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Short Communication

# Application of centrifugal partition chromatography to the separation of Lauraceous alkaloids

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**Abstract**

A thorough and facile separation of polar phenolic alkaloids from Lauraceous plants was achieved by application of centrifugal partition chromatography using $CHCl_3$–MeOH–$H_2O$ (containing 0–1% HOAc) (2:2:1 or 5:5:3) as the delivery system, and subsequently in combination with conventional methods. This method solves the problems of low resolution and poor recovery in the application of general adsorption chromatography to the separation of polar phenolic alkaloids. The most polar alkaloid, laurolitsine, was isolated easily and several additional alkaloids were obtained from the investigation or reinvestigation of three Lauraceous plants.

## Introduction

Lauraceous plants are widely distributed in Taiwan and it has been demonstrated that they are rich in isoquinoline alkaloids, especially of the aporphine type. The phenolic alkaloids are amphoteric natural products and hence are less stable and relatively polar. By using conventional adsorption chromatography to separate these compounds, two problems, poor resolution and poor recovery, are generally encountered. Reversed-phase liquid chromatography might be an alternative to solve the recovery problem. However, the low capacity, high cost and resolution problem need to be settled. Considering these, modern partition chromatographic techniques possessing the properties of high capacity and complete recovery were applied to resolve these problems. Among these, centrifugal partition chromatography (CPC) has the advantage of a high flow velocity, which greatly shortens the separation time [1]. We report here the results of the application of CPC to the separation and fractionation of the polar phenolic isoquinolines from three Lauraceous plants, *Litsea cubeba*, *Phoebe formosana* and *Neolitsea konishii*.

## 2. Experimental

### 2.1. Instrumentation and chemicals

The following instrumentation was used: for CPC, LLN Model, six preparative cartridges (Type 1000E, radius 11 cm), void volume 425 ml (Sanki Engineering, Nagaokakyo, Kyoto, Japan); for droplet counter-current chromatography) (DCCC), EYELA D.C.C.-A, 300 tubes (O.D. 0.5 cm, length 40 cm) (Tokyo Rikakikai, Tokyo, Japan); Chromatotron (a preparative centrifugally accelerated radial, thin-layer chromatograph), Model 7924T, coated with silica gel (Merck Kieselgel 60 $PF_{254}$ gipshaltig, 2 mm thick) (Harrison Research, Palo Alto, CA,

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USA); TLC analytical system, silica gel TLC plate (Merck, 0.20 mm thick) with developing solvent systems (A) chloroform–methanol (9:1) and (B) acetone–toluene (3:2), both saturated with ammonia solution.

Ammonia solution (27%), glacial acetic acid, sodium hydroxide, bismuth subcarbonate and sodium iodide were purchased from Wako (Osaka, Japan). The last two reagents were used for preparing Dragendorff's reagent for detection of alkalodes. Other solvents were obtained locally and were distilled before use.

### 2.2. General procedure for extraction and separation of Lauraceous alkaloids

The dried and powdered plant material was extracted with MeOH or 95% EtOH. The alcoholic extract was triturated with citric acid (2%) or HOAc (2%). The combined acidic solution was partitioned with $CHCl_3$ to remove neutral or acidic components and was then adjusted to pH 9 with ammonia solution. In this process, the precipitate, if any, which generally contained laurolitsine (**1**) in several Lauraceous plants, was filtered. The filtrate was extracted with $CHCl_3$ and $CHCl_3$ layer, after drying over $MgSO_4$, was evaporated to give total free bases. The total alkaloids were divided into non-phenolic bases and phenolic bases by partitioning between $CHCl_3$ and NaOH (0.5 *M*).

By this procedure, the dried powdered stems of *Litsea cubeba* (Lour.) Persoon (8.50 kg) yielded non-phenolic bases (9.00 g) and phenolic bases (11.36 g) [2] and from 2.5 kg of roots 8.27 g of total free alkaloids were obtained. The dried, powdered roots of *Phoebe formosana* (Hayata) Hayata (10.5 kg) yielded non-phenolic bases (0.23 g), phenolic bases (15.5 g) and a precipitate (209 g, containing **1** as the major component) [3]. From the dried powdered stem woods (16.80 kg) of *Neolitsea konishii* (Hayata) Kanehira & Sasaki, non-phenolic alkaloids (0.58 g) and phenolic alkaloids (8.09 g) were obtained [4].

