fluent from conditioned BaP columns. Appropriate corrections were made for their emission and/or absorbance contributions to those of the BaP.

Registry No. Anthracene, 120-12-7; benz[a]anthracene, 56-55-3; benzo[a]pyrene, 50-32-8; water, 7732-18-5.

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RECEIVED for review March 11, 1983. Accepted June 27, 1983. Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Extraction and Fluorometric Determination of Organotin Compounds with Morin

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Morin produces a green fluorescence with various organotin compounds in organic solvent. The reagent is especially sensitive to dialkyltin compounds. The excitation and emission spectra show peaks at ca. 415 nm and ca. 495 nm, respectively, for each alkyltin–Morin complex and at ca. 405 nm and ca. 520 nm for the triphenyltin–Morin complex. The maximum fluorescence requires a ratio of 3 to 9 mol of Morin for 1 mol of dialkyl- and triphenyltin and a 6–12 to 1 molar ratio for trialkyltin. Detection limits are 1×10^{-9} M for dialkyltins, 1×10^{-7} M for monoalkyltin, 5×10^{-7} M for trialkyltins, and 1×10^{-7} M for triphenyltin. The fluorometric procedure can be used for the determination of individual organotin, especially a dialkyltin compound in environmental and biological samples. Recoveries of organotins added to various tissues at the 1.0–100 nmol level ranged from 91.0 to 99.7% depending upon the organotin species.

Organotin compounds have been widely used not only as plastic stabilizers or catalytic agents in industry but also as biocidal compounds, i.e., bactericides, fungicides, anthelminthics, and insecticides, in agriculture and medicine and antifouling agents for ships. Recently, deep concern has been expressed concerning the safety of these compounds in the environmental cycle. Consequently, the development of better analytical methods has been needed for the determination of the various organotin compounds present in environmental and biological samples.

In previous reports, we described gas chromatographic methods for the simultaneous determination of tetraalkyl- (*1*) and trialkyltins (*2*) in various kinds of biological material. Unfortunately, the gas chromatographic determination of dialkyltin homologues was not easy because of their adsorption and decomposition during chromatography.

During the course of this study, we have also investigated the possibility of using spectrofluorometry in order to develop a simple and rapid method which can be used for the determination of organotin compounds themselves. Previously, a few fluorometric methods have been recommended for organotin, but most of them have been confined to triphenyltin derivatives. Coyle and White showed that 3-hydroxyflavone could be used to determine submicrogram amounts of inorganic tin (*3*), and then Vernon used the reagent to determine triphenyltin compounds in potatoes (*4*). On the basis of this procedure, Blunden and Chapman spectrofluorometrically determined triphenyltin compounds in water (*5*). Further, they showed that chloride ions quenched the fluorescence but that on shaking with aqueous sodium acetate solution a stable complex was formed, although the instability to light of the triphenyltin chloride–3-hydroxyflavone complex had been initially pointed out by Aldridge and Cremer (*6*).

The present study proposed that Morin (2′,3,4′,5,7-pentahydroxyflavone) can be used as a fluorescence reagent for organotin, especially dialkyltin compounds. Although quer-

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cetin and 3-hydroxyflavone are similar to Morin in structure, they are unsuitable because of their sensitivity and instability.

