(*R*)-(+)-Isotembetarine, a Quaternary Alkaloid from *Zanthoxylum nitidium*

Masataka Moriyasu,* Momoyo Ichimaru, Yumi Nishiyama, and Atsushi Kato

Department of Natural Medicinal Chemistry, Kobe Pharmaceutical University, Motoyamakita-machi, Higashinada-ku, Kobe 658, Japan

Jie Wang, Hong Zhang, and Gui-Bao Lu

Department of Chinese Medicine, Tianjin Institute for Drug Control, Nanjin-Lu, Tianjin, China

Received April 29, 1996[⊗]

Quaternary alkaloids in *Zanthoxylum nitidium* were examined using ion-pair HPLC. A new benzylisoquinoline alkaloid named (*R*)-(+)-isotembetarine (**1**) was isolated together with seven known alkaloids.

In the course of isolating alkaloids from plant materials, we have found that quaternary alkaloids may be efficiently purified by a combination of ion-pair extraction and preparative ion-pair HPLC using sodium perchlorate.[1−3] In this study, the method was applied to *Zanthoxylum nitidium* DC. (Rutaceae), a medicinal plant used in the Southern part of Asia as an antiinflammatory and analgesic agent.[4,5] Recently the extract of *Z. nitidium* has been added to toothpaste in China, due to the strong bactericidal activity of its benzo[*c*]-phenanthridine alkaloids.[6] This plant is the source for nitidine, a benzo[*c*]phenanthridine alkaloid reported to have antitumor activity.[7] To date, many alkaloids have been isolated, including chelerythrine, oxynitidine, oxychelerythrine, dihydronitidine, 6-methoxy-5,6-dihydrochelerythrine, 6-ethoxy-5,6-dihydrochelerythrine, des-*N*-methylchelerythrine, bocconoline, decarine, oxyterihanine, α-allocryptopine, skimianine, arnottianamide, isoarnottianamide, integriamide, liriodenine, and magnoflorine.[8−13] Although tertiary and amide alkaloid constituents in *Z. nitidium* have extensively been examined, the water-soluble quaternary alkaloids have not been investigated well.

In the present study, the Et_2O, $CHCl_3$, and acidic quaternary alkaloid fractions (fractions 1, 2, and 4) of the stems of *Z. nitidium* each showed almost a single peak in the HPLC. Chelerythrine, nitidine, and (+)-magnoflorine were obtained, respectively, from fractions 1, 2, and 4. A number of previously reported alkaloids must be present in the Et_2O- and $CHCl_3$-soluble fractions 1 and 2, but their contents were too small to identify them. The quaternary alkaloid fraction extractable under alkaline conditions (fraction 3) was subjected to preparative HPLC separation leading to five components being purified. Among these, three were identified as (+)-tembetarine (**2**), (+)-menisperine, and (−)-*cis*-*N*-methylcanadine by comparison with authentic samples stored in our laboratory.[2,14] The fourth alkaloid was determined to be *N*,*N*,*N*-trimethyltryptamine, which was confirmed by synthesis of *N*,*N*,*N*-trimethyltryptamine perchlorate from tryptamine. *N*-Methyltryptamine and *N*,*N*-dimethyltryptamine have both been found in plant kingdom and are reported as strong hallucinogens,[15] but *N*,*N*,*N*-trimethyltryptamine, which has not been found in plant material, has no hallucinogenic action.[16]

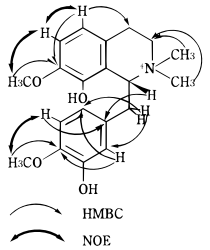


Figure 1. HMBC and NOE interactions observed for (*R*)-(+)-isotembetarine.

