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Fluorescent Probe Studies of Metal Salt Effects on Bile Salt Aggregation

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Abstract: The effects of metal salts on the aggregation behavior of two trihydroxy bile salts, anionic sodium taurocholate (NaTC) and zwitterionic 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), were studied with use of benzo[*k*]fluoranthene (BkF) as a fluorescent probe. Fluorescence spectra, intensity, and anisotropy, as well as relative scattered light intensity, were measured as a function of the bile salt concentration. In NaTC, the metal cations lowered the critical micelle concentration (cmc) and increased BkF fluorescence intensity and anisotropy, with trivalent cations showing larger effects than divalent and univalent cations. The electrolytes had much less effect on the aggregation behavior of CHAPSO and the fluorescence of BkF in CHAPSO.

Introduction

Micellar media have been used in chemical analysis to solubilize hydrophobic molecules in aqueous solution.[1-3] We have been exploring bile salt media as an alternative to conventional micelles to modify the luminescent properties of solubilized analytes. Although the aggregation behavior of bile salts has been well-studied,[4-6] considerable controversy still exists over values of critical micelle concentration (cmc) and aggregation number. Bile salt aggregation is quite different from that of conventional detergents; sharp, well-defined cmc's do not exist, and aggregation occurs over wide concentration ranges.[7]

The effects of Tb^{3+}, Eu^{3+}, and Al^{3+} on the fluorescence intensity of polycyclic aromatic hydrocarbons (PAH's) in aqueous solutions of sodium taurocholate (NaTC), which is an anionic trihydroxy bile salt, at 10 mM concentration have been recently studied;[8] the fluorescence of the more soluble PAH's was quenched by the heavy lanthanide ions, whereas fluorescence enhancement was observed for highly insoluble PAH's. Greatest fluorescence enhancement, almost 18-fold, was observed for benzo[*k*]fluoranthene (BkF).

In this paper, we describe further studies of the effects of metal salts on the fluorescence of solubilized BkF probe molecules in solutions of NaTC and 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), which is a zwitterionic analogue of NaTC. We selected BkF for these studies because it showed the greatest enhancement in the previous studies. The effects of the metal salts on the aggregation properties of NaTC and CHAPSO, as indicated by probe fluorescence and relative scattered light intensity in the absence of probe, are also described.

Experimental Section

The NaTC and CHAPSO compounds (ULTROL grade, >98%) were purchased from Calbiochem (La Jolla, CA), and benzo[*k*]fluoranthene (BkF) (98% purity) was from UltraScientific (North Kingstown, RI). The nitrate salts (>99.99%) of terbium(III), aluminum(III), gallium(III), indium(III), magnesium(II), calcium(II), copper(II), and sodium and the chloride salts (99.999%) of zinc(II) and sodium were purchased from Aldrich. All of the compounds were used as received. Absolute ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY) and deionized, HPLC-grade water were used for all solution preparations.

Stock solutions of the metal salts were prepared in water. Stock solutions of NaTC and CHAPSO, with or without metal, were prepared fresh daily by dissolution of the solid in metal stock solution or water. Lower concentrations of NaTC and CHAPSO, with or without metal, were prepared by serial dilution of the stock solutions with metal salt stock solution or water. Micellar solutions containing 2.0 μM BkF were prepared by evaporating the ethanol from an appropriate volume of a stock solution of BkF in absolute ethanol with a gentle stream of nitrogen, followed by dissolution of the remaining solid in the appropriate bile salt solution in a volumetric flask and sonication for at least 1 h. The solutions were not deoxygenated prior to measurement.

Fluorescence measurements were made with an SLM 48000S multifrequency, phase-modulation spectrofluorometer (SLM Instruments, Inc., Urbana, IL) with a 450-W xenon arc lamp source, excitation and emission monochromators for wavelength selection, and photomultiplier tube detectors. The sample chamber was maintained at 25.0 ± 0.1 °C. Fluorescence excitation and emission spectra were collected at 1-nm scanning intervals, in the "5-average" mode, where each measurement is the average of 5 samplings over a 1.5-s interval. Fluorescence anisotropy was measured at an excitation wavelength of 402 nm and an emission wavelength of 430 nm in the L-format, with use of the appropriate correction factors.[9]

Relative scattered light intensity was also measured with the SLM 48000S by setting the excitation and emission monochromators to 500 nm and detecting the scattered light emission at 90° relative to the excitation beam.