EXPERIMENTAL SECTION

Reagents. Trimethyltin chloride (Me_3SnCl), triethyltin chloride (Et_3SnCl), tripropyltin chloride (Pr_3SnCl), tributyltin chloride (Bu_3SnCl), triphenyltin chloride (Ph_3SnCl), dioctyltin dichloride (Oc_2SnCl_2) and diphenyltin dichloride (Ph_2SnCl_2) were obtained from the Aldrich Chemical Co., Inc. (Milwaukee, WI) Dimethyltin dichloride (Me_2SnCl_2), diethyltin dichloride (Et_2SnCl_2), dipropyltin dichloride (Pr_2SnCl_2), dibutyltin dichloride (Bu_2SnCl_2), monomethyltin trichloride ($MeSnCl_3$), monoethyltin trichloride ($EtSnCl_3$), monopropyltin trichloride ($PrSnCl_3$), monobutyltin trichloride ($BuSnCl_3$), and monophenyltin trichloride ($PhSnCl_3$) were purchased from K & K Laboratories (Plainview, NY). Triethyllead chloride, diethyllead dichloride, methylmercury chloride, ethylmercury chloride, and dimethyl arsenide were obtained from the Alfa Division, Ventron Corp. (Danvers, MA). The purity of these compounds was not less than 98%. Morin (2′,3,4′,5,7-pentahydroxyflavone) was obtained from Wako Pure Chemical Industries, Osaka, Japan. Other regents included special grade organic solvents such as *n*-hexane, ethyl acetate, and ethanol (each provided by Wako Pure Chemicals Co., Tokyo, Japan). Silica gel plates were purchased from Brinkmann (Polygram Sil-G). A stock solution of each organotin was prepared by dissolving 1 mmol of organotin in 100 mL of *n*-hexane. Me_2SnCl_2 solution was similarly prepared in 95% ethyl alcohol. These stock solutions proved to be stable for 4 to 5 weeks in flasks that were covered with black paper. A reagent solution of Morin (0.005%) was made in 95% ethyl alcohol; the 0.005% solution of Morin contains about 0.15 μmol of Morin/mL.

Apparatus. All fluorescence measurements were made with a Shimadzu Model RF-500 fluorescence spectrophotometer (Japan).

General Procedure. To 3 mL of *n*-hexane solution containing 0.001–1.0 nmol/mL of dialkyltin or 1.0–100 nmol/mL of monoalkyltin or 1.0–1000 nmol/mL of trialkyltin or 1.0–100 nmol/mL of triphenyltin, add 0.5 mL of a 0.005% solution of Morin in absolute ethanol. After mixing thoroughly, measure the fluorescence intensity at ca. 495 nm, using an excitation wavelength of ca. 415 nm. A reagent blank should be run concurrently.

RESULTS AND DISCUSSION

Formation of the Fluorescent Organotin–Morin Complex. Morin alone had very little fluorescence. However, the reagent produced a strong green fluorescence with various organotin compounds in organic solvent. Although the fluorescent intensity varied only slightly depending upon the organosolvent species, i.e., benzene, toluene, hexane, ether, ethyl acetate, chloroform, and ethanol, *n*-hexane was the most satisfactory as a medium for this test because of the greater solubility and stability of the complexes. Ethanol was suitable for some methyltin compounds, however, because of their polarity. The excitation and emission spectra for each alkyltin–Morin complex show peaks at ca. 415 nm and ca. 495 nm, respectively. Similarly both spectra for triphenyltin–Morin complex show peaks at ca. 405 nm and ca. 520 nm (Figure 1). The formation of the organotin–Morin complexes progressed very rapidly at room temperature and the fluorescence intensities remained constant for hours. Particularly, the dialkyltin complexes were stable over a number of hours. Less than 10% of acetic acid in the reaction solution had no effect on the fluorescence intensity readings, although the presence of hydrochloric and sulfuric acids resulted in lower fluorescent readings. A quenching of the fluorescence by chloride ions and the instability of the complexes to light were not found under the conditions of the test. For a maximum fluorescence, the Morin concentration should be at least in a 3–9 to 1 molar ratio to each dialkyl- and triphenyltin, and in a 6–12 to 1 molar ratio to each trialkyltin (Figure 2).

