Two *Alangium* Alkaloids from *Alangium lamarckii*

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Received January 14, 2000

Two new *Alangium* alkaloids, 1′,2′-dehydrotubulosine (**1**) and alangine (**2**), were isolated from the dried fruits of *Alangium lamarckii* along with tubulosine (**3**), isotubulosine (**4**), deoxytubulosine, cephaeline, isocephaeline, psychotrine, neocephaeline, 10-*O*-demethylcephaeline, 2′-*N*-(1″-deoxy-1″-*β*-D-fructopyranosyl)cephaeline, protoemetine, protoemetinol, salsoline, and alangiside. The structures of the new alkaloids (**1** and **2**) were determined by spectroscopic and chemical means.

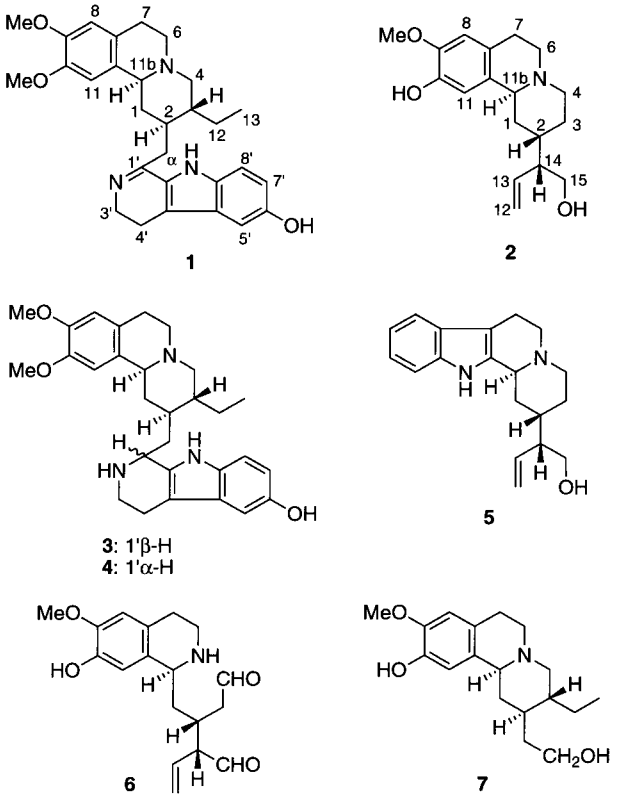
Alangium lamarckii Thwaites (Alangiaceae) is a deciduous shrub of wide distribution in India and South East Asia. The root, root-bark, and bark of this plant have been used in the indigenous Indian systems of medicine for a long time.[1] Previous phytochemical studies of *A. lamarckii* resulted in the isolation of numerous classes of alkaloids, i.e., emetan, tubulosan, 3-ethyl-2*H*-benzo[*a*]quinolizine (protoemetinol), and 8*H*-isoquino[2,1-*b*][2,7]naphthyridine-8-one (alangimaridine).[1] Recently, several new nitrogenous glycosides closely related to the *Alangium* alkaloids were isolated from the fruits of this plant.[2−5] We have reexamined the alkaloidal fraction of the fruits of *A. lamarckii* and report here the isolation and characterization of two additional novel *Alangium* alkaloids (**1** and **2**).

The dried and crushed fruits of *A. lamarckii* were extracted with hot MeOH, and the MeOH extract was successively partitioned between H_2O/$CHCl_3$ and H_2O/*n*-BuOH. The H_2O layer was basified and extracted with Et_2O and then $C_2H_4Cl_2$. The organic layers were separated by a combination of chromatographic procedures, affording alkaloids **1** and **2** along with the known compounds tubulosine (**3**),[6] isotubulosine (**4**),[6] deoxytubulosine,[6] cephaeline,[6] isocephaeline,[6] psychotrine,[6] 10-*O*-demethylcephaeline,[7] protoemetinol,[6] (±)-salsoline,[8] alangiside,[9] neocephaeline,[7] 2′-*N*-(1″-deoxy-1″-*β*-D-fructopyranosyl)cephaeline,[7] and protoemetine.[6] The last three alkaloids were isolated for the first time from this plant species.