Results and Discussion

Effects of Metal Cations on NaTC Aggregation. Figure 1 shows the fluorescence intensity of BkF as a function of NaTC concentration in the absence of added metal salt and in the presence of the nitrate salts of Tb^{3+}, Al^{3+}, Mg^{2+}, and Na^{+}. The ionic strength contribution for each of the added salts was kept constant at 60 mM. Measurements were made at $\lambda_{ex} = 380$ nm and λ_{em} = (0-0) band maximum. Error bars indicating the standard deviation in each of the measured intensity points are plotted in Figure 1, but the magnitudes of most are smaller than the symbols used to plot the points. Above the cmc region (>10 mM NaTC), relative standard deviations (rsd's) were within 2% for Tb^{3+}, 4% for Al^{3+}, 4% for Mg^{2+}, 7% for Na^{+}, and 8% for NaTC alone. At lower concentrations (<10 mM NaTC), the rsd's were proportionately larger, but never greater than ±14% in all cases. The results clearly indicate that the metal cations lower the cmc for NaTC, resulting in increased solubilization of BkF. It also appears that BkF fluorescence is slightly more intense in the presence of the trivalent cations than in the other solutions, even at NaTC concentrations well above the critical region. This suggests that the trivalent cations may influence the structure of the NaTC aggregates.

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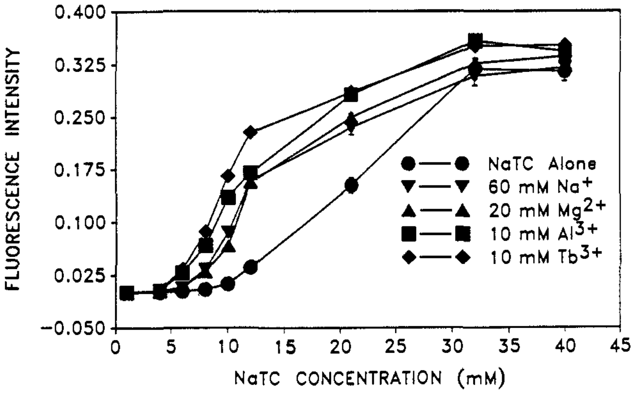

Figure 1. BkF fluorescence intensity, measured at λ_{ex} = 380 nm and λ_{em} = (0–0) band maximum vs NaTC. Error bars are plotted for all points.

Table I. Metal Cation Enhancement of BkF Fluorescence in NaTC Solutions[a]

[NaTC], mM	Na^+	Mg^{2+}	Al^{3+}	Tb^{3+}
1	1.1	1.1	1.1	1.2
4	1.4	1.3	2.9	2.9
6	3.1	3.7	12.5	15.0
8	7.0	6.1	13.6	17.6
10	6.9	5.2	10.6	14.8
12	4.4	4.2	4.6	6.0
21	1.5	1.6	1.8	1.8
32	1.0	1.0	1.1	1.1
40	1.0	1.0	1.1	1.1

[a] Metal cation concentrations of 60 mM Na^+, 20 mM Mg^{2+}, 10 mM Al^{3+}, and 10 mM Tb^{3+}; all nitrate salts were used.

In the absence of metal, the cmc (or "quasi-cmc"[7]) for NaTC occurs at approximately 12 mM. Although aggregation occurs at lower concentrations, our previous studies show that only the aggregates formed above the critical region are able to completely solubilize the probe molecules into interior, hydrophobic sites.[10] As shown in Figure 1, the addition of the metal salts enhances NaTC aggregation and sharpens the breaking points in the curves used to determine the cmc. The cmc of NaTC is lowered to ~4 mM in the presence of Tb^{3+}, ~5 mM in the presence of Al^{3+}, and ~6 mM in the presence of Mg^{2+} and Na^+. The BkF excitation and emission spectra correspondingly indicate the formation of hydrophobic microenvironments at the lower critical concentrations. Similar cmc behavior for NaTC micelles in borate buffer solutions containing 60 mM Na^+ has been demonstrated by spin-label measurements.[11]

Electrolytes have been known to lower the cmc of conventional ionic detergents by binding to the charged head groups and decreasing the electrical repulsion between them.[12] Similar effects have been observed for bile salts in the presence of high concentrations of Na^+ (>100 mM).[5,13,14] Lanthanide shift ^1H NMR studies have shown that Dy^{3+}, Ca^{2+}, and Na^+ bind with premicellar NaTC monomers at the anionic sulfonate group on the hydrophobic tail.[15] We have observed that the sulfonate group is the preferred binding site in micellar NaTC solutions as well, with the apparent association constant being 3–4 times greater than in premicellar NaTC solutions.[16]

The lower cmc for NaTC in the presence of Tb^{3+}, Al^{3+}, Mg^{2+}, and Na^+ can be attributed to binding of the metal cation to the sulfonate functional group. Electrostatic forces are important in the binding process, and cationic charge appears to be a primary factor since the trivalent cations show greater effects on the cmc than the divalent and univalent cations.