Specificity of Dialkyltin Compounds. Dialkyltin compounds produced a much stronger fluorescence than other

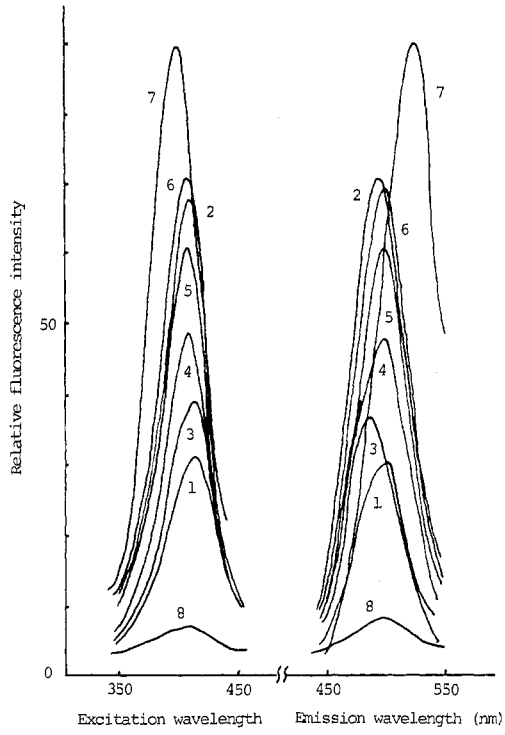

Figure 1. Excitation and emission spectra of each organotin (1×10^{-6} M)–Morin complex drawn on a relative scale intensity, reduced by the value in parentheses: 1, Me_2Sn (1/60); 2, Et_2Sn (1/60); 3, Bu_2Sn (1/60); 4, Et_3Sn (1/2); 5, Pr_3Sn (1/2); 6, Bu_3Sn (1); 7, Ph_3Sn (1/6); 8, Morin alone (1).

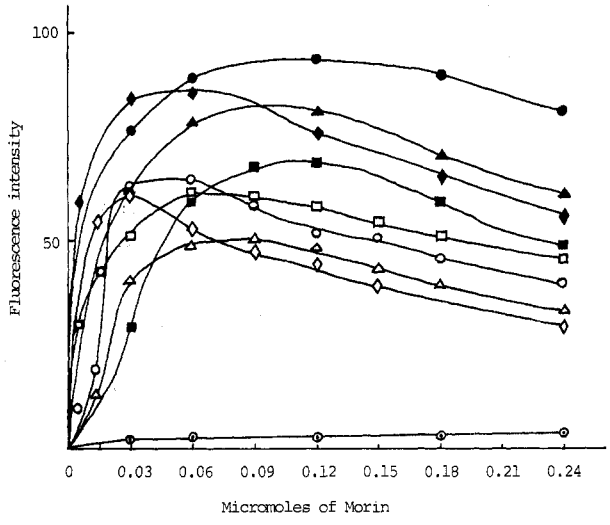

Figure 2. Variation of fluorescence intensity of organotin–Morin complexes with varying amounts of Morin for 0.01 μmol of each organotin in 3.5 mL of reaction solution: (○) Me_2SnCl_2; (△) Et_2SnCl_2; (□) Bu_2SnCl_2; (◇) $BuSnCl_3$; (●) Pr_3SnCl; (▲) Et_3SnCl; (■) Bu_3SnCl; (◆) Ph_3SnCl; ⊙, reagent blank

organotin compounds with Morin. For 1×10^{-6} M of each organotin compound the relative fluorescence intensity was 10.2 for $BuSnCl_3$, 42.5 for Me_2SnCl_2, 99.2 for Et_2SnCl_2, 99.4 for Pr_2SnCl_2, 51.7 for Bu_2SnCl_2, 2.2 for Et_3SnCl, 2.8 for Pr_3SnCl, 1.6 for Bu_3SnCl, and 12.7 for Ph_3SnCl at the same instrument setting (Figure 1). The concentration detection limits for dialkyltin compounds were in the 10^{-8} to 10^{-9} M range, at which other organotin compounds could not be detected. This large difference in fluorescent intensities among different organotin–Morin complexes appears to be dependent on the valence state of the metal.