Alkaloid **1** was obtained as an amorphous powder. HREIMS revealed the molecular formula $C_{29}H_{35}N_3O_3$. It showed UV maxima at 230, 291, 328, and 370 nm, and IR bands at 3432, 2833, 2748, 1612, and 1514 cm^{-1}. Its ^1H NMR spectrum exhibited signals for an ethyl group at *δ* 1.00, 1.24−1.32, and 1.85, singlets for two aromatic protons at *δ* 6.19 and 6.61, an AMX spin system for three aromatic protons at *δ* 6.90, 6.92, and 7.28, and singlets for methoxyl groups at *δ* 3.27 and 3.72. These spectral features were similar to those of tubulosine (**3**), except for absence of the H-1′ signal, which appeared at *δ* 4.12 in **3**, suggesting that **1** was the 1′,2′-dehydrogenated analogue of tubulosine (**3**). The proposed structure of **1** was consistent with its ^{13}C NMR spectrum, where C-1′ was observed at *δ* 166.4, instead of at *δ* 48.4 in **3**. Finally, **1** was treated with $NaBH_4$ to afford **3** and isotubulosine (**4**). Thus, alkaloid **1** was determined to be 1′,2′-dehydrotubulosine.

The second new alkaloid, **2**, alangine, was obtained as a white powder and analyzed for $C_{18}H_{25}NO_3$ (HREIMS). It showed UV maxima at 225 and 285 nm and IR bands at 3569, 1613, and 1516 cm^{-1}. The signals for a methoxyl group (*δ* 3.86) and two aromatic protons (*δ* 6.57 and 6.77) in its ^1H NMR spectrum, a NOESY correlation between OMe (*δ* 3.86) and H-8 (*δ* 6.57) which correlated with H-7, a fragment ion peak at *m*/*z* 230,[10] and its ^{13}C NMR spectrum (see Experimental Section) all suggested that **2** contained a 9-methoxy-10-hydroxybenzo[*a*]quinolizine moiety. Its ^1H NMR spectrum showed signals for a terminal vinyl group at *δ* 5.17, 5.23, and 5.60, signals for a hydroxymethyl group at *δ* 3.57 and 3.79, and a methine proton at *δ* 2.35. COSY correlation between the methine proton at *δ* 2.35 (H-14) and the hydroxymethyl proton at *δ* 3.57 (H-15), as well as HMBC correlations from H_2-15 to C-13 and C-2 and from H-13 to C-14 and C-15 defined the side chain, and the sequence of correlations of H_2-1 (*δ* 2.14), H-2 (*δ* 1.68), H_2-3 (*δ* 1.71−1.81), and H_2-4 (*δ* 2.84) in the COSY spectrum indicated the substitution at C-2.

The *cis* quinolizine ring junction was indicated by the absence of Bohlmann bands in its IR spectrum and the presence of a deshielded proton resonance at *δ* 4.07, attributed to H-11b.[11] The relative configurations of C-2,



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Published on Web 04/12/2000

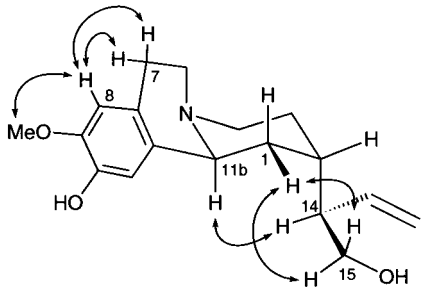

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Figure 1. Selected NOESY correlations of **2**.

-11b, and -14 were suggested by the NOESY correlations between H-11b and H-14 and between H-1 and H_2-15 (Figure 1). This assumption was supported by comparative studies of its ^{13}C NMR spectral data with those of antirhine (**5**).[12] Biogenetic considerations that the non-dopamine portion of **2** originated from secologanin, and thereby the chirality of C-2 should be *S*, allowed assignment of the absolute stereochemistry of **2**. Thus, alangine was determined to be structure **2**.

