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Cytotoxic naphthoquinones and plumbagic acid glucosides from *Plumbago zeylanica*

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Abstract

Two plumbagic acid glucosides, 3′-*O*-β-glucopyranosyl plumbagic acid and 3′-*O*-β-glucopyranosyl plumbagic acid methylester along with five naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone), and five coumarins (seselin, 5-methoxyseselin, suberosin, xanthyletin and xanthoxyletin) were isolated from the roots of *Plumbago zeylanica*. All coumarins were not previously found in this plant. Cytotoxicity of these compounds to various tumor cells lines was evaluated, and plumbagin significantly suppressed growth of Raji, Calu-1, HeLa, and Wish tumor cell lines.

Keywords: *Plumbago zeylanica*; Plumbaginaceae; Plumbagic acid glucoside; Naphthoquinone; Coumarin; Cytotoxicity

1. Introduction

Plumbago zeylanica Linn. (Plumbaginaceae) is a semi-climbing subshrub distributed in thickets or grassland at low elevations of Taiwan (Li, 1998). The whole plant and its root have been used as a folk medicine in Taiwan for the treatment of rheumatic pain, menostasis, carbuncle, and injury by bumping (Chiu and Chang, 1986). Several naphthoquinones, binaphthoquinones, flavonoids and β-sitosterol have been isolated from this plant (Gunaherath and Gunatilaka, 1988; Gunaherath et al., 1983; Dinda and Saha, 1989; Sankaram et al., 1976). Preliminary screening demonstrated that EtOAc and *n*-BuOH extracts of the root of *P. zeylanica* exhibited cytotoxic activities against cancer cells in cultures. Two plumbagic acid glucosides (**1** and **2**) and plumbagic acid (**3**) (Dinda et al., 1998) were identified from the *n*-BuOH fraction, whereas, chromatography of the EtOAc extract gave five naphthoquinones [plumbagin (**4**) (Gunaherath et al., 1983), chitranone (**5**) (Sankaram et al., 1976), maritinone (**6**) (Tezuka et al., 1973), elliptinone (**7**) and isoshinanolone (**8**) (Gunaherath et al., 1983)] and five coumarins [seselin (**9**) (Murray and Zeghdi, 1989), 5-methoxyseselin (**10**) (Wu et al., 1983a), suberosin (**11**) (Nayar and Bhan, 1972; Wu et al., 1983b), xanthyletin (**12**) (Wu et al., 1983b) and xanthoxyletin (**13**) (Ito et al., 1990)]. The spectral evidence leading to structures for (**1**) as 3′-*O*-β-glucopyranosyl plumbagic acid and (**2**) as 3′-*O*-β-glucopyranosyl plumbagic acid methyl ester are reported herein. Furthermore, the results of cytotoxic assays of **1**–**13** are presented.

2. Results and discussion

The molecular formula of **1** was established as $C_{17}H_{22}O_{10}$ by HR-FAB–MS, ^{13}C NMR and DEPT analyses. The UV spectrum showed λ_{max} (log ε) at 214 (4.19), 259 (3.76) and 333 (3.19) nm similar to that of plumbagic acid (**3**). The IR spectrum exhibited bands at 3465 (OH), 1720 (C=O) and 1635 (chelated >C=O) cm^{-1}. The ^1H NMR spectrum displayed signals for one methyl (δ1.25, 3H, *d*, *J* = 7.0 Hz, Me-3), one methylene (δ2.39, 1H, *dd*, *J* = 16.5, 5.0 Hz, Hα-2; 2.91, 1H, *dd*, *J* = 16.5, 9.0 Hz, Hβ-2), one methine (δ4.03, 1H, *m*, H-3), three aromatic protons (δ6.94, 1H, *t*, *J* = 8 Hz, H-5′; 7.45, 1H, *d*, *J* = 8 Hz, H-4′; 7.66, 1H, *d*, *J* = 8 Hz, H-6′) and an anomeric proton of sugar (δ4.91, 1H, *d*, *J* = 8 Hz, H-1″). In negative ion ESIMS of **1** showed a

