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TECHNICAL NOTE

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Determination of Luminescent Europium β-Diketonates Used as Tracers for Shadowing Pursuits

ABSTRACT: Markers with red luminescence under ultraviolet light were used as tracers for shadowing pursuits in a variety of criminal cases. The luminescent markers consisted of a mixture of 1% europium β-diketonates in Vaseline as the carrier. The visual detection limit under ultraviolet light was 1–100 ppm. Six types of europium β-diketonates were extracted with acetonitrile and promptly identified using both fluorescence spectrophotometry and electrospray ionization mass spectrometry at the detection limits of 10–100 ppb. Vaseline was readily analyzed by gas chromatography. The markers were scientifically identified for criminal proof in the field of forensic science. Three examples of the use of luminescent markers are described.

KEYWORDS: forensic science, electrospray ionization mass spectrometry, europium β-diketonates, fluorescence spectrophotometry, luminescent marker, Vaseline

In Japan, fluorescent compounds such as anthracene have been conventionally used for shadowing pursuits by emitting pale fluorescence under ultraviolet light (1,2). However, their applications were limited to tagging items that do not contain optical whitening agents since their fluorescence is similar to that of the tagging compounds. Recently, the luminescent markers using europium β-diketonates were developed as tracers for shadowing the pursuit of suspects in the field of forensic science. Europium β-diketonates are used as red light emitters and markers in various fields (3–8) and have a sharp luminescent band at wavelength of about 610 nm under ultraviolet light. These compounds have the advantage of being identified easily under ultraviolet light and quantitatively analyzed by fluorescence (FL) spectrophotometry (3–6,8,9). However, since the FL spectrophotometry analysis shows similar emission spectra with featureless and broad excitation spectra for the compounds, the *qualitative* analysis contains large errors. Furthermore, europium β-diketonates were identified using electron ionization and chemical ionization mass spectrometries (10–13), which require solvent removal after extracting the samples and in which the nonvolatile europium complexes can be hardly detected. Although X-ray analysis (14) is used to analyze pure crystal samples, a trace of the samples in a matrix was not able to be determined. In this study, six types of the tris forms of europium β-diketonates mixed in Vaseline as a matrix were prepared as sticky jelly samples. Vaseline was used as a carrier in order to increase their adhesiveness as markers. The extracts from the luminescent markers with acetonitrile were promptly and sensitively analyzed by both FL spectrophotometry and electrospray ionization mass spectrometry (15,16). Examples of the luminescent markers are also described.

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Methods

Chemicals and Luminescent Markers

Europium (III) chloride hexahydrate (CP 99.9%), benzoyltrifluoroacetone (CP 98%), pivaloyltrifluoroacetone (CP 90%), thenoyltrifluoroacetone (CP 98%), 28% NH_3 aqueous solution, methanol (HPLC grade), acetonitrile (HPLC grade), distilled water (HPLC grade), Vaseline (EP), and hexane (GR) were purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). 4,4,4-Trifluoro-1-(2-naphthyl)-1,3-butanedione was from Kanto Chemical Co., Inc. (Tokyo, Japan). 2-Furoyltrifluoroacetone (CP 99%) and 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione (CP 95%) were from Tokyo Kasei Kogyo Co. Ltd (Tokyo, Japan).

Six types of europium β-diketonates with Vaseline were prepared by the conventional method (17). Six tris forms were prepared as powders of the europium β-diketonates coordinate-bonded with three molecules to one europium atom: pivaloyltrifluoroacetone (PTA), benzoyltrifluoroacetone (BFA), furoyltrifluoroacetone (FTA), 4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione (thenoyltrifluoroacetone, TTA), 4,4,4-trifluoro-1-(2-naphthyl)-1,3-butanedione (naphtyltrifluoroacetone, TFNB), or 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione (heptafluorobutanoylpivaloylmethane, DMHFO), such as shown in Fig. 1. The solutions of the europium β-diketonates in approximately 1 mL of chloroform were added to Vaseline (1%/99%), stirred in a water

