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Short communication

Determination of lauraceous aporphine alkaloids by high-performance liquid chromatography[1]

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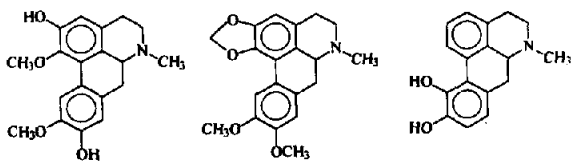
1. Introduction

Aporphine alkaloids, commonly present in lauraceous plants [1], have been shown to possess various pharmacological activities, e.g. choleretic [2] and smooth-muscle relaxing [3] for boldine (Fig. 1) and hypotensive and hyperlipidaemia-reducing in tested animals [4,5] for dicentrine (Fig. 1).

In view of the large number of species of the lauraceous plants (e.g. 16 and 11 species in *Litsea* and *Neolitsea* genuses, respectively, in Taiwan [6]), an efficient analytical method was required to facilitate the study of the alkaloidal constituents in these plants.



Boldine Dicentrine Apomorphine

Fig. 1. Structures of boldine, dicentrine and apomorphine.

In 1988, Pietta et al. [7] reported a high-performance liquid chromatographic (HPLC) determination of three aporphine alkaloids (boldine, isocorydine and *N*-methyllaurotetanine) in Boldo extracts from the herb *Peumus boldus*. In 1990, Betts [8] modified Pietta et al.'s procedure and recorded the separation of six components (boldine, isoboldine, laurotetanine, *N*-methyllaurotetanine, isocorydine and isocorydine-N=O) in processed Boldo; however, some peaks overlapped with the isocratic solvent system used as the authors intention was only to obtain a quick view of the constituents of the extract.

The present work was undertaken to develop a rapid and reliable HPLC method for the determination of eight aporphine alkaloids found in many lauraceous plants; glaziovine (a proaporphine), laurolitsine, boldine, isoboldine, isocorydine, laurotetanine, *N*-methyllaurotetanine and isodomesticine (Fig. 2). The method involves

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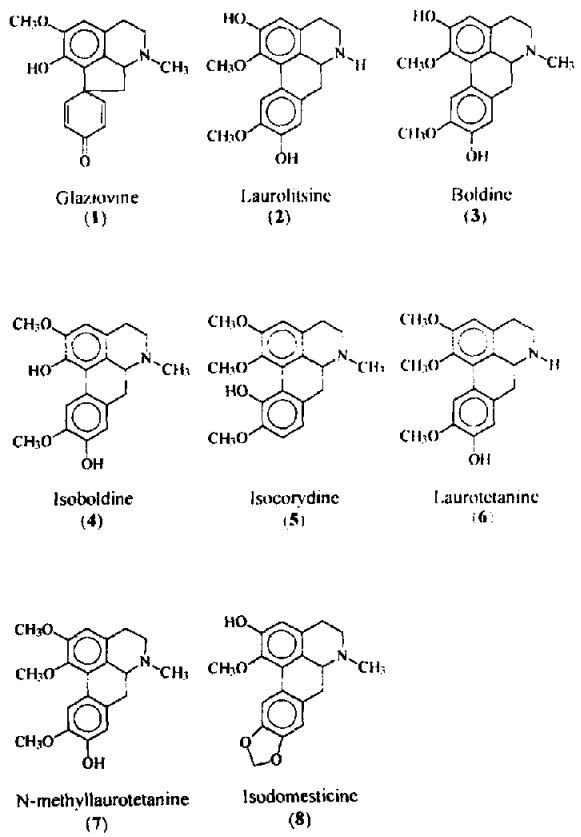

Fig. 2. Aporphine alkaloids used for the analysis of lauraceous plants.

treatment of stem wood and leaf parts of the plant with a conventional extraction procedure [1] to remove interfering substances, followed by isocratic reversed-phase HPLC.

