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Quinoline alkaloids from *Acronychia laurifolia*

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Abstract

Bioassay-directed fractionation of a root extract of *Acronychia laurifolia* (Rutaceae) using the KB-V1$^+$ human tumor cell line led to the isolation of six quinoline alkaloids. One of these alkaloids is novel, namely, 2,3-methylenedioxy-4,7-dimethoxyquinoline and the other five were identified as the known compounds, evolitrine, γ-fagarine, skimmianine, kokusaginine and maculosidine. Two known bis-tetrahydrofuran lignans, sesamolin and yangambin, were also identified. The structure of the new alkaloid was determined by spectroscopic methods. All of the isolates were evaluated against a panel of human cancer cell lines; four of the alkaloids showed weak cytotoxic activity.

Keywords: *Acronychia laurifolia*; Rutaceae; Quinoline alkaloids; 2,3-methylenedioxy-4,7-dimethoxyquinoline; Lignans; Cytotoxic activity

1. Introduction

As part of our continuing search for novel plant-derived anticancer agents, the roots of *Acronychia laurifolia* Blume (Rutaceae) were investigated. Several quinoline alkaloids have been documented previously as constituents of plants classified in the genus *Acronychia* (Svoboda, 1966; Svoboda, Poore, Simpson, & Boder, 1966; Lamberton, 1966; Lahey, & McCamish, 1968; Fong, Farnsworth, & Svoboda, 1969; Lahey, McCamish, & McEwan, 1969; De Silva, De Silva, Mahendran, & Jennings, 1979; Funayama, & Cordell, 1984; Xu, & Xue, 1984; Bowen, Dennis, & Osborne, 1985; Kumar, Karunarantne, & Meegalle, 1989; Bissoue et al., 1996). The alkaloid fraction of *A. laurifolia* leaves was reported to have CNS-depressant activity in a rat model (Chowrashi, Mukherjee, & Sikdar, 1969). Flavonols from *A. pedunculata* and the known benzenoid, acrovestone, from *A. porteri*, have been reported to be cytotoxic against the KB cell line (Wu et al., 1989; Lichius et al., 1994). The isolation of β-sitosterol, seselin, norbraylin and acrovestone has been previously reported from *A. laurifolia* (Govindachari et al., 1969; Seccombe, & Kennard, 1974; Rahman, Taufiq-Yap, & Sukari, 1996).

In the present paper, activity-guided fractionation of the chloroform-soluble extract of the roots of *A. laurifolia* using the KB-V1$^+$ human cancer cell line led to the isolation of one new and five known quinoline alkaloids (**1**–**6**), along with two known bis-tetrahydrofuran lignans (**7**,**8**). Alkaloids **2** and **4**–**6** showed weak cytotoxic activity when evaluated against a panel of human cancer cell lines, while compounds **1**, **3**, **7** and **8** were inactive in this regard (Table 1).

2. Results and discussion

Compounds **2**–**8** were identified as evolitrine (Narasimhan, & Mali, 1974), γ-fagarine (Collins,

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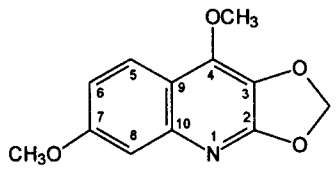
Table 1
Evaluation of the cytotoxic potential of isolates **2**, **4**, **5** and **6** obtained from *A. laurifolia*[a]

Compound	Cell line[b]							
	BC1	Lu1	Col2	KB	KB-V1$^+$	KB-V1$^-$	LNCaP	ASK[c]
2	5.8	>20	>20	>20	2.9	2.8	>20	–
4	5.6	>20	>20	>20	4.4	4.3	>20	–
5	>20	>20	>20	>20	4.4	14.4	>20	–
6	>20	>20	>20	>20	4.5	10.2	>20	–

[a] Results are expressed as ED_{50} values (μg/ml) (Likhitwitayawuid et al. 1993).
[b] Key: BC1 = human breast cancer; Lu1 = human lung cancer; Col2 = human colon cancer; KB = human epidermoid carcinoma; KB-V1$^+$ = drug-resistant KB assessed in presence of vinblastine (1μg/ml); KB-V1$^-$ = drug-resistant KB assessed in absence of vinblastine; LNCaP = hormone-dependent human prostate cancer; ASK = rat glioma.
[c] = Reversal of astrocyte formation was not observed when tested at a concentration of 100 μg/ml for the ASK cell line.