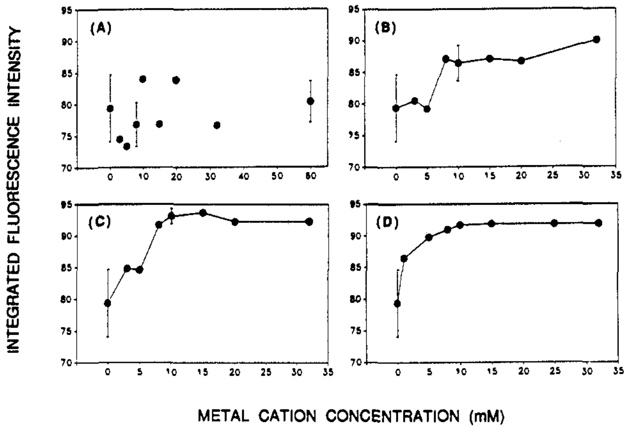

Figure 2. Integrated fluorescence intensity of BkF in 32 mM NaTC vs metal cation: (A) Na^+, (B) Mg^{2+}, (C) Al^{3+}, and (D) Tb^{3+}.

It is interesting that cationic charge is not the only factor influencing NaTC aggregation. As shown in Figure 1 and Table I, the intensities and intensity enhancements for BkF are greater with Tb^{3+} than with Al^{3+}. The different effects can be interpreted in terms of the acid–base properties of the metal cation–NaTC system with use of hard–soft acid–base (HSAB) theory.[17] Although Tb^{3+} and Al^{3+} are both hard Lewis acids, Tb^{3+} is the larger of the two (0.92 vs 0.51 Å)[18] and should therefore be softer and more polarizable. The sulfonate functional group is a soft, weak base, and consequently, the Tb^{3+}–NaTC interaction should be more favorable than the Al^{3+}–NaTC interaction.

Effects of Metal Cations on BkF Fluorescence Intensity in NaTC. The effects of Tb^{3+}, Al^{3+}, Mg^{2+}, and Na^+ on the fluorescence intensity of BkF in solutions of NaTC were shown in the cmc curves of Figure 1. The ratio of the fluorescence intensity of BkF in the presence of metal relative to the intensity in the absence of metal, calculated from the data of Figure 1, is shown in Table I for each metal.

Over the range of NaTC concentrations studied, greatest enhancement of BkF fluorescence intensity was observed in the region between the cmc in the absence of the metal salts (12 mM) and the cmc in the presence of metal salts. The enhancement is due, at least in part, to increased solubilization of the BkF probe as the cmc of NaTC is lowered.

Enhancements of BkF fluorescence were also measured for several divalent and univalent cations at 10 mM metal salt concentration in the critical region (10 mM NaTC). Nitrate salts of the cations were used, except for Zn^{2+}, where the chloride salt was used. Enhancements varied for different metal cations, including 4.7 for Mg^{2+}, 4.2 for Zn^{2+}, 3.8 for Ca^{2+}, 3.0 for Cu^{2+}, and 1.6 for Na^+. Addition of Ga^{3+} and In^{3+} to the NaTC solutions gave a precipitate (most likely heavy metal salts of taurocholate), and enhancements were not obtained.

