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Isolation of Alkaloids from Plant Materials by Combination of Ion-Pair Extraction and Preparative Ion-Pair HPLC Using Sodium Perchlorate. III[1)]

Chinese Magnoliae Cortex

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Chinese Magnoliae Cortices (commercial Chinese Magnoliae Cortex and cortices of *Magnolia officinalis* var. *biloba* and *Magnolia officinalis*) were examined for their quaternary alkaloids and the results were compared with those of Japanese Magnoliae Cortex. Six alkaloids, (−)-magnocurarine, (−)-magnoflorine, (+)-laurifoline, (±)-oblongine, (+)-menisperine, and (+)-xanthoplanine, were found in both Chinese and Japanese samples. The quaternary alkaloid contained in Chinese Magnoliae Cortex but not in Japanese Magnoliae Cortex (cortex of *M. obovata*) was purified by preparative ion-pair HPLC using sodium perchlorate, and characterized mainly by the two-dimensional NMR techniques to be (−)-10-demethylcryptaustoline, a new alkaloid having dibenzopyrrocoline skelton.

Keywords——ion-pair HPLC; dibenzopyrrocoline alkaloid; (−)-10-demethylcryptaustoline; Chinese Magnoliae Cortex; *Magnolia officinalis* var. *biloba*

In our previous report, we examined the quaternary alkaloids in Japanese Magnoliae Cortex (和厚朴, cortex of *Magnolia obovata*) by preparing a quaternary alkaloidal fraction by means of ion-pair extraction using sodium perchlorate,[2,3)] and efficiently separating the resulting quaternary alkaloidal fraction by ion-pair HPLC, in which sodium perchlorate was also utilized.[3,4)] In the present study, we applied the method to the assay of Chinese Magnoliae Cortex, commercial Chinese Magnoliae Cortex, and cortices of *M. officinalis* and *M. officinalis* var. *biloba*. The Chinese and Japanese Magnoliae Cortices were found to contain similar quaternary alkaloids, but commercial Chinese Magnoliae Cortex and *M. officinalis* var. *biloba* cortex contained a large peak which was not detected in Japanese Magnoliae Cortex. This alkaloid was isolated and its structure was determined.

EXPERIMENTAL

Plant materials and ion-pair extraction of alkaloids Finely cut Chinese Magnoliae Cortex purchased in Tianjin market were assayed according to the procedures reported previously.[4)] The plant material (1 kg) was extracted with hot MeOH (3 *l*×3). The extract was acidified with 1% aqueous citric acid (500 ml), and treated with diethyl ether (500 ml×2) to remove fat-soluble substances. The remaining aqueous layer was basified with sodium carbonate, and treated with $CHCl_3$ (500 ml×3) to extract tertiary alkaloids. The solvent was evaporated off (Fr-1) and to the residual aqueous layer was added sodium perchlorate to the perchlorate concentration of about 0.5 M. The aqueous layer was extracted with 1,2-dichloroethane (500 ml×3) and the solvent was evaporated off. The quaternary alkaloidal fraction extracted under weakly alkaline conditions (Fr-2) was 500 mg. The remaining aqueous layer was then acidified by adding perchloric acid, and extracted with 1,2-dichloroethane (500 ml×3). Evaporation of 1,2-dichloroethane gave quaternary alkaloids which were not extractable with organic solvent under alkaline conditions but were extractable under acidic conditions (Fr-3, 469 mg). A small amount of Cortices of *M. officinalis* and *M. officinalis* var. *biloba* (each 2 g) were treated with similar manner and analyzed by HPLC.

Ion-pair HPLC Analytical HPLC was performed on a Shimadzu LC-10A apparatus equipped with a UV detector (280 nm). For the measurement of three dimensional HPLC (3D-HPLC), a photodiode-array detector (Model 991 Waters) was used. Either a Cosmosil 5C18-AR column (5 μm, 6 mm×15 cm, Nacalai Tesque, Kyoto) or "Irregular-shaped ODS" column (10 μm, 4 mm×25 cm, carbon content 16%, bore-size 60 Å, Tiajin Secondary Chemical Reagent Co., Ltd.) was used, both giving similar HPLC results. Preparative HPLC was carried out

