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Case reports

Identification of species of weeds using High Performance Liquid Chromatography in three crime cases*

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Summary. Stains on clothing from two victims which were later on determined as grass stains, and a blade of dried grass taken from another were examined to identify the sites of crimes. The species of each sample was identified based on the characteristic distribution pattern of flavonoids seen on high performance liquid chromatography. The scene of the crime was clearly traced from the evidential material.

Key words: Plant pigments – Flavonoid – HPLC – Stain identification

Zusammenfassung. Spuren an der Bekleidung zweier Opfer, welche sich als Grasspuren herausstellten und ein Halm getrockneten Grases von einem weiteren Kriminalfall wurden mit dem Ziel untersucht, die Orte der Verbrechen zu identifizieren. Die Spezies von jeder Probe wurde durch eine Methode identifiziert, welche auf der charakteristischen Verteilung der Flavonoid-Muster basiert, wie sie in der HPLC nachzuweisen sind. Der Ort des Verbrechens wurde mit Hilfe dieses Beweismaterials klar identifiziert.

Schlüsselwörter: Pflanzenpigmente – Flavonoide – HPLC – Spurenidentifikation

Introduction

A sensitive method for analyzing flavonoids by high performance liquid chromatography (HPLC) has been reported whereby plant species could be identified from as little as 100~200 µg of powdered leaves [1]. Although there are reports concerning the chemical analysis of flavonoids from taxonomic viewpoints [2–6], the criminological application has been given less attention. We report here on an accurate tracing of scenes of crimes by identifying plant species based on the characteristic distribution pattern of flavonoids seen on HPLC.

Case reports

Case 1. A 34-year-old barmaid was raped and manually strangled to death in a parking lot. A suspect was arrested 2 weeks later, but denied the crime.

A 0.5 × 1 cm dark brown stain was found on the left sleeve of the suspect's suit. A blood test of this stain was negative.

Case 2. A 17-year-old girl riding her bike home was suddenly pulled down by a man hiding in a bush. She lost consciousness after her head was covered with a sack and she was raped. The victim was taken by the assailant in his car to a place remote from the scene and was set free.

A dark brown-greenish stain with the size of a fingertip was found on the back of her slacks. Blood tests were negative.

Case 3. A 27-year-old male gangster was found tightly bound with rope and floating in a river. There was a piece of a dried weed firmly inserted between the rope and the body. This specimen proved to belong to a family of true grasses. The method previously reported [1] was used to search the grassy area where the crime was presumed to have occurred.

Materials and methods

1. Preliminary examination. Following the negative blood test on the stains of cases 1 and 2 a preliminary examination was made using Harborne's method [7]. One or 2 fibers, ca. 0.5 cm long, were ground in 3 drops of methanol on a welled glass slide. The extract was spotted onto a silica gel TLC plate, exposed to ammonia vapor, and radiated with UV light (wavelength 365 nm). A greenish-violet fluorescence was observed in the samples.

2. Confirmatory examination. Approximately 0.5 × 0.5 cm pieces were cut from stains on clothing worn by the victims in cases 1 and 2, and a small portion of the ear of the weed in case 3 was ground to a powder. Each sample was mixed with 0.2 ml 2N hydrochloric acid in a 10 ml test tube. The preparation was hydrolysed by heating

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at 100°C for 60 min, cooled and extracted twice with 1 ml of ethyl acetate. The organic layer was evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 50 µl of ethanol, passed through a filter unit (Columnguard FH 4, Millipore Ltd., USA) and 2 µl aliquots of the solution were applied to HPLC. The flavonoids were selectively identified by an absorption detector adjusted to a 365 nm wavelength.

3. HPLC conditions. The apparatus used was a Shimadzu LC-6A model high performance liquid chromatograph equipped with an absorption detector and a computerized integrator, a Shimadzu chromatopac C-R3A. The column was a 15 × 0.6 cm i.d. stainless tube packed with Shim-pack CLC-ODS (octadecyl silica). The eluent was water:acetic acid:methanol = 47:10:43 (v/v/v), with the flow rate of 1.0 ml/min. All chromatographic procedures were carried out at room temperature.

4. Calculation of retention indices (RIs) for flavonoids. To solve the variations of retention times (RTs) in HPLC, the idea of RI in gas chromatography [8] was introduced with modifications for the flavonoids [1].

Rutin with the smallest RT and rhamnetin with the largest were set at 0 and 100 in RIs, respectively. Each value of RI was calculated according to the following formula, and set as a whole number between 0 and 100.

$$RI(A) = [RI(Y) - RI(X)] \times \frac{\log t(A)/t(X)}{\log t(Y)/t(X)} + RI(X)$$

where

$t(A)$ = RT of flavonoid A, $t(X)$ = RT of rutin, $t(Y)$ = RT of rhamnetin, RI(A) = RI of flavonoid A, RI(X) = RI of rutin = 0, and RI(Y) = RI of rhamnetin = 100.