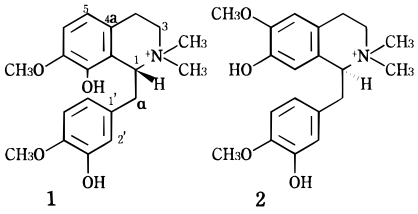
The structure of **1**, the fifth isolate, has the molecular formula of $C_{20}H_{26}NO_4^+$ by HRLSIMS (calcd 344.1861, found 344.1866), which was identical with that of **2**. The UV spectra of **1** and **2** were also similar. The ^{1}H-NMR spectrum of **1** also resembled that of **2**, suggesting that **1** was a benzylisoquinoline alkaloid. Further support for the structure of this compound was provided by measuring various 2D-NMR spectra. All methylene and methine protons and carbons were assigned from ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. The presence of two *o*-substituted aromatic doublet protons (6.83 and 7.08 ppm) and an HMBC correlation (Figure 1) from one of the protons (6.83 ppm) to C-4 (23.69 ppm) revealed that these protons bound with C-5 and C-6. HMBC correlations were observed from H-α (3.28 and 3.52 ppm) to two aromatic carbons (116.73 and 121.12 ppm), and the presence of three protons combined with C-2′, C-5′, and C-6′ was disclosed. Therefore, two methoxyl and two hydroxyl groups are substituents at C-7, C-8, C-3′, and C-4′. One of the methoxyl groups (3.89 ppm) was proved to bind with C-7 by an HMBC correlation from these protons to C-7. Another methoxyl group (3.82 ppm) should bind with either C-3′ or C-4′ from an HMBC correlation, but due to the proximity of chemical shifts of C-3′ and C-4′ (147.52 and 147.64 ppm), these carbons were not distinguished. However, this problem was resolved by the NOESY spectrum. Because an NOE was observed between H-5′ (6.87 ppm) and the methoxyl protons, the methoxyl group must bind with C-4′. This compound (**1**), an isomer of tembetarine, is a new benzylisoquinoline alkaloid, which we named (+)-isotembetarine. The absolute configuration of **1** concerning C-1 remains in question. A small positive optical rotation ($[\alpha]_D$ +3.5°) was observed for **1**, differing from **2**, which has a large positive value ($[\alpha]_D$ +126°).

* To whom correspondence should be addressed. E-mail: moriyasu@kobepharma-u.ac.jp.

⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1997.

S0163-3864(96)00420-X CCC: $14.00

We previously isolated (−)-oblongine ($[\alpha]_D$ −11.6°) from *Z. usambarense* and elucidated its configuration to be (1*R*) by measuring its CD and comparing the data with several benzylisoquinoline alkaloids.[17,18] In a similar analysis the CD curve of **1**, which showed negative Cotton effects at 285 and 236 nm, ($[\theta]_{285}$ −7400, $[\theta]_{250}$ −220, $[\theta]_{236}$ −6100), resembled that of (−)-oblongine ($[\theta]_{284}$ −6900, $[\theta]_{253}$ −500, $[\theta]_{237}$ −11 700)[2], thus suggesting that **1** has the same configuration as (−)-oblongine (1*R*), and not (+)-tembetarine (1*S*). When both H-7 and H-8 of benzylisoquinolines are substituted, the $[\alpha]_D$ is close to zero.



Experimental Section

General Experimental Procedures. NMR spectra, including ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY, were obtained using a Varian VXR 500 instrument. LRLSIMS and HRLSIMS were measured with a Hitachi M-4100 instrument using glycerol as matrix. The primary ion was Cs^+, and the accelerating voltages of primary and secondary ions were 15 and 6kV, respectively. The IR spectrum, optical rotation, and CD spectrum were measured with a FT-IR 8200 spectrometer (Shimadzu), DIP-370 polarimeter (JASCO), and J-500 spectropolarimeter (JASCO), respectively. The HPLC apparatus used included a dual-pump system (Model 510, Waters), a gradient controller (Model 680, Waters), and a photodiode array detector (Model 990, Waters). Cosmosil 5C18-AR (5 μm, ODS-type, Nacalai Tesque) columns of a smaller size (150 × 6 mm i.d.) and larger size (250 × 20 mm i.d.) were utilized for analysis and preparative HPLC, respectively. The mobile phase was the mixture of A [0.2 M sodium perchlorate, 60% perchloric acid (1000:0.2)] and B (MeOH). The use of MeOH gave a better result for the separation of fraction 3 than did MeCN. The gradient conditions were as follows: A/B = 80/20 to 60/40, 40 min, 2.0 mL/min (for analysis), and A/B = 80/20 to 60/40, 160 min, 9.0 mL/min (for preparative HPLC).