The phenolic fraction was subjected to CPC separation, 3.5–8.0 g in each run, using the upper layer (aqueous layer) and lower layer (organic layer) of chloroform–methanol–water (containing 0–1% of acetic acid) in the ratio of 2:2:1 or 5:5:3 (see Figs. 2–5) as mobile phase and stationary phase, respectively. The parameters set for optimum pressure (35–50 bar) were flow-rate 2.5–5.3 ml/min and centrifugal force 78.7–123.0 *g* (corresponding to 800–1000 rpm). The eluent was collected in 6.0–10.6-ml fractions. The fractions were monitored directly by TLC. The combined fractions were evaporated at 45°C under reduced pressure to remove the solvents and, if necessary, toluene or water was added to remove acetic acid azetropically. The fractions containing mixtures were further separated via a silica gel gravity column or flash column chromatography (Merck, 230–400 mesh) with sample–gel (1:40, w/w), or via preparative TLC plates (Merck, $SiO_2$, 1–2 mm thick, 20 × 20 cm) eluted with solvent A or B, or DCCC using the upper layer and lower layer of the solvent system $CHCl_3$–MeOH–1% HOAc (5:5:3) as mobile and stationary phase, respectively, at a flow-rate of 0.5 ml/min, or via the Chromatotron (2 mm thick) eluted with acetone–toluene (3:2) at a flow-rate of 4 ml/min. All the isolated alkaloids were characterized on the basis of spectral data and were found to be known bases.

## 3. Results and discussion

From the stem woods of *Litsea cubeba*, a novel phenanthrene alkaloid, litebamine, together with five known bases, liriotulipiferine, reticuline, boldine (**2**), N-methyllaurotetanine (**9**) and isocorydine (**10**), have been isolated [5–7]. *Phoebe formosana* is an abundant source of the aporphine laurolitsine [**1**, 0.84% (w/w) of the root] [8]. From the roots of *P. formosana*, two other phenolic bases, reticuline (**11**) and coreximine (**12**), were isolated [9]. Structures of all the compounds are shown in Fig. 1.

By using the reversed-phase partition model CPC with $CHCl_3$–MeOH–$H_2O$ (containing 0–1% HOAc) (2:2:1 or 5:5:3; see Figs. 2–5) in the separation of phenolic alkaloids from *L. cubeba*, seven fractions from roots (Fig. 2) and eight fractions from stems (Fig. 3) were obtained.

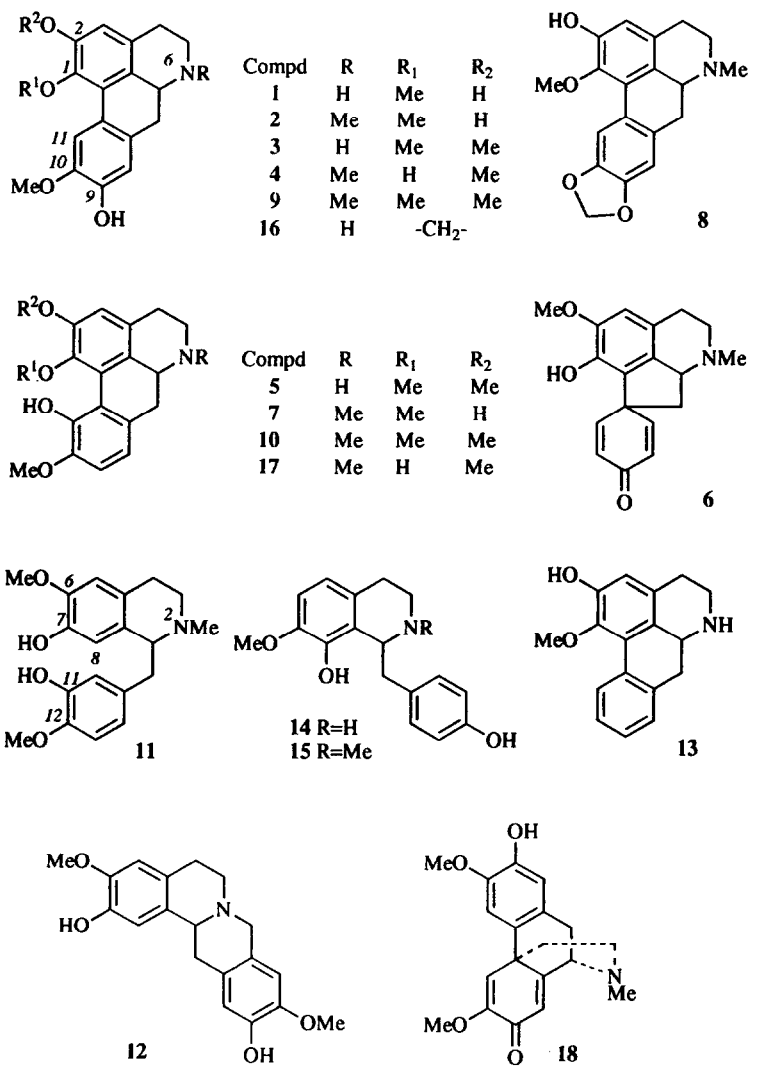


Fig. 1. Structures of compounds **1–18**.