Integrated fluorescence intensity of BkF in 32 mM NaTC as a function of metal cation concentration is shown for Na^+, Mg^{2+}, Al^{3+}, and Tb^{3+} in Figure 2. Since 32 mM is well above the cmc for NaTC, the BkF probe is well solubilized in all cases. Those metal cations that show greatest binding to NaTC have the greatest effect on total intensity and are also most effective in stabilizing the NaTC aggregates; relative standard deviations determined for the BkF intensity measurements are greatest for Na^+ (±7%) and smallest with Tb^{3+} (±1%). Clearly, the presence of 60 mM Na^+ has no significant effect on the aggregates formed at 32 mM NaTC. These observations are consistent with our earlier studies of energy transfer in 30 mM NaTC micellar media,

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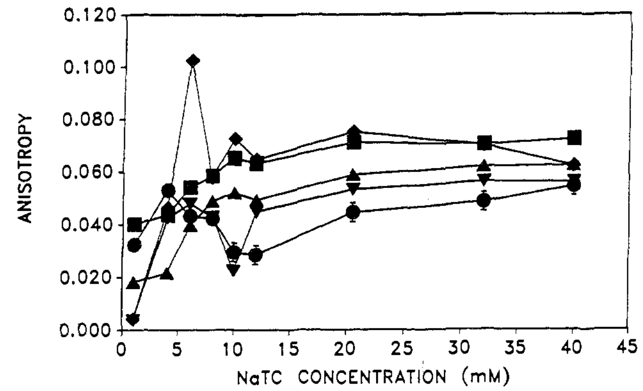

Figure 3. BkF anisotropy vs NaTC, measured at λ_{ex} = 402 nm and λ_{em} = 430 nm, in the presence of the following: ●, no added metal salt; ▼, 60 mM Na^+; ▲, 20 mM Mg^{2+}; ■, 10 mM Al^{3+}; and ◆, 10 mM Tb^{3+}.

which was shown to be relatively insensitive to NaCl concentrations as high as 1.0 M.[19]

Extrapolation of the linear portions of the curves in Figure 2 indicates a binding stoichiometry of 1 mol of di- or trivalent cation to 4 mol of taurocholate anion. A similar binding ratio was reported for the binding of Na^+ with the dihydroxy salt sodium taurodeoxycholate (NaTDC).[13,20] Approximately 1-2 Na^+ ions were shown to bind per NaTDC micelle, which was reported to have an aggregation number of 6; thus, there was an average of 1.5 Na^+ per 6 NaTDC monomers, or a 1:4 stoichiometry.

BkF Fluorescence Anisotropy in NaTC Solutions. Fluorescence anisotropy is a measure of the relative viscosity of the probe microenvironment and is commonly used to study freedom of rotation in micelles.[9,21] Figure 3 shows anisotropy as a function of NaTC concentration for BkF in NaTC alone as well as in the presence of Tb^{3+}, Al^{3+}, Mg^{2+}, and Na^+. Below the cmc, each curve is complicated by various maxima that may reflect an increased rigidity due to interaction of NaTC monomers or small aggregates with colloidal BkF probe aggregates.[10] Anisotropy levels off at a maximum value at NaTC concentrations above the cmc region, indicating a relatively constant microviscosity around the BkF probe molecule in the presence of a given metal cation. Uncertainty in the anisotropy data was determined to be approximately ±0.0035 anisotropy unit for BkF in NaTC at all concentrations above the cmc region. Precision for solutions with added metal cations is expected to be similar. Below the cmc, interactions between partially solubilized probe molecules occur and values of anisotropy become more variable; however, the various maxima shown in Figure 3 are consistently observed at low NaTC concentrations.[10] The maximum anisotropy depends on the metal cation present in solution: largest values were observed in the presence of Tb^{3+} and Al^{3+}, followed by decreasing values in the order Mg^{2+} > Na^+ > NaTC alone.

The anisotropy measurements indicate that the BkF probe is held more rigidly in the presence of the metal cations. Metal cation enhancement of NaTC aggregation is probably partly responsible for the increase in the observed anisotropy values; however, comparison of the anisotropy measurements with the fluorescence intensity curves of Figure 1 suggests that changes in the probe microenvironment are not simply a result of increased NaTC aggregation. While the cmc is similar in the Mg^{2+} and Na^+ salt solutions, the microviscosity of the probe environment in the presence of these two salts appears to be different; above the cmc, the trends in the anisotropy curves suggest that the microviscosity is greater with Mg^{2+} than with Na^+.