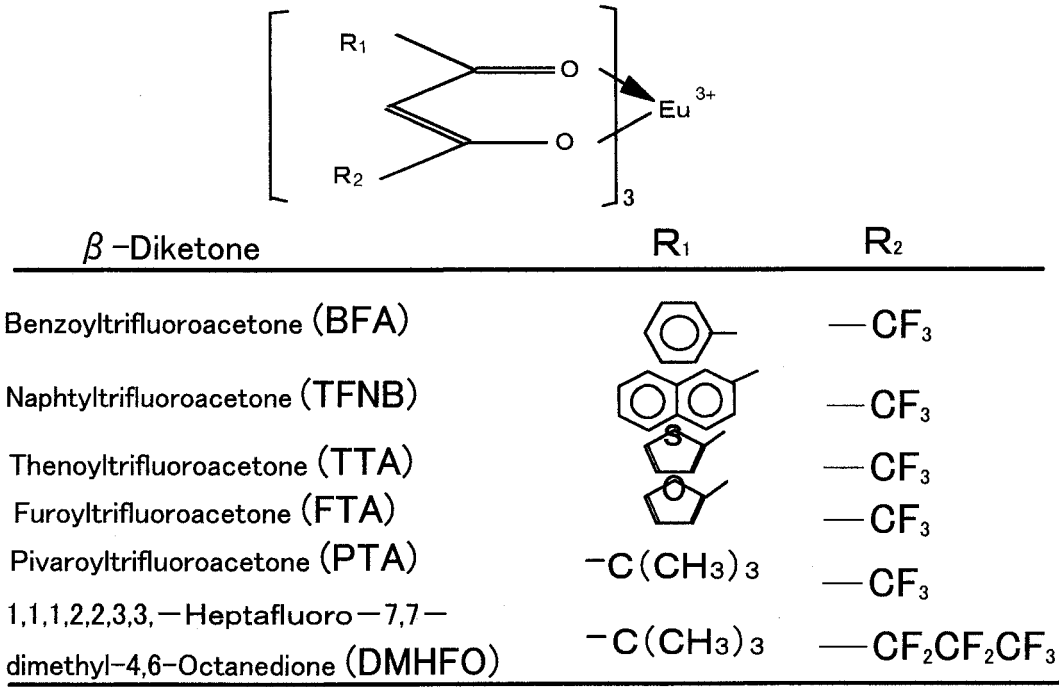

FIG. 1—*Molecular structures of six types of europium β-diketonates.*

bath at 70°C, and then gradually cooled to room temperature. The six types of europium β-diketonates with Vaseline were used as the samples in this study.

Apparatus for Analyses of Luminescent Markers

A mineral light lamp, model UVGL-58 (UVP Inc.), with multi-ultraviolet (UV) bands at the wavelengths of 254 and 366 nm and a chromato-VUE lamp, model UVM-57 (UVP Inc.), with a UV band at 302 nm were used for observing the emissions of the europium complexes under ultraviolet light.

The emissions of each europium complex in both acetonitrile and Vaseline were measured using a Hitachi F-4500 fluorescence spectrophotometer equipped with a 150-W CW xenon lamp excitation source. The band passes for the excitation and emission monochromators were set to 5 nm. Each emission spectrum of the europium complexes with Vaseline excited at 365, 302, and 254 nm was analyzed using the fluorescence spectrophotometer in order to study the visual observation results of the marker under ultraviolet light. The europium complexes with Vaseline (1%) were measured as they were put in test tubes. In this case, a test tube had no influence on the emission. The acetonitrile solutions were placed in a 1-cm path-length quartz cell.

A Hitachi U-6500 model microscope spectrometer with a fluorescence detector at the excitation wavelength of about 365 nm (330 to 380 nm) was used for the degradation measurement of the emission intensity of the $^5D_0 \rightarrow {}^7F_2$ band of the europium complexes in the markers after application. The emission spectra of the samples on a glass microscope slide were measured by the microscope spectrometer. The light source is a 100-W high pressure mercury lamp. The microscope with the object lens having a magnifying power of 10 and a 0.3 mm pin hall were used for the measurement.

GC-FID analyses for the detection of Vaseline were performed on a Hewlett- Packard 5890 series II gas chromatograph (Hewlett-Packard, CA) equipped with a flame ionization detector and a split/splitless injector in the split mode. The split ratio was 50:1. The chromatographic conditions were as follows: column type—J&W Scientific DB-5MS fused silica capillary column, 6 m × 0.25 mm × 0.25 μm film thickness; carrier gas—He; flow rate—1.0 mL/min; injector temperature—320°C; detector temperature—300°C; temperature of the GC column was programmed as follows: initial temperature 50°C, ramp 10°C/min to 320°C, final time 10 min. The peak measurement and integration were performed by a chromatographic recorder HP3396 Series II Integrator (Hewlett-Packard, CA).

Infusion electrospray ionization mass spectrometry (ESI-MS) analysis of the europium complexes was carried out using a micromass ZMD in the Waters Alliance systems. Acetonitrile was chosen as the solvent and the solutions were prepared in the concentration range of 10 ppb–10 ppm. A Harvard Apparatus 11 syringe pump delivered the solutions at 30 μL/min to the ESI probe connected to a 100-μm-i.d. fused-silica capillary (phenylmethyl-deactivated GC guard column, Restek Corp., Bellefonte, PA). The samples were injected using a 250 μL syringe (Hamilton Co., Reno, NV). Nitrogen was introduced into the capillary region at the flow rate of 420 L/h. The ESI conditions were as follows: electrospray capillary, 4.0 kV; cone, 60 V; Extractor, 2 V; Rf lens, 0.2 V; source block temperature, 130°C; desolvation temperature 400°C; LM resolution, 14.7; HM resolution, 14.7; ion energy, 0.5 V; multiplier, 650 V. The mass spectra were determined in the negative-ion mode with a mass to charge range of 100–1500.

