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Cytotoxic *Alangium* alkaloids from *Alangium longiflorum* ☆

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Abstract

Seven alkaloids (**1**–**7**) were isolated from the stem bark of *Alangium longiflorum*. Compound **1**, (−)-10-*O*-demethylisocephaeline, was isolated for the first time as a naturally occurring product from a plant source. All structures were elucidated by detailed spectroscopic analysis. Biological evaluation showed that **2**, 10-*O*-demethylcephaeline, exhibited potent cytotoxic activity against human lung carcinoma (A549) and breast adenocarcinoma (MCF-7) with ED_{50} values of 0.013 and 0.062 μM, respectively. The stereoisomer **1** was less potent than **2**, and related compounds with different hydroxy/methoxy substitution patterns were also less potent or inactive. Thus, compound **2** merits attention as a cytotoxic lead for further study.

Keywords: *Alangium longiflorum*; Alangiaceae; Cytotoxic activity; 10-*O*-Demethylcephaeline; 10-*O*-Demethylisocephaeline

1. Introduction

In a continuing collaboration with the National Cancer Institute (NCI) to discover potent antitumor agents from rain forest plants, an extract of *Alangium longiflorum* (sample number N33611) showed significant in vitro cytotoxicity against human tumor cell lines A549 and MCF-7. Subsequent bioassay-directed fractionation of this extract led to the isolation of seven tetrahydroisoquinoline monoterpene alkaloids **1**–**7**. Their structures were established from analysis of spectroscopic data, including MS, ^{1}H and ^{13}C NMR, DEPT, COSY, HETCOR, HMBC and NOESY. Among them, **1** has been isolated as a new naturally occurring compound from this plant, although it has been prepared previously synthetically (Fujii and Ohba, 1985).

A. longiflorum is found in West Kalimantan, Indonesia, and the related *Alangium lamarckii* Thwaites is distributed widely throughout India and southeast Asia and has been used as a herbal dewormer in livestock (Iqbal et al., 2003). Although there are no reports of chemical or biological research on the former species, it has been reported (Itoh et al., 2001; Xu et al., 2003) that *A. lamarckii* contains numerous alkaloids, most of which are characterized by the presence of a tetrahydroisoquinoline monoterpene skeleton. Biological activities for other *Alangium* plants are diverse and include DNA damaging activity (Xu et al., 2003) and dihydrofolate reductase inhibition (Rao and Venkatachalam, 1999). Herein, we report the isolation, structure elucidation, and biological evaluation of constituents of *A. longiflorum*.

2. Results and discussion

Ground stem bark of *A. longiflorum* was extracted with a 1:1 mixture of CH_2Cl_2 and aqueous MeOH, which was then concentrated to dryness. The residue was dissolved

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in MeOH, and this MeOH extract was further partitioned successively with *n*-hexane, $CHCl_3$, EtOAc, and *n*-butyl alcohol. The fractionation and chromatographic isolation were guided by cytotoxic activity against human A549 and MCF-7 tumor cell lines, and led to the isolation of seven tetrahydroisoquinoline monoterpene alkaloids.