The occurrence of **1** and **2** is of great interest from the viewpoint of biosynthesis of *Alangium* alkaloids.[13] Alkaloid **1** could be derived from tubulosine (**3**) or isotubulosine (**4**). Two plausible mechanisms could be proposed for the formation of **3** and **4**. Two epimeric alkaloids might be independently biosynthesized through condensation of a protoemetine type alkaloid with tryptamine (or serotonin) in a manner similar to the biosynthesis of deacetylipecoside and deacetylisoipecoside.[14] Another possibility is an oxidation−hydrogenation mechanism as observed in the conversion of (*S*)-reticuline to (*R*)-reticuline via the 1,2-dehydroreticulinium ion.[15] In the latter case **1** should be an intermediate between **3** and **4**. Alkaloid **1** could also be oxidized to 1′,2′,3′,4′-tetradehydrotubulosine in *Pogonopus speciosus*.[16] On the other hand, alkaloid **2** is the first compound with a new basic skeleton, which could be formed from **6**, a common intermediate to 10-*O*-demethylprotoemetinol (**7**).[17]

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra on a Shimadzu FTIR-8200 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter and CD spectra on a Shimadzu-AVIV 62 A DS circular dichroism spectrometer. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Varian VXR-500 spectrometer with TMS as an internal standard. MS and HRMS were obtained with a Hitachi M-4100 mass spectrometer. MPLC was carried out with Wakogel FC-40. TLC was performed on precoated Kieselgel $60F_{254}$ plates (Merck).

Plant Material. The dried fruits of *Alangium lamarckii*, collected in India, were purchased from Mikuni, Osaka, Japan. A voucher specimen (KPFY-921) is deposited in our laboratory.

Extraction and Isolation. The dried fruits (4.5 kg) of *A. lamarckii* were crushed and extracted with hot MeOH, and the extracts were fractionated as described previously.[2] A part (426 g) of the residue (556 g) from the H_2O layers was redissolved in H_2O, basified with Na_2CO_3, and extracted with Et_2O and $C_2H_4Cl_2$ successively. The residue (8.8 g) from the Et_2O layer was subjected to MPLC, and elution with $CHCl_3$/MeOH mixtures of the indicated MeOH content gave 5 fractions: 1 (2%, 171 mg), 2 (2−5%, 2.49 g), 3 (8%, 1.75 g), 4 (10−15%, 2.44 g), 5 (20−25%, 192 mg). Fraction 1 was purified by preparative TLC ($CHCl_3$/MeOH/NH_4OH, 85:15:1.5) to afford protoemetine (15.2 mg) and deoxytubulosine (11.6 mg). Fraction 2 was purified by MPLC ($CHCl_3$/MeOH/NH_4OH, 98:2:0.2 to 90:9:1) and preparative TLC ($CHCl_3$/MeOH/NH_4OH, 90:9:1), affording protoemetine (4.8 mg), deoxytubulosine (83.9 mg), protoemetinol (8.8 mg), neocephaeline (97.0 mg), 2′-*N*-(1″-deoxy-1″-*β*-D-fructopyranosyl)cephaeline (25.5 mg), cephaeline (1.39 g), and isocephaeline (109 mg). In the same way, the following fractions were purified by a combination of MPLC with $CHCl_3$/MeOH/NH_4OH (98:2:0.2 to 90:9:1) and preparative TLC with $CHCl_3$/MeOH/NH_4OH (90:9:1) or C_6H_6/EtOAc/Et_2NH (2:7:1). Fraction 3 yielded deoxytubulosine (28.0 mg), neocephaeline (28.6 mg), cephaeline (1.15 g), and isocephaeline (154 mg); fraction 4, **3** (224 mg), **4** (23.6 mg), 2′-*N*-(1″-deoxy-1″-*β*-D-fructopyranosyl)cephaeline (4.7 mg), cephaeline (345 mg), isocephaeline (899 mg), psychotrine (37.9 mg), and 10-*O*-demethylcephaeline (24.5 mg); fraction 5, **3** (57.0 mg), **1** (10.9 mg), **4** (16.2 mg), cephaeline (10.2 mg), and isocephaeline (119 mg). The $C_2H_4Cl_2$ layer (6.4 g) was also subjected to MPLC, and elution with $CHCl_3$/MeOH mixtures of the indicated that the MeOH content gave 7 fractions: 1 (2%, 69.3 mg), 2 (2%, 245 mg), 3 (5%, 1.90 g), 4 (5−8%, 2.01 g), 5 (8%, 587 mg), 6 (8−20%, 826 mg), 7 (20%, 335 mg). Each fraction was purified in a manner similar to that for the Et_2O layer to yield protoemetine (41.4 mg), cephaeline (1650 mg), isocephaeline (985 mg), deoxytubulosine (26.1 mg), neocephaeline (16.1 mg), 2′-*N*-(1″-deoxy-1″-*β*-D-fructopyranosyl)cephaeline (74.9 mg), psychotrine (596 mg), alangiside (96.0 mg), **3** (6.2 mg), **4** (3.4 mg), salsoline (2.8 mg), and **2** (3.0 mg). The known alkaloids were identified by comparisons ($[\alpha]_D$, UV, IR, NMR, and MS) with pure standards.