Gray, Grundon, Harrison, & Spyropoulos, 1973), skimmianine (Liu, Wei, Wang, & Gao, 1991), kokusaginine (Lahey, & McCamish, 1968), maculosidine (Xu, & Xue, 1984), sesamolin (Haslam, 1970) and yangambin (MacRae, & Towers, 1985), respectively, by comparison with reported data.



1

Alkaloid **1** was obtained as plates (MeOH), mp 176–8° and its molecular formula of $C_{12}H_{11}O_4N$ was determined by HREIMS. Analysis of the ^{1}H- and ^{13}C-NMR spectra of **1** indicated that it was a quinoline alkaloid biogenetically related to alkaloids **2**–**6**, differing in the absence of a furan ring functionalized at the C-2 and C-3 positions. In the ^{1}H-NMR spectrum of **1**, three aromatic proton signals were observed at δ 7.85 (1H, d, J = 9.1 Hz), 7.16 (1H, d, J = 2.5 Hz) and 6.99 (1H, dd, J = 9.1, 2.5 Hz) and two methoxy groups at δ 4.25 (3H, s) and 3.88 (3H, s) were comparable in their chemical shifts to those of evolitrine (**2**). These five resonances in **1** were assigned to H-5, H-8, H-6, CH_3O-4 and CH_3O-7, respectively. The ^{1}H- and ^{13}C-NMR signals at δ 6.00 (2H, s) and δ 99.0 (t) and a HETCOR experiment indicated the presence of a methylenedioxy group (OCH_2O) in **1**. In a HMBC NMR experiment performed on **1**, the methylenedioxy proton signal at δ 6.00 (2H, s) showed cross-correlation peaks with the ^{13}C-NMR resonances at δ 160.1 (s) and 121.9 (s), assignable to C-2 and C-3, respectively. The above evidence suggested that the location of the methylenedioxy group should be at the C-2 and C-3 positions. Assignments of all of the protons and carbons for **1** were made through ^{1}H–^{1}H COSY, NOESY, APT, HETCOR and HMBC NMR experiments. Consequently, the structure of **1** was identified as the new alkaloid 2,3-methylenedioxy-4,7-dimethoxyquinoline.

Compounds **1–8** were evaluated against a panel of human cancer cell lines as summarized in Table 1. Compounds **1**, **3**, **7** and **8** were inactive in all test systems. Alkaloids **2** and **4** showed cytotoxic activity against the BC1 and drug-resistant KB-V1 cell lines. With the multidrug-resistant cell line, no advantage was noted in the presence of vinblastine for these compounds. On the other hand, alkaloids **5** and **6** were found to be weakly active against the KB-V1$^+$ cell line in the absence of vinblastine, but activity was enhanced 2–3-fold when vinblastine was added to the incubation medium. All compounds were inactive against the ASK cell line.

In the present investigation, *A. laurifolia* was collected from a marked plot in the tropical rain forest. The rationale for using this approach to plant selection in drug discovery programs has been described previously (Soejarto, 1991).

3. Experimental

MPs. uncorr.; IR: film; ^{1}H and ^{13}C NMR spectra were recorded on either a 300 or 360 MHz NMR instrument with tetramethylsilane (TMS) as an internal standard in $CDCl_3$. HETCOR spectra were recorded on 300 MHz NMR instrument. HMBC data were obtained using a 500 MHz NMR instrument. Low- and high-resolution MS were measured with a Finnigan MAT-90 instrument (70 eV). Column chromatography was carried out with Merck silica gel G (70–230 and 230–400 mesh) and Aldrich RP-C_{18} silica gel.

3.1. Plant material

The roots of *A. laurifolia* [syn. *A. pedunculata* (L.) Miq.] (Rutaceae) were collected from a marked plot in

a forest on the northeast base of Thumb Peak, Barangay Simpocan, Municipality of Puerto Princesa, Palawan, Philippines, in September, 1992 by F.D.H. (*D. Horgen, R. Majaducon and E. Burlaza 45*) and identified by one of us (B.H.). A voucher specimen (FM2167711) has been deposited at the Field Museum of Natural History, Chicago, Il.