Organolead compounds such as di- and triethyllead and organosilane compounds such as di- and monomethylsilane

Table I. Typical Conditions for the Determination of Organotin Compounds[a]

compound	approximate wavelength, nm: excitation	approximate wavelength, nm: emission	concn range, nmol/mL	detection limit, nmol/mL
$BuSnCl_3$	420	495	1.0-100	0.1
Me_2SnCl_2	420	500	0.01-1.0	0.001
Et_2SnCl_2	415	490	0.01-1.0	0.001
Pr_2SnCl_2	420	490	0.01-1.0	0.001
Bu_2SnCl_2	420	490	0.01-1.0	0.001
Et_3SnCl	415	495	1.0-1000	0.5
Pr_3SnCl	415	495	1.0-1000	0.5
Bu_3SnCl	415	495	1.0-1000	0.5
Ph_3SnCl	405	520	1.0-100	0.1

[a] 3 mL of organotin solution with 0.5 mL of 0.005% solution of Morin in ethanol.

did not interfere at 1×10^{-3} M under the conditions used for the determination of organotin. Other organometal compounds such as dimethyl arsenide and methyl- and ethylmercury chlorides did not fluoresce at all. Although aluminum(III), zinc(II), tin(IV), magnesium(II), and cadmium(II) produced a strong fluorescence, and manganese(II), selenium(IV), and mercury(II) produced a very weak fluorescence with Morin in water solutions, these inorganometal compounds did not interfere at 1×10^{-3} M under the conditions of the organotin determination. Arsenic(III or V), lead(II), chromium(III or VI), copper(II), and iron(II or III) did not fluoresce even in water solutions.

Calibration Curves, Precision, and Detection Limits. Calibration curves for monobutyltin, dimethyltin, diethyltin, dipropyltin, dibutyltin, triethyltin, tripropyltin, tributyltin, and triphenyltin compounds obtained by using the procedure given above were linear within the concentration ranges studied as shown in Table I. The detection limits reached 1×10^{-9} M for dialkyltin compounds, 1×10^{-7} M for monoalkyltin compounds, 5×10^{-7} M for trialkyltin compounds, and 1×10^{-7} for triphenyltin compounds (Table I).

Precision of the method averaged ±1.2% relative over the range of each calibration curve. Reproducibility of the procedure for the replicate analyses of standard samples was found to be an average of ±1.5% relative for samples containing 0.01–1.0 nmol of dialkyltin, ±2.1% relative for 10–500 nmol of trialkyltin, and ±2.5% relative for 1.0–100 nmol of triphenyltin compounds.

Isolation of Organotin Compound from Various Samples. In general, organotin compounds in environmental and biological samples were isolated by extraction with *n*-hexane or ethyl acetate. An aqueous sample containing 0.01–1.0 nmol of dialkyltin, 1.0–100 nmol of monoalkyltin, 0.01–1.0 μmol of trialkyltin, or 1.0 to 100 nmol of triphenyltin compound in up to 100 mL of solution was directly shaken with 10 mL of *n*-hexane in a separatory funnel for 10 min, and the two layers were allowed to separate. A tissue sample (2) weighing between 1.0 and 5.0 g (wet weight) was first homogenized in 10 mL of normal saline solution. Hydrochloric acid (8 mL) was carefully added to the homogenate, and the contents were mixed thoroughly. After the mixture was allowed to stand for 5 min, ethyl acetate (20 mL) and sodium chloride (2 g) were further added and shaken for 10 min. The extraction procedure was repeated twice; the recovery from double extractions was about 98%. The combined ethyl acetate layers were then concentrated under reduced pressure at about 20 °C to 0.5–1.0 mL. Loss of organotin compounds through the concentration procedure was not seen. *n*-Hexane (10 mL) was added to the concentrated solution and the precipitate produced was removed by centrifugation. By this replacement of the extraction solution with *n*-hexane, ethyl acetate soluble and *n*-hexane insoluble substances are eliminated.

Each *n*-hexane layer was directly subjected to the fluorometric procedure for the determination of individual organotin compounds after being concentrated to a suitable volume and being made up to 3 mL with *n*-hexane finally.

For the analysis of organotin mixtures, the individual organotin compound must be preseparated from each other by an appropriate chromatography technique for subsequent determination by the fluorometric procedure.

