Five New Flavone 5-*O*-Glycosides from *Lethedon tannaensis*: Lethedosides and Lethediosides

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Received June 26, 1998

Five new 7-methoxy-flavone 5-*O*-glycosides were isolated from a cytotoxic MeOH extract of *Lethedon tannaensis*, and the structures were elucidated by 2D NMR spectral analysis and by chemical methods. Lethedosides A (**1**), B (**2**), and C (**3**) were 5-*O*-glucosides of 7,3′,4′-tri-*O*-methylluteolin, 7,3′,4′,5′-tetra-*O*-methyltricetin, and 7,3′,4′-tri-*O*-methytricetin, respectively; lethediosides A (**4**) and B (**5**) and a known compound **6** were 5-*O*-xylosylglucosides of 7,3′,4′-tri-*O*-methylluteolin, 7,3′,4′,5′-tetra-*O*-methyltricetin, and 7,4′-di-*O*-methylapigenin, respectively. These flavonoids were either inactive or weakly active against KB tumor cells, in contrast to previously isolated flavones from the same plant.

Lethedon tannaensis (Thymelaeaceae) is a tree growing in New Caledonia, where it is used as an antibacterial in folk medicine. In our search for antitumor agents from plants, we found that a MeOH extract of the leaves of *L. tannaensis* displayed cytotoxic activity against murine leukemia (P-388) cells, and inhibited DNA topoisomerase I.[1,2]

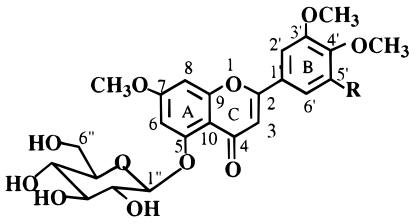
We previously isolated, from EtOAc and MeOH extracts of the leaves, seven 5-hydroxy-7-methoxyflavones substituted at various positions of the B-ring. These flavones showed cytotoxic activity against human nasopharynx carcinoma KB cells (IC_{50} 4.8−47.6 μM).[1,3]

Five new 5-*O*-glycosylflavones, named lethedosides A (**1**), B (**2**), and C (**3**), and lethediosides A (**4**) and B (**5**), besides the known 7,4′-dimethylapigenin 5-*O*-xylosylglucoside (**6**), were further isolated from the MeOH extract. We report herein the structure determination of these flavonoids.

Results and Discussion

Bioassay-directed fractionation by reversed-phase chromatography of the MeOH extract of the leaves of *L. tannaensis* provided two groups of active fractions. The less polar active fractions and the EtOAc extract yielded seven flavones.[1] The polar fractions of the MeOH extract furnished, by chromatography on Sephadex LH20 and on Si gel, followed by TLC purification monitored by C8 HPLC analysis, six flavonoids: lethedosides A (**1**), B (**2**), and C (**3**); lethediosides A (**4**) and B (**5**); and the known flavonoid **6**.[4]

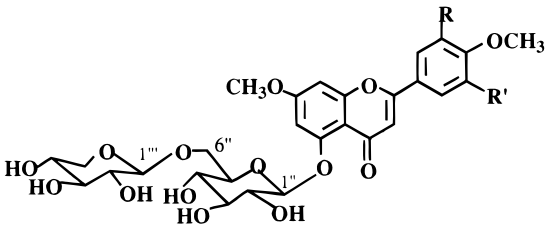
Lethedoside A (**1**) was obtained as an amorphous solid. The HRFABMS showed the protonated molecular ion $[MH]^+$ at *m*/*z* 491.1565 corresponding to the molecular formula $C_{24}H_{26}O_{11}$. UV, IR, and NMR data (Tables 1 and 2) suggested a highly conjugated flavone ring system.[5] The ^{1}H NMR spectrum displayed signals for three methoxy groups at δ 3.91, 3.94, and 3.95; six aromatic protons: one singlet at δ 6.68; two doublets at δ 6.95 and 6.97 characteristic of *meta*-protons on a tetrasubstituted benzene ring; and three protons at δ 7.11, 7.63, and 7.52 in an ABX system. The 18 carbon signals of the ^{13}C NMR spectrum



1 : R=H

2 : R=OCH$_3$

3 : R=OH



4 : R=OCH$_3$, R'=H

5: R=R'=OCH$_3$

6 : R=R'=H

were assigned, by analysis of the ^{1}H−^{13}C COSY and HMBC spectra, to those of 5-hydroxy-7,3′,4′-trimethoxyflavone.

