J Forensic Sci, May 2004, Vol. 49, No. 3
Paper ID JFS2003360
Available online at: www.astm.org

TECHNICAL NOTE

Hikoto Ohta,[1] Ph.D.; Yasuhiro Suzuki,[1] M.S.; Ritsuko Sugita,[1] M.S.; Masaaki Kasamatsu,[1] M. Pharm.; Yoshireru Marumo,[1] Ph.D.; and Shinichi Suzuki,[1] Ph.D.

Evaluation of 5-(4-Nitrophenyl)-2,4-pentadien-1-al (NPPD)as a Tracer for Shadowing Pursuits

ABSTRACT: The chemical compound 5-(4-Nitrophenyl)-2,4-pentadien-1-al (NPPD), called "spy dust," was used in the Soviet Union as a shadowing pursuit, the act of following someone secretly, for investigating the activities of diplomatic personnel. It is also useful for counter-terrorism, and some criminal cases in the forensic science field. In this paper, it was synthesized and evaluated as a tracer for shadowing pursuits. The method for utilizing this reagent was very simple: it was dissolved in methanol (1 mg/mL) and sprayed on the restricted area. If the suspect was to enter this area or touched the sprayed material, NPPD was attached to the suspect's shoe surface or hands. The color examination was a two-steps process: first was the addition of 1 mL of a 0.1% naphthoresorcinol methanol solution to the methanol extracts of a methanol-contained cotton swab used to smear some surfaces of the suspect, and second, the addition of 1 mL of concentrated hydrochloric acid, which turned the solution dark red. The λ_{max} of the colored solution was 510 nm, measured by ultraviolet-vis spectroscopy. Detection limits for three methods were determined: a visual method (detection limit 100 ng/3 mL), an ultraviolet-visible spectrometric method (detection limit 10 ng/3 mL), and a selected-ion-monitoring gas chromatographic/mass spectrometric method (detection limit 300 pg/injection). The forensic utility of NPPD was demonstrated for two simulated cases: a theft case and a case where NPPD was used as a tracer to prove that an automobile had entered a restricted area.

These examinations prove NPPD is a useful shadowing pursuit (spy dust) for the forensic science field.

KEYWORDS: forensic science, 5-(4-nitrophenyl)-2,4-pentadien-1-al (NPPD), spy dust, shadowing pursuit, selected-ion mass spectrometry

In theft, arson, and public security cases in Japan, tracers for shadowing pursuits have been limited to polyaromatic hydrocarbons (PAHs; for example, anthracene or phenanthrene) and phenolphthalein (1,2). However, these tracers have serious disadvantages. PAHs are present in the asphalt of road surfaces and may be carcinogenic. Phenolphthalein is colored under basic conditions, and therefore visual detection methods using this reagent suffer low sensitivity under basic atmospheric conditions.

Recently, luminescent markers using europium β-diketonates have been developed (3–8). Methods using these markers avoid the disadvantages of the conventional PAH and phenolphthalein methods. However, the luminescence methods suffer low selectivity because the colors of light emitted from europium β-diketonate complexes are similar (9). Kurata et al. attempted to resolve this problem by using a mass spectrometer to accurately identify the diketonate complexes (10–14).

In this paper, we used selected-ion-monitoring (SIM) gas chromatography/mass spectrometry (GC/MS) to evaluate 5-(4-nitrophenyl)-2,4-pentadien-1-al (NPPD) as a tracer for shadowing pursuits; NPPD has been referred to be "spy dust." Though this reagent was considered to be nonmutagenic and noncarcinogenic (15), very low mutagenic activity for Salmonella typhimurium was predicted (16–18). This activity is found only in Salmonella and marrow (18). By these reports, NPPD was considered to be a safe reagent compared with PAHs. The classic method for detecting this reagent involves two steps: colorization using naphthoresorcinol and hydrochloric acid, followed by either visual or ultraviolet-visible (UV-vis) spectrometric measurement of the intensity at 510 nm. This reagent had no fluorescence in this region.

Experimental

Chemicals

In this study, the reaction of NPPD and acidified naphtoresorcinol was utilized. Naphthoresorcinol was purchased from Aldrich Chemical Co. Ltd. (Milwaukee, WI). NPPD was synthesized using the following method, and other solvents were analytical grade.

Synthesis of 5-(4-nitrophenyl)-2,4-pentadien-1-al (NPPD, (1)*, (Fig. 1).*

Wittig reaction of monoene 4-bromo-2-buten–1-oic acid methyl ester (2) gave an unsaturated diene (3). The compound (3) was treated with *p*-nitrobenzoic acid, and the resulting product (4) was reduced with isobutylaluminum hydride, yielding 5-(4-nitrophenyl)-2,4-pentadien-1-ol (5). NPPD (1) was obtained by oxidizing (5) with manganese dioxide.