Solvent for Extraction of Luminescent Markers

One mg of the europium complexes (1% of concentration) with Vaseline was applied on one side of the filter papers (a 5 × 20 mm oblong), each paper was put on the bottom of the test tubes, and then 1 mL of acetonitrile was added to the test tubes. The solubility of the Vaseline in acetonitrile was determined by the gas chromatography detection intensity of *n*-tricosane detected as the strongest peak in

the Vaseline and that of the europium complex in the solvent was done with the intensity of the $^5D_0 \rightarrow {}^7F_2$ emission band detected at about 610 nm by fluorescence spectrophotometry, after supersonic washing for 0, 1, 3, 5, and 10 min using an ultrasonic Branson Model B12 cleaner (Branson Cleaning Equipment Co., Danbury, CT). It is sufficient to efficiently extract the europium complexes and Vaseline using a supersonic washing time of 5 min.

Analysis of Luminescent Markers

One mg of the luminescent markers applied on the filter papers was used as the sample. After the compounds applied on the papers were visually confirmed under the ultraviolet light at about 365 nm, the extractives with acetonitrile were analyzed as the samples. The emission and excitation spectra of the europium complexes in the solution samples were measured by fluorescence spectrometry. Parts of the solution samples were analyzed by GC in order to obtain the chromatogram of Vaseline. The remaining solution samples were analyzed by electrospray ionization mass spectrometry in order to identify the europium complexes.

Degradation of Luminescent Markers after Application

After about 1 mg of the luminescent markers were applied on one side of the filter papers (5 × 5 mm square), the papers were stored in three places: by a sunny window, in a shady drawer, and in water (10 mL) in a beaker in the shady drawer. These samples were stored at room temperature. The degradation of the emission strength of the $^5D_0 \rightarrow {}^7F_2$ band of the europium complexes was studied using the microscope spectrometer with the fluorescence detector.

Results and Discussion

Visual Observation under Ultraviolet Light

The six components in Vaseline even at a 1% concentration emitted a strong red light under ultraviolet light with wavelengths at about 302 and 365 nm. The excitation wavelength of 302 nm was clearly observed by the emitted red light. The concentration of the detection limit for the europium complexes with Vaseline for the visual observation of the red luminescence was as follows: tris-(thenoyltrifluoroacetonato) europium ($EuTTA_3$) ~ tris-(naphtyltrifluoroacetonato) europium ($EuTFNB_3$) (1–10 ppm) < tris-(benzoyltrifluoroacetonato) europium ($EuBFA_3$) (10–100 ppm) < tris-(furoyltrifluoroacetonato) europium ($EuFTA_3$) ~ tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato) europium ($EuDMHFO_3$) ~ tris-(pivaloyltrifluoroacetonato) europium ($EuPTA_3$) (100 ppm - 0.1%). Furthermore, only a weak luminescence was visually detected from the 1% component under ultraviolet light at 254 nm.

Detection of Vaseline by Gas Chromatography

Vaseline, a petroleum jelly, is slightly soluble in acetonitrile. The gas chromatogram of Vaseline as C_{19}—C_{31} paraffin hydrocarbons with a C_{23} top peak from the extracts with acetonitrile is shown in Fig. 2. The detection limits of Vaseline extracted with acetonitrile were about 100 ng. The concentration of the luminescent markers was about 1% (the ratio of the europium complexes to the Vaseline was 1 to 99) and the detection limit for the visual observation was greater than 1 ng (13). The detection limit of Vaseline for GC is about 100 ng and enough to analyze the marker.

Emissions of Each Europium Complex Measured by Fluorescence Spectrophotometry

The emission spectra and excitation spectra for the $^5D_0 \rightarrow {}^7F_2$ emission bands of the 1% europium complexes with Vaseline are shown in Figs. 3*a* and 4. The peak intensities at 365, 302, and 254 nm in the excitation spectra for the $^5D_0 \rightarrow {}^7F_2$ emission bands increased in the following order: 254 < 302 < 365 nm. The intensity of the red emission for the visual observation under ultraviolet light increased in the following order: 254 < 365 < 302 nm. Although the red light at 302 nm had a weaker intensity than that at 365 nm, the light at 302 nm was more visible than that at 365 nm because the relative fluorescence intensity of Vaseline at 302 nm was considerably lower than that at 365 nm. The emission spectra and excitation spectra for the $^5D_0 \rightarrow {}^7F_2$ emission band at about 610 nm for each 10 ppm of the europium complex in acetonitrile are shown in Figs. 3*b* and 4. The detection limit in acetonitrile was 10–100 ppb.

