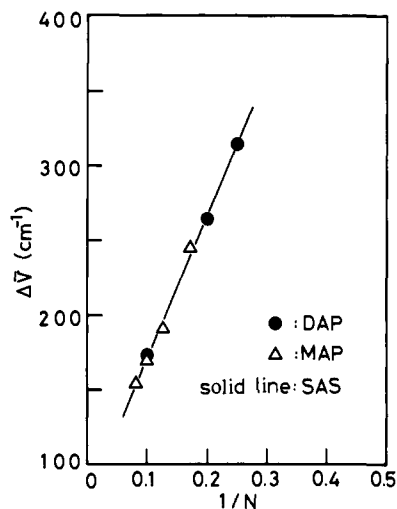


Figure 5. Dependence of accordion frequencies upon the carbon number (N) of the alkyl group for DAP, MAP, and SAS.

We also investigated the ^{31}P NMR spectra of DBP in aqueous solutions at various concentrations and obtained some evidence for complex formation between barium ion and DBP anions at a concentration higher than the cmc.¹⁸ This is consistent with the general tendencies of alkali- or alkaline-earth metal ions in an aqueous solution.¹⁹⁻²³ Crutchfield et al.²⁴ have also suggested such complex formation from their ^{31}P NMR study of polyphosphate anions. These observations also support the above explanation for the multiplicity of the $\nu_s(\text{PO}_2^-)$ vibrational mode.

Concentration Dependence of Raman Spectra of DMP and DEP. For the DMP and DEP molecules in aqueous solutions, the barium cations would form a complex with the phosphate anions similar to DBP. We examined the concentration dependence of the Raman spectra of DMP and DEP in aqueous solutions in the 1000–1150- cm^{-1} range. The Raman spectra of DMP and DEP in the solid state and aqueous solutions were in agreement with

Table II. Accordion Frequencies (cm^{-1}) of Barium Dialkyl Phosphates (DAP) and Disodium Monoalkyl Phosphates (MAP) in the Crystals

N^a	MAP	DAP	SAS ^b
3			340
4		313	315
5		265	
6	245		236
8	190		196
10	170	170	169
12	150		

^a N : carbon number. ^b SAS: sodium n -alkyl sulfates (see ref 25).

those in other work.⁴ It is noted that the DMP aqueous solution exhibited both 1086- and 1063- cm^{-1} lines, and their relative intensity changed markedly with the concentrations (I_{1086}/I_{1063} is 2.4 for 50 mg/mL and 3.0 for 600 mg/mL). Since DMP has no $\text{CH}_2\text{-CH}_2$ bond, this observation shows that the intensity change occurs without a conformational change of an alkyl group from the all-trans to gauche form. For DEP, a similar feature was also observed; the I_{1082}/I_{1060} is 3.5 at 50 mg/mL, but it is 4.6 at 480 mg/mL. Generally, the number of the complexes between Ba^{2+} and the phosphate anions would increase at an increased concentration. Therefore, the intensity increase of the 1086- cm^{-1} line would be reasonable if the ion-pair form gave rise to the 1086- cm^{-1} line and its population increased at higher concentration.

Accordion Vibrations. The accordion vibrations of DBP, DPP, DDP, and a series of monoalkylphosphates (carbon number = 6, 8, 10, and 12) were measured in the crystalline states. Their frequencies together with those of sodium alkylsulfates (SAS) are listed in Table II and are plotted against a reciprocal of the carbon number in Figure 5. It should be emphasized that the accordion frequencies of the crystalline DBP, DPP, and DDP fall on the same straight line as derived from those of monoalkylphosphates. This line is also in accord with the line derived from crystalline SAS molecules having a carbon number of from four to ten.¹⁸ Therefore, the accordion frequency is useful to estimate the length of the all-trans part of the alkyl chains of phospholipids as pointed out previously for normal hydrocarbon chains.^{19,26}

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Registry No. DPP Ba salt, 68698-63-5; DBP Ba salt, 3716-34-5; DMP Ba salt, 17323-82-9; DEP Ba salt, 14506-44-6.

- (19) Van Wazer, J. R.; Callis, C. F. *Chem. Rev.* **1958**, *58*, 1011.
 (20) Lambert, S. M.; Watters, J. I. *J. Am. Chem. Soc.* **1957**, *79*, 5606.
 (21) Watters, J. I.; Lambert, S. M. *J. Am. Chem. Soc.* **1959**, *81*, 3201.
 (22) Irani, R. R.; Callis, C. F. *J. Phys. Chem.* **1960**, *64*, 1398.
 (23) Irani, R. R. *J. Phys. Chem.* **1961**, *65*, 1463.
 (24) Crutchfield, M. M.; Irani, R. R. *J. Am. Chem. Soc.* **1965**, *87*, 2815.
 (25) Okabayashi, H.; Okuyama, M.; Kitagawa, T.; Miyazawa, T. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 1075.
 (26) Schaufele, R. F.; Shimanouchi, T. *J. Chem. Phys.* **1967**, *47*, 3605.