Apparatus for Confirmation of the Structure of NPPD

The structure of NPPD was confirmed by proton nuclear magnetic resonance spectroscopy (^{1}H NMR), carbon-13 nuclear magnetic

[1] National Research Institute of Police Science, 6-3-1 Kashiwano-ha, Kashiwa-shi, Chiba 277-0882, Japan.

Received 28 Oct. 2003; and in revised form 16 Jan. 2004; accepted 16 Jan. 2004; published 7 April 2004.

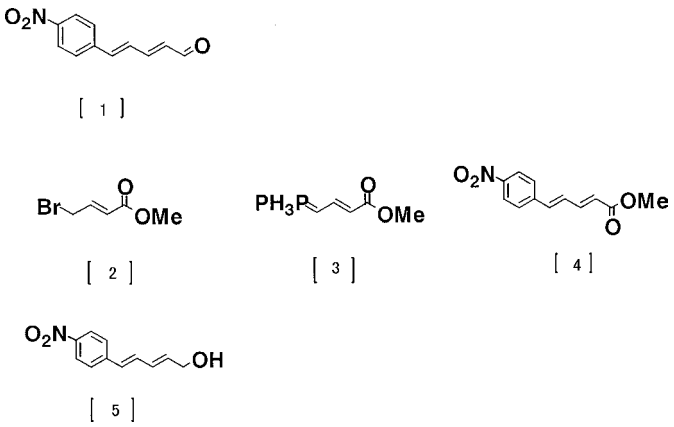

FIG. 1—*Structures of 5-(4-nitrophenyl)-2,4-pentadien-1-al and its intermediates.* (1) *5-(4-nitrophenyl)-2,4-pentadien-1-al and its intermediates,* (2) *4-bromo-2-butene-1-oic acid methyl ester,* (3) *Wittig reaction intermediate,* (4) *5-(4-nitrophenyl)-2,4-pentadien-1-oic acid methyl ester,* (5) *5-(4-nitrophenyl)-2,4-pentadien-1-ol.*

resonance spectroscopy (^{13}C NMR), and direct-inlet electron-impact mass spectrometry (DI-EI-MS). ^{1}H NMR and ^{13}C NMR spectra were acquired with a 600-MHz JNM-ECP600 NMR spectrometer (JEOL Ltd., Tokyo, Japan) using 5 mg of NPPD powder dissolved in $CDCl_3$. DI-EI-MS analysis of 1 mg of NPPD dissolved in 10 mL of methanol and 1 μL of this solution was analyzed with a JEOL JMS-GCmate mass spectrometer (ionization voltage, 30 eV; ionization current, 70 μA; ion source and sample inlet temperatures, 280°C). The purity of the NPPD sample was certified by high-performance liquid chromatography (HPLC) (Agilent 1100 series chromatograph (Agilent Co. Ltd., Palo Alto, CA)); column, Hypersil ODS, 2.0 mm inside diameter × 125 mm; mobile phase, 90:10 (v/v) methanol-H_2O (1 mL/min); column temperature (controlled by a column oven), 25°C; detection wavelength, 254 nm).

Visual Detection Method

One milliliter of a 0.1% naphthoresorcinol methanol solution was added to 1 mL of a 0.1 mg/mL solution of NPPD in methanol. The resulting solution was colorless at this point. After the addition of 1 mL of concentrated hydrochloric acid, the solution became dark red. The λ_{max} of the colored solution was 510 nm, measured by UV-vis spectroscopy (UV-1700 Pharmaspec, Shimadzu, Kyoto, Japan).

Visual and UV-vis detection limits were determined according to the above procedure using standard methanol solutions of NPPD (10 ng/mL, 100 ng/mL, 1 μg/mL, 10 μg/mL, and 100 μg/mL of solution).

Selected-ion Monitoring

The base peak of NPPD, *m/z* 128 [M^+, $-NO_2$, $-C{=}O$], was used for selected-ion monitoring of NPPD. Selected-ion monitoring was performed with a JEOL JMS-GCmate mass spectrometer under the following conditions: Agilent 6890 gas chromatograph; column, DB-5, 30 m × 0.25 mm inside diameter, 0.25 μm thickness (J&W Scientific, Folsom, CA); temperature program: 10°C/min from 200 to 300°C, 5 min at 300°C; carrier gas, helium; linear velocity, 22.5 mm/s; ion source, interface, and injection temperatures, 280°C; injection method, splitless; ionization voltage, 30 eV; ionization current, 70 μA.