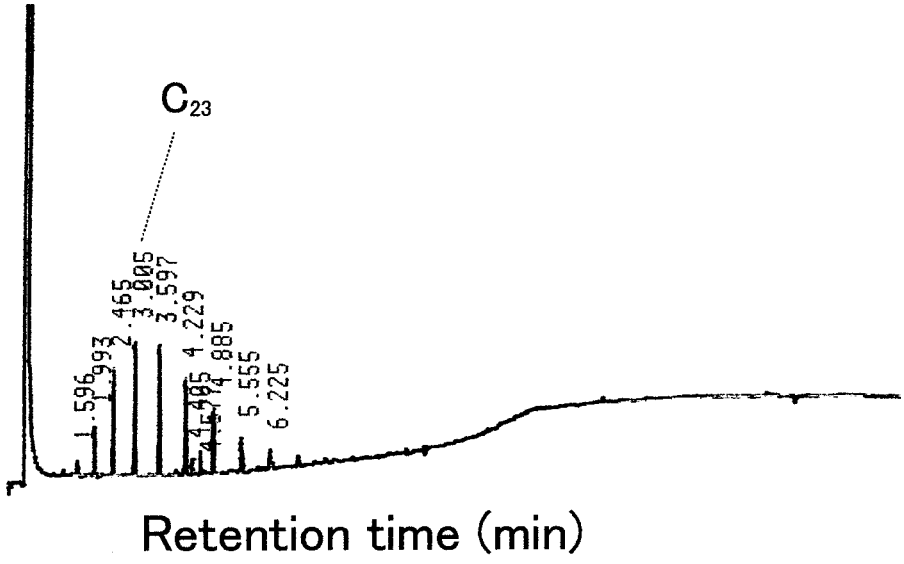

FIG. 2—*Gas chromatogram of 1 μL of 0.1% Vaseline in acetonitrile.*

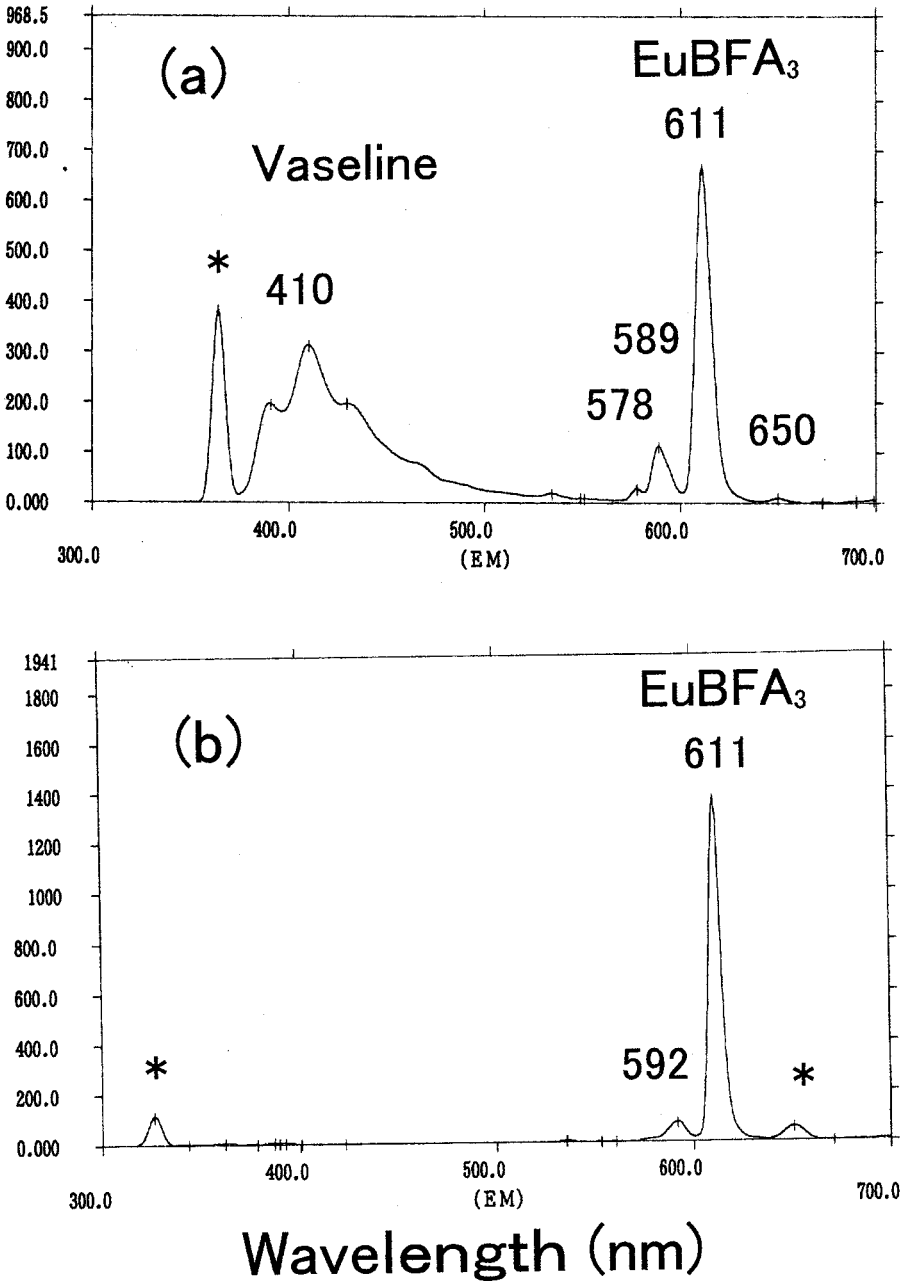

FIG. 3—*Emission spectra of tris-(benzoyltrifluoroacetonato) europium. (a) 1% Vaseline solution; and (b) 1 ppm acetonitrile solution. The peaks with an asterisk are scattering lines.*

ESI-MS Spectra of Europium β-Diketonates by ESI-MS Spectrometry

The ESI-MS spectra of 10 ppm for the six types of europium complexes in acetonitrile are shown in Fig. 5. Since the europium isotopes in the natural abundance are ^{151}Eu (47.77%) and ^{153}Eu (52.23%), each ion containing one europium atom in a molecule was characteristically detected as a pair of strong peaks due to the isotope effect. The ions formed from the europium complexes can be distinguished in the ESI-MS spectra in the negative-ion mode. The complexes (M) were identified on the basis of an adduct ion ($[M + L - H]^-$) formed by adding a deprotonated molecular ion ($[L - H]^-$) of a ligand (L) to the neutral molecule of the complex, a radical anion ($M^{\cdot -}$) of the molecule of the complex, and $[L - H]^-$; the six types of complexes were easily distinguished. The mass to charge ratios (m/z) of $[M + L - H]^-$, $M^{\cdot -}$, and $[L - H]^-$ of $EuBFA_3$, $EuPTA_3$, $EuTTA_3$, $EuFTA_3$, $EuDMHFO_3$, and $EuTFNB_3$ are shown in Table 1 and Fig. 5. The detection limit of the compounds in acetonitrile was about 10–100 ppb. The detection sensitivities between the peak intensity of the $M^{\cdot -}$ by the ESI-MS and the emission intensity of the $^5D_0 \rightarrow {}^7F_2$ band by the fluorescence spectrophotometry of $EuDMHFO_3$ in the range of 0.1–10 ppm in acetonitrile solvent are shown in Fig. 6. The detection sensitivities of both analyses were similar. This relation was similarly obtained even with the other five complexes. In this case, $[M + L - H]^-$ was detected mainly together with the radical anion $M^{\cdot -}$ in the spectra. As the concentration of the complexes in the acetonitrile solvent decreased, the relative peak intensity of the radical anion $M^{\cdot -}$ increased.