Plant Materials and Ion-Pair Extraction of Alkaloids. The root of *Z. nitidium* of Chinese pharmacopoeial quality was purchased in a Tianjin market and identified by two of the authors (J.W. and G.-B.L.). A voucher specimen is deposited at the Tianjin Institute for Drug Control. The powdered plant material (44.6 g) was extracted with three 500-mL portions of hot MeOH. After the MeOH was evaporated, a 200-mL portion of 2% aqueous tartaric acid was added. Fat-soluble substances were removed with two 200-mL portions of Et_2O. The aqueous layer was basified with Na_2CO_3, and tertiary alkaloidal fractions were obtained by conventional procedures extracting first with three 200-mL portions of Et_2O (fraction 1, 226 mg) and then with three 200-mL portions of $CHCl_3$ (fraction 2, 97 mg). To the residual aqueous layer was added sodium perchlorate so that the concentration of perchlorate was about 0.5 M. Quaternary alkaloids in the aqueous layer formed an ion-pair with perchlorate and were extracted with three 200-mL portions of 1,2-dichloroethane (fraction 3, 257 mg). The aqueous layer was then acidified by perchloric acid and extracted with three 200-mL portions of 1,2-dichloroethane (fraction 4, 121 mg). These fractions were purified by preparative ion-pair HPLC using sodium perchlorate. Thus, fractions 1, 2, and 4 gave chelerythrine (170.7 mg, yield 0.30%), nitidine (57.4 mg, 0.10%), and (+)-magnoflorine (110.0 mg, 0.19%), respectively. Fraction 3 gave five quaternary alkaloids including (+)-menisperine (125.3 mg, 0.22%), (+)-tembetarine (25.1 mg, 0.044%), (−)-*cis*-*N*-methylcanadine (23.4 mg, 0.041%), (+)-isotembetarine (21.6 mg, 0.038%), and *N*,*N*,*N*-trimethyltryptamine (22.8 mg, 0.034%).

***(R)*-(+)-Isotembetarine Perchlorate (1).** (*R*)-(+)-Isotembetarine perchlorate, (1*R*)-1,2,3,4-tetrahydro-8-hydroxy-1-(3-hydroxy-4-methoxyphenyl)methyl-7-methoxy-2,2-dimethyl isoquinolinium perchlorate, was obtained as a white crystalline from Me_2CO−hexane: mp 172 °C; $[\alpha]^{25}_D$ +3.5° (*c* 0.43, MeOH), CD (MeOH); $[\theta]_{310}$ 0, $[\theta]_{285}$ −7400, $[\theta]_{250}$ −220, $[\theta]_{236}$ −6100, $[\theta]_{228}$ −3700; IR (KBr) ν_{max} 3400(OH), 1460, 1085, 750, 625 cm^{-1}; ^{1}H NMR [$(CD_3)_2CO$, 500 MHz] δ 3.15 (1H, m, H-4), 3.28 (1H, m, H-α), 3.30 (1H, m, H-4), 3.31 (3H, s, NCH_3), 3.34 (1H, s, NCH_3), 3.52 (1H, dd, J = 16.0, 7.5 Hz, H-α), 3.72 (1H, m, 3-H), 3.82 (3H, s OCH_3-4′), 3.89 (3H, s, OCH_3-7), 4.04 (1H, ddd, J = 13.5, 12.5, 6.5 Hz, H-3), 5.22 (1H, m, H-1), 6.81 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 6.83 (1H, d, J = 8.5 Hz, H-5), 6.87 (1H, d, J = 8.5 Hz, H-5′), 6.91 (1H, d, J = 2.0 Hz, H-2′), 7.08 (1H, d, J = 8.5 Hz, H-6), 7.65 (1H, s, OH), 8.53 (1H, s, OH); ^{13}C NMR [$(CD_3)_2CO$, 125 MHz], 23.69 (C-4), 37.83 (c-α), 51.74 (NCH_3), 54.11 (NCH_3), 55.08 (C-3), 56.30 (OCH_3-4′), 56.67 (OCH_3-7), 69.84 (C-1), 112.47 (C-5′), 113.03 (C-6), 116.73 (C-2′), 120.23 (C-8a), 120.39 (C-5), 121.12 (C-6′), 122.07 (C-4a), 131.40 (C-1′), 143.80 (C-8), 146.87 (C-7), 147.52 (C-3′ or C-4′), 147.64 (C-4′ or C-3′); LRLSIMS *m*/*z* [M^+] 344(100), 133(13), 58(33); HRLSIMS [M^+] 344.1866 (344.1861 calcd for $C_{20}H_{26}NO_4^+$).