The sample was prepared as follows. The target surface was swabbed with a cotton ball (about 1 cm diameter) moistened with methanol or acetone. The cotton ball was extracted four times with 5 mL of methanol by centrifugation, and the supernatants were combined and dried at 60°C under a moderate flow of nitrogen for 1 h. The residue was redissolved in 20 μL of ethyl acetate, and 1 μL of this solution was introduced into the GC/MS.

Results and Discussion

Structure of NPPD

The structure of NPPD was confirmed spectroscopically. The ^{1}H NMR, ^{13}C NMR, and DI-EI-MS spectra of NPPD are shown in Figs. 2–4, respectively. Figure 5 shows the HPLC chromatogram of NPPD.

Visual and UV-vis Spectrometric Detection of Colored NPPD

Visual and UV-vis detection limits at $\lambda_{max} = 510$ nm (λ_{max} determined by UV-vis spectroscopy (Fig. 6)) were determined according to the colorization procedure described in the experimental section. The visual detection limit was 100 ng of NPPD in 3 mL of solution, and the UV-vis detection limit ($S/N = 5$) was 10 ng in 3 mL of solution.

The dark red color was stable for 5 h, after which the color slowly changed to pale yellow (Fig. 7).

Selected-ion-monitoring Detection of NPPD

The GC/MS/SIM method was much more sensitive than the visual and UV-vis detection methods. The GC/MS/SIM detection limit was initially estimated by injecting 1 μL of a standard methanol solution of NPPD (1 μg/μL, 100 ng/μL, 10 ng/μL, 1 ng/μL, 100 pg/μL) into the GC/MS. An obvious peak (retention time of NPPD, 14.5 min) was observed for the 1-ng sample, but the 100-pg sample did not satisfy the qualitative detection limit regulation ($S/N = 3$). Further study showed that the $S/N = 3$ requirement was satisfied at 300 pg/injection.

Selected-ion-monitoring chromatograms (monitoring ion: *m/z* 128, base peak) for 50 ng/injection and 1 ng/injection are shown in Fig. 8.

Simulated Experiments

Case One

A theft case in which the suspect intended to steal an expensive computer was simulated. To identify the suspect's behavior and prevent the theft, 0.1 mg/mL of NPPD methanol solution was applied to the exit and entrance doors of the target room, and the surfaces were allowed to air-dry. After the suspect entered and exited the room, dried NPPD was transferred to the suspect through the sweat on the hands. NPPD tests were carried out according to the visual colorization protocol. A dark red color was observed, proving that the suspect entered the room (Fig. 9).