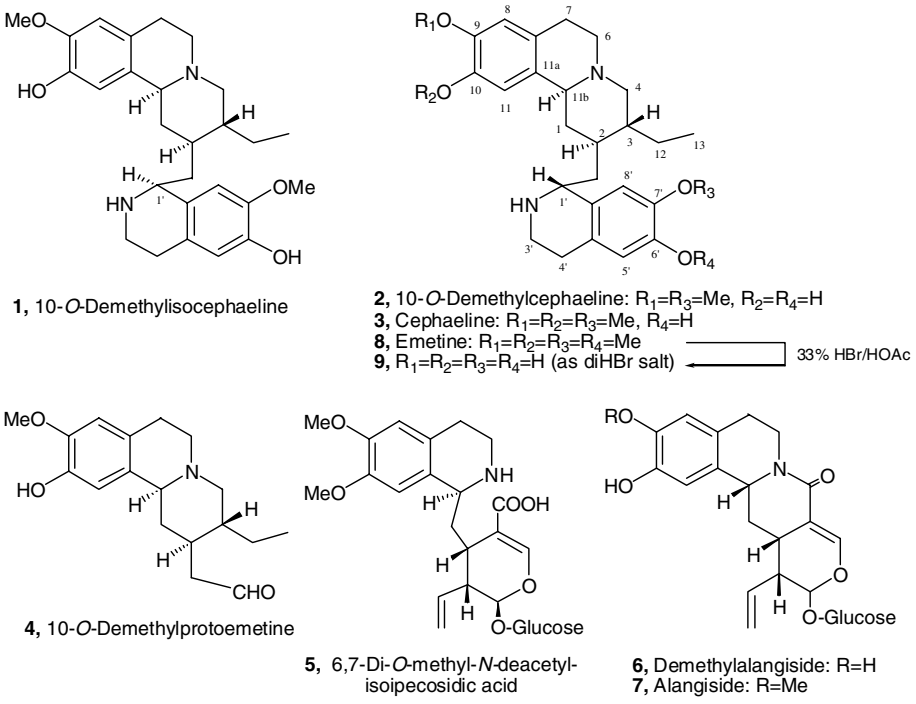
Compound **1**, colorless needles, had a molecular weight of 452 by ESI-MS. It was identified as (−)-10-*O*-demethylisocephaeline by comparison of its ^1H and ^{13}C NMR spectroscopic data with those in the literature (Fujii and Ohba, 1985). The NOESY correlations of H-8 and H-8′ with respective OMe groups provided additional support for the structural assignments. The coupling between H-1′ and the α-CH_2 was observed as a broad triplet ($J = 6$ Hz). This is the first report of isolation of **1** as a naturally occurring compound.

Compound **2**, a pale yellow powder, was isomeric with **1** from ESI-MS data. Its spectral features closely resembled those of **1**, except for the C-1, C-2 and C-1′ signals. 2D-NMR experiments definitively established the structure of **2** as 10-*O*-demethylcephaeline (Itoh et al., 1999).

Compounds **3** and **4** were identified as cephaeline (Itoh et al., 1999) and 10-*O*-demethylprotoemetine (Itoh et al., 2001), respectively. Compound **5**, colorless crystals, had a molecular weight of 537 by MS, and was identified as 6,7-di-*O*-methyl-*N*-demethylisoipecosidic acid by a comparison of ^1H and ^{13}C NMR spectroscopic data with literature values (Itoh et al., 2001). Compounds **6** and **7** were identified as demethylalangiside and alangiside, respectively (Itoh et al., 1994).

Compounds **1**–**3** and **5**–**7** were assayed for cytotoxicity using a reported procedure (Rubinstein et al., 1990). Compound **2** showed notable potency with ED_{50} values of 0.013 and 0.062 μM against A549 and MCF-7 cell lines, respectively. Compound **1**, which has the opposite stereochemistry at C-1′, was ca. 10-fold less active ($ED_{50} = 0.15$ and 0.55 μM against A549 and MCF-7, respectively). Compounds **3** and **5**–**7** showed no toxicity. The extreme activity of **2** was particularly notable as it varies in structure from the inactive cephaeline (**3**), only by the presence of a hydroxy (**2**) rather than methoxy (**3**) group at C-10.

Accordingly, based on the strong activity of **2**, we evaluated two related compounds. Emetine, which contains four methoxy substituents, is most well known as an emetic and amebicide, but also showed good in vitro activity in murine tumor systems, particularly against L-1210 and P-388 leukemia (Auletta et al., 1974). Phase I clinical trials of emetine against lung cancer showed some partial remissions (Panettiere and Coltman, 1971) and led to phase II trials against various tumor systems (Siddiqui et al., 1973). In our study, emetine (**8**) showed good potency ($ED_{50} = 0.048$ μM), but was ca. 4-fold less active than **2** in the A549 cell line. Demethylation of emetine with 33% HBr in HOAc gave the tetraol (**9**) as the HBr salt. The tetrahydroxy substituted **9** showed no cytotoxic activity against A549 cells.