2. Experimental

2.1. Chemicals and reagents

Boldine was purchased from Sigma, St. Louis, MO and the other seven alkaloids, glaziovine, laurolitsine, isoboldine, isocorydine, laurotetanine, *N*-methyllaurotetanine and isodomesticine, were isolated from the lauraceous plant *Litsea cubeba* [9]. The identities and purities of the substances were verified by TLC and HPLC and by UV, IR, ^{1}H NMR and mass spectrometry. Codeine phosphate was supplied by the Narcotic Bureau of the Government (Taiwan). Ethanol (95%) an chloroform used in the extraction process for sample preparations were obtained locally and distilled before use. Hydrochloric acid used in the same process was purchased from Carlo Erba (Milan, Italy). All organic solvents and chemicals used in HPLC analysis were of chromatographic or analytical grade. Acetonitrile and methanol were purchased from Mallinckrodt (Paris, KY). Ammonia solution (25%) and phosphoric acid (85%) were obtained from Merck (Darmstadt, Germany). Water was purified in a Barnstead water-purification system (Dubuque, IA, USA).

2.2. Preparation of samples [1]

The ground powders of stem woods of the plant *Neolitsea sericea* var. *aurata* (collected in August 1992 in Lanyu, Taitung County, Taiwan) were extracted with 95% ethanol. Concentration of the ethanolic extract afforded a residue from which the alkaloids were extracted with 0.1 N hydrochloric acid. The neutral compounds in the acidic aqueous layer were removed by partitioning with chloroform. The pH of the aqueous layer was adjusted to 9.0 with ammonia solution and the free bases were extracted with chloroform. The chloroform extract and the precipitate were taken as samples for the determination of the aporphine alkaloids. The preparation of the samples from the leaves of the plant followed the same procedure.

2.3. Apparatus

The chromatographic equipment consisted of a Waters Model 501 HPLC pump, coupled with a Waters U6K manual injector, a Waters photodiode-array (PDA) detection system and an NEC CP6 printer. The PDA system consisted of a Waters Model 990 diode-array detector, an NEC PowerMate 2 computer and PDA software (all from Waters, Milford, MA). The loop volume was 20 μl.

Separations were carried out on a μBondapak C_{18} column (300 × 3.9 mm i.d., 10 μm) (Waters).

UV absorption spectra were obtained in the range 220–400 nm.

2.4. Chromatographic conditions

The mobile phase was ammonium phosphate buffer–acetonitrile–methanol (45:12:1, v/v/v). The buffer was prepared with 0.02 M phosphoric acid solution adjusted to pH 4.3 by addition of ammonia solution.

The mobile phases were filtered through a 0.45 μm filter (Millipore) and degassed ultrasonically before use. The flow rate was 1.0 ml min^{-1} and the column was operated at room temperature. The detection wavelength was set at 280 nm and the injection volume was 10 μl.

A reference solution containing eight standards, glaziovine (60 μg ml^{-1}), laurolitsine (30 μg ml^{-1}), boldine (60 μg ml^{-1}), isoboldine (60 μg ml^{-1}), isocorydine (60 μg ml^{-1}), laurotetanine (30 μg ml^{-1}), *N*-methyllaurotetanine (60 μg ml^{-1}) and isodomesticine (30 μg ml^{-1}), in methanol was prepared and analysed. Calibration graphs for laurolitsine, boldine, isoboldine and laurotetanine were prepared with the addition of codeine phosphate as an internal standard.

3. Results and discussion

Phenolic aporphines are amphoteric and more stable in acidic than in alkaline solutions. Considering these properties, Pietta et al. [7] pointed out that acidic amine phosphate buffers could be useful in reversed-phase HPLC for the determination of alkaloids by reducing the tailing of peaks, thus improving peak separations.

In this work, a mobile phase of ammonium phosphate buffer (0.02 M phosphoric acid solution adjusted to the desired pH with ammonia solution)–acetonitrile (80:20, v/v) was initially tested. The eight standards were separated but all the peaks were strongly tailed. With the addition of a minor amount of methanol (the resulting mobile phase was phosphate buffer–acetonitrile–methanol (45:12:1, v/v/v)), the peak shapes were much improved and a near-baseline separation was achieved (Fig. 3).