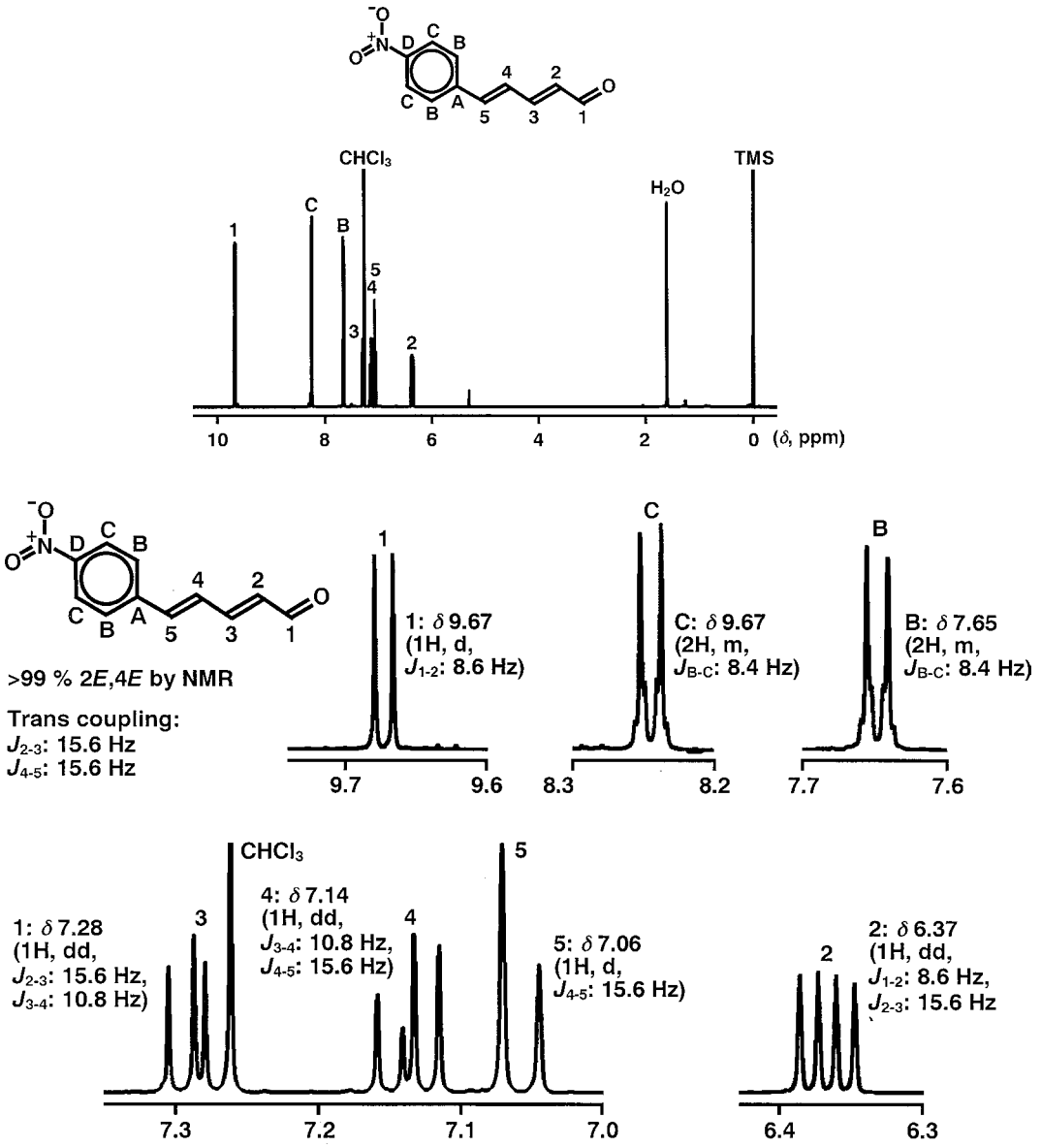

FIG. 2—*Spectrum of NPPD by* ^{1}*H-NMR. Peaks are assigned to the number at NPPD structure. TMS: tetramethylsilane.*

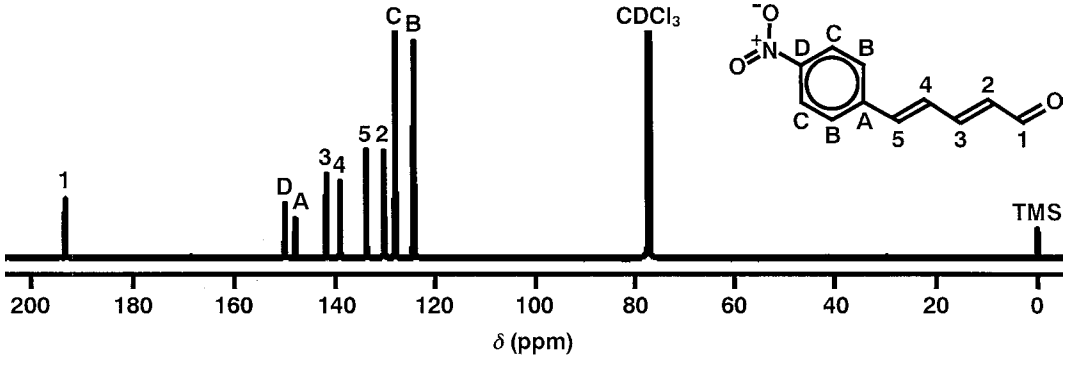

FIG. 3—*Spectrum of NPPD by* ^{13}*C-NMR. Peaks are assigned to the number at NPPD structure. TMS: tetramethylsilane.*