1′,2′-Dehydrotubulosine (1): amorphous powder; $[\alpha]^{18}_D$ +2.1° (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ϵ) 230sh (4.20), 291 (3.69), 328 (4.04), 370sh (3.67) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 211 (+8.7), 223 (−2.1) nm; IR (KBr) ν_{max} 3432, 2833, 2748, 1612, 1514 cm^{-1}; ^1H NMR (CD_3OD) δ 1.00 (3H, t, J = 7.5 Hz, H_3-13), 1.17 (1H, dt, J = 13.5, 11.5 Hz, H-1), 1.24−1.32 (3H, m, H-12, H_2-α), 1.55 (1H, m, H-3), 1.81 (1H, m, H-2), 1.85 (1H, dqd, J = 13.5, 7.5, 3.0 Hz, H-12), 2.03 (1H, ddd, J = 13.5, 4.0, 3.0 Hz, H-1), 2.10 (1H, t, J = 11.5 Hz, H-4), 2.50 (1H, m, H-6), 2.65 (1H, dt, J = 14.0, 4.0 Hz, H-7), 2.96 (2H, m, H_2-4′), 2.99−3.18 (3H, m, H-6, H-7, H-11b), 3.11 (1H, dd, J = 11.5, 4.0 Hz, H-4), 3.27 (3H, s, 10-OMe), 3.72 (3H, s, 9-OMe), 3.78 (1H, m, H-3′), 3.92 (1H, dt, J = 15.0, 7.0 Hz, H-3′), 6.19 (1H, s, H-11), 6.61 (1H, s, H-8), 6.90 (1H, dd, J = 8.5, 2.5 Hz, H-7′), 6.92 (1H, dd, J = 2.5, 0.5 Hz, H-5′), 7.28 (1H, dd, J = 8.5, 0.5 Hz, H-8′); ^{13}C NMR (CD_3OD) δ 11.5 (C-13), 20.5 (C-4′), 24.5 (C-12), 29.3 (C-7), 30.8 (C-α), 37.2 (C-1), 42.0 (C-2), 43.4 (C-3), 47.3 (C-3′), 53.6 (C-6), 56.1 (10-OMe), 56.4 (9-OMe), 61.8 (C-4), 63.6 (C-11b), 104.0 (C-5′), 109.1 (C-11), 113.1 (C-8), 114.4 (C-8′), 118.7 (C-7′), 119.4 (C-4′a), 127.0 (C-5′a), 127.5 (C-7a), 130.3 (C-11a), 130.6 (C-9′a), 135.5 (C-8′a), 148.6 (C-10), 149.2 (C-9), 153.0 (C-6′), 166.4 (C-1′); NOESY correlations H-11/OMe (δ 3.27); H-11/H-1 (δ 2.03); H-8/OMe (δ 3.78); H-8/H-7 (δ 2.65); EIMS *m*/*z* 473 $[M]^+$, 272, 270, 244, 201, 200, 192, 176, 146; HR-EIMS *m*/*z* 473.2648 (calcd for $C_{29}H_{35}N_3O_3$, 473.2680).