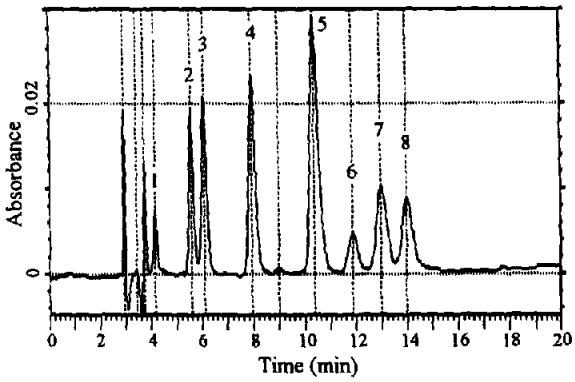

Fig. 3. Chromatogram of the standard mixture of aporphine alkaloids. Eluent, ammonium phosphate buffer (pH 3.7, 0.02 M)–acetonitrile–methanol (45:12:1, v/v/v); flow rate, 1.0 ml min^{-1}; UV detection at 280 nm. For compound identities, see Fig. 2.

With an acidic mobile phase, the elution order of the alkaloids remains unchanged, i.e. glaziovine > laurolitsine > boldine > isoboldine > isocorydine > laurotetanine > *N*-methyllaurotetanine > isodomesticine (Fig. 4). Glaziovine, although having one less phenolic group in comparison with the diphenolics laurolitsine, boldine and isoboldine, was eluted ahead of the latter. This irregular order might be attributed to the

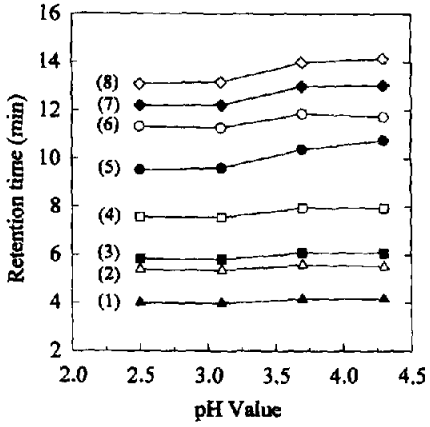

Fig. 4. Retention times of the aporphine standards vs. pH. Eluent, ammonium phosphate buffer (0.02 M)–acetonitrile–methanol (45:12:1, v/v/v); flow rate, 1.0 ml min^{-1}; UV detection at 280 nm. For compound identities, see Fig. 2.

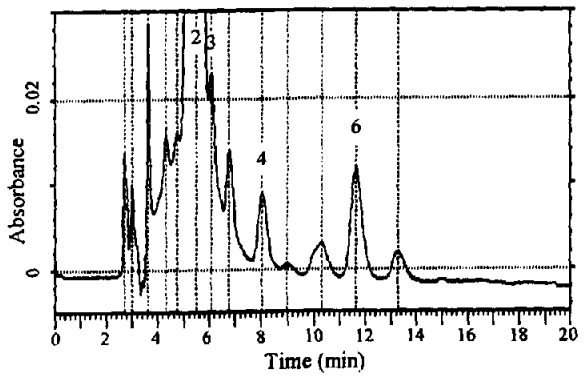

Fig. 5. Chromatogram of the chloroform extract preparation from the stem woods of *N. sericea* var. *aurata*. Eluent, ammonium phosphate buffer (pH 4.3, 0.02 M)–acetonitrile–methanol (45:12:1, v/v/v); flow rate, 1.0 ml min^{-1}; UV detection at 280 nm. Laurolitsine (2), boldine (3), isoboldine (4) and laurotetanine (6) were detected in the sample.

larger charge density of smaller bases such as glaziovine in the acidic mobile phase. The charge density arising from the protonation of the bases plays a dominant role in polarity over those contributed by other functional groups [1]. The three diphenolics were eluted before the four monophenolics isocorydine, laurotetanine, *N*-methyllaurotetanine and isodomesticine, with the noraporphines being eluted earlier than the corresponding aporphines (laurolitsine > boldine and laurotetanine > *N*-methyllaurotetanine).

