HETEROCYCLES, Vol. 78, No. 3, 2009, pp. 763 - 770. © The Japan Institute of Heterocyclic Chemistry
Received, 3rd September, 2008, Accepted, 10th November, 2008, Published online, 11th November, 2008
DOI: 10.3987/COM-08-11540

CHEMICAL CONSTITUENTS FROM THE LEAVES OF *XYLOPIA POILANEI* AND THEIR BIOACTIVITY

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Abstract – A new flavonoid glycoside, xylopoillin A (**1**), and nine known compounds were isolated from the methanolic extracts of the leaves of *Xylopia poilanei*. The structure of this new compound was completely elucidated using a combination of 2D NMR techniques (COSY, NOESY, HMQC and HMBC) and HR-ESI-MS analyses. The other chemical structures of known compounds were identified by comparison of their spectroscopic and physical data with those reported in the literature.

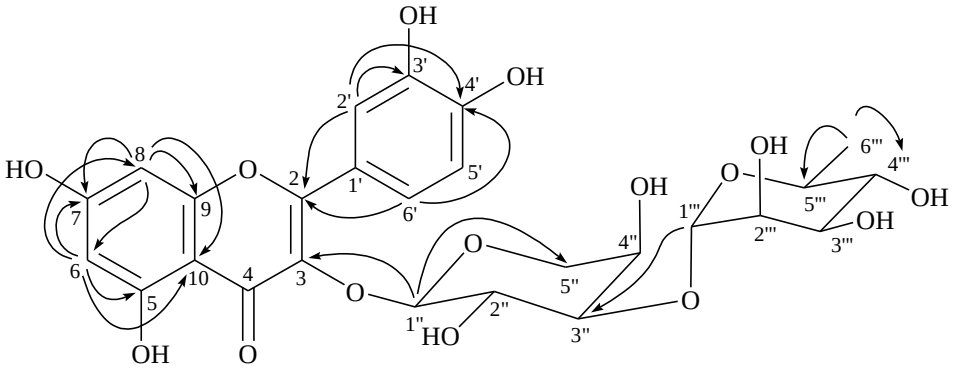
The *Xylopia* genus (Annonaceae) comprises about 160 species with occurrence in South and Central America, Africa and Asia.[1] Approximately four *Xylopia* species were identified in Vietnam.[2] These species produce a wide variety of metabolites including alkaloids,[3,4,5,6,7,8] lignans,[9] acetogenins,[10,11] diterpenoids,[12,13,14,15,16] and sesquiterpenoids.[17,18,19] Termite antifeedant, cardiovascular and diuretic activities were reported for this genus.[20-21] *Xylopia poilanei* is utilized traditionally in the regions of India, Malaya, Philippine islands, and Indo-China for the medicinal uses as febrifuge, anthelmintic, anti-emetic, anti-inflammatory, and in urinary and uterine discharges, piles, and lumbago.[22] In our investigations of phytochemical diversity of this plant, the structure of a new flavonoid, xylopoillin A (**1**), was established by spectroscopic methods

including 1D and 2D NMR and MS techniques. In addition, nine known compounds, including four alkaloids, three flavonoids, and two carbohydrates, were also characterized by comparison of their spectral and physical data with those reported in the literatures. The present study wishes to report the structural assignments of this new compound, and their cytotoxic activity of the isolates.

The air-dried and powdered leaves of *X. poilanei* were extracted with hot methanol and concentrated to give a dark brown syrup. The methanol extract was suspended in H_2O and partitioned with ethyl acetate and *n*-butanol. The successive purification of *n*-butanol soluble extracts by a combination of conventional chromatographic techniques afforded a new compound (**1**). In addition, nine known compounds including of dicentrine (**2**),[23] dicentrinone (**3**),[24] nordicentrine (**4**),[25] oxoanolobine (**5**),[26] rutin (**6**),[27] quercitrin (**7**),[28] naringenin 7-neohesperidoside (**8**),[29] *myo*-inositol (**9**),[30] and sucrose (**10**)[31] were also characterized from this plant. The structure of this new flavonoid glycoside, xylopoillin A (**1**), was established on the basis of 1D and 2D NMR and mass spectroscopic analyses.