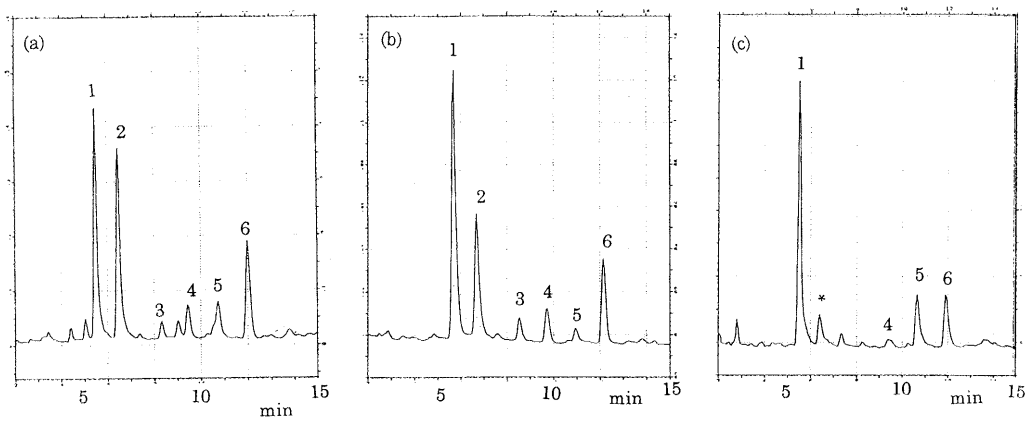

Fig. 1. HPLC Chromatograms of Alkaloidal Fractions (Fr-2) of Chinese Magnoliae Cortices
(a) Chinese Magnoliae Cortex obtained in Tianjin market, (b) Cortex of *Magnolia officinalis* var. *biloba*, (c) Cortex of *Magnolia officinalis*.
HPLC conditions: column, Cosmosil 5C18-AR (6 mm ×15 cm); eluent, gradient elution of (A) 0.2 M sodoium perchlorate: 60% perchloric acid (1,000 : 0.2) and (B) acetonitrile, from A : B=80 : 20 to A : B=65 : 35 (linear gradient for 15 min); flow rate, 2.0 ml/min; detection, photodiode-array detector at 280 nm.
1. (−)-magnocurarine, 2. (−)-10-demethylcryptaustoline, 3. (+)-laurifoline, 4. (±)-oblongine, 5. (+)-menisperine, 6. (+)-xanthoplanine, *. unknown (see text).

on a Shimadzu LC-10A apparatus equipped with a semi-preparative scale column of "Irregular-shaped" ODS (10 μm, 10 mm×25 cm). In both analytical and preparative HPLCs, a gradient elution system composed of (A) 0.2 M $NaClO_4$: 60% $HClO_4$ (1,000 : 0.2) and (B) acetonitrile was used unless otherwise stated. The precise gradient conditions for analytical HPLC are shown in footnote to Fig. 1. The gradient conditions for preparative HPLC were as follows; (A)/(B) 85/15 to 75/25 (linear gradient for 32 min); flow rate: 4.5 ml/min. A part of Fr-2 was submitted to preparative HPLC several times to give 5 mg of (**2**) in addition to (**1**), (**3**), (**4**), (**5**), and (**7**) obtained previously.[4]

NMR and other apparatus ^{1}H-, ^{13}C-, and various 2D-NMR spectra were obtained by using a Varian VXR 500 instrument. Low- and high-resolution SIMSs were measured with a Hitachi M-4100 instrument using glycerol as a matrix. The primary ion was Cs^+ and the accelerating voltages of primary and secondary ions were 15 and 6 kV, respectively. IR and optical rotation were measured with a FTIR 8200 spectrometer (Shimadzu) and a DIP-370 polarimeter (JASCO), respectively.