Results and discussion

Case 1

The chromatogram of the stain extract showed a single, small peak with a retention index (RI) of 48 (Fig. 1), which was identical with that of luteolin. Since the stain originated from plant material, a number of different species of weeds were collected at the assumed site 2 months after the crime. The reference grass samples did not show the same pattern with a single peak of luteolin as seen in the stain sample. However, *Equisetem arvense* (horse-tail) showed a marked high peak of luteolin together with 3 other smaller peaks corresponding to quercetin, kaempherol and apigenin and was apparently the origin of the stain on the sleeve. Peaks other than luteolin disappeared or became too low to be identified when analyzing minute amounts.

The defendant confessed to the crime.

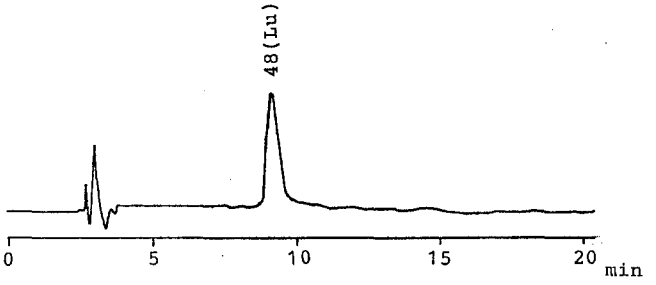

Fig. 1. Chromatogram of the extract from the stain on the sleeve (case 1). *Lu*, luteolin

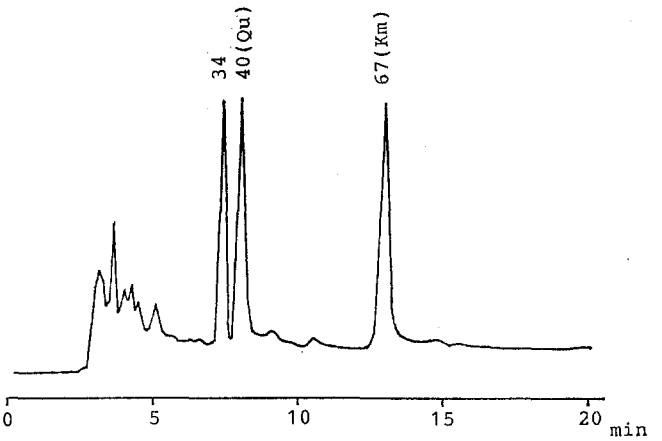

Fig. 2. Chromatogram of the extract from the stain on the slacks (case 2). *Qu*, quercetin; *Km*, kaempferol

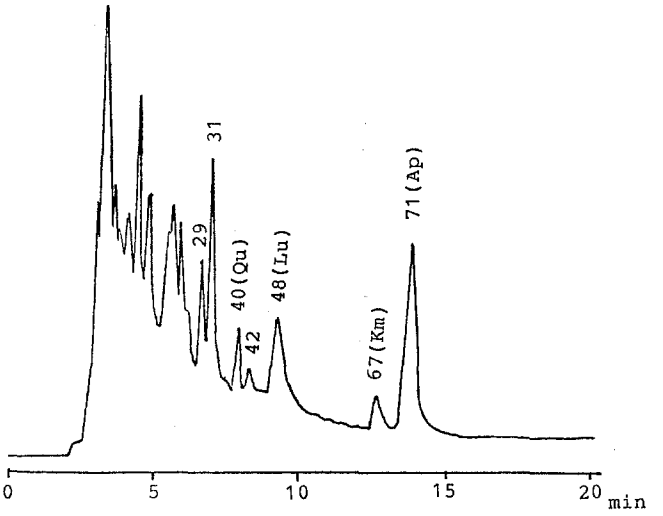

Fig. 3. Chromatogram of the extract from the weed (case 3). *Qu*, quercetin; *Lu*, luteolin; *Km*, kaempferol; *Ap*, apigenin

Case 2

The chromatogram of the stain on the slacks showed one unidentified peak and 2 others corresponding to quercetin and kaempferol, with RIs of 34, 40 and 67, respectively (Fig. 2). This chromatographic pattern was identical with that of *Trifolium repens* (white clover) which was present at the suspected scence of crime. The suspect confessed to the crime.

Case 3

The chromatogram of the extract from the weed blade is shown in Fig. 3. The characteristic peaks with RIs of 29, 31, 40 (quercetin), 42, 48 (luteolin), 67 (kaempherol) and 71 (apigenin) were present. For purposes of comparison, various species of wild weeds were collected from a wide area along the river where the body had been found. The flavonoid patterns of the sample coincided with that of *Lolium perenne* (a type of wild rice: Hoso-mugi in Japanese) present at a localized upstream

area of the river bank. The crime was allegedly committed at the designated site.

Conclusion

Plant fragments and plant stains examined using HPLC led to prosecution of criminals, thus indicating that this method for analyzing flavonoids can be of value in criminal cases.

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