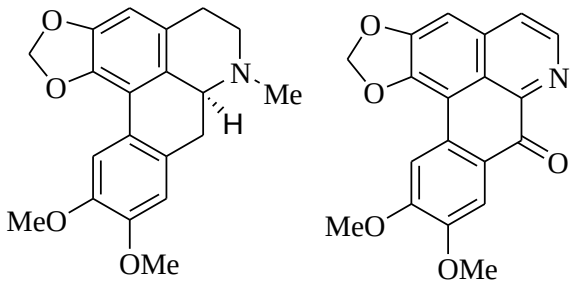
The HR-ESI-MS of the new compound (**1**) displayed a sodium adduct ion peak at *m/z* 603.1328 $[M+Na]^+$, corresponding to the pseudomolecular formula of $C_{26}H_{28}O_{15}Na$ (calcd. 603.1326). The UV absorption maxima at 360, 269, and 257 nm were characteristic of a flavone skeleton.[32] The IR absorption bands at 3405 and 1656 cm^{-1} displayed the presence of a hydroxyl group and a hydrogen-bonded carbonyl group. In the ^1H NMR spectrum, a typical set of ABX signals at δ 7.74 (1H, d, *J* = 2.0 Hz), 7.60 (1H, dd, *J* = 8.5, 2.0 Hz), and 6.90 (1H, d, *J* = 8.5 Hz) were attributed to the trisubstituted B-ring. Two mutually coupled doublet at δ 6.42 (1H, d, *J* = 2.0 Hz) and 6.23 (1H, d, *J* = 2.0 Hz) was assumed to be H-6 and H-8 since they displayed ^2J ,^3J-HMBC correlations with the carbon signals at δ 165.9 (C-7), 158.4 (C-5), 105.7 (C-10), 99.9 (C-8); and δ 165.9 (C-7), 163.0 (C-9), 105.7 (C-10), 94.7 (C-6), respectively. Two anomeric proton signals at δ 5.19 (d, *J* = 6.5 Hz) and 5.13 (d, *J* = 1.5 Hz) suggested the presence of two sugar units. In addition, there are oxygenated methine and methylene protons at δ 3.44 (1H, t, *J* = 9.5 Hz), 3.47 (1H, dd, *J* = 12.5, 1.5 Hz), 3.74 (1H, dd, *J* = 8.5, 3.0 Hz), 3.82 (1H, dd, *J* = 12.5, 4.0 Hz), 3.86 (2H, m), 3.93 (1H, m), 4.05 (1H, dd, *J* = 3.5, 1.5 Hz), and 4.08 (1H, dd, *J* = 8.5, 6.5 Hz), which were identified as the proton signals of the sugar moieties. Moreover, the upfield methyl doublet at δ 1.28 (3H, d, *J* = 6.5 Hz) was the characteristic absorption for the rhamnose fragment. In the ^{13}C NMR and DEPT spectral analyses, the sugar portion of **1** displayed one methyl (δ 18.1), one oxymethylene (δ 67.1), seven oxymethine (δ 68.9, 70.2, 71.8, 72.1, 72.2, 74.1, 80.0), and two anomeric signals (δ 103.0 and 104.7), which were identified as L-arabinose (δ 104.7, 80.0, 71.8, 68.9, and 67.1)[33] and D-rhamnose (δ 103.0, 74.1, 72.2, 72.1, 70.2, and 18.1)[34] by comparison with the literature values and the assistance of comprehensive 2D NMR spectroscopic analyses, including the COSY, NOESY, HMQC, and HMBC experiments. The sugar fraction of acid hydrolysate of compound **1** was further analyzed with HPLC by comparison of their retention times and optical rotations with those of authentic samples as described in the reference.[35] In the

HMBC spectrum, long range correlations from H-1" (δ 5.19) to C-3 (δ 135.8) and H-1"' (δ 5.13) to C-3" (δ 67.1) confirmed the presence of 3-*O*-α-D-rhamnosyl-(1→3)-β-L-arabinose moiety and the configurations of arabinose and rhamnose were determined as β and α, respectively, according to their coupling constants of anomeric protons. In addition, the proton signals at δ 7.74 (H-2') and 7.60 (H-6') displayed ^{3}J-HMBC correlations with the carbon signal at δ 158.6 (C-2), respectively, confirmed the substitution pattern of B-ring to be 1,3,4-trisubstituted and the groups attached at C-3' and -4' were hydroxyl functions with the assistance of molecular formula and ^{13}C NMR spectroscopic analysis. The successive NOESY and HMBC experiments furnished the complete assignments of proton and carbon signals of **1** and thus its chemical structure was established as shown in Figure 1 and named trivially as xylopoillin A.