Spectral data of (−)-10-demethylcryptaustoline perchlorate (2) (7*S*, 12a*R*)-5, 6, 12, 12a-tetrahydro-2, 10-dihydroxy-3, 9-dimethoxy-7-methylindolo [2, 1-*a*] isoquinolinium perchlorate. White powder, $[\alpha]_D$ −112° (*c*=0.33, MeOH), low-resolution SIMS *m/z*: [M^+] 328 (58), 207 (99), 115 (100), high-resolution SIMS 328.1539 (328.1547 calcd. for $C_{19}H_{22}NO_4^+$), UV λ_{max} (HPLC eluent) nm : 285, IR ν_{max}^{KBr} cm^{-1} : 3250 (OH), 1518, 1146, 1120, 1090, 636, ^{1}H-NMR ($(CD_3)_2CO$) δ : 3.11 (1H, m, H-5), 3.29 (1H, ddd, *J*= 16.0, 9.0, 0.5 Hz, H-12), 3.34 (1H, m, H-5), 3.74 (3H, s, NCH_3), 3.87 (3H, s, OCH_3-3), 3.90 (1H, m, H-6), 3.92 (1H, dd, *J*= 16.0, 8.0 Hz, H-12), 3.97 (3H, s, OCH_3-9), 4.10 (1H, m, H-6), 5.40 (1H, dd, *J*= 9.0, 8.0 Hz, H-12a), 6.87 (1H, s, H-1), 6.93 (1H, s, H-4), 6.98 (1H, s, H-11), 7.63 (1H, s, H-8), ^{13}C-NMR ($(CD_3)_2CO$) δ : 24.87 (C-5), 37.08 (C-12), 50.29 (NCH_3), 56.37 (OCH_3-3), 57.05 (OCH_3-9), 59.50 (C-6), 75.76 (C-12a), 102.14 (C-8), 112.06 (C-4), 112.61 (C-11), 113.79 (C-1), 120.92 (C-4a), 123.14 (C-12b), 125.85 (C-11a), 139.25 (C-7a), 147.42 (C-2), 148.84 (C-3), 149.40 (C-9), 150.13 (C-10).

RESULTS AND DISCUSSION

The HPLC chromatograms of alkaloidal fractions showed that Chinese and Japanese Magnoliae Cortices had similar alkaloidal constituents. Because Fr-1 gave only very small peaks in all cases, no further investigation was made on Fr-1. Fr-3s of Japanese Magnoliae Cortex and Chinese Magnoliae Cortices contained (−)-magnoflorine and a part of (−)-magnocurarine (**1**). Identification of (−)-magnoflorine and (**1**) was done by co-chromatography with respective authentic samples: Not only the retention times but also the absorption spectra measured with a photodiode-array detector were compared. Majority of the quaternary alkaloids were found in Fr-2 (Fig. 1). As reported previously, from Fr-2 of Japanese Magnoliae Cortex were isolated six quaternary alkaloids, (**1**), (+)-laurifoline (**3**), (±)-oblongine (**4**), (+)-menisperine (**5**), (+)-xanthoplanine (**6**), and (+)-*N*-methylglaucine (**7**).[4] Three Chinese Magnoliae Cortices contained **1**, **3**, **4**, **5**, and **6**, but **7** was not found in any of the Chinese Magnoliae Cortices. On the other hand, the HPLC chromatograms of Fr-2 of commercial Chinese Magnoliae Cortex and Cortex of

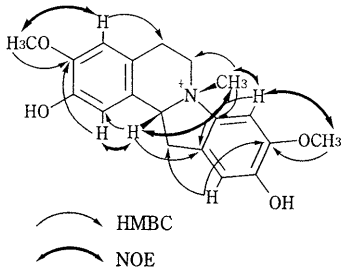

Fig. 2. HMBC and NOE Interactions Observed for (−)-10-Demethylcryptaustoline

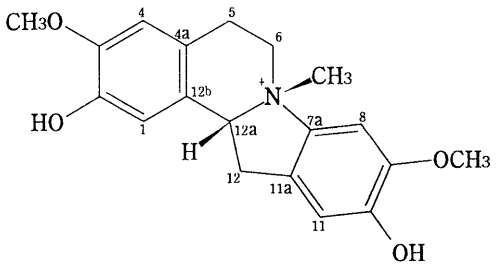

Chart 1.

M. officinalis var. *biloba* showed a large peak of compound **2** appearing next to the peak of **1** (Fig. 1). We isolated the compound by preparative HPLC and determined its structure as follows.

HR-SIMS showed that the molecular formula of **2** was $C_{19}H_{22}NO_4^+$. The UV absorption spectrum of **2** resembled those of benzylisoquinolines and tetrahydroprotoberberines with the absorption maximum at 285 nm. The structural features of **2** were determined by ^{1}H-^{1}H-COSY, HMQC, HMBC, and 2D-NOESY experiments. The ^{1}H-NMR spectrum of **2** resembled that of tetrahydroprotoberberines except that the H-8 methylene signals of tetrahydroprotoberberines were absent in **2**. The HMBC spectrum of **2** (Fig. 2) showed a correlation between NCH_3 protons (δ 3.74) and an aromatic quaternary carbon (δ 139.25), suggesting that the nitrogen atom was linked directly to the aromatic ring, and thus confirming **2** to be a dibenzopyrrocoline alkaloid.

