Journal of Chromatography, 644 (1993) 43–48
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 128

Determination and quantitative analysis of *Aconitum* alkaloids in plants by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

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(First received January 4th, 1993; revised manuscript received March 30th, 1993)

ABSTRACT

A high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry method was useful for the simultaneous determination of *Aconitum* alkaloids, kobusine, pseudokobusine, dehydroluciduscuIine, luciduscuIine, delcosine and 14-acetyldelcosine, found in the *Aconitum yesoense* var. *macroyesoense*. These compounds were chromatographed by definite elution in 10 min and were quantitated by selected-ion monitoring of the protonated molecules. The method is linear over the range 10 ng to 10 μg of alkaloids per injection. The detection limits of alkaloids were *ca.* 1–5 ng per injection.

INTRODUCTION

Various *Aconitum* (Ranunculaceae) plants produce C_{19}- and C_{20}-diterpenoid alkaloids [1]. The tubers of *Aconitum* plants, called "busi", have been employed as an oriental drug in Japan and China. The crude drug "busi" has been consumed clinically in large quantities in East Asia.

A number of reports on the analysis of trimethylsilylated aconitine-type alkaloids by gas chromatography [2] and toxic C_{19}-diterpenoid alkaloids by high-performance liquid chromatography (HPLC) have appeared in recent years [3–7]. These alkaloids have a moiety of aromatic ester (such as benzoyl and anisoyl groups), and can be applied directly to the method with UV detection. However, no report exists on the investigation of the separation and the simultaneous determination of C_{19}- (no aromatic ester group) and C_{20}-diterpenoid alkaloids.

Recently, the use of high-performance liquid chromatography–mass spectrometry (HPLC–MS) for analysis in various fields has been reported. HPLC–MS has proved effective for the detection of various alkaloids, for example strychnine [8], nicotine [9], indole alkaloids [10–13], ergot alkaloids [14–16] and tropane alkaloids [17].

In the present paper we report on the analytical conditions for the determination and the quantitative analysis of kobusine (KB), pseudokobusine (PK), dehydroluciduscuIine (DL), luciduscuIine (LD), delcosine (DC) and 14-acetyldelcosine (AD) (Fig. 1), using high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (HPLC–APCI-MS). A method using selected-ion monitoring (SIM) of protonated molecules ($[M + H]^+$) was developed for the verification of the HPLC peak and for the quantitation of

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0021-9673/93/$06.00

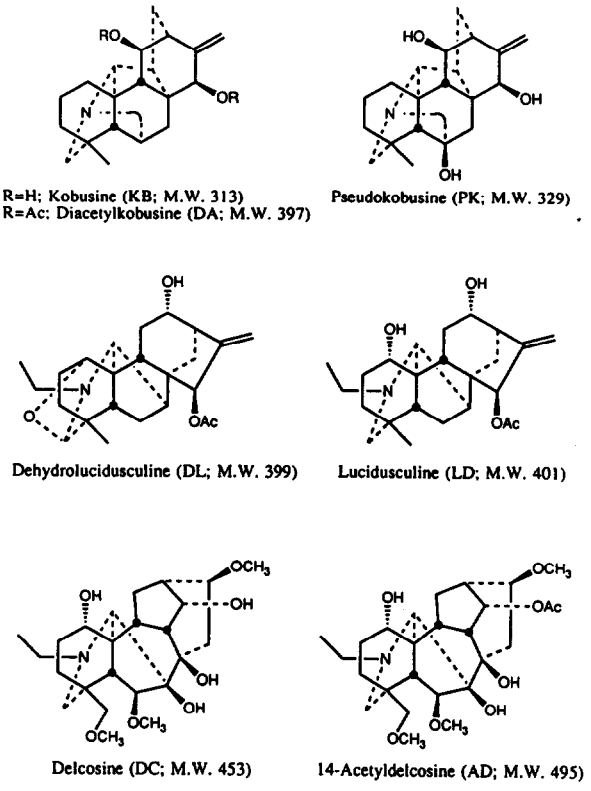

Fig. 1. Structures and molecular mass (M.W.) of the investigated *Aconitum* alkaloids and internal standard alkaloid.

diterpenoid alkaloid in plant extracts of *Aconitum yesoense* var. *macroyesoense* (Nakai) Tamura.