The acidic and basic strengths of the tested aporphines have not been reported in the literature. By analogy with apomorphine (Fig. 1) [10], the pK_b's and pK_a's of these aporphines were estimated to be about 7 and 9, respectively. In acidic mobile phases, the aporphines existed as the protonated amines. The retention times changed only slightly over the tested acidic pH range (2.5–4.3) (Fig. 4); however, when the pH was raised above 5, the retention times increases abruptly (data not shown). This is due to the predominance of the non-ionized form of aporphines in neutral eluents, which can be verified by the high methanol and/or acetonitrile contents (> 45%, v/v) in the mobile phases for the analysis of boldine at pH 7.4 and 9.0, respectively [11,12].

In the analysis of the samples prepared from the stem woods and leaves of *N. sericea* var. *aurata*, four aporphines, laurolitsine, boldine, isoboldine and laurotetanine, were identified in the chloroform extract preparation, whereas only laurolitsine was detected as the aporphine constituent in the precipitate preparation. Figs. 5 and 6 show the chromatograms obtained for the two preparations from stem woods of the plant. Sets of five standard alkaloid solutions covering the range 15–200 $\mu g\ ml^{-1}$ were run, with codeine phosphate as internal standard, and the following relationships between peak-height ratios (y) and concentrations injected ($\mu g\ ml^{-1}$) (x) were obtained:

laurolitsine:
$y = 0.0183x + 0.3184$ $r = 0.995$
boldine:
$y = 0.0180x + 0.0434$ $r = 0.997$
isoboldine:
$y = 0.0220x + 0.0295$ $r = 0.995$
laurotetanine:
$y = 0.0077x + 0.0505$ $r = 0.997$

The quantitation of the four aporphines in stem wood and leaf parts of *N. sericea* var. *aurata* was carried out by internal standardization (Table 1) with good repeatability (RSD = 1%, $n = 6$). The procedure can be successfully used to determine

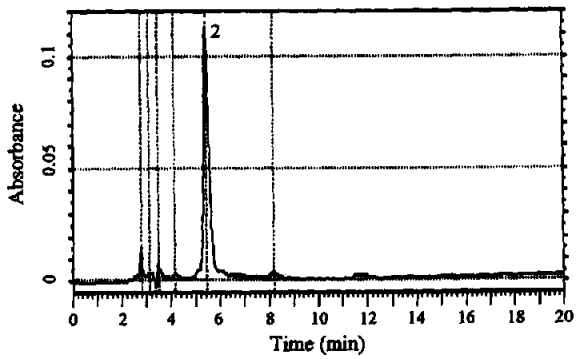

Fig. 6. Chromatogram of the precipitate preparation from the stem woods of *N. sericea* var. *aurata*. Eluent, ammonium phosphate buffer (pH 4.3, 0.02 M)–acetonitrile–methanol (45:12:1, v/v/v); flow rate, 1.0 ml min^{-1}; UV detection at 280 nm. Laurolitsine (2) was detected in the sample.

Table 1
Contents of laurolitsine, boldine, isoboldine and laurotetanine in stem woods and leaves of *N. sericea* var. *aurata*[a]

Part	Compound (mg-%)			
	Laurolitsine	Boldine	Isoboldine	Laurotetanine
Stem woods	135	1.6	0.4	1.8
Leaves	53	12	1.1	6.8

[a] Chromatographic conditions: eluent, ammonium phosphate buffer (pH 4.3, 0.02 M)–acetonitrile–methanol (45:12:1, v/v/v); flow rate, 1.0 ml min^{-1}; UV detection at 280 nm.

the contents of these compounds in other lauraceous plants.

The content of boldine in the leaves (12 mg-%, i.e. 120 ppm on a w/w basis) of the plant *N. sericea* var. *aurata* is comparable to that in the leaf extracts of *Peumus boldus* found by Pietta et al. [7] (8, 14 and 16 mg-%). Total aporphine alkaloids calculated as boldine form 133 mg-% of the stem woods and 71 mg-% of the leaves for *N. sericea* var. *aurata*. This suggests that the lauraceous plants, as an abundant source of aporphine alkaloids, deserve extensive studies for practical reasons.

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