***N*,*N*,*N*-Trimethyltryptamine Perchlorate.** *N*,*N*,*N*-Trimethyltryptamine perchlorate was synthesized[16] as follows. To a solution of tryptamine (500 mg) dissolved in Me_2CO, MeI was added, and the mixture was left standing for a day. To the resultant crude *N*,*N*,*N*-trimethyltryptamine iodide was added aqueous sodium perchlorate in excess. The mixture was then extracted with 1,2-dichloroethane. After evaporating the solvent, *N*,*N*,*N*-trimethyltryptamine perchlorate was recrystallized from MeOH−Et_2O (131 mg, yield 14%). The spectral data (i.e., ^{1}H-NMR and HRLSIMS) were identical to those of the isolate.

References and Notes

(1) Moriyasu, M.; Ichimaru, M.; Nishiyama, Y.; Kato, A. *Bunseki Kagaku* **1993**, *42*, 659−665.
(2) Kato, A.; Moriyasu, M.; Ichimaru, M.; Nishiyama, Y.; Juma, F. D.; Nganga, J. N.; Mathenge, S. G.; Ogeto, J. O. *Phytochem. Anal.* **1995**, *6*, 89−95.
(3) Kato, A.; Moriyasu, M.; Ichimaru, M.; Nishiyama, Y.; Juma, F. D.; Nganga, J. N.; Mathenge, S. G.; Ogeto, J. O. *J. Nat. Prod.* **1996**, *59*, 316−318.
(4) Yang, H.-C.; Chang, H.-H.; Weng, T.-C. *J. Formosan Med. Assoc.* **1953**, *52*, 109−112; *Chem. Abstr.* **1953**, *47*, 8175d.
(5) *Zhang yao da ci dan* (*Encyclopedia of Chinese Medicines*); Jiang su xin yi yuan, Ed. Shanghai Renmin: Shanghai, 1977; pp 40−41.

(6) Onda, M.; Takiguchi, K.; Hirakura, M.; Fukushima, H.; Akagawa, M.; Naoi, F. *Nippon Nogei Kagaku Kaishi* **1965**, *39*, 168−170; *Chem. Abstr.* **1965**, *63*, 18964f.
(7) Wall, M. E.; Wani, M. C.; Taylor, H. L. *Abstracts of Papers*, 162nd National Meeting of the American Chemical Society, Washington DC, American Chemical Society: Washington, DC 1971; MEDI 34.
(8) Arthur, H. R.; Hui, W. H.; Ng, Y. L. *J. Chem. Soc.* **1959**, 1840−1845.
(9) Huang, Z.; Li, Z. *Hua Hsueh Hsueh Pao* **1980**, *38*, 535−542; *Chem. Abstr.* **1980**, *94*, 99773e.
(10) Wang, M. C. *Yao Hseuh Tung Pao* **1981**, *16*, 48; *Chem. Abstr.* **1981**, *95*, 192260r.
(11) Morita, N.; Shimidzu, M.; Arisawa, M. *Yakugaku Zasshi* **1968**, *88*, 1214−1217.
(12) Ishii, H.; Ishikawa, T.; Akaike, M.; Tohjoh, T.; Toyoki, M.; Ishikawa, M.; Cheng, I.; Lu, S. *Yakugaku Zasshi* **1984**, *104*, 1030−1042.
(13) Ishii, H.; Iwai, M.; Johji, S.; Tan, S.; Cheng, I.; Ishikawa, T. *Chem. Pharm. Bull.* **1994**, *42*, 108−110.
(14) Moriyasu, M.; Ichimaru, M.; Nishiyama, Y.; Kato, A. *Nat. Med.* **1994**, *48*, 282−286.
(15) Dowing, D. F. *Quart. Rev.* **1962**, *16*, 133−162.
(16) Takahashi, T.; Takahashi, K.; Ido, T.; Yanai, K.; Iwata, R.; Ishiwata, K.; Nozoe, S. *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 965−969.
(17) Bhacca, N. S.; Cymerman, C. J.; Manske, R. H. F.; Roy, S. K.; Shamma, M.; Slusarchyk, W. A. *Tetrahedron* **1966**, *22*, 1467−1475.
(18) Albonico, S. M.; Comin, J.; Kuck, A. M.; Sanchez, E.; Scopes, P. M.; Swan, R. J.; Vernengo, M. J. *J. Chem. Soc. C* **1966**, 1340−1342.

NP960420V