Biogenetic Conversion of Tetrahydroisoquinoline−Monoterpene Glucosides into Benzopyridoquinolizine Alkaloids of *Alangium lamarckii*

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The biogenetic conversion of alangiside (**6**), a major tetrahydroisoquinoline−monoterpene glucoside of *Alangium lamarckii*, into (+)-alangimaridine (**1**), one of the benzopyridoquinolizine alkaloids of the same plant, was carried out confirming its absolute configuration. Isoalangimarine (**3**) and its possible precursor (+)-isoalangimaridine (**19**) were synthesized from 3-*O*-demethyl-2-*O*-methylalangiside (**11**).

Alangimaridine (**1**), alangimarine (**2**), and isoalangimarine (**3**) are unique benzo[*a*]pyrido[3,4-*g*]quinolizine alkaloids isolated from *Alangium lamarckii* Thwaites (Alangiaceae), a medicinal plant used in Indian folk medicine in the treatment of various diseases.[1,2] The previous phytochemical studies demonstrated that these alkaloids coexist in the plant along with ipecac alkaloids, represented by emetine (**4**) and cephaeline (**5**),[3] and a nitrogenous glucoside, alangiside (**6**).[4] It is of biogenetic interest to note that the stereochemistry of C-13a of alangimaridine[5] (**1**) is the same as that of alangiside (**6**) but different from the stereochemistry at the corresponding chiral center of emetine (**4**). Previous biosynthetic investigations demonstrated that emetine (**4**) and cephaeline (**5**) are not derived from deacetylipecoside (**7**) but from deacetylisoipecoside (**8**) with the opposite chiral center of alangiside (**6**).[6] In contrast to the results, Pakrashi *et al.*[2] proposed, on the basis of the stereochemical relationships, that the benzopyridoquinolizine alkaloids **1**−**3** could be biosynthesized via hypothetical intermediates **9** or **10** (Scheme 1). However, the absolute configuration of **1** was deduced only from comparison of its specific optical rotation with those of the tetrahydroprotoberberine alkaloids[2] and was not definitely established by chemical evidence, although some benzopyridoquinolizine alkaloids have been chemically synthesized as racemic forms.[7,8] As a part of our phytochemical studies on nitrogenous glycosides, we recently investigated the fruits of *A. lamarckii* and isolated for the first time 3-*O*-demethyl-2-*O*-methylalangiside (**11**), a possible precursor of isoalangimarine (**3**).[9] Furthermore, isoalangiside (**12**) and 3-*O*-demethyl-2-*O*-methylisoalangiside (**13**) were also obtained as the first natural glucosides with the same absolute configurations as deacetylisoipecoside (**8**), a key intermediate in the biosynthesis of ipecac alkaloids.[10] The isolation of these new glucosides prompted us to reexamine the stereochemistry of alangimaridine (**1**). Thus, we have undertaken a biogenetic synthesis of this type of alkaloid from tetrahydroisoquinoline-monoterpene glucosides to confirm their absolute stereochemistry.

An attempt to introduce a nitrogen atom into the dihydropyrane ring of an iridoid glucoside moiety to construct a pyridine ring has been made by Brown's group in the synthesis of the dihydro derivative of angustine (**14**), an indolic analogue of alangimaridine (**1**).[11] Because alangiside (**6**) was expected to be susceptible to ring closure between its vinyl group and an aldehyde group arising from the dihydropyran ring after deglucosidation,[12] we undertook a preliminarily partial synthesis of dihydroalangimaridine (**15**) from dihydroalangiside (**16**) by a method similar to that used for dihydroangustine. Dihydroalangiside (**16**) was prepared from dihydroalangiside pentaacetate (**17**) by Zemplen deacetylation. Cleavage of the glucose with β-glucosidase in citrate−phosphate buffer gave aglycon (**18**) (36%), which was characterized by [M]+ at *m/z* 345. Hydrolysis of **16** with 3% HCl afforded the same product, **18**, in a higher yield (64%). On standing for 24 h with concentrated NH₃ the aglycon **18** was converted to a product, which was allowed to stand in TFA overnight, to yield (+)-dihydroalangimaridine (**15**), [α]D +279°, in 59% yield. Its MS and ¹H-NMR spectral data were identical with those of synthetic (±)-dihydroalangimaridine,[8] establishing the structure of the product.
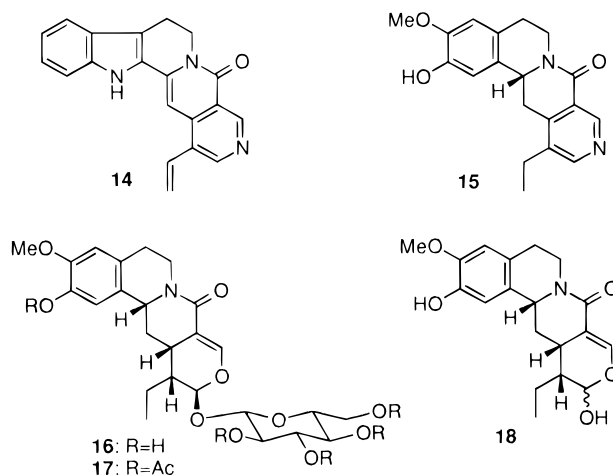
Inasmuch as this conversion proceeded with a satisfactory yield, alangiside (**6**) with a vinyl group was subjected to the same reactions. Hydrolysis with β-glucosidase yielded the aglycon of alangiside with an [M]+ ion peak at *m/z* 343. This product was treated with concentrated NH₃ followed by TFA to furnish (+)-alangimaridine (**1**) (12% overall yield from **6**). Its UV, MS, and ¹H-NMR spectral data were identical with those reported for the natural product.[2] Its specific