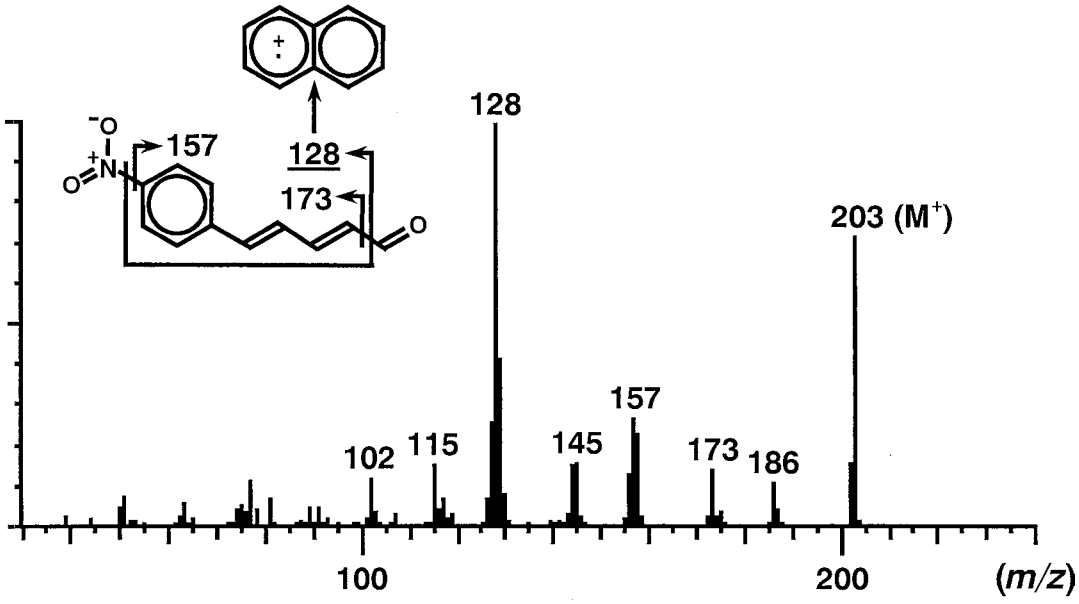

FIG. 4—*Mass spectrum of NPPD by DI-EI-MS.*

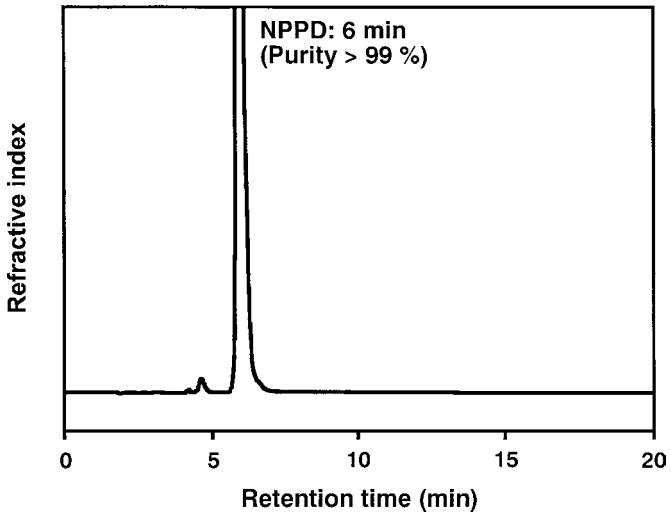

FIG. 5—*Chromatogram of NPPD by HPLC.*

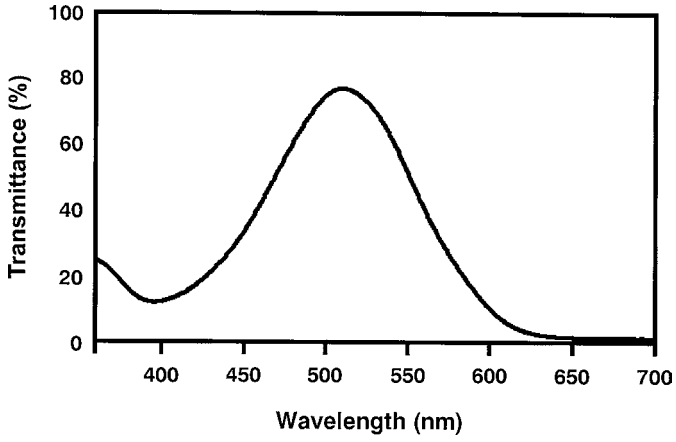

FIG. 6—*Ultraviolet visible spectrum of colored NPPD in methanol.*

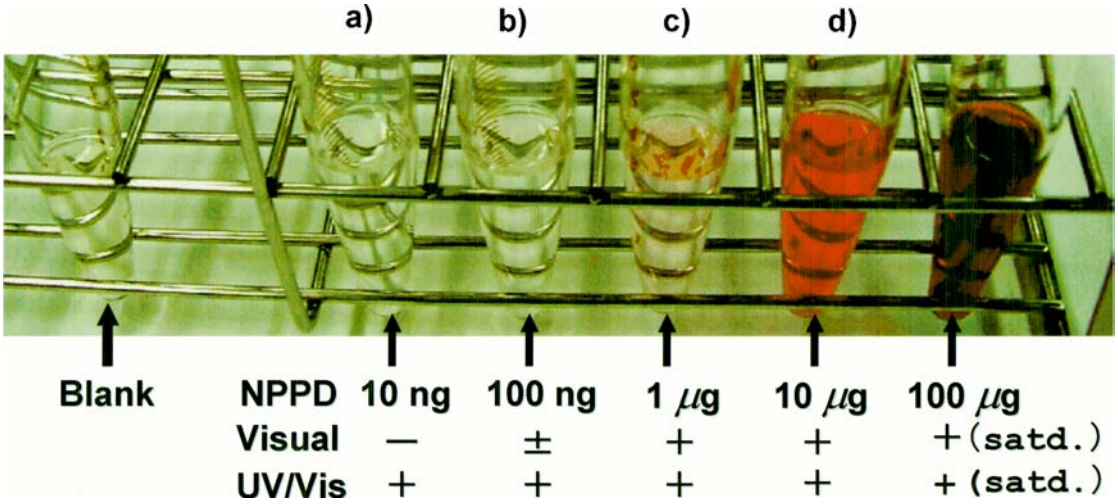

FIG. 7—*Colorization of NPPD by visual detection protocol.* (a) *10 ng/3 mL,* (b) *100 ng/3 mL,* (c) *1 μg/3 mL,* (d) *10 μg/3 mL of NPPD final colorization solution.*