EXPERIMENTAL

Materials

Kobusine (KB), pseudokobusine (PK), dehydroluciduscusline (DL), luciduscusline (LD), delcosine (DC) and 14-acetyldelcosine (AD) were purified from *Aconitum yesoense* var. *macroyesoense* roots and identified as described previously [18]. Diacetylkobusine (DA) used as an internal standard was synthesized from kobusine (KB). Ammonium acetate of reagent grade was purchased from Kanto Chemicals (Tokyo, Japan), and acetonitrile and tetrahydrofuran of HPLC grade were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Apparatus

IR spectra in potassium bromide discs were taken with a Model IRA-2 spectrometer (Jasco, Tokyo, Japan). NMR spectra were measured in deuterated trichloromethane solution with a Model GX-270 spectrometer (JEOL, Tokyo, Japan) using TMS as an internal standard. MS was performed with a Model M-2000 mass spectrometer (Hitachi, Tokyo, Japan). A Model M-2000 mass spectrometer through the APCI interface was used as the HPLC–APCI-MS system.

Diacetylkobusine

M.p., 139–141°C (from ether–hexane); IR, 1730, 1370 cm^{-1}; ^1H NMR, δ 0.97 (3H, s, 18-CH_3), 2.00 and 2.09 (each 3H, s, OAc), 5.00 and 5.18 (each 1H, s, 17-H_2), 5.08 (1H, d, $J = 5$ Hz, 11-H), 5.42 (1H, s, 15-H); MS, m/z 397 (M^+, base peak), 354, 338.

HPLC–APCI-MS conditions

The HPLC system consisted of a Model L-6200 chromatographic pump (Hitachi, Tokyo, Japan) and a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 20-μl loop. The HPLC column was an Inertsil ODS-2 (150 × 4.6 mm I.D., 5 μm; GL Sciences, Tokyo, Japan), and the definite eluent consisted of 0.05 *M* ammonium acetate–acetonitrile–tetrahydrofuran (60:25:15, v/v/v) at a flow-rate of 0.8 ml/min. The interface consisted of nebulizing and vaporizing unit. The temperature of the nebulizer was set to 370°C to give the strong intensity of target ions. The desolvater temperature was set to 400°C.

Vaporized sample and solvent molecules were led into the ion source of the APCI–MS system, the solvent molecules were ionized by corona discharge and then the sample molecules and ionized solvent molecules underwent ion–molecule reactions.

The mass spectrometer was operated in the APCI mode, the drift voltage was set to 143 V and the scan ranges were 280–510 u.

Sample preparation

The *A. yesoense* var. *macroyesoense* samples were obtained from the root of Jozankei, the city

of Sapporo, Hokkaido, Japan. The root samples were freeze-dried and ground. The samples (1 g) were stirred with organic solvents (ether, chloroform or methanol, 10 ml) containing 10% aqueous ammonia (0.5 ml) at room temperature for 30 min [6]. Water (0.5 ml) was added to the solution. The liquid phase was filtered and the residue was further extracted twice in the same manner with the same solvent (10 ml each), but no water was added. The combined solutions were evaporated to dryness. The extract was dissolved with HPLC mobile phase (5 ml), and filtered with a 0.5-μm filter. Internal standard (10 μg per 20 μl of diacetylkobusine) was added to the filtrated solution (180 μl), and this solution (10 μl) was injected into the HPLC–APCI-MS system. The content of alkaloids was calculated.

The recovery of extracted alkaloids from root samples was determined by addition of kobusine, lucidusculine and delcosine at 400 μg to the root samples. The recoveries of alkaloids were compared with those from root extract samples to which no alkaloids had been added. The extraction procedure was the same as above.