Scheme 1. Proposed Sequence for the Biosynthesis of Alkaloids and Nitrogenous Glucosides in *A. lamarckii*
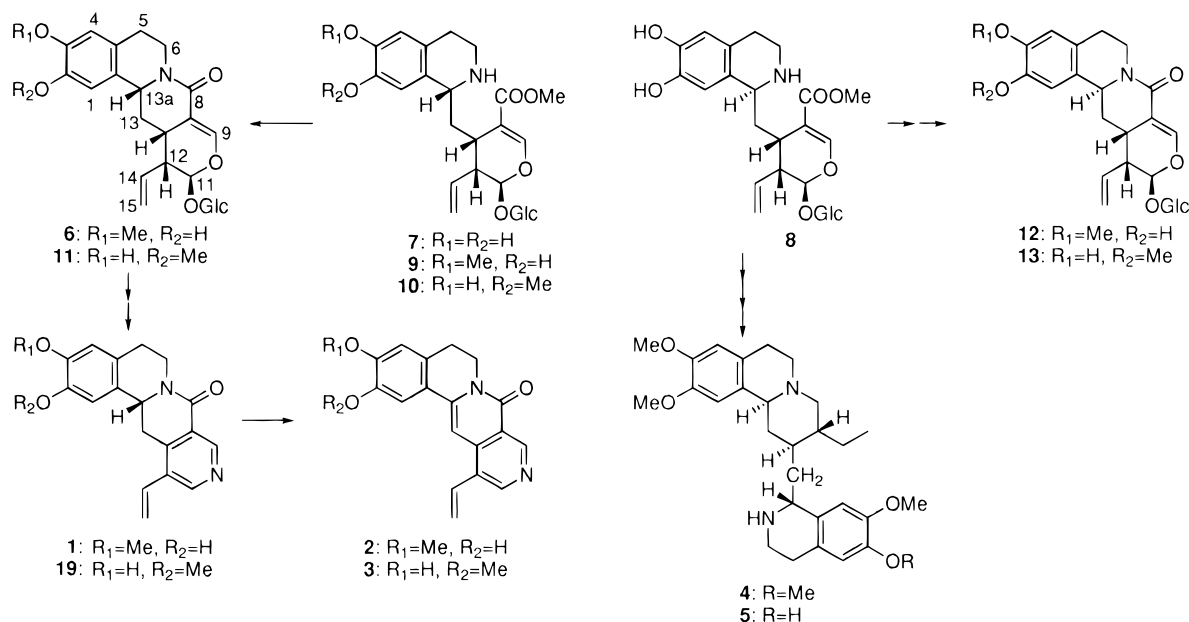
optical rotation showed the same sign as that of natural (+)-alangimaridine (**1**), but the values of the two compounds were rather different (synthetic **1**: $[\alpha]_D$ +235°; natural **1**:[2] $[\alpha]_D$ +429°). Nevertheless, our results seem reasonable because the specific optical rotation of synthetic (+)-alangimaridine (**1**) as well as that of (+)-dihydroalangimaridine (**15**) and (+)-isoalangimaridine (**19**) described below were in the same range of +220° to +280°. Finally, the CD spectrum of synthetic (+)-alangimaridine (**1**) showed positive Cotton effects as in the case of the natural product, confirming that the absolute configuration at C-13a of (+)-alangimaridine is *R*, as Pakrashi's group proposed.[2]