Recovery of Organotin Compound from Aqueous and Tissue Samples. The application of the procedure to the determination of individual organotin compounds in aqueous and tissue samples was studied by conducting recovery tests on both human urine and rat tissues, to which were added 10 nmol of monoalkyltin, 1.0 nmol of dialkyltin, 100 nmol of trialkyltin, or 10 nmol of triphenyltin, separately (Table II). Average recoveries of monoalkyltin, dialkyltin, trialkyltin, and triphenyltin compounds ranged from 91.0 to 96.5%, 96.7 to 99.3%, 91.3 to 94.7%, and 93.6 to 97.5% for human urine samples and from 92.0 to 95.9%, 97.5 to 99.7%, 93.4 to 97.1%, and 92.8 to 96.9% for rat tissues, respectively. No difference in recoveries was seen among different organs.

Analysis of Dibutyltin Compound in Rats after Oral Administration. Tissue samples (1–5 g) from weanling rats (weighing 40–50 g) fed a diet containing 100 ppm of dibutyltin dichloride for 1 week and after that period a normal diet for 1 week were analyzed for dibutyltin content to demonstrate application of the procedure (Figure 3). Dibutyltin was distributed to every organ, although the levels in the liver and kidney were much higher than those in other organs during the administration period of dibutyltin. However, the concentration of dibutyltin in each organ except the brain was rapidly decreased not necessarily linearly with time by feeding of normal diet for 1 week after the dibutyltin treatment period. Particularly, the severe reduction of thymus weight (40% of control) which was observed during the dibutyltin treatment period was rehabilitated with the decrease of dibutyltin content in the thymus.

Application to Spot Test. The fluorometric procedure can be used for a spot test on silica gel plates. The procedure is as follows.

Table II. Recovery of Organotin Compound Added to Human Urine and Rat Tissues

	% recovery[a]							
	$BuSnCl_3$ (10 nmol)[b]	Et_2SnCl_2 (1.0 nmol)	Pr_2SnCl_2 (1.0 nmol)	Bu_2SnCl_2 (1.0 nmol)	Et_3SnCl (100 nmol)	Pr_3SnCl (100 nmol)	Bu_3SnCl (100 nmol)	Ph_3SnCl (10 nmol)
human urine	93.2 ± 0.6	96.7 ± 0.3	97.1 ± 0.7	99.3 ± 0.4	93.5 ± 0.8	94.7 ± 0.8	91.3 ± 0.6	94.8 ± 0.8
rat organ								
liver	93.7 ± 0.5	98.9 ± 0.7	98.3 ± 0.5	99.1 ± 0.7	94.7 ± 0.7	96.1 ± 0.6	93.2 ± 0.9	94.6 ± 0.9
kidney	93.8 ± 0.6	98.6 ± 0.7	98.2 ± 0.8	98.2 ± 0.6	94.2 ± 0.6	95.9 ± 0.9	94.3 ± 0.8	93.9 ± 0.7
spleen	94.2 ± 0.6	99.0 ± 0.5	99.1 ± 0.5	98.9 ± 0.6	95.2 ± 0.7	97.0 ± 0.9	93.8 ± 0.6	94.7 ± 0.6
brain	92.0 ± 0.7	97.8 ± 0.9	97.5 ± 0.9	98.6 ± 0.6	92.9 ± 0.5	93.4 ± 0.8	93.4 ± 1.0	92.8 ± 0.9
thymus	95.9 ± 0.5	99.2 ± 0.6	98.9 ± 0.4	99.7 ± 0.5	95.6 ± 0.8	97.1 ± 0.8	95.2 ± 0.8	96.9 ± 0.7

[a] Data are average and average deviation of five replicates. [b] Amount of compound added.