3.2. Extraction and isolation

The air-dried roots (500 g) of *A. laurifolia* were extracted with three changes of MeOH (1 × 2 l, 2 × 1.5 l). The resultant extracts were combined, concentrated under a vacuum, dissolved in MeOH–H_2O (4:1, 400 ml) and washed with hexanes (3 × 200 ml). The lower layer was concentrated under reduced pressure and partitioned between 10% MeOH (300 ml) and $CHCl_3$ (3 × 200 ml). The $CHCl_3$-soluble extract (2.2 g) was subjected to silica gel column chromatography and eluted with hexanes–acetone–MeOH mixtures in a gradient. Fractions 8–12, eluted with hexanes–acetone (20:1), were purified over a silica gel column using mixtures of hexanes–EtOAc (8:1) to afford **7** (10 mg). Fractions 14–18, eluted with hexanes–acetone–MeOH (12:1:0.1), were combined and purified by silica gel column chromatography using mixtures of hexanes–EtOAc–MeOH (6:1:0.1) to afford **3** which was crystallized from MeOH (colorless needles, 82 mg). Fraction 20, eluted with hexanes–acetone–MeOH (10:1:0.1), was purified over a silica gel column using mixtures of hexanes–EtOAc–MeOH (5:1:0.1) to afford **1** which was crystallized from MeOH (colorless plates, 102 mg). Fractions 7–11, eluted from the first column with hexanes–acetone–MeOH (15:1:0.1), were combined and chromatographed over a silica gel column eluted with mixtures of hexanes–acetone (10:1 → 6:1) to yield **2** (8 mg). Subfractions 7–10, eluted with hexanes–acetone (8:1), represented a mixture of **2** and **4**. These alkaloids were purified by reversed-phase C_{18} silica gel column chromatography eluted with MeOH–CH_3CN–H_2O mixtures (45:5:50 → 60:5:35) to provide **2** (6 mg) and **4** (2 mg). Subfractions 53–57, eluted with hexanes–acetone (6:1), were purified by a silica gel column using mixtures of $CHCl_3$–acetone (80:1 → 20:1) to afford **5** (14 mg), **6** (8 mg) and **8** (23 mg).

3.3. 2,3-Methylenedioxy-4,7-dimethoxyquinoline (*1*)

Plates, mp 176–8°; UV λ_{max}^{MeOH} nm (log ε): 242 (1.5), 255 (2.8), 308 (3.5), 335 (3.8); IR ν_{max} (film) cm^{-1}: 2915, 1620, 1586, 1320, 1200, 1143; ^1H NMR (300 MHz, $CDCl_3$): δ 7.85 (1H, d, J = 9.1 Hz, H-5), 7.16 (1H, d, J = 2.5 Hz, H-8), 6.99 (1H, dd, J = 9.1, 2.5 Hz, H-6), 6.00 (2H, s, OCH_2O), 4.25 (3H, s, CH_3-4), 3.88 (3H, s, CH_3-7); ^{13}C NMR (360 MHz, $CDCl_3$): δ 160.1 (s, C-2), 159.7 (s, C-7), 144.7 (s, C-10), 142.2 (s, C-4), 122.5 (d, C-5), 121.9 (s, C-3), 115.3 (d, C-8), 115.2 (s, C-9), 107.2 (d, C-6), 99.0 (t, OCH_2O), 59.3 (q, CH_3-4), 55.3 (q, CH_3-7); main HMBC correlations: OCH_2O/C-2,C-3, CH_3O-4/C-4, H-5/C-4,C-6,C-7,C-10, H-6/C-8,C-9, CH_3O-7/C-7, H-8/C-6,C-9; EIMS (70 eV) m/z (rel. int.): $[M]^+$ 233 (65), 160 (100), 117 (16), 77 (7), 57 (6); HREIMS m/z: 233.0916 (calcd for $C_{12}H_{11}O_4N$, 233.0912).

Evolitrine (**2**), *γ-fagarine* (**3**), *skimmianine* (**4**), *kokusaginine* (**5**), *maculosidine* (**6**), *sesamolin* (**7**) and *yangambin* (**8**) were identified by comparison with reported data (Lahey, & McCamish, 1968; Haslam, 1970; Collins et al., 1973; Narasimhan, & Mali, 1974; Xu, & Xue, 1984; MacRae, & Towers, 1985; Liu et al., 1991).

3.4. Bioassay evaluation

Compounds **1**–**8** were evaluated for cytotoxic activity against a panel of human cancer cell lines according to established protocols (Likhitwitayawuid, Angerhofer, Cordell, Pezzuto, & Ruangrungsi, 1993). ED_{50} values of < 5 μg/ml for pure compounds were regarded as being significantly active. Antimitotic activity was assessed using cultured rat glioma cells (Swanson, Jiang, De Souza, & Pezzuto, 1988). Results for compounds exhibiting significant activity in one or more cell lines are given in Table 1.

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