The signals of five oxymethine protons in *trans* diaxial conformations (J = 7.2−8.0 Hz) and one oxymethylene group indicated the presence of a β-glucopyranosyl group. The glucosyl residue was located at the 5-*O*-position of the flavone skeleton according to long-range HMBC correlations between C-5 at δ 159.7 and the anomeric H-1″ at δ 4.81, as well as H-6 at δ 6.95. Subsequently, lethedoside A (**1**) was hydrolyzed by β-D-glucosidase, furnishing a flavone and D-glucose. Thus, lethedoside A (**1**) was confirmed to be 5-*O*-β-D-glucopyranosyl-7,3′,4′-tri-*O*-methylluteolin.

Lethedoside B (**2**) was an amorphous solid, $[\alpha]^{20}_D$ −33.4° (pyridine). The HRFABMS exhibited the protonated molecular ion $[MH]^+$ at *m*/*z* 521.1694, allowing us to deduce the molecular formula $C_{25}H_{28}O_{12}$, indicating that compound

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10.1021/np980284p CCC: $18.00
Published on Web 01/21/1999

Table 1. ^{1}H NMR Data for Flavonoids **1**−**6** (δ ppm; *J* Hz)[a]

carbon	1	2	3	4	5	6
3	6.68 s	6.83 s	6.49 s	6.98 s	6.95 s	6.76 s
6	6.95 d, 2.4	6.83 d, 2.2	6.82 d, 2.3	7.42 d, 2.4	7.03 d, 2.2	6.86 d (2.2)
8	6.97 d, 2.4	7.02 d, 2.2	6.76 d, 2.3	6.96 d, 2.4	7.01 d, 2.2	7.03 d (2.2)
2′	7.52 d, 2.1	7.24 s	6.93 d, 2.1	7.56 d, 2.1	7.29 s	8.01 m
5′	7.11 d, 8.6			7.10 d, 8.6		7.09 m
6′	7.63 dd, 8.6, 2.1	7.24 s	7.00 d, 2.1	7.65 dd, 8.6, 2.1	7.29 s	8.01 m
OMe-7	3.95 s	3.86 s	3.84 s	3.84 s	3.82 s	3.89 s
OMe-3′	3.94 s	3.86 s	3.88 s	3.87 s	3.90 s	
OMe-4′	3.91 s	3.72 s	3.89 s	3.84 s	3.92 s	3.84 s
OMe-5′		3.86 s			3.90 s	
1″	4.81 d, 7.2	4.78 d, 7.3	4.80 d, 7.3	5.42 d, 7.3	4.80 d, 7.5	4.80 d, 7.0
2″	3.57 dd, 8.0, 7.2	3.38 dd, 7.3, 8.0	3.54 dd, 9.0; 7.3	4.39 dd, 7.3; 9.0	3. 57 dd, 9.0; 7.5	2.97 dd, 9.0; 7.0
3″	3.52 t, 8.0	3.32 t, 8.0	3.56 t, 9.0	4.30 t, 9.0	3.49 t, 9.0	3.11 t, 9.0
4″	3.41 t, 8.0	3.18 t, 8.0	3.42 t, 9.0	4.22 t, 9.0	3.41 t, 9.0	3.27 t, 9.0
5″	3.47 m	3.38 m	3.47 m	4.35 m	3.71 m	3.30 m
6″	3.73 dd, 12.0; 6.0	3.50 dd, 11.5; 6.0	3.72 dd, 12.0; 5.0	4.31 m	3.87 dd, 11.0; 5.0	3.65 dd, 11.0; 5.6
	3.94 dd, 12.0; 2.0	3.72 dd, 11.5; 2.0	3.93 dd, 12.0; 2.0	4.81 m	4.13 dd, 11.0; 2.0	3.97 dd, 11.0; 2.0
1‴				4.95 d, 7.8	4.32 d, 7.5	4.19 d, 7.8
2‴				4.02 dd, 8.5; 7.8	3.22 dd, 9.0; 7.5	3.37 dd,9.0; 7.8
3‴				4.13 t,8.5	3.31 t, 9.0	3.56 t,9.0
4‴				4.21 m	3.52 m	3.23 m
5‴				3.65 dd,11.2; 10.0	3.18 dd, 11.2; 10.0	3.02 m
				4.33 m	3.84 dd, 11.0; 6.5	3.68 m