3. Concluding remarks

From the data above, the presence or absence of the methyl groups on the C-10 and C-6′ hydroxyls played a role in the activity of these tetrahydroisoquinoline monoterpene alkaloids – demethylation of the C-6′ methoxy abolished activity (**8** → **3**), while demethylation also at C-10 gave the most potent compound evaluated (**3** → **2**). Further studies on the optimal methylation pattern are

warranted to develop **2** as a new cytotoxic lead compound.

4. Experimental

4.1. General

Melting points were determined on a Fisher–Johns melting apparatus and are uncorrected. The optical rotation was measured on a JASCO DIP-1000 digital polarimeter. NMR spectra were measured on Varian Gemini 2000 300 MHz and JEOL A600 600 MHz NMR spectrometers with TMS as internal reference. The MS spectra were recorded on an APE-Sciex API-3000 LC/MS/MS instrument equipped with a turbo Ions Spray source.

4.2. Plant material

Stem bark of *Alangium longiflorum* was collected in the Bentuang area of West Kalimantan, Indonesia on June 1989 by J.S. Burley and Tukirin and identified by Dr. John Burley. A voucher specimen is located in the Botany Department, Museum on Natural History, Smithsonian Institution, Washington, DC.

4.3. Extraction and isolation

Air-dried ground bark (478 g) of *A. longiflorum* was extracted with a 1:1 mixture of CH_2Cl_2 and aqueous MeOH and concentrated in vacuo to give a crude extract (35.6 g: sample number N33611). A part (9.8 g) of this extract was dissolved in MeOH–H_2O (1–4) and extracted with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH, successively. A part (2.74 g) of the residue (5.74 g) from the *n*-BuOH layers was fractionated on SiO_2 MPLC with $CHCl_3$–MeOH (10%–100%) to give 7 fractions, frs. 1 (10%, 44 mg), 2, 3 (20%, 356 mg and 219 mg, respectively), 4 (25%, 151 mg), 5 (30%, 843 mg), 6 (50%, 471 mg), 7 (100%, 497 mg). Fr. 3 was purified by HPLC (YMC ODS-A; C18, 5 μ, 120 Å) with MeOH:H_2O (6:4) to afford compound **6** (9.7 mg). Frs 5–7 were submitted to MPLC (RP-8) and prep. TLC [SiO_2, $CHCl_3$:MeOH (4:1) or benzene:MeOH (9:1), repeatedly] to give compounds **1** (8.2 mg), **2** (27 mg) and **7** (6.6 mg). A part (2.32 g) of the residue (2.40 g) from the water layer and a part (2.96 g) of the residue (5.74 g) from the *n*-BuOH layer were combined and dissolved in H_2O, basified with Na_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated in vacuo to give a $CHCl_3$ extract (1.31 g). The H_2O layer was concentrated to 500 ml, and was subjected to DIAION HP-20 cc with H_2O:MeOH (1:0–2:8), Sephadex LH-20 with H_2O:MeOH (1:0–2:8), ODS with H_2O:MeOH (1:0–2:8) and silica gel cc with $CHCl_3$:MeOH:H_2O (60:25:7) to give compound **5** (8 mg). $CHCl_3$ extract was subjected to silica gel cc with $CHCl_3$:MeOH:NH_4OH (90:10:1–85:15:1.5) to give compounds **4** (8 mg), **3** (18 mg), **2** (314 mg) and **1** (127 mg).

The known compounds (**2**–**7**) were identified by comparison of their physical and spectral data with those reported in the literature (Itoh et al., 1994, 1999, 2001).