1

Figure 1. Structure and significant HMBC correlations of compound **1**



2

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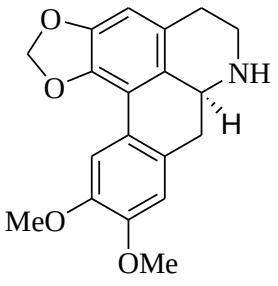

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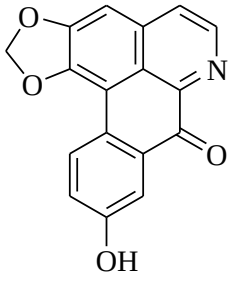

5

Figure 2. Structures of alkaloids **2-5**

Some of the isolated compounds were assayed for cytotoxic activity against tumor cell lines Daoy (human medulloblastoma), Hep-2 (human laryngeal carcinoma), MCF-7 (human breast adenocarcinoma), and Hela (human cervical epitheloid carcinoma), as described previously,[36] and the EC_{50} values are summarized in Table 1. Only compound **4** displayed moderate cytotoxicity with the EC_{50} values of 3.84, 2.57, 2.16, 1.28 μg/mL against the Daoy, Hep-2, MCF-7, and Hela tumor cell lines, respectively.

Tested compounds	ED_{50} (μg/mL)			
	Daoy	Hep2	MCF-7	Hela
1	(-)	(-)	(-)	(-)
2	(-)	(-)	(-)	(-)
3	(-)	(-)	(-)	(-)
4	3.84	2.57	2.16	1.28
5	(-)	(-)	(-)	(-)
6	(-)	(-)	(-)	(-)
Mitomycin *C*	0.13	0.15	0.14	0.15

(-): $ED_{50} > 40$ μg/mL

Table 1. EC_{50} values of tested compounds against human tumor cell line panel

EXPERIMENTAL

General Melting points were determined using an Electrothermal IA-9200 melting points measuring apparatus. The UV spectra were determined on an Agilent UV-VIS recording spectrophotometer. The IR spectra were obtained, as KBr discs, on a Hitachi 270-30 type spectrometer. Optical rotations were determined on a Jasco DIP-1000 KUY polarimeter. The electrospray ionization (ESI) mass spectra were obtained using an AGILENT 1100 LC-MSD Trap spectrometer. The HR-ESI-MS spectra were measured on a Bruker APEX II mass spectrometer. ^{1}H- and ^{13}C-NMR, COSY, NOESY, HMQC, and HMBC spectra were recorded on the Bruker Avance-500 NMR spectrometer, using tetramethylsilane (TMS) as the internal standard. Standard pulse sequences and parameters were used for the NMR experiments and all chemical shifts were reported in parts per million (ppm, δ). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, E. Merck).

Plant Materials The leaves of *Xylopia poilanei* (Annonaceae) was collected from Nghe An, Vietnam, during May 2007 and the plant materials were identified and authenticated by Assoc. Prof. Dr. Vu Xuan Phuong, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology.

A voucher specimen (20070505) was deposited in the herbarium of the Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hanoi, Vietnam.

Extraction and Isolation The leaves of *Xylopia poilanei* (4.4 Kg) were powdered and soaked with MeOH (5 L × 3) at rt, and the combined extracts were concentrated under reduced pressure to give a deep brown syrup (260 g). This was partitioned between H_2O and EtOAc followed by *n*-BuOH. The *n*-BuOH soluble residue (105 g) was chromatographed over a silica gel column, which was developed by gradient elution with $CHCl_3$ and increasing concentrations of MeOH to afford seven fractions. Chromatography of fraction 3 on silica gel column by eluting with mixture of $CHCl_3$-MeOH (19:1) and step gradient with MeOH led to the isolation of pure compound **2** (35 mg), **3** (42 mg), **4** (23 mg), **5** (21 mg). Separation of fraction **4** by silica gel column chromatography with $CHCl_3$-MeOH (9:1) afforded compound **1** (55 mg), **6** (26 mg), **7** (20 mg), and **8** (27 mg), successively. Fraction 6 was subjected to a series of silica gel column chromatographic separation using $CHCl_3$-MeOH-H_2O (9:1:0.05) yielded compounds **9** (43 mg) and **10** (38 mg).