Alangine (2): amorphous powder; $[\alpha]^{30}_D$ −0.95° (*c* 0.21, MeOH); $[\alpha]^{23}_D$ −2.5° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225sh (3.74), 285 (3.45) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 206 (−4.8), 219 (+1.4), 235 (+0.7) nm; IR (KBr) ν_{max} 3569, 2975, 1613, 1516, 1457 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.68 (1H, m, H-2), 1.71−1.81 (2H, m, H_2-3), 2.14 (2H, m, H_2-1), 2.35 (1H, m, H-14), 2.71 (1H, m, H-7), 2.84 (2H, m, H_2-4), 3.07 (1H, m, H-6), 3.09 (1H, m, H-7), 3.19 (1H, m, H-6), 3.57 (1H, dd, J = 10.5, 7.5 Hz, H-15), 3.79 (1H, dd, J = 10.5, 4.5 Hz, H-15), 3.86 (3H, s, OMe), 4.07 (1H, m, H-11b), 5.17 (1H, ddd, J = 17.0, 1.5, 0.5 Hz, H-12), 5.23 (1H, dd, J = 10.0, 1.5 Hz, H-12), 5.60 (1H, ddd, J = 17.0, 10.0, 9.5 Hz, H-13), 6.57 (1H, s, H-8), 6.77 (1H, s, H-11); ^{13}C NMR ($CDCl_3$) δ 25.0 (C-7), 27.4 (C-3), 31.0 (C-2), 31.5 (C-1), 47.8 (C-4), 49.0 (C-14), 50.7 (C-6), 55.9 (OMe), 56.8 (C-11b), 63.5 (C-15), 111.0 (C-8), 111.2 (C-11), 118.8 (C-12), 124.5 (C-7a), 127.0 (C-11a), 138.1 (C-13), 144.5 (C-10), 145.7 (C-9); NOESY correlations H-1/H-11; H_2-7/H-8; H_2-15/H_2-1; H-11b/H-14; H-14/H-12 (δ 5.23); OMe/H-8; H-12 (δ 5.17)/H-14; EIMS *m*/*z* 303 $[M]^+$, 302, 272, 232, 230, 191, 178, 176; HREIMS *m*/*z* 303.1858 (calcd for $C_{18}H_{25}NO_3$, 303.1836).

Reduction of 1′,2′-Dehydrotubulosine (1). A methanolic solution (1 mL) of 1′,2′-dehydrotubulosine (**1**) (4.9 mg) was

stirred with $NaBH_4$ (75 mg) for 10 min at room temperature. The mixture was then diluted with H_2O and extracted with $CHCl_3$, and the extract was washed, dried, and concentrated. The residue (5.0 mg) was purified by preparative TLC ($CHCl_3$/MeOH/NH_4OH, 85:15:1.5) to give **3** (1.7 mg) and **4** (1.1 mg). UV, IR, ^1H NMR, EIMS, optical rotation ($[\alpha]^{27}_D$ −60° (*c* 0.16, MeOH)), and CD ((MeOH) λ_{max} ($\Delta\epsilon$) 229 (−9.5), 242 (+1.6) nm) spectra of **3** were identical with those of the authentic tubulosine. UV, IR, ^1H NMR, EIMS, optical rotation ($[\alpha]^{27}_D$ −74° (*c* 0.10, MeOH)), and CD ((MeOH) λ_{max} ($\Delta\epsilon$) 220 (−15.5), 241 (+2.6) nm) spectra of **4** were identical with those of the authentic isotubulosine.

Acknowledgment. Our thanks go to Dr. M. Sugiura (Kobe Pharmaceutical University) for the NMR spectra and to Dr. K. Saiki (Kobe Pharmaceutical University) for the MS measurements.

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