Among these, **1** (fraction 1, Figs. 2 and 3), **2** (fraction 3, Fig. 3), norisocorydine (**5**) (fraction 6, Fig. 2) and **10** (fraction 8, Fig. 3) were directly obtained from these two separations as pure compounds. Similar treatment of the phenolic alkaloids from the roots of *P. formosana* yielded six fractions (Fig. 4). Of these, fraction 1 yielded the pure laurolitsine, appearing also as the major alkaloid.

From *L. cubeba*, six other known alkaloids, laurotetanine (**3**), isoboldine (**4**), glaziovine (**6**), N-methyllindcarpine (**7**), isodomesticine (**8**) and N-methyllaurotetanine (**9**), were isolated from the other fractions by the combination of flash column chromatography and preparative $SiO_2$ TLC [2]. Compounds **1** and **3–8** were isolated for the first time from this species. Similarly, four additional known phenolic bases, **3**, asimilobine (**13**), norjuziphine (**14**) and juziphine (**15**), in addition to the reported three, were separated from the root of *P. formosana* [3]. The last two benzylisoquinolines, **13** and **14**, were isolated for the first time from Lauraceous plants.

By application of this technique, we carried

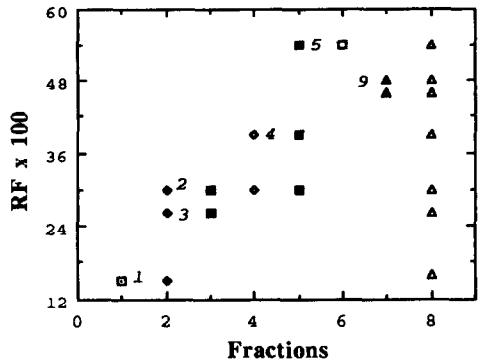


Fig. 2. TLC of fractions obtained by CPC separation of the phenolic alkaloids from the roots of *Litsea cubeba*. Sample: crude phenolic alkaloids (4.32 g). CPC conditions: $CHCl_3$–MeOH–0.5% HOAc (2:2:1); stationary phase, lower layer; mobile phase, upper layer; flow-rate, 4 ml/min; pressure, 40–42 bar; centrifugal force, 123.0 *g*; volume per tube, 10 ml. Fraction 1 (tubes 8–13), 407 mg; fraction 2 (tubes 14–23), 551 mg; fraction 3 (tubes 24–53), 2.63 g; fraction 4 (tubes 54–57), 445 mg; fraction 5 (tube 58), 82 mg; fraction 6 (tube 59), 73 mg; fraction 7 (tubes 60–69), 487 mg; 8 = sample. TLC developing system, solvent A; detection, Dragendorff's reagent.

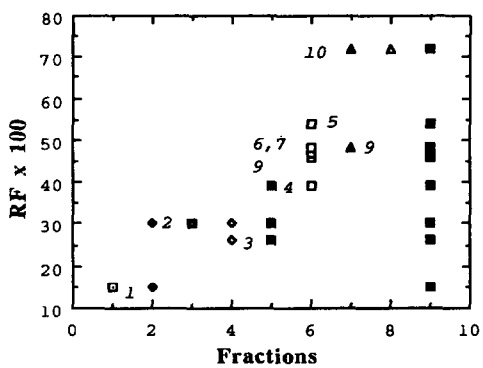


Fig. 3. TLC of fractions obtained by CPC separation of phenolic alkaloids from the stems of *Litsea cubeba*. Sample: crude phenolic alkaloids 3.78 g. CPC conditions: $CHCl_3$–MeOH–$H_2O$ (pH 6.5) (2:2:1); stationary phase, lower layer; mobile phase, upper layer; flow-rate, 3 ml/min; centrifugal force, 123.0 *g*; volume per tube, 6 ml. Fraction 1 (tubes 22–94), 464 mg; fraction 2 (tubes 95–128), 360 mg; fraction 3 (tubes 129–140), 314 mg; fraction 4 (tubes 141–150), 552 mg; fraction 5 (tubes 151–162), 793 mg; fraction 6 (tubes 163–170), 297 mg; fraction 7 (tubes 171–180), 629 mg; fraction 8 (tube 181), 9 mg; 9 = sample. TLC developing system, solvent A; detection, Dragendorff's reagent.