Xylopoillin A (1): yellow powder, mp 265−268 °C; $[\alpha]_D^{30}$ −76.7° (*c* 1.0, MeOH); UV λ_{max} (MeOH) (log ε): 360 (4.20), 270 (4.25, sh), 257 (4.35) nm; IR (KBr) ν_{max}: 3405, 2924, 1656, 1607, 1563, 1500, 1358, 1292, 1211 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) δ : 1.28 (3H, d, *J* = 6.5 Hz, Me-6'''), 3.44 (1H, t, *J* = 9.5 Hz, H-4'''), 3.47 (1H, dd, *J* = 12.5, 1.5 Hz, H-5''), 3.74 (1H, dd, *J* = 8.5, 3.0 Hz, H-3''), 3.82 (1H, dd, *J* = 12.5, 4.0 Hz, H-5''), 3.86 (2H, m, H-3''' & -5'''), 3.93 (1H, m, H-4''), 4.05 (1H, dd, *J* = 3.5, 1.5 Hz, H-2'''), 4.08 (1H, dd, *J* = 8.5, 6.5 Hz, H-2''), 5.13 (1H, d, *J* = 1.5 Hz, H-1'''), 5.19 (1H, d, *J* = 6.5 Hz, H-1''), 6.23 (1H, d, *J* = 2.0 Hz, H-8), 6.42 (1H, d, *J* = 2.0 Hz, H-6), 6.90 (1H, d, *J* = 8.5 Hz, H-5'), 7.60 (1H, dd, *J* = 8.5, 2.0 Hz, H-6'), 7.74 (1H, d, *J* = 2.0 Hz, H-2');□^{13}C-NMR (125 MHz, CD_3OD) δ : 18.1 (C-6'''), 67.1 (C-5''), 68.9 (C-4''), 70.2 (C-5'''), 71.8 (C-2''), 72.1 (C-3'''), 72.2 (C-2'''), 74.1 (C-4'''), 80.0 (C-3''), 94.7 (C-6), 99.9 (C-8), 103.0 (C-1'''), 104.7 (C-1''), 105.7 (C-10), 116.2 (C-5'), 117.4 (C-2'), 122.9 (C-1'), 123.2 (C-6'), 135.8 (C-3), 146.0 (C-3'), 149.9 (C-4'), 158.4 (C-5), 158.6 (C-2), 163.0 (C-9), 165.9 (C-7), 179.4 (C-4); ESI-MS *m/z*: 581 ($[M+H]^+$, 5). HR-ESI-MS *m/z*: 603.1328 $[M+Na]^+$ (calcd for $C_{26}H_{28}O_{15}Na$, 603.1326).

Acid hydrolysis of Compound 1 Compound **1** (12.0 mg) was refluxed at 100 ºC for 1 h with 2*N* HCl (10 mL). The acid hydrolysate was extracted with EtOAc and evaporated to dryness to yield a colorless amorphous solid which on recrystallization from MeOH afforded 2.0 mg of quercetin,[37] identified by mp, UV, IR, ^{1}H-, ^{13}C-NMR and mass spectral analysis, while the sugars in the aqueous layer were identified as L-arabinose and D-rhamnose, respectively, with HPLC analysis by comparison of their retention times and optical rotations with those of authentic samples as described in the reference.[35]

In Vitro Cytotoxicity Assay. All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500-7500 cells per well with

compounds added from DMSO-diluted stock. After 3 days in culture, attached cells 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 mean EC_{50} is the concentration of agent that reduces cell growth by 50 % under the experimental conditions and is the average from at least three independent determinations that were reproducible and statistically significant. Four human cancer cell lines, Daoy, Hep2, MCF-7, and Hela were used in the assay. Mitomycin *C* (5 μM, final concentration) and DMSO (0.3 %, final concentration) were used as positive and vehicle controls. Results were expressed as percent of DMSO control.

ACKNOWLEDGEMENTS

The authors would like to thank Assoc. Prof. Dr. Vu Xuan Phuong, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, for the plant identification. Authors also want to thank the National Science Council, Taiwan (NSC 97-2323-B-006-002) for partial financial support of this research. We are also grateful to the National Center for High-performance Computing, Taiwan, for computer time and facilities.

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