[a] Compounds **1** and **2** (CD_3OD−20% DMSO-d_6); **3** and **5** (CD_3OD); **4** (Py-d_5); and **6** (DMSO-d_6).

Table 2. ^{13}C NMR Data of Flavonoids **1**−**6**[a]

carbon	1	2	3	4	5	6
2	163.3	161.3	163.2	161.9	163.1	160.9
3	107.7	108.1	108.0	107.7	108.5	106.5
4	179.5	177.8	179.8	177.0	179.8	176.9
5	159.7	158.5	159.5	159.3	159.4	158.1
6	104.4	103.4	104.3	103.8	104.5	102.9
7	165.7	164.3	165.7	164.4	166.0	163.6
8	97.7	96.9	97.4	96.9	97.3	96.6
9	160.3	159.1	160.0	159.3	160.4	158.4
10	110.5	109.5	110.2	110.4	110.3	109.2
1′	124.3	126.4	126.9	123.8	127.4	122.7
2′	110.3	104.2	102.4	109.7	104.9	128.0
3′	153.7	153.7	154.7	150.0	154.8	114.5
4′	150.7	141.1	141.1	152.8	141.5	162.1
5′	112.7	153.7	152.4	112.0	154.8	114.5
6′	121.2	104.2	108.8	120.3	104.9	128.0
OCH_3-7	56.9	56.4	56.6	55.9	56.9	56.1
OCH_3-3′	56.7	56.6	56.6	56.0	56.9	
OCH_3-4′	56.6	60.6	61.6	55.8	61.2	55.5
OCH_3-5′		56.6			56.9	
1″	105.3	104.0	105.0	105.4	104.7	103.7
2″	74.8	73.8	74.7	74.8	74.7	73.4
3″	77.2	76.0	77.2	77.5	77.2	76.6
4″	71.4	70.3	71.4	71.1	71.5	69.5
5″	78.8	77.9	78.6	77.4	77.4	75.7
6″	62.5	61.3	62.6	70.0	70.4	68.7
1‴				105.8	105.6	104.1
2‴				74.8	74.2	73.4
3‴				78.0	77.7	76.0
4‴				70.9	71.1	69.8
5‴				66.9	66.8	65.6

[a] Compounds **1** and **2** (CD_3OD−20% DMSO-d_6); **3** and **5** (CD_3OD); **4** (Py-d_5); **6** (DMSO-d_6).

2 had one additional methoxy group with respect to lethedoside A (**1**). The ^{1}H NMR spectrum (Table 1) displayed, for the B-ring of flavonoid **2**, the resonances of three methoxy groups at δ 3.86, 3.72, and 3.86, and two aromatic protons as one singlet at δ 7.24. Hence, the flavone moiety was tetra-*O*-methyltricetin. The 5-*O*-position of the glucosyl was also ascertained by HMBC correlations. Consequently, lethedoside B (**2**) was identified as 5-*O*-β-D-glucopyranosyl-7,3′,4′,5′-tetra-*O*-methyltricetin, and this result was further substantiated by β-D-glucosidase hydrolysis.