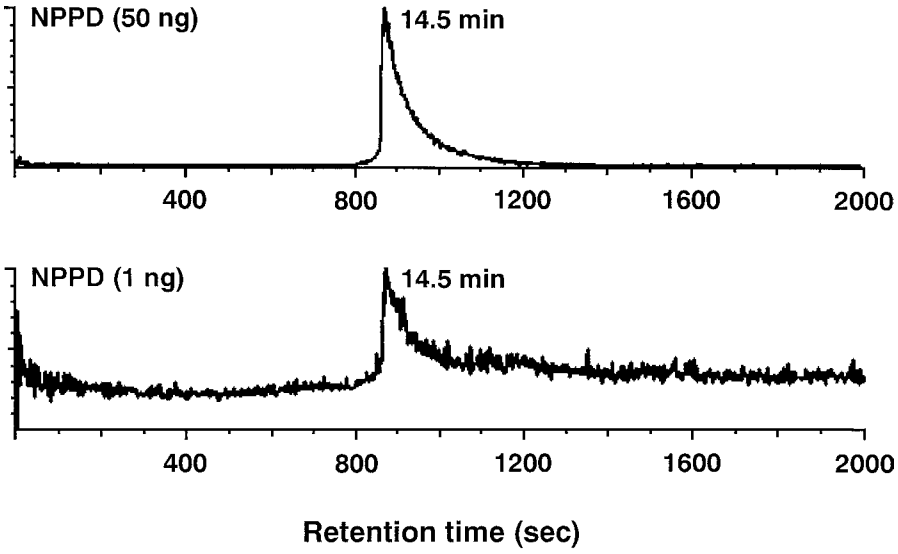

FIG. 8—*Selected ion chromatogram at m/z 128 of NPPD* (top) *50 ng/injection,* (bottom) *1 ng/injection.*

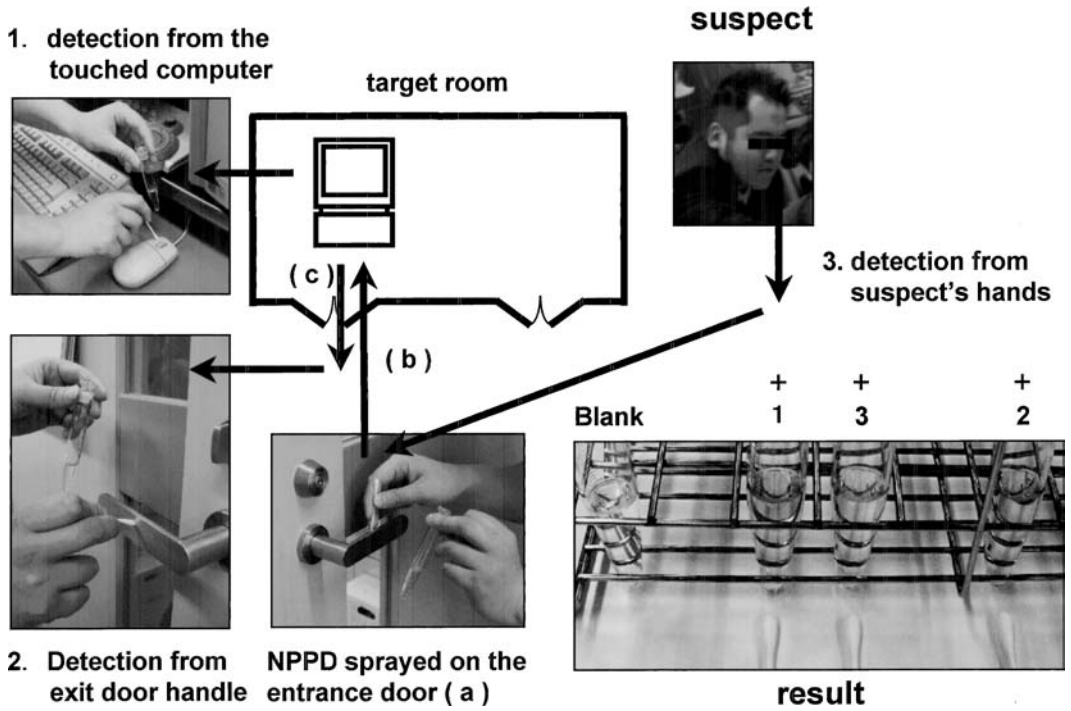

FIG. 9—*Theft simulation experiment results.* (a) *0.1% NPPD methanol solution was applied to the entrance side of the door handle.* (b) *Suspect entered the room, touched the door handle and target computer.* (c) *Colorization result by NPPD from* (1) *computer,* (2) *suspect hands, and* (3) *exit side of the door handle.*