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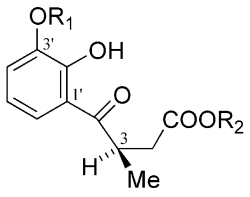
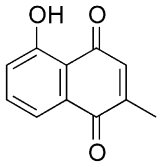


1 R_1= β-glucopyranosyl R_2= H
2 R_1= β–glucopyranosyl R_2= Me
3 R_1= H R_2= H



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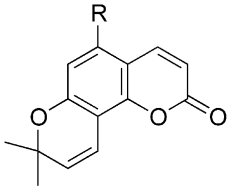
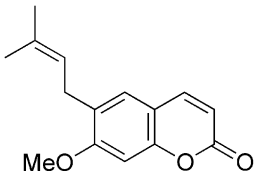


9 R= H
10 R= OMe



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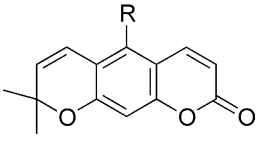

12 R= H
13 R= OMe

quasi-molecular ion $[M–H]^-$ at *m*/*z* 385 and a fragment peak at *m*/*z* 223 $[M-162-H]^-$ indicated the loss of a hexosyl moiety from the quasi-molecular ion. Upon acid hydrolysis of **1**, glucose was detected by HPLC, and the aglycone was identified as plumbagic acid (**3**). In the HMBC experiments, the signals at δ147.6 (C-3′) and 153.6 (C-2′) correlated with δ4.91 (H-1″), 6.94 (H-5′) and 7.45 (H-4′), and with δ7.45 and 7.66 (H-6′), respectively, indicating the presence of glucosyl group at the C-3′ position. The NOESY spectrum also confirmed the above observation, where cross-peaks were observed between H-4′ and H-1″, and between H-4′ and H-5′. The circular dichroism (CD) spectrum exhibited a positive Cotton effect (Peak: 254 nm, [Δε] 0.56; Trough: 209 nm, [Δε]−4.20), similar to that of plumbagic acid (**3**) (Dinda et al., 1998). Therefore, C-3 was assigned the *S* configuration and **1** was astablished to be 3′–*O*-β-glucopyranosyl plumbagic acid.

The IR spectrum and the UV absorption maxima of **2** were very similar to those of **1**. The ^{1}H NMR spectrum of **2** showed a close resemblance to that of **1** except for the additional presence of a signal at δ3.63 (3H, *s*). The ^{13}C NMR spectrum of **2** also resembled that of **1** except for an additional oxygenated methyl carbon at δ52.2, suggesting it to be a methyl ester derivative of **1**. In agreement with these observations, the negative APCIMS revealed a pseudomolecular ion at *m*/*z* 399 $[386+14-H]^-$. Hence, **2** was assigned as 3′-*O*-β-glucopyranosyl plumbagic acid methylester.

All isolated compounds (**1**–**13**) were tested against a panel of cancer cell lines according to established protocols (Kuo et al., 1994). Compound **4** significantly inhibited Raji, Calu-1, HeLa, and Wish cell growth, with IC_{50} values 8.1±3.9, 25.0±8.8, 21.5±2.6, and 21.2±5.0 μM, respectively. By contrast, **4** had a less suppressory activity on Jurkat (IC_{50} =77.5±10.6 μM), K562 (IC_{50}=85.0±9.0 μM), and Vero (IC_{50}=65.0±7.4 μM) tumor cell lines. All other test compounds (**1**–**3**, **5**–**13**) had IC_{50} values higher than 100 μM against all cell lines. These results suggest that **4** plays a significat role in the cytotoxicity of this plant.

3. Experimental section

3.1. General

IR spectra were obtained as KBr pellets on a Perkin-Elmer 781 IR spectrometer. CD spectra were recorded on a JASCO J-715 spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter in MeOH. UV spectra were obtained on a Hitachi U-3200 spectrophotometer in MeOH. ^{1}H, ^{13}C and 2D NMR spectra were measured with a Varian Inova-500 spectrometer with deuterated solvent as internal standard. APCIMS and ESIBMS were recorded on a Finnigan LCQ spectrometer. HR-FAB–MS were recorded on a JEOL-SX102A spectrometer.