By a procedure similar to that described for (+)-alangimaridine (**1**), 3-*O*-demethyl-2-*O*-methylalangiside (**11**) was converted to isoalangimaridine (**19**). Compound **19** was not isolated as a natural compound, but it is a plausible intermediate between **11** and **3**. Thus, 3-*O*-demethyl-2-*O*-methylalangiside (**11**) was hydrolyzed with 3% HCl. This aglycon was treated with concentrated NH₃ and subsequently with TFA to furnish (+)-isoalangimaridine (**19**), $[\alpha]_D$ +220° (3.7% overall yield from **11**). The structure of the product was characterized by $[M]^+$ at *m/z* 322 and mass fragment ions at *m/z* 176 arising from A/B rings and at *m/z* 145 from C/D rings.[2] Its ¹H-NMR spectrum showed a singlet for a methoxyl group at δ 3.94, two singlets for aromatic protons on the A ring at δ 6.68 and 6.79, two singlets for aromatic protons on the pyridine ring at δ 8.80 and 9.21, and signals for a terminal vinyl group at δ 5.56 (dd, *J* = 11.0 and 1.0 Hz), 5.79 (dd, *J* = 17.5 and 1.0 Hz) and 6.87 (dd, *J* = 17.5 and 11.0 Hz). This compound was oxidized with iodine to isoalangimarine (**3**). Its MS and ¹H-NMR spectral data agreed well with those reported for the natural product.

The present work describes the first biogenetic synthesis of benzopyridoquinolizine alkaloids from tetrahydroisoquinoline-monoterpene glucosides and chemically confirms the absolute configuration of alangimaridine.

Experimental Section

General Experimental Procedures. Mps were recorded on a Büchi melting point apparatus and were

uncorrected. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra on a Hitachi 270-30 infrared spectrophotometer. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. CD spectra were recorded on a JASCO J-500C spectropolarimeter. MS and HREIMS were obtained with a Hitachi M-80 mass spectrometer. The ¹H-NMR spectra were taken on a Varian XL-200 spectrometer, with TMS as an internal standard. TLC was performed on a precoated Kieselgel 60F₂₅₄ plate (Merck), and the spots were visualized under UV light.

Dihydroalangiside (16). Dihydroalangiside pentaacetate (**17**) was prepared from alangiside (**6**) according to the method of Shoeb *et al.*[4] Methanolic NaOMe (0.06 M, 2 mL) was added to a solution of **17** (200 mg; 0.279 mmol) in dry MeOH (20 mL), and the mixture was stirred for 2.25 h at room temperature. After neutralization with dry ice, the reaction mixture was concentrated *in vacuo*. The resulting residue (156.6 mg) was chromatographed on a Si gel column with CHCl₃–MeOH (95:5) as eluent to afford dihydroalangiside (**16**) (116.4 mg, 82%) as an amorphous powder; $[\alpha]^{28}_D$ −74° (*c* 1.0, MeOH); UV (EtOH) λ max (log ε) 232 (4.32), 283 (3.67) nm; IR (KBr) ν max 3400, 1660, 1584, 1516 cm⁻¹; ¹H NMR (CD₃OD) δ 0.97 (3H, t, *J* = 7.0Hz, H₃-15), 3.84 (3H, s, OMe), 4.69 (1H, d, *J* = 7.9Hz, H-1′), 5.63 (1H, d, *J* = 1.5Hz, H-11), 6.71, 6.73 (2H, each s, H-1, H-4), 7.36 (1H, d, *J* = 2.5Hz, H-9); EIMS *m/z* $[M]^+$ 507, 345, 274, 178.

(+)-Dihydroalangiside Aglycon (18). (a) To a solution of dihydroalangiside (**16**) (68.3 mg; 0.135 mmol) in citrate–phosphate buffer (4 mL, pH 5.0) was added β-glucosidase (10 mg), and the whole was incubated for 17 h at 37 °C. The resulting precipitate (16.9 mg, 36%) was collected by filtration, the filtrate was extracted successively with CHCl₃ and EtOAc, and the combined extracts were evaporated *in vacuo* to leave aglycon **18** (9.6 mg) as a colorless crystalline solid (EtOAc): mp 195−196 °C; ¹H NMR (DMSO-*d₆*) δ 0.94 (3H, t, *J* = 7.5Hz, H₃-15), 3.74 (3H, s, OMe), 5.37 (1H, br s, H-11), 6.68, 6.74 (2H, each s, H-1, H-4), 7.23 (1H, d, *J* = 2.0Hz, H-9); EIMS *m/z* $[M]^+$ 345, 317, 274, 178, 162.