Calibration curves and quality control of assay

The calibration curves (10, 100 and 500 ng and 1 and 10 μg per injection) were prepared using standard solutions of each alkaloid. At same time, the internal standard (500 ng per injection of diacetylkobusine) was added to each solution, and 10-μl aliquots of the solutions were injected into the HPLC–APCI-MS system. The calibration curves were calculated by the internal standard method for each peak area. Relative standard deviations (R.S.D.s) were determined using 10 ng and 100 ng of alkaloids.

RESULTS AND DISCUSSION

The mass spectra show protonated molecules ($[M + H]^+$) for kobusine at m/z 314, for pseudokobusine at m/z 330, for dehydroluciduscuiline at m/z 400, for lucidusculine at m/z 402, for delcosine at m/z 454, for 14-acetyldelcosine at m/z 496 and for diacetylkobusine (internal standard) at m/z 398 (Fig. 2). However, the characteristic clusters produced by water or ammonia

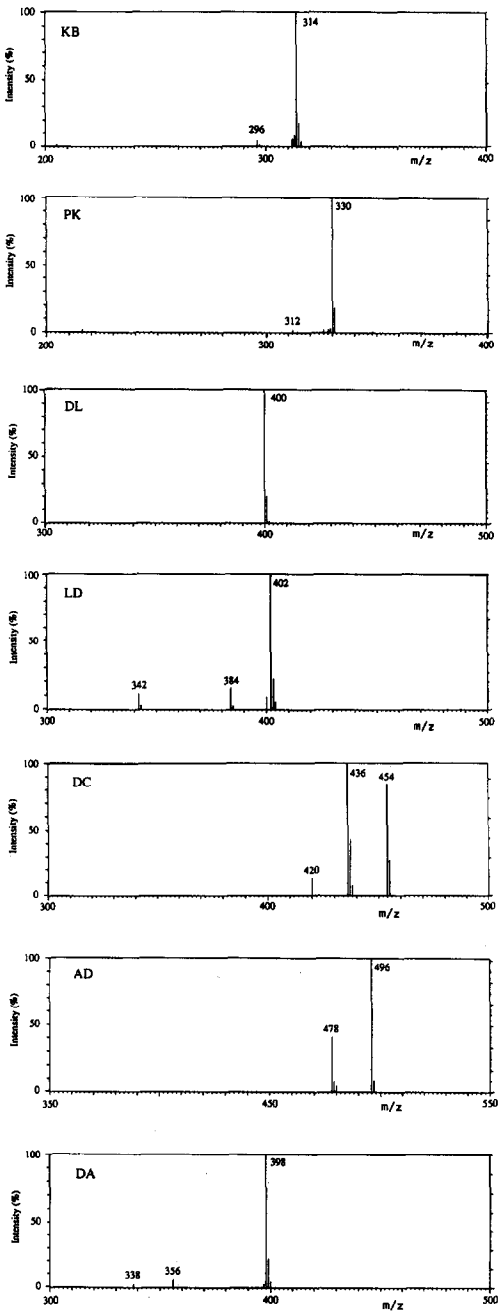

Fig. 2. Mass spectra for kobusine (KB), pseudokobusine (PK), dehydroluciduscuiline (DL), lucidusculine (LD), delcosine (DC), 14-acetyldelcosine (AD) and diacetylkobusine (DA; internal standard). The compounds (2 μg) were injected via the column. Conditions: Inertsil ODS-2 column, 150 × 4.6 mm I.D., 5 μm; mobile phase, 0.05 *M* ammonium acetate–acetonitrile–tetrahydrofuran (60:25:15, v/v); flow-rate 0.8 ml/min; nebulizer temperature, 370°C; desolvater temperature, 400°C.