3.2. Plant material

Roots of *P. zeylanica* were collected at Wulai, Taipei, Taiwan, in June, 2001. A voucher specimen (No. 1732) has been deposited in the herbarium of the Department of Botany of the National Taiwan University.

3.3. Extraction and isolation

The roots of *P. zeylanica* (2.2 kg) were extracted with 95% EtOH (40 l×3). The solvent was concentrated in vacuo and the residue was successively partitioned between H_2O (1 l) and *n*-hexane (1 l×3), followed by EtOAc (1 l×3) and *n*-BuOH (1 l×3). The EtOAc extract (21 g) was subjected to silica gel cc with a gradient of EtOAc in *n*-hexane, and 10 fractions were collected. Fraction 1 was applied to a Si gel column, eluting with *n*-hexane-EtOAc (9:1) to yield a solid, which on crystallization from an *n*-hexane–EtOAc mixture gave plumbagin (**4**, 1.54 g). Fraction 3 was further purified by Sephadex LH-20 column (acetone) and prep. HPLC (Column: Inersil 10 μ ODS, 22×250 mm, flow rate: 12 ml/min; eluent: 85% MeOH–H_2O) to give seselin (**9**, 25.8 mg), 5-methoxyseselin (**10**, 3.6 mg), suberosin (**11**, 26.4 mg), xanthyletin (**12**, 76.2 mg) and xanthoxyletin (**13**, 16.5 mg). Fraction 7 upon concentration yielded a precipitate, which was separated and recrystallized from acetone to give elliptinone (**7**, 19.0 mg). The filtrate was repeatedly subjected to Sephadex LH-20 cc using acetone as eluent to give **7** and isoshinanolone (**8**, 205.6 mg). Fraction 5 gave chitranone (**5**, 129.5 mg) and maritinone (**6**, 18.9 mg) after Sephadex LH-20 and prep. HPLC (Column: Inersil 10 μ ODS, 22×250 mm, flow rate: 12 ml/min; eluent: 90% MeOH–H_2O). The *n*-BuOH fraction (16 g) was repeatedly applied to a Sephadex LH-20 column with MeOH/H_2O as eluent to give **1** (65.7 mg), **2** (4.2 mg) and **3** (55.4 mg).

3.4. 3′*O*-β-Glucopyranosyl plumbagic acid (*1*)

Amorphous powder; UV λ_{max} (MeOH) (log ε) 214 (4.19), 259 (3.76) and 333 (3.19) nm; $[\alpha]_D^{27}$ −51° (MeOH; *c* 1.1); IR ν_{max} (KBr), 3465, 1720, 1635, 1456, 1238, 1071 cm^{-1}; ES–IMS *m*/*z* 385 $[M-H]^-$, 223 $[M-162-H]$; HR–FAB–MS *m*/*z* 385.1133 $[M-H]^-$(calc. 385.1131 for $C_{17}H_{21}O_{10}$); ^1H NMR (CD_3OD) δ1.25 (3H, *d*, *J* = 7.0 Hz, Me-3), 2.39 (1H, *dd*, *J* = 16.5, 5.0 Hz, Hα-2), 2.91 (1H, *dd*, *J* = 16.5, 9.0 Hz, Hβ-2), 4.03 (1H, *m*, H-3), 4.91 (1H, *d*, *J* = 8 Hz, H-1″), 6.94 (1H, *t*, *J* = 8 Hz, H-5′), 7.45 (1H, *d*, *J* = 8 Hz, H-4′), 7.66 (1H, *d*, *J* = 8 Hz, H-6′); ^{13}C NMR (CD_3OD)δ18.5 (3-$\underline{C}H_3$), 38.2 (C-2), 39.0 (C-3), 62.5 (C-1″), 71.3 (C-4″), 74.8 (C-2″), 77.7 (C-3″), 78.3 (C-5″), 103.2 (C-1″), 120.0 (C-5″), 121.0 (C-1′), 124.1 (C-4′), 125.1 (C-6′), 147.6 (C-3′), 153.6 (C-2′), 175.8 (C-1), 210.8 (C-4).