Relative Scattered Light Intensity of NaTC Solutions. Plots of relative scattered light intensity vs NaTC concentration in the absence of BkF probe (Figure 4) provide further evidence for enhanced aggregation in the presence of the metal cations. The

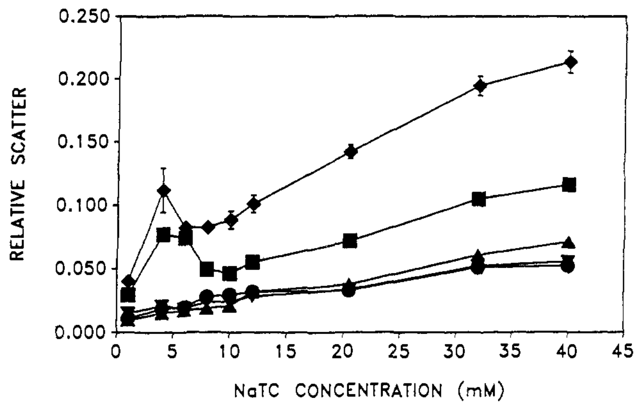

Figure 4. Relative scattered light intensity vs NaTC, in the presence of the following: ●, no added metal salt; ▼, 60 mM Na^+; ▲, 20 mM Mg^{2+}; ■, 10 mM Al^{3+}; and ◆, 10 mM Tb^{3+}.

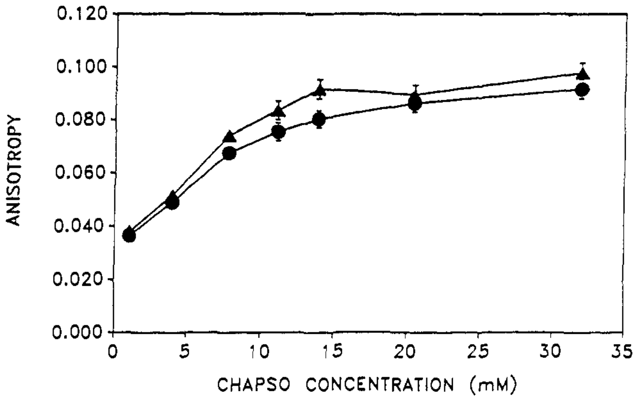

Figure 5. BkF anisotropy vs CHAPSO, measured at λ_{ex} = 402 nm and λ_{em} = 430 nm, in the presence of the following: ●, no added metal salt, and ▲, 10 mM Tb^{3+}.

relative standard deviations in the scattered light measurements with Tb^{3+} were in the range of ±1-8%. Similar rsd's are obtained for the other solutions.

Greatest scattering occurs in the presence of the trivalent cations, with divalent Mg^{2+} and univalent Na^+ showing much smaller increases in scattered light intensities relative to NaTC alone. Thus, the largest aggregates appear to be formed by NaTC in the presence of Tb^{3+} and the smallest aggregates in NaTC alone. Since the metal salts were all added at the same ionic strength, the differences in aggregate size must be due to differences between the metal-NaTC structures.

Effects of Metal Salt Anions on NaTC Aggregation. BkF fluorescence intensity and anisotropy were measured as a function of NaTC concentration in the presence of 60 mM NaCl and 60 mM $NaNO_3$ in order to determine the influence of the metal salt counterion on NaTC aggregation. Fluorescence intensities were similar in the presence of both the chloride and nitrate salts, and no significant difference in BkF anisotropy was indicated when NaCl rather than $NaNO_3$ was used. The results clearly indicate that the cmc effects reported for NaTC (Figure 1 and Table I) must be independent of the ionic strength contribution of the metal salt anions.

Effects of Tb^{3+} on CHAPSO Aggregation and BkF Fluorescence. While conventional anionic and cationic detergents exhibit great sensitivity to electrolyte effects,[12] zwitterionic detergents are much less affected.[22] In order to compare electrolyte effects for the bile salts, BkF fluorescence was studied in CHAPSO, a zwitterionic analogue of NaTC.

Fluorescence intensity of BkF as a function of CHAPSO concentration indicates no statistical difference in the absence or presence of Tb^{3+}, and the cmc occurs at ~8 mM in both cases. Thus, Tb^{3+} does not appear to enhance the aggregation of

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CHAPSO, which is in contrast to the pronounced effects of Tb^{3+} on NaTC aggregation. This is consistent with our lanthanide-induced ^1H NMR shift studies, which indicate that the association constant for Tb^{3+}–CHAPSO is much smaller than that for Tb^{3+}-NaTC.[16]

Fluorescence anisotropy of BkF in CHAPSO (Figure 5) showed a small increase in the presence of Tb^{3+} in the 13–14 mM CHAPSO region. As shown in the figure, uncertainty in the anisotropy values is in the range of ±0.003–0.004 at concentrations of CHAPSO above 10 mM. The increase in the viscosity of the probe microenvironment probably results from association of Tb^{3+} with CHAPSO micelles. Structural changes in the aggregate upon binding of the metal cation, which were indicated for NaTC, may be occurring for CHAPSO as well, despite the absence of cmc effects.