Four singlet aromatic proton signals (δ 6.87, 6.93, 6.98, and 7.63) suggest the presence of two sets of para-substituted protons in two aromatic rings, and three of the four were assigned by the HMBC correlations. An HMBC correlation was observed between NCH_3 protons and a methine carbon (C-12a, δ 75.76), and H-12a (δ 5.40) was assigned. An HMBC connectivity from an aromatic proton (δ 6.87) to C-12a was found, and this proton was assigned as H-1. HMBC correlations observed between the singlet proton at δ 6.98 and C-7a (δ 139.25) and C-12 (δ 37.08) showed that this proton was H-11. An HMBC correlation between NCH_3 protons and the methylene carbon (δ 59.50) showed that the methylene group was at C-6. The $-CH_2-CH_2-$ moiety of benzylisoquinoline was assigned by the ^{1}H-^{1}H-COSY and HMQC spectra. A connectivity was observed between an aromatic proton (δ 6.93) and C-5 (δ 24.87) in HMBC spectrum. Consequently this proton was assigned to H-4. The remaining singlet hydrogen (δ 6.98) was attributed to H-8.

Substituents, i.e. two methoxyl groups and two hydroxyl groups, were on C-2, C-3, C-9, and C-10. As regard substitution positions of these groups, an HMBC correlation was observed between one methoxyl protons (δ 3.87) and C-3, and an NOE between the methoxyl group and H-4. This confirms that the methoxyl group binds to C-3. The other methoxyl group (δ 3.97) linked either to C-9 (δ 149.40) or to C-10 (δ 150.13) according to HMBC correlation, but due to the proximity of chemical shift of C-9 and C-10, it was difficult to determine to which carbon the methoxyl group is combined. However, 2D-NOESY measurements showed an NOE between the methoxyl group and H-8, suggesting that the methoxyl group was on C-9.

A large negative optical rotation ($[\alpha]_D$ −112°) was observed for **2**, as in (−)-cryptaustoline ($[\alpha]_D$ −151°) and (−)-acutupyrrocoline ($[\alpha]_D$ −134°). The absolute configuration of **2** was determined to be (7*S*, 12a*R*), the same as (−)-cryptaustoline.[6] An NOE was observed between N-CH_3 and H-12a, which suggested the *cis* configuration between H-12a and N-CH_3, and supported the (7*S*, 12a*R*) configuration. Thus, the structure of compound **2** was determined to be 10-demethyl derivative of (−)-cryptaustoline (Chart 1).

Not many dibenzopyrrocoline alkaloids are known in plant kingdom; (−)-cryptaustoline and (−)-cryptowoline from *Cryptocara bowiei*[5] and (−)-acutupyrrocoline from *Sinomenium acutum*.[1] **2** is the fourth dibenzopyrrocoline reported from plants.

In China two *Magnolia* species, *Magnolia officinalis* Rehd et Wils and *M. officinalis* Rehd et Wils var. *biloba* Rehd et Wils have been utilized for the preparation of Magnoliae Cortex. These two plants quite resemble each other, and it is difficult to distinguish both cortices anatomically. In the present study their HPLC profiles were compared. In addition to commercial Magnoliae Cortex we isolated alkaloids, we collected four samples of Chinese Magnoliae Cortice, one was *M. officinalis*, two were *M. officinalis* var. *biloba*, and the fourth was unidentified. In HPLC, the presence of **2** was confirmed in all the samples except in *M. officinalis*. The small asterisked peak in HPLC of *M. officinalis* (Fig. 1(c)) had a retention time very close to that of **2**. However, this peak is not of **2**, because it has absorption spectrum different from that of **2**. These two peaks were better separated when methanol was used as HPLC solvent in place of acetonitrile. We may safely concluded that *M. officinalis* var. *biloba* contains a new dibenzopyrrocoline alkaloid **2** and that **2** may be used as a key substance to distinguish the two Chinese *Magnolia* species, though it is necessary to analyze more samples to confirm this fact.

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