Lethedoside C (**3**), amorphous, possessed molecular formula $C_{24}H_{26}O_{12}$, obtained from protonated molecular ion

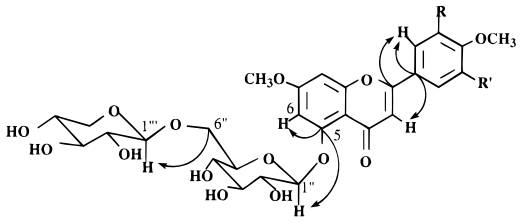

Figure 1. Selected HMBC correlations of lethediosides A (**4**: R=OCH_3, R′=H) and B (**5**: R=R′=OCH_3).

$[MH]^+$ at *m/z* 529.1339 by HRFABMS (one more oxygen atom with regard to flavonoid **1**). Two *meta* proton resonances at δ 6.93 and 7.00 were assigned to H2′ and H6′ on the flavone B-ring, while 3′- and 4′-methoxy groups were evident from the signals at δ 3.88 and 3.89. The flavone part of **3**, thus, was lethedocin.[1] HMBC experiments and β-D-glucosidase hydrolysis allowed to establish the structure of lethedoside C (**3**) as 5-*O*-β-D-glucopyranosyl-7,3′,4′-tri-*O*-methyltricetin.

The ^{1}H and ^{13}C NMR spectra of lethediosides A (**4**) and B (**5**) and of the compound **6** displayed the signals of two sugar residues in δ_H 3−5 and δ_C 65−105 regions, in addition to flavone resonances. The nature of hexose and pentose, revealed by ^{1}H−^{1}H and ^{1}H−^{13}C COSY, were glucose and xylose, respectively, in view of the *trans* diaxial ^{1}H−^{1}H coupling constants (7−10 Hz). HMBC experiments provided evidence for a connection of the 1‴-β-xylosyl residue to C″-6 of glucose, while 1″-β-glucosyl group was bonded to C-5 of the flavone as shown by cross-peaks between H-1‴ and C-6″; H-1″ and C-5, respectively (Figure 1). The lethediosides could not be hydrolyzed by β-D-glucosidase but gave rise to glucose, xylose, and the corresponding flavones by acid hydrolysis. Lethediosides A (**4**) and B (**5**) were identified as the 5-*O*-1-β-xylopyranosyl (1→6)-β-glucopyranoside of 7,3′,4′-tri-*O*-methylluteolin and 7,3′,4′,5′-tetra-*O*-methylytricetine, respectively, while the previously isolated compound **6** proved to be the 5-*O*-xylosylglucoside of 7,4′-di-*O*-methylapigenin.[4]

The *in vitro* IC_{50} values of flavonoids **1**−**6** against human nasopharynx carcinoma (KB) cells were >100, 37, 43, >100, 32, and >100 μg/mL, respectively. The compounds are much less cytotoxic than velutin (= 5,4′-dihydroxy-7,3′-dimethoxylflavone), which displayed an IC_{50} value of 1.5

μg/mL (4.8 μM).[1] The loss of cytotoxicity of the flavone 5-*O*-glycosides corroborates the fact that both the cytotoxicity and the inhibition of topoisomerase I of *Lethedon* flavones require the presence of a 5-hydroxy group as well as 4′-hydroxy and 7- and 3′-methoxy groups.[1]

Experimental Section

General Experimental Procedures. NMR spectra were recorded at 300 MHz (^{1}H) and 75 MHz (^{13}C) on a Bruker AC 300 spectrometer. CIMS were obtained from a Nermag R 10−10 mass spectrometer and HRFABMS from a V.G. Analytical ZAB−HF mass spectrometer. TLC was carried out on Si gel 60 F_{254} (Merck) with detection by UV (254 nm) and vanillin−H_2SO_4 spray.

Plant Material. The leaves of *L. tannaensis* Forst. were collected in New Caledonia in 1992. A voucher specimen (COPI 842) was deposited at the Herbarium of the Forest Montravel Park of Nouméa.