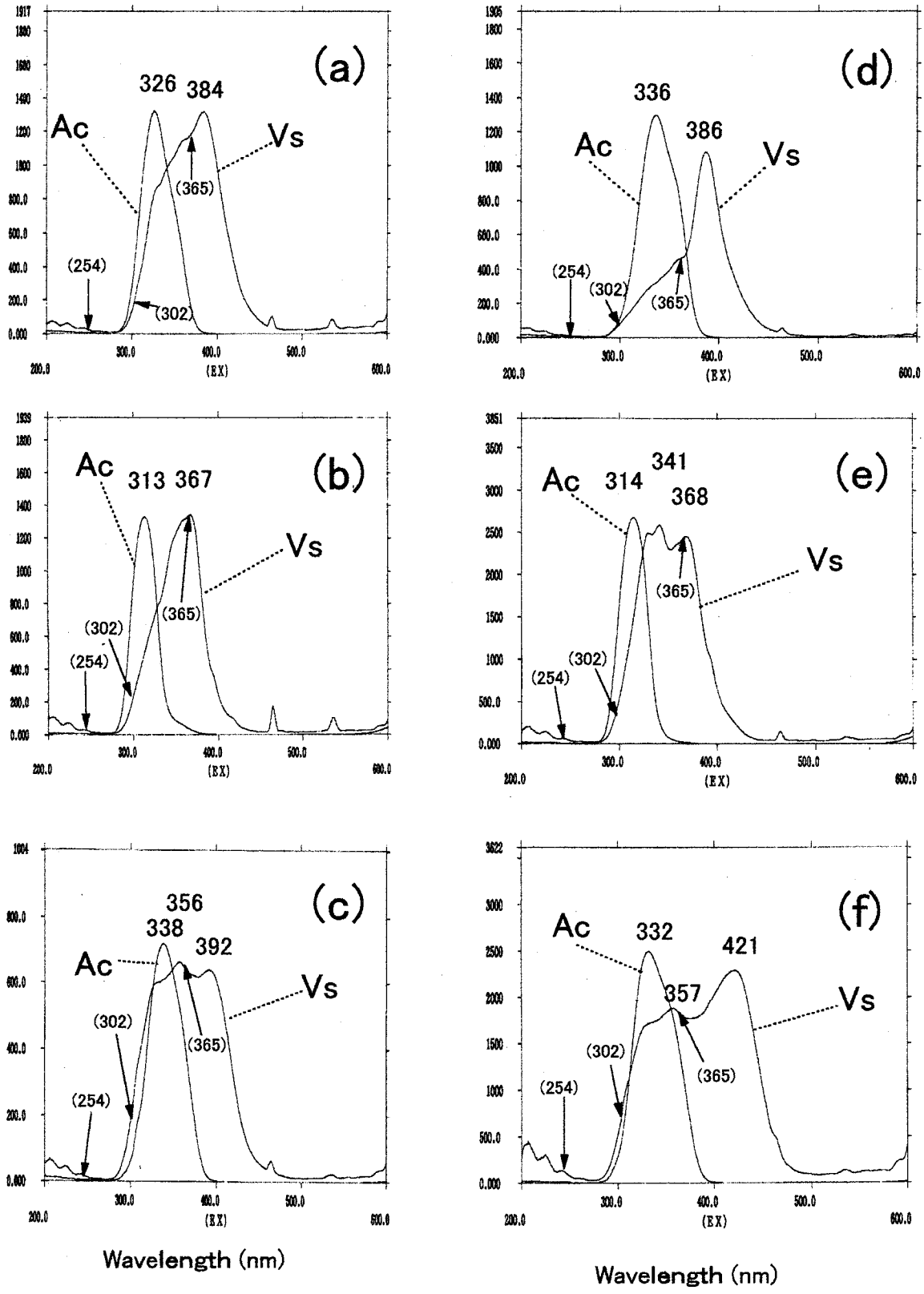

FIG. 4—*Excitation spectra of tris-(benzoyltrifluoroacetonato) europium (a), tris-(pivaloyltrifluoroacetonato) europium (b), tris-(thenoyltrifluoroacetonato) europium (c), tris-(furoyltrifluoroacetonato) europium (d), tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato) europium (e), and tris-(naphtyltrifluoroacetonato) europium (f). Ac, 1 ppm acetonitrile solution; and Vs, 1% Vaseline solution.*

TABLE 1—*Mass to charge ratio (m/z) of six types of europium β-diketonates.*

Compounds	$[L - H]^-$	$M^{\cdot -}$	$[M + L - H]^-$
$EuBFA_3$	215	796 and 798	1011 and 1013
$EuPTA_3$	195	736 and 738	931 and 933
$EuTTA_3$	221	814 and 816	1035 and 1037
$EuFTA_3$	205	766 and 768	971 and 973
$EuDMHFO_3$	295	1036 and 1038	1332 and 1334
$EuTFNB_3$	265	946 and 948	1212 and 1214

Degradation of Luminescent Markers after Application

The degradation of the 1% europium complexes in Vaseline was clearly detected from the compounds, which were kept in a sunny window in comparison with those preserved in the dark drawer due to photodegradation. The compounds in the sunny window emitted a red luminescence for a few weeks. The red luminescence from the compounds in the shade was easily detected without decreasing even after one month. The 1% concentration of the europium complexes with Vaseline were readily degraded in the water (10 mL) in