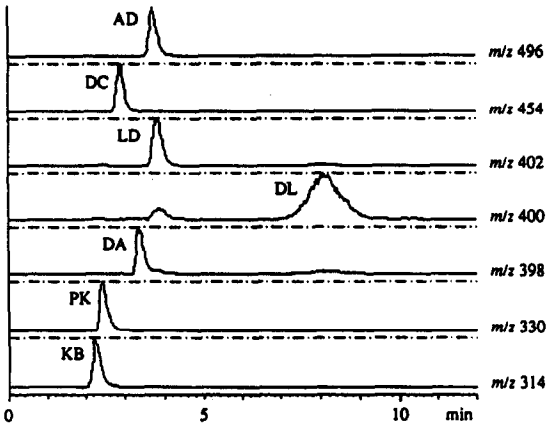

Fig. 3. Mass fragmentograms of kobusine (KB), pseudokobusine (PK), dehydroluciduscuiline (DL), luciduscuiline (LD), delcosine (DC), 14-acetyldelcosine (AD) and diacetylkobusine (DA; internal standard) using the selected-ion monitoring method. Conditions as in Fig. 2.

ion attachment to each $[M+H]^+$ were not observed. The mass spectrum of kobusine shows a fragment ion at m/z 296 ($[M+H-water]^+$). The mass spectrum of pseudokobusine shows a fragment ion at m/z 312 ($[M+H-water]^+$). The mass spectrum of dehydroluciduscuiline shows no fragment ion. The mass spectrum of luciduscuiline shows fragment ions at m/z 384 ($[M+H-water]^+$) and 342 ($[M+H-acetic\ acid]^+$). The mass spectrum of delcosine shows fragment ions at m/z 436 ($[M+H-water]^+$) and 420 ($[M+H-CH_4-water]^+$). The mass spectrum of 14-acetyldelcosine shows a fragment ion at m/z 478 ($[M+H-water]^+$). The mass spectrum of diacetylkobusine (as an internal standard) shows fragment ions at m/z 356 ($[M+H-CH_2CO]^+$) and 338 ($[M+H-acetic\ acid]^+$).

The $[M+H]^+$ ion showed high intensity in the mass spectra of kobusine, pseudokobusine, dehydroluciduscuiline, luciduscuiline, delcosine and 14-acetyldelcosine, and selected-ion monitoring (SIM) of the $[M+H]^+$ ions was a useful technique for the quantitation of their compounds. *Aconitum* alkaloids were unambiguously determined by the SIM method under these conditions (Fig. 3). Good linearity was observed ($r > 0.997$ in all instances) when *Aconitum* alkaloids and internal standard peak-area ratios were plotted for the range 10 ng to 10 μg per injection. The reproducibility of the measurement was investigated by analysing known amounts (10 and 100 ng) of *Aconitum* alkaloids. The relative standard deviations (%) were obtained by calculating the ratio of the peak intensities of *Aconitum* alkaloids against the intensity of internal standard (Table I). The reproducibility of the result was found to be within the acceptable limit.

The HPLC–APCI-MS method with SIM was applied to the determination of *Aconitum* alkaloids in plants, and the elution at a flow-rate of 0.8 ml/min resulted in good chromatographic separation of the alkaloids within 10 min using the definite solvent system, and buffer ionization

TABLE I

REGRESSION PARAMETERS OF THE HPLC–APCI-MS ASSAY OF *ACONITUM* ALKALOIDS

r = Coefficient of correlation; R.S.D. = relative standard deviation; x = amount of analyte in ng; y = peak-area ratio of analyte and internal standard.

Compound	Curve equation	r	R.S.D. (%, $n=7$)	
			10 ng	100 ng
Kobusine	$y = 6.607 \cdot 10^{-3}x + 9.462 \cdot 10^{-4}$	0.9967	10.1	11.1
Pseudokobusine	$y = 6.487 \cdot 10^{-3}x - 1.678 \cdot 10^{-4}$	0.9990	9.1	11.7
Dehydroluciduscuiline	$y = 1.511 \cdot 10^{-3}x + 2.641 \cdot 10^{-3}$	0.9962	13.3	10.4
Luciduscuiline	$y = 6.532 \cdot 10^{-4}x + 6.715 \cdot 10^{-3}$	0.9994	10.6	2.9
Delcosine	$y = 1.906 \cdot 10^{-4}x + 8.192 \cdot 10^{-4}$	0.9976	17.3	6.4
14-Acetyldelcosine	$y = 1.681 \cdot 10^{-4}x - 4.458 \cdot 10^{-5}$	0.9972	9.1	11.7