(b) A solution of dihydroalangiside (**16**) (39.9 mg; 0.079 mmol) in 3% HCl (1 mL) was heated for 5 h at 90–95 °C. The resulting crystalline solid was filtered to furnish an aglycon (17.4 mg, 64%). Its MS and ^{1}H-NMR spectra were identical with **18**; mp 193–195 °C (H_2O, dec).

(+)-Dihydroalangimaridine (15) from (+)-Dihy-droalangiside Aglycon (18). The aglycon **18** (25.1 mg; 0.073 mmol) was dissolved in a mixture of MeOH (1 mL) and 28% NH_4OH (1 mL), and the whole was allowed to stand at 4 °C for 24 h. The reaction mixture was concentrated with a stream of N_2 gas, and the residue (15.9 mg), after purification by preparative TLC (EtOAc, 2 developments), was redissolved in TFA (1 mL) and set aside for 20 h at room temperature. After dilution with ice–water, the reaction mixture was made basic with 14% NH_4OH and extracted with $CHCl_3$. The washed and dried $CHCl_3$ layers were concentrated *in vacuo* to leave dihydroalangimaridine (**15**) (14.0 mg, 59%), which was recrystallized from EtOH, giving rise to (+)-dihydroalangimaridine (**15**) (4.4 mg) as colorless crystalline solid: mp 230–232 °C; $[\alpha]^{26}_D$ +279° (*c* 0.68, $CHCl_3$); UV (EtOH) λ max (log ϵ) 222 sh (4.26), 279 (3.86) nm; ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, $J = 7.5$Hz, H_3-15), 2.73 (2H, q, $J = 7.5$Hz, H_2-14), 3.33 (1H, dd, $J = 16.2, 3.8$Hz, H-13), 3.93 (3H, s, OMe), 4.82 (1H, dd, $J = 13.5, 3.8$Hz, H-13a), 5.70 (1H, br s, OH, disappeared on addition of D_2O), 6.70 (1H, s, H-4), 6.82 (1H, s, H-1), 8.55 (1H, s, H-11), 9.16 (1H, s, H-9); EIMS m/z [M]$^+$ 324, 323, 309, 178, 162, 147, 119; HREIMS m/z [M]$^+$ 324.1481 (calcd for $C_{19}H_{20}N_2O_3$, 324.1473); CD (EtOH) $\Delta\epsilon$ (nm) +38.1 (207), +10.9 (233), +9.0 (266).

(+)-Alangimaridine (1) from Alangiside (6). Alangiside (**6**) (91.4 mg; 0.181 mmol) was treated with β-glucosidase (20 mg) in citrate–phosphate buffer (5 mL, pH 5.0) for 19 h at 37 °C and an aglycon (28.9 mg, 47%) was collected by centrifugation. To the supernatant was added further β-glucosidase (10 mg), and the whole was incubated for an additional 24 h at 37 °C and worked up to give an aglycon (23.1 mg, 37%). ^{1}H NMR ($CDCl_3$) δ 1.22 (1H, br q, $J = 12.0$Hz, H-13), 2.04 (1H, ddd, $J = 12.0, 9.0, 2.0$Hz, H-13), 3.86 (3H, s, OMe), 5.19 (1H, dd, $J = 17.0, 1.5$Hz, H-15), 5.24 (1H, dd, $J = 10.0, 1.5$Hz, H-15), 5.40 (1H, d, $J = 2.0$Hz, H-11), 5.81 (1H, ddd, $J = 17.0, 10.0, 9.5$Hz, H-14), 6.59, 6.75 (2H, each s, H-1, H-4), 7.52 (1H, d, $J = 2.0$Hz, H-9); EIMS m/z [M]$^+$ 343, 328, 272, 178.

The aglycon (37.9 mg; 0.110 mmol) was dissolved in a mixture of MeOH (1 mL) and 28% NH_4OH (1 mL), and the solution was allowed to stand at 4 °C for 16 h. The reaction mixture was concentrated with a stream of N_2 gas, and the residue redissolved in TFA (1.5 mL) was set aside for 19 h at room temperature. After dilution with ice–water, the reaction mixture was made basic with 14% NH_4OH and extracted with $CHCl_3$. The washed and dried $CHCl_3$ layers were concentrated *in vacuo* to leave a residue, which was subjected to preparative TLC ($CHCl_3$–MeOH–NH_4OH, 95:4.5:0.5), giving rise to (+)-alangimaridine (**1**) (5.3 mg, 14%), which was recrystallized from MeOH to give a colorless crystalline solid: mp 253–254.5 °C; $[\alpha]^{28}_D$ +235° (*c* 0.22, $CHCl_3$); UV (EtOH) λ max (log ϵ) 218 (4.49), 251 sh (4.03), 283 (3.83) nm; ^{1}H NMR ($CDCl_3$) δ 3.41 (1H, dd, $J = 16.7, 3.5$Hz, H-13), 3.92 (3H, s, OMe), 4.82 (1H, dd, $J = 13.2, 3.5$Hz, H-13a), 5.56 (1H, dd, $J = 11.3, 1.0$Hz,