Case Two

In this case, NPPD was used as a tracer to prove that an automobile had entered a restricted area. The protocol was slightly changed, because the adhered period of tire and road surface was short, 5 mg/mL NPPD solution was applied, and the extraction method was also changed. The absence on the surface proves that 10 km of tire motion removed NPPD. A simulated restricted area (outlined by tape in Fig. 10) was sprayed with a 5 mg/mL NPPD methanol solution, and the target automobile passed over this area once. After a 10-km drive (simulating a police chase), the tire surface and tire tread were swabbed with cotton swabs moistened with methanol. The cotton swab was sonicated in acetone five times, and the supernatants were combined and then dried under a moderate flow of nitrogen at 60°C for 1 h. The residue was completely dissolved in 10 μL of ethyl acetate, and 1 μL of this solution was introduced into the GC/MS. NPPD was not detected in the sample collected from the tire surface, even by the SIM method. However, NPPD was detected by GC/MS/SIM (Fig. 11) in the sample collected from the tire tread, proving that the automobile had entered the restricted area.

Conclusions

The chemical compound 5-(4-Nitrophenyl)-2,4-pentadien-1-al (NPPD) is an excellent shadowing pursuit from two standpoints. First, NPPD is a colorless, odorless, harmless, nonmutagenic compound. Second, NPPD exhibits a higher sensitivity and

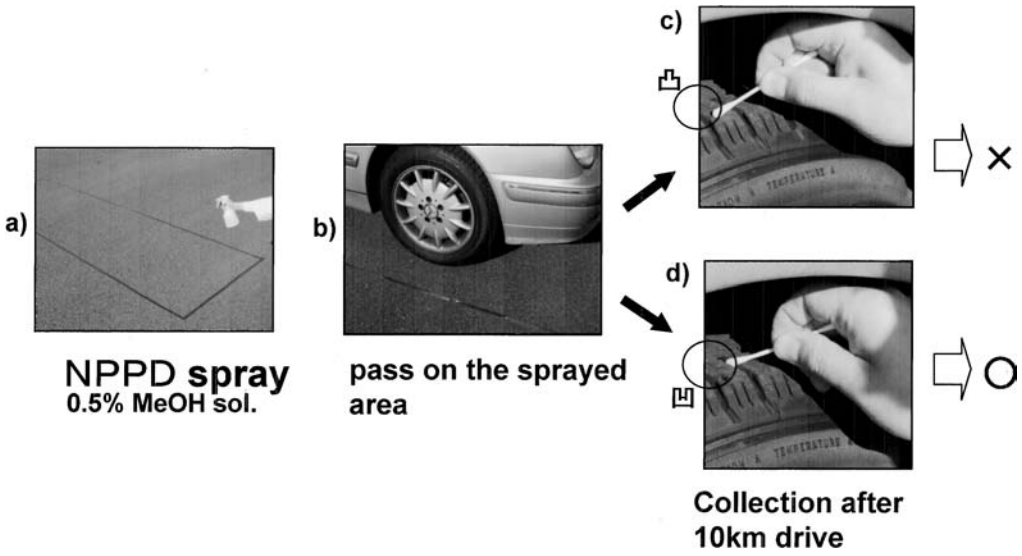

FIG. 10—*Automobile pursuit experiment.* (a) *Inside the red square, 0.5% of NPPD was sprayed.* (b) *Automobile passed over this area once.* (c) *After a 10 km drive, NPPD was collected from the tire surface.* (d) *After a 10 km drive, NPPD was collected from the tire tread.*

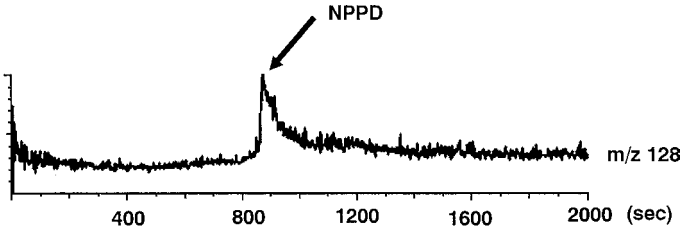

FIG. 11—*Selected ion monitoring at* m/z *128 of tire tread swab.*

selectivity compared with conventional PAH shadowing pursuits. Visual detection of NPPD involves only two steps, and NPPD does not exist in common biological systems, so false positives are excluded. NPPD was detected with a very high selectivity and sensitivity in the GC/MS/SIM method. Mixing NPPD with Vaseline™ for better adhesion (15) may result in improved traceability.