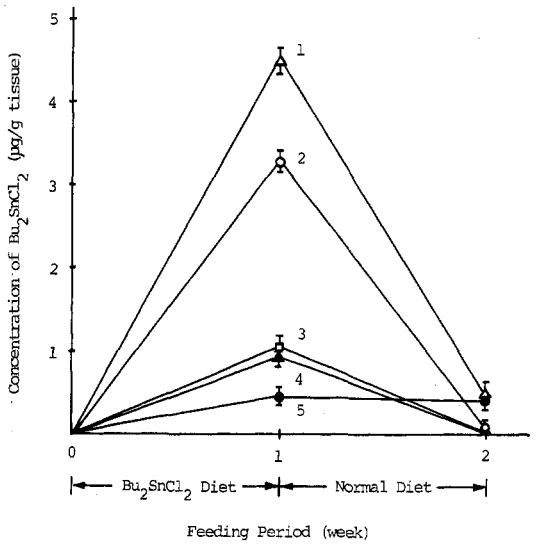

Figure 3. Distribution patterns of dialkyltin compounds in rat organs after oral administration: (1) kidney, (2) liver, (3) spleen, (4) thymus, (5) brain.

A silica gel G plate is previously sprayed with 0.1% Morin in ethanol and dried. A drop of dialkyltin solution dropped on the plate produces a bright green fluorescence when exposed to 360-nm radiation. The test is sensitive to 0.001 nmol of dialkyltin in 1 mL.

By these experiments, it has been confirmed that the fluorometric procedure can be used for the determination of individual organotin, especially a dialkyltin compound in the quantitative scheme of analysis. The applications of this procedure to high-performance liquid chromatography (7) and thin-layer chromatography for the simultaneous determination of organotin compounds are now in progress and will be published elsewhere.

Registry No. Morin, 480-16-0; Pr_3SnCl, 2279-76-7; Bu_3SnCl, 1461-22-9; Ph_3SnCl, 639-58-7; Et_2SnCl_2, 866-55-7; Pr_2SnCl_2, 867-36-7; Bu_2SnCl_2, 683-18-1; $BuSnCl_3$, 1118-46-3; Et_3SnCl, 994-31-0; Me_2SnCl_2, 753-73-1.

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Fluorescence Quenching Method for Determination of Two or Three Components in Solution

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The Stern–Volmer equation is extended for cases of two or more dynamic quenchers being present in one solution. It is shown that the determination of *n* quenchers requires *n* indicators whose Stern–Volmer constants have to be different. The concentration of *n* quenchers can then be computed by solving an $n \times n$ matrix. The validity of the equations is demonstrated by the precise fluorimetric microdetermination of chloride and bromide in an organic material after combustion and by the determination of chloride, bromide, and iodide in a synthetic mixture. The method is expected to be generally applicable for the quantitation of a variety of dynamic quenchers (ions as well as neutral molecules), provided they act independently. Specific indicators are no longer necessary.

The Stern–Volmer equation describes one of the fundamental processes in photophysics, namely, dynamic quenching. Their equation relates the ratio of the fluorescence intensities in the absence and presence of a quencher (F°/F) to the quencher concentration [Q] as follows:

$$F^\circ/F = 1 + K[Q] \qquad (1)$$

The constant can be shown to be the product of two components (the decay time and the bimolecular rate constant of the quenching process), but this refinement is without significance for this work.

On the basis of this relation we have developed (*1*) a fluorimetric method for the microassay of halides in organic matter after combustion. An interesting feature is its increasing sensitivity on going from chloride to iodide. Further, the equation allows the determination of various photophysical parameters (*2*) and—of particular interest in biosciences—the estimation of tertiary and quarternary structures of polymers such as proteins (*3*).

Lehrer (*4*) has presented a modified Stern–Volmer equation for cases where *one dynamic quencher* can interact with several fluorophores in different environments. For instance, the tryptophans in a protein can be surrounded by amino acids of different polarity and spatial requirements, which results in different quenching of each tryptophan.

Here we report a modification of the Stern–Volmer equation which, unlike the one given by Lehrer (*4*), is valid for cases where *one fluorophore* interacts with *several dynamic quenchers*. Its analytical application allows the determination of two or more components in one solution.

THEORY

If the fluorescence of a fluorophore is quenched by several components present in concentrations [Q_n], their contribution to the overall quenching process is taken into account by adding additional terms to the Stern–Volmer equation (eq 2).

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