3.5. Hydrolysis of *1*

Compound **1** (13 mg) was dissolved in 3% HCl/EtOH (10 ml) and the solution was heated until reflux began, then being maintained for 2 h. The reaction solution was cooled, then concentrated under reduced pressure to give a syrup which was partitioned between EtOAc/H_2O. Plumbagic acid (**3**) was isolated from the EtOAc layer by prep. HPLC (Column: Inersil 10 μ ODS, 22×250 mm, flow rate: 10 ml/min; eluent: 50% MeOH–H_2O), whereas glucose was identified from the H_2O layer by HPLC (Column: Luna 5 μ NH_2, 4.6×250 mm, flow rate: 1 ml/min; eluent: 75% CH_3CN–H_2O; Detector: RI).

3.6. 3′-*O*-β-Glucopyranosyl plumbagic acid methylester (*2*)

UV λ_{max} (MeOH) (log ε) 214 (4.19), 259 (3.76), 333 (3.19) nm; $[\alpha]_D^{27}$ −37° (MeOH; *c* 0.35); IR ν_{max} (KBr), 3390, 1728, 1638, 1456, 1245, 1071 cm^{-1}; APCI–MS *m*/*z* 399 $[M-H]^-$, 237 $[M-162-H]^-$; HR-FAB–MS *m*/*z* 401.1462 $[M+H]^+$ (calc. 401.1476 for $C_{18}H_{25}O_{10}$); ^1H NMR (CD_3OD) δ1.24 (3H, *d*, *J* = 6.5 Hz, Me-3), 2.54 (1H, *dd*, *J* = 17.0, 5.0 Hz, Hα-2), 2.93 (1H, *dd*, *J* = 17.0, 9.5 Hz, Hβ-2), 3.63 (3H, *s*, -OMe), 4.05 (1H, *m*, H-3), 4.92 (2H, *d*, *J* = 7.5 Hz, H-1″), 6.94 (1H, *t*, *J* = 8 Hz, H-5′), 7.46 (1H, *d*, *J* = 8 Hz, H-4′), 7.65 (1H, *d*, *J* = 8 Hz, H-6′); ^{13}C NMR (CD_3OD) δ 18.5 (3-$\underline{C}H_3$), 37.8 (C-2), 39.1 (C-3), 52.2 (–O$\underline{C}H_3$), 62.5 (C-1″), 71.3 (C-4″), 74.9 (C-2″), 77.7 (C-3″), 78.3 (C-5″), 103.2 (C-1″), 120.1 (C-5′), 121.1 (C-1′), 124.1 (C-4′), 125.1 (C-6′), 147.6 (C-3′), 153.5 (C-2′), 174.2 (C-1), 210.4 (C-4).

3.7. Cell lines

The K562, Raji, Jurkat, Vero, Calu-1, HeLa and Wish cell lines were utilized as target cells in the cytotoxic assay. K562, Raji, and Jurkat cells are erythroleukemia, EBV-transformed B, and T leukemia cell lines, respectively (American Type Culture Collection, ATCC, Rockville, MD). They were cultured in RPMI-1640 Medium (Hyclone, Logan, UT) containing 10% fetal calf serum (FCS, Gibco, Grand Island, NY), 100 u/ml penicillin, and 100 μg/ml streptomycin. The Vero cell is a green monkey kidney tumor cell line (ATCC, Rockville, MD). The Wish cell is a transformed epithelial cell line and the Calu-1 cell is a human lung carcinoma cell line (ATCC, Rockville, MD). The HeLa cell is a human cervical carcinoma cell line (ATCC, Rockville, MD). The Vero, Wish, Calu-1, and HeLa cell lines were cultured in MEM medium containing 10% FCS, 100 μg/ml streptomycin, and 100 u/ml penicillin. These cell lines were cultured at 37 °C in an atmosphere of humidified 5% CO_2.

3.8. Growth inhibition assay

Growth inhibition was assessed as described previously (Kuo et al., 1994). Each tumor cell line was cultured with or without various test compounds for 3 days, after which tritiated thymidine was incubated for 16 h before harvest. Radioactivity was determined by a scintillation counter, and inhibitory activity was calculated. The IC_{50} was calculated as inhibition at 50% cell proliferation.

Acknowledgements

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