Acknowledgment

We gratefully acknowledge Dr. Shoji Kurata, Forensic Science Laboratory, Tokyo, Japan, for his suggestion of an improved detection method protocol.

References

1. Kurata S, Hirano H. Study on fluorescent substances for development of multipurpose markers. Acta Criminol Med Leg Jpn [Japanese] 1994;60:95–100.
2. Kurata S, Hirano H, Nagai M. Development of fluorescence markers using polycyclic aromatic hydrocarbons with vaniline. J Forensic Sci [PubMed] 2002;47(2):244–53.
3. Lui L, Li W, Hong Z, Peng, Lui X, et al. Europium complexes as emitters in organic electroluminescence devices. Synth Met 1997;91: 267–9.
4. Ohmori Y, Ueta H, Kurosawa Y, Hamada Y, Yoshino K. Enhanced emission from europium complex utilizing quantum-well structure in organic electroluminescent device. Jpn J Appl Phys 1998;37:798–801.
5. Uekawa M, Miyamoto Y, Ikeda H, Kaifu K, Nakaya T. Synthesis and properties of europium complexes with β-diketone ligands for organic electroluminescent devices. Bull Chem Soc Jpn 1998;71: 2253–8.
6. Lock ERA, Mazzella WD, Margot P. A new europium chelate as a fluorescent dye for cyanoacrylate pretreated fingerprints-EuTTAPhen: europium thenoyltrifluoroacetone ortho-phenanthroline. J Forensic Sci 1995;40:654–8.
7. Allred CE, Menzel ER. A novel europium-bioconjugate method for latent fingerprint detection. J Forensic Sci 1995;85:83–94.
8. Yuan J, Wang G, Kimura H, Matsumoto K. Highly sensitive detection of bensulfuron-methyl by time-resolved fluoroimmunoassay using a tetradentate β-diketonate europium chelate as a label. Anal Sci 1999;15:125–8.
9. Takatsu T. Spectrophotofluorometric determination of terbium, europium and samarium with pivaloyltrifluoroacetone and tri-*n*-octylphosphine oxide in micellar solution of nona-oxyethylene dodecyl ether. Talanta 1982;29:397–400.
10. Kurata S, Hirano H, Nagai M. Determination of luminescent europium β-diketones used as tracers for shadowing pursuits. J Forensic Sci 2002;47(4):797–804.
11. Kowalski BR, Isenhour TL, Sievers RE. Ultra-trace mass spectrometric metal analysis using heptafluorodimethyloctanedione chelates. Anal Chem 1974;46:726–8.
12. Risby TH, Jurs PC, Lampe FW, Yergey AL. Isobutane chemical ionization mass spectra of volatile lanthanide chelates. Anal Chem 1974;46:998–1003.
13. Risby TH, Jurs PC, Lampe FW, Yergey AL. Isobutane chemical ionization mass spectra of lanthanide perfluorinated β-diketonates. Anal Chem 1974;46:161–4.
14. Kurata S, Nagai M. Identification of luminescent europium β-diketonates using high resolution emission spectroscopy and direct-inlet electron ionization mass spectrometry. Anal Sci 2001;17:1079–84. [PubMed]
15. Jameson CW, Moseman RF, Collins BJ, Hooper ND. Spy dust, detecting chemical tracking agent. Anal Chem 1986;58(8):915A–6A.
16. Klopman G, Kalos A, Frierson M, Herbert SR. NPPD (spy dust) is predicted to be a mutagen. Environmental Mutagenesis 1986;8: 637–40.
17. Klopman G, Kalos A, Frierson M, Herbert SR. Mutagenecity of spy dust. Science 1986;231:442. [PubMed]
18. Zeiger E, Shelby MD, Ivett J, McFee AF. Mutagenicity testing 5-(4-nitrophenyl)-2,4-pentadien-1-al (spy dust) and its metabolites in vitro and in vivo. Environmental Mutagenesis 1987;9:269–80. [PubMed]

Additional information and reprint requests:
Shinichi Suzuki, Ph.D.
National Research Institute of Police Science
6-3-1 Kashiwano-ha, Kashiwa-shi,
Chiba 277-0882, Japan
E-mail: suzukis@nrips.go.jp