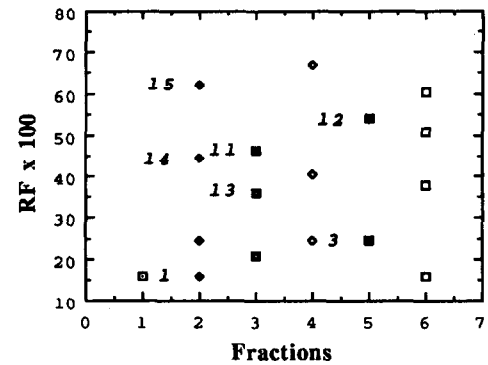


Fig. 4. TLC of fractions obtained by CPC separation of phenolic alkaloids from the root barks of *Phoebe formosana*. Sample: crude phenolic alkaloids, 7.00 g × 2. CPC conditions: $CHCl_3$–MeOH–1% HOAc (5:5:3); stationary phase, lower layer; mobile phase, upper layer; flow-rate, 2.5 ml/min; centrifugal force, 123.0 *g*; volume per tube, 10 ml; fraction 1, 9.018 g; fraction 2, 299 mg; fraction 3, 868 mg; fraction 4, 139 mg; fraction 5, 228 mg; fraction 6, 265 mg. TLC developing system, solvent A; detection, Dragendorff's reagent.

out the first chemical investigation of *Neolitsea konishii* (Hayata) Kanehira & Sasaki [4]. The CPC separation of the phenolic alkaloids from the woods (Fig. 5) resulted in the isolation of pure **1** (fraction 1), **11** (fraction 3) and **4** (fraction 7). Further separation of other fractions with DCCC, Chromatotron, flash column chromatography or preparative TLC gave **2**, **3**, actinodaphnine (**16**), corytuberine (**17**), pallidine (**18**) and N-methyllaurotetanine (**9**).

These results reveal that reversed-phase CPC is very useful for the separation of polar phenolic aporphine alkaloids. The results are reproducible under similar conditions. This technique not only separates the most polar constituent, laurolitsine (**1**), exclusively well, but also rearranges the elution order, which is not the reverse order of that given by the adsorption chromatography. The latter property facilitates to a great extent the further separation of the mixture in the same fraction because of their large $R_F$ differences, such as between N-methyllaurotetanine (**9**, $R_F$ 0.55, solvent B) and pallidine (**18**, $R_F$ 0.30, solvent B) in fraction 9 (Fig. 5). The reason for this irregular elution order might be attributable to the protonation of the bases in the acidic

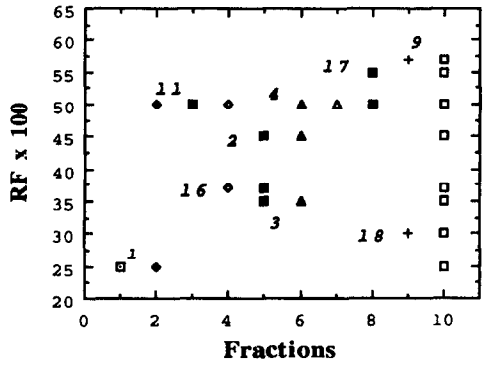


Fig. 5. TLC of fractions obtained by CPC separation of the phenolic alkaloids from the woods of *Neolitsea konishii*. Sample: crude phenolic alkaloids, 8.08 g. CPC conditions: $CHCl_3$–MeOH–1% HOAc (5:5:3); stationary phase, lower layer; mobile phase, upper layer; flow-rate, 5.3 ml/min; centrifugal force, 78.7 *g*; volume per tube, 10.6 ml. Fraction 1 (tubes 1–19), 2.09 g; fraction 2 (tubes 20–25), 393 mg; fraction 3 (tubes 26–49), 3.29 g; fraction 4 (tubes 50–52), 296 mg; fraction 5 (tube 53), 114 mg; fraction 6 (tube 54), 103 mg; fraction 7 (tubes 55–58), 351 mg; fraction 8 (tubes 59–61), 196 mg; fraction 9 (tubes 63–65), 99 mg; 10 = sample. TLC developing system, solvent B; detection, Dragendorff's reagent.

mobile phase, which increases the water solubility of alkaloids and plays a dominant role in polarity over those contributed by other functional groups. From the elution order, **1** > **11** > **16** > **2** > **3** > **4** > **17** > **18** > **5** ≈ **9** ≈ **7** > **10**, we observed that nor-aporphines (**1**, **3**, **5**) always eluted earlier than the corresponding aporphines (**2**, **9**, **10**). We also find that the elution of isomeric phenolic aporphines follows the order 2-OH (**2**) > 1-OH (**4**) > 11-OH (**17**) and 9-OH (**9** or **3**) > 11-OH (**10** or **5**). These elution properties will be of value for the purification of other related isoquinolines.

## 4. Conclusions

These studies demonstrate a good separation model for solving the conventional problems in dealing with complicated phenolic isoquinolines. With this approach, more thorough studies of alkaloidal contents in the Lauraceous plants will become more facile. We hope that these studies will lead to the establishment of a chemotaxonomy of Formosan Lauraceous plants.

## 5. Acknowledgement

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