Conclusions

These studies indicate that metal cations can lower the cmc and increase the size of NaTC aggregates in aqueous solution. Trivalent cations show the greatest effect, while the smallest effects are observed with univalent Na^+. Other factors besides metal cation charge are also important: Tb^{3+} shows greater effects than Al^{3+}, suggesting that the larger and relatively softer acid Tb^{3+} is more strongly associated with the soft-base sulfonate groups on the hydrophilic NaTC tail than is the harder Al^{3+} ion. The microenvironment of the BkF probe is most viscous in the presence of Tb^{3+}, again suggesting a stronger binding and structural rigidity for NaTC aggregates with Tb^{3+} than with the other cations. The results suggest a 1:4 metal cation to NaTC binding stoichiometry above the cmc, which is consistent with other reports.[13,20] Scattered light intensity shows that the largest NaTC aggregates form in the presence of Tb^{3+}, followed by $Al^{3+} > Mg^{2+} > Na^+$, NaTC alone. The counterion of the metal salt appears to have no effect on the cmc behavior of NaTC.

In contrast to NaTC, CHAPSO is relatively insensitive to the metal cations. No significant cmc effects are observed, although the viscosity of the BkF microenvironment is slightly higher in the presence of Tb^{3+}. The absence of strong metal cation effects on CHAPSO aggregation is consistent with the nonionic behavior reported for zwitterionic bile salt derivatives.[23,24]

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Photochemistry of Tetraarylborate Salts (Ar_4B^-): Formation of 2,5,7,7-Tetraphenyl-7-boratabicyclo[4.1.0]hepta-2,4-diene (a Boratanorcaradiene) by Irradiation of (*p*-Biphenylyl)triphenyl Borate

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Abstract: Irradiation of tetramethylammonium (*p*-biphenylyl)triphenylborate with UV light gives three isomeric boratanorcaradiene anions by a process analogous to the di-π-methane rearrangement of hydrocarbons. One of the boratanorcaradienes, tetramethylammonium 2,5,7,7-tetraphenyl-7-boratabicyclo[4.1.0]hepta-2,4-diene (**1**), was isolated as a deep red crystalline solid, and its structure was determined by X-ray crystallography. Borate **1** is a fluxional compound at room temperature; an electrocyclic rearrangement exchanges the two boron-bound phenyl groups. A mechanism for the photochemical rearrangement of (*p*-biphenylyl)triphenylborate is proposed, and the structural, chemical, and physical properties of **1** are discussed.

Williams and co-workers were the first to report a systematic study of the photochemistry of tetraarylborates.[1] They found that irradiation of sodium tetraphenylborate in oxygen-free aqueous solution produces 1-phenyl-1,4-cyclohexadiene in nearly quantitative yield. Under these conditions, the ultimate boron-containing product is the sodium salt of diphenylborinic acid. The mechanism of this unusual rearrangement was investigated by means of labeling experiments. These studies showed that the new carbon–carbon bond is formed intramolecularly between carbon atoms originally bound to boron. Photolysis of deuterium-labeled potassium dimesityldiphenylborate in water-containing dimethoxyethane (DME) solution gives a triarylborane. The substitution pattern observed in this product led Williams and co-workers to propose a reaction mechanism that proceeds through the formation of the bicyclic borate-containing intermediate(s) shown in Scheme I.

Scheme I

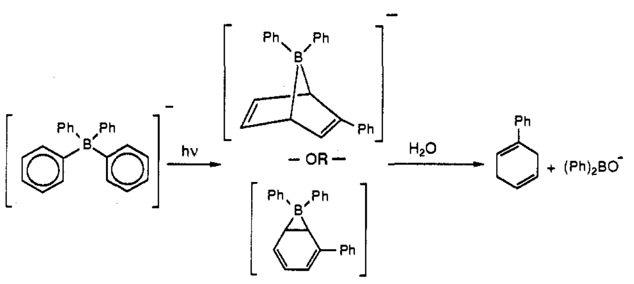
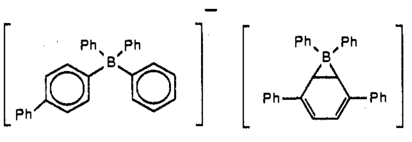


Chart I



(p-Biphenylyl)triphenylborate

Borate 1

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