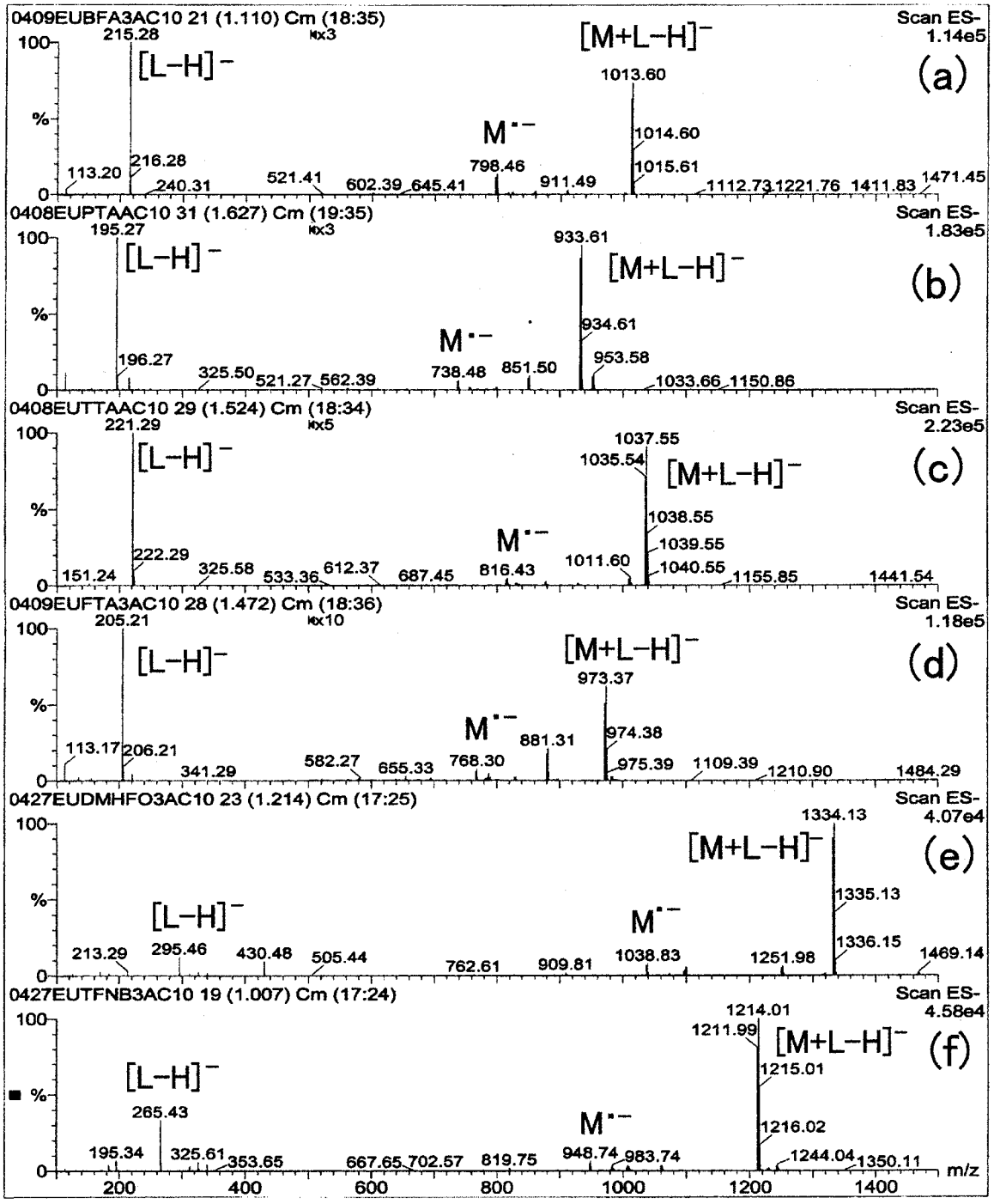

FIG. 5—*Electrospray ionization mass spectra of 10 ppm tris-(benzoyltrifluoroacetonato) europium (a), tris-(pivaloyltrifluoroacetonato) europium (b), tris-(thenoyltrifluoroacetonato) europium (c), tris-(furoyltrifluoroacetonato) europium (d), tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato) europium (e), and tris-(naphtyltrifluoroacetonato) europium (f) in acetonitrile.*