4.4. 10-O-Demethylisocephaeline (1)

Colorless needles, mp 114–116 °C, $[\alpha]_D^{30}$ −12.8 (*c* 0.81, MeOH); IR ν_{max} 3520, 1450, 1393 cm^{-1}; ^1H NMR (CD_3OD) δ 0.93 (3H, *t*, $J = 7.7$ Hz, H-13), 3.80, (3H, *s*, 9-OCH_3), 3.81 (3H, *s*, 7′-OCH_3), 3.90 (1H, *br t*, $J = 6$ Hz, H-1′), 6.53, 6.63, 6.69, 6.70 (1H each, *s*, aromatic H); ^{13}C NMR (CD_3OD) δ 11.6 (*q*, C-13), 24.9 (*t*, C-12), 29.2 (*t*, C-4′), 29.5 (*t*, C-7), 39.4 (*t*, C-1), 39.5 (*d*, C-2), 42.2 (*t*, C-α), 43.7 (*d*, C-3), 53.8 (*t*, C-6), 53.9 (*d*, C-1′), 56.3 (*q*, 9-OCH_3), 56.6 (*q*, 7′-OCH_3), 62.3 (*t*, C-4), 63.9 (*d*, C-11b), 110.9 (*d*, C-8′), 112.6 (*d*, C-8), 116.7 (C-5′), 126.2 (*s*, C-7a), 128.3 (*s*, C-4a′), 131.0 (*s*, C-11a), 131.4 (*s*, C-8′a), 145.8 (*s*, C-10), 146.3 (*s*, C-6′), 147.4 (*s*, C-7′), 147.8 (*s*, C-9). ESI-MS $m/z = 451$ $[M-1]^-$; Anal. Calcd for $C_{27}H_{36}Br_2N_2O_4 \cdot H_2O$: C, 68.91; H, 8.14; N, 5.95. Found: C, 68.89; H, 7.99; N, 5.83.

4.5. Tetrahydroxy emetine diHBr salt (9)

HBr (33%) in HOAc (1.0 mL) was added to emetine (79 mg, 0.14 mmol), then the mixture was refluxed for 1 h. The volatile solvent was removed under reduced pressure to provide 85 mg (quant.) of tetrahydroxy emetine as the diHBr salt. The brown amorphous material was obtained using CH_2Cl_2. ^1H NMR (CD_3OD): δ 6.94 (*s*, 1H), 6.69 (*s*, 1H), 6.64 (*s*, 1H), 6.63 (*s*, 1H), 4.75 (*d*, 1H, $J = 10.0$ Hz), 4.58 (*br s*, 1H), 4.44 (*d*, 1H, $J = 10.0$ Hz), 3.88–3.83 (*m*, 1H), 3.74–3.63 (*m*, 2H), 3.63–3.52 (*m*, 1H), 3.50–3.37 (*m*, 2H), 3.30–3.20 (*m*, 1H), 3.17–2.98 (*m*, 5H), 2.36–2.24 (*m*, 1H), 2.06–1.94 (*m*, 1H), 1.94–1.68 (*m*, 3H), 1.58–1.44 (*m*, 1H), 1.42–1.26 (*m*, 2H), 1.00 (*t*, 3H, $J = 7.3$ Hz). MS $m/z = 425$ (M^+−2HBr). Anal. Calcd for $C_{25}H_{34}Br_2N_2O_4 \cdot H_2O$: C, 49.68; H, 6.00; N, 4.64. Found: C, 49.96; H, 5.83; N, 4.14.

4.6. Cytotoxicity assay

All stock cultures were grown in T-25 flasks (5 mL of RPMI-1640) medium supplemented with 25 mM HEPES, 0.25% $NaHCO_3$, 10% fetal bovine serum, and 100 μg/mL kanamycin. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500–7500 cells per well with test compounds from DMSO-diluted stock. After three days in culture, cells attached to the plastic substratum were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The ED_{50} is the concentration of test compound that reduced cell growth by 50% over a 3-day assay period (see Table 1).

Table 1
Cytotoxic activity of *Alangium* alkaloids against human tumor cell lines[a]

Compound	ED_{50}(μM)[b]/cell Line	
	A549	MCF-7
1	0.15	0.55
2	0.013	0.062
3	NA[c]	NA
5	NA	NA
6	>2.0	>2.0
7	<2.0	>2.0
8	0.048	ND[d]
9	NA	ND

[a] Cell lines in RPMI-1640, 10% (V/V) FBS, 100 μg/mL kanamycin.
[b] ED_{50} is the concentration of compound that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using SRB assay.
[c] NA, not active at 10 μM.
[d] ND, not determined.

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