H-15), 5.61 (1H, br s, OH), 5.79 (1H, dd, $J = 17.7, 1.0$Hz, H-15), 6.70 (1H, s, H-4), 6.81 (1H, s, H-1), 6.85 (1H, dd, $J = 17.7, 11.3$Hz, H-14), 8.78 (1H, s, H-11), 9.20 (1H, s, H-9); EIMS m/z [M]$^+$ 322, 321, 307, 176, 145, 117; HREIMS m/z [M]$^+$ 322.1295 (calcd for $C_{19}H_{18}N_2O_3$, 322.1318); CD (EtOH) $\Delta\epsilon$ (nm) +19.0 (209), +17.1 (233), +13.1 (256), +7.03 (291 sh).

(+)-Isoalangimaridine (6) from 11. A solution of 3-*O*-demethyl-2-*O*-methylalangiside (**11**) (99.7 mg; 0.197 mmol) in 3% HCl (6 mL) was heated for 1 h at 90–95 °C. The reaction mixture was extracted with $CHCl_3$, and the combined $CHCl_3$ layers were rinsed with H_2O, dried, and evaporated *in vacuo* to give a residue (25 mg), which was purified by preparative TLC ($CHCl_3$–MeOH, 19:1) to yield an aglycon (14.7 mg, 22%).

The aglycon (7.2 mg; 0.021 mmol) was dissolved in a mixture of MeOH (0.5 mL) and 28% NH_4OH (0.5 mL), and the solution was allowed to stand at 4 °C overnight. The reaction mixture was concentrated with a stream of N_2 gas, and the residue redissolved in TFA (0.5 mL) was set aside at 4 °C overnight. After dilution with ice-water, the reaction mixture was made basic with 14% NH_4OH and extracted with $CHCl_3$. The washed and dried $CHCl_3$ layers were concentrated *in vacuo* to leave a residue (5.3 mg), which was subjected to preparative TLC ($CHCl_3$–MeOH–NH_4OH, 95:4.5:0.5), giving rise to (+)-isoalangimaridine (**19**) (1.2 mg, 17.8%). $[\alpha]^{23}_D$ +220° (*c* 0.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.37 (1H, dd, $J = 17.0, 4.0$Hz, H-13), 3.94 (3H, s, OMe), 4.85 (1H, dd, $J = 13.0, 4.0$Hz, H-13a), 5.56 (1H, dd, $J = 11.0, 1.0$Hz, H-15), 5.59 (1H, br s, OH), 5.79 (1H, dd, $J = 17.5, 1.0$Hz, H-15), 6.68, 6.79 (2H, each s, H-1, H-4), 6.87 (1H, dd, $J = 17.5, 11.0$Hz, H-14), 8.80 (1H, s, H-11), 9.21 (1H, s, H-9); EIMS m/z [M]$^+$ 322, 176, 145, 117; HREIMS m/z [M]$^+$ 322.1308 (calcd for $C_{19}H_{18}N_2O_3$, 322.1318).

Isoalangimarine (3). A solution of (+)-isoalangimaridine (**19**) (2.4 mg; 0.007 mmol) in EtOH (2 mL) was refluxed with I_2 (5 mg) for 40 min. The reaction mixture was concentrated *in vacuo* and subjected to preparative TLC ($CHCl_3$–MeOH–NH_4OH, 95:4.5:0.5), giving iso-alangimarine (**3**) (1.5 mg, 63%) with a recovery of **19** (0.5 mg). ^{1}H NMR ($CDCl_3$-CD_3OD) δ 2.93 (2H, t, $J = 6.0$Hz, H_2-5), 4.03 (3H, s, OMe), 4.33 (2H, t, $J = 6.0$Hz, H_2-6), 5.60 (1H, dd, $J = 11.0, 1.0$Hz, H-15), 5.88 (1H, dd, $J = 17.0, 1.0$Hz, H-15), 6.84, 6.95 (2H, each s, H-1, H-4), 7.17 (1H, dd, $J = 17.0, 11.0$Hz, H-14), 7.27 (1H, s, H-13), 8.74 (1H, s, H-11), 9.48 (1H, s, H-9); EIMS m/z [M]$^+$ 320, 176, 117; HREIMS m/z [M]$^+$ 320.1154 (calcd for $C_{19}H_{16}N_2O_3$, 320.1162).

References and Notes

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