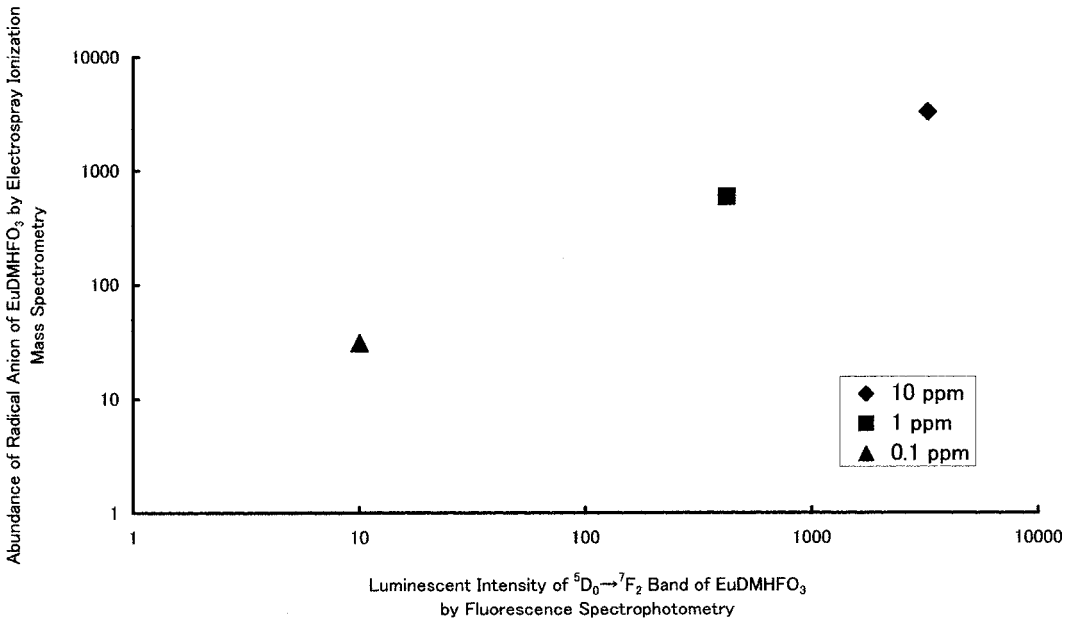

FIG. 6—*Relation between analyses of tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato) europium by fluorescence spectrophotometry and electrospray ionization mass spectrometry.*

comparison to those stored in the atmospheric closure of the shade. The red luminescence from the compounds preserved in the water was detected for a few weeks. Therefore, the 1% luminescent markers were readily detected by visually observing the red luminescence under ultraviolet light for a few weeks in the sunny place and water and for longer than one month in the shade after application. Furthermore, if the luminescent marker in the tube was stored in the freezer for one year and then measured at room temperature, it was used just like a newly prepared marker.

Cases Involving Luminescent Markers

These luminescent markers are used by only a particular set of investigators. The investigators must keep the following promises strictly: a same type of luminescent marker must not be used in any investigations within geographically neighboring areas at the same period of time. Since the markers are degraded after a period of several weeks of exposure to light, the applied compounds naturally disappear in the sun during the period. The markers applied in the dark such as a safe and a keyhole must be removed within a few weeks at least after application by exchanging the applied parts or washing with detergent, even if the markers had no effect on the investigation. If the promises are not kept, it may be difficult to know with certainty that the marker detected was that which was planted by the investigators in their given investigation. The markers used under these promises became crime evidence at the adjudicative stage of the following three cases and the problems concerning the markers have never arisen in Japan.

Case 1—Safecrackers using bars were arrested in Tokyo. A suspected group came under investigation. The investigators decided to use the luminescent marker in order to prove their crime. The investigators found the store where the suspects often purchased the bars for safecracking and applied the luminescent marker to the ends of the bars. Another safecracker robbery occurred with a similar modus operandi a few days later after they bought the bars. The investigators then arrested the robbers. The luminescent marker was detected from both the wall of the safe and their bars based on forensic laboratory analyses. The crime was proved by the resulting identification.

Case 2—A ransom kidnapping occurred in Tokyo. Investigators urgently prepared a ransom at the request of the criminal, and gave him money with no time to record the serial numbers of the bank notes. The investigators gave the ransom in exchange for a hostage soon after they applied the luminescent marker to the money. About one month later, the police arrested the suspect. When the investigators searched the house where he lived, they found the wads of money with a trace of red luminescent substances under ultraviolet light. The compound was identified by forensic laboratory analyses. As a result, most of money was safely returned.

Case 3—Serial theft cases have occurred in the city. Although a suspect was identified by the investigators, they had no conclusive evidence. He committed thefts after riding on a bicycle. The investigators scattered many small dumplings composed of a mixture of the marker and soil in front of his house. The suspect stepped on them, and then rode on the bicycle during the thefts. The red luminescent substance under ultraviolet light and his footmarks were detected at the crime scene. The luminescent substance was the same compound as the marker as a result of the laboratory analyses. Based on this evidence the suspect was caught and arrested.

Conclusions

Luminescent markers were developed that emitted red light under ultraviolet light. They were colorless and sticky. The markers were promptly and efficiently searched without misjudging during the investigation at a crime scene in comparison with the pale fluorescence compounds such as anthracene conventionally used in Japan. Although the markers gradually photodegraded, they were sufficiently detected even for longer than one month in the shade. The luminescent europium β-diketonates extracted with acetonitrile solvent were promptly and sensitively discriminated by both

fluorescence spectrometry and electrospray ionization mass spectrometry. Vaseline used as the base material was also easily detected by gas chromatography. Accordingly, the luminescent markers were easily identified. Various types of luminescent markers were produced by changing the types of ligands in the europium complexes. Furthermore, more types of markers can be prepared by changing the types of rare earth metals in the complexes.

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