Extraction and Isolation. The air-dried and pulverized leaves of *L. tannaensis* (460 g) were extracted at room temperature with EtOAc and then MeOH, and the extracts were concentrated to dryness under reduced pressure, yielding residues (56 and 45 g, respectively).

The MeOH extract was fractionated by reversed-phase chromatography (RP 2) eluted with a H_2O−MeOH gradient (100/0, 90/10, 80/20, 60/40, 20/80, and 0/100) and affording 29 fractions. Fractions 17−23 and 24−29 were cytotoxic for P-388 and KB cells. The polar fractions 17−22 (1.4 g) were separated on Sephadex LH20 eluted with MeOH into eight fractions. Fractions 3−6 were further purified by preparative TLC (CH_2Cl_2−MeOH 85:15) to furnish the pure flavonoids: **1** (31 mg), **2** (38 mg), **3** (24 mg), **4** (15 mg), **5** (22 mg), and **6** (81 mg).

HPLC Analysis. The purity of the respective fractions was monitored by HPLC performed on a reversed-phase C8 Kromasil column (Φ: 7.8 mm, L: 30 cm) eluted by MeOH−H_2O (46:54), with 2 mL/min of flow rate and detection at 220 nm. The retention times were 58, 47, 30, 32, 37, and 75 min for compounds **1**−**6**, respectively.

Lethedoside A (1): amorphous solid; $[\alpha]^{20}_D$ −51° (pyridine, *c* 0.35); UV (MeOH) λ_{max} (log ϵ) 334 (4.4), 260 (4.3), 239 (4.5), 207 (4.7) nm; IR (KBr) ν_{max} 3450, 1646, 1627, 1602 cm^{-1}; CIMS in NH_3, *m*/*z* 328 (100); HRFABMS *m*/*z* 491.1565 $[MH]^+$ (calcd for $C_{24}H_{27}O_{11}$, 491.1553).

Lethedoside B (2): amorphous solid, $[\alpha]^{20}_D$ −33.4° (pyridine, *c* 0.32); UV (MeOH) λ_{max} (log ϵ) 323 (4.2), 265 (4.1), 237 (4.4), 207 (4.6) nm; IR (KBr) ν_{max} 3430, 1636 cm^{-1}; CIMS in NH_3, *m*/*z* 358 (100); HRFABMS *m*/*z* 521.1694 $[MH]^+$ (calcd for $C_{25}H_{29}O_{12}$, 521.1659).

Lethedoside C (3): amorphous solid, $[\alpha]^{20}_D$ −61.6° (pyridine, *c* 0.36); UV (MeOH) λ_{max} (log ϵ) 330 (4.3), 263 (4.1), 239 (4.4), 207 (4.7) nm; IR (KBr) ν_{max} 3430, 1650, 1636 cm^{-1}; CIMS in NH_3, *m*/*z* 344 (100); HRFABMS *m*/*z* 529.1339 $[MH]^+$ (calcd for $C_{24}H_{27}O_{12}$, 521.1322).

Lethedioside A (4): amorphous solid, $[\alpha]^{20}_D$ −50.6° (pyridine, *c* 0.31); UV (MeOH) λ_{max} (log ϵ) 335 (4.3), 263 (4.1), 237 (4.4), 207 (4.6) nm; IR (KBr) ν_{max} 3430, 1636 cm^{-1}; CIMS in NH_3, *m*/*z* 358 (100); HRFABMS *m*/*z* 623.1991 $[MH]^+$ (calcd for $C_{29}H_{35}O_{15}$, 623.1976).

Lethedoside B (5): amorphous solid, $[\alpha]^{20}_D$ −51.5° (pyridine, *c* 0.34); UV (MeOH) λ_{max} (log ϵ) 325 (4.5), 262 (4.4), 239 (4.5), 213 (4.8) nm; IR (KBr) ν_{max} 3437, 1646 cm^{-1}; CIMS in NH_3, *m*/*z* 358 (100); HRFABMS *m*/*z* 675.1917 $[MH]^+$ (calcd for $C_{30}H_{37}O_