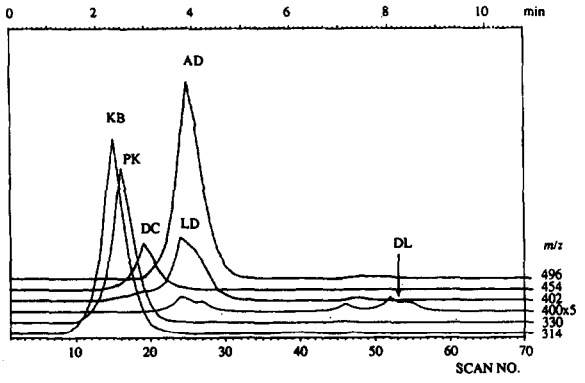

Fig. 4. Mass chromatograms of a plant sample of *A. yesoense* var. *macroyesoense*. Compounds monitored: kobusine (KB) at *m/z* 314, pseudokobusine (PK) at *m/z* 330, dehydrolucidusculine (DL) at *m/z* 400, lucidusculine (LD) at *m/z* 402, delcosine (DC) at *m/z* 454, 14-acetyldelcosine (AD) at *m/z* 496. Conditions as in Fig. 2.

was stable and the background noise was a low enough to allow good net peaks for 1–5 ng samples of the alkaloids.

In order to verify the applicability of this procedure, *Aconitum* root was assayed by employing the above procedure. The mass chromatograms of a plant sample are shown in Fig. 4. A comparative assessment of various solvents for extraction of *Aconitum* alkaloids from aconite was made to examine the efficiency of different extraction procedures. The results are shown in Table II. In the chloroform extraction procedure, the recovery of kobusine, lucidusculine and delcosine was found to be 95.4, 97.4 and 94.3%, respectively, and the extraction method was considered to be the most satisfactory. The contents of 14-acetyldelcosine was found to be the highest in *Aconitum* alkaloids.

CONCLUSIONS

It has been demonstrated that HPLC–APCI-MS can give $[M + H]^+$ ions species of *Aconitum* alkaloids, and that the method is useful for the separation and simultaneous determination of *Aconitum* alkaloids in plant samples. The wide linear range of the assay is applicable to determination with respect to a variety of contents in samples from different sources. The precision of the method is adequate for screening the *Aconitum* alkaloid content of plant extracts and can be also applied to the identification of *Aconitum* alkaloid metabolites in biological samples.

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TABLE II

CONTENTS OF ALKALOIDS IN *A. YESOENSE* VAR. *MACROYESOENSE*

Compound	Extracting solvents (μg/g ± S.D.)			Recovery (%)		
	Ether	Chloroform	Methanol	Ether	Chloroform	Methanol
Kobusine	409 ± 27.7	443 ± 41.0	449 ± 17.9	39.5	95.4	79.8
Pseudokobusine	263 ± 9.85	384 ± 21.2	344 ± 18.5	–	–	–
Dehydrolucidusculine	186 ± 12.9	44.8 ± 1.77	7.15 ± 0.699	–	–	–
Lucidusculine	$1.28 \cdot 10^3 \pm 51.6$	$1.53 \cdot 10^3 \pm 108$	$1.35 \cdot 10^3 \pm 76.9$	97.2	97.4	98.3
Delcosine	632 ± 30.0	778 ± 58.8	723 ± 59.7	94.6	94.3	94.3
14-Acetyldelcosine	$7.07 \cdot 10^3 \pm 632$	$6.06 \cdot 10^3 \pm 742$	$5.87 \cdot 10^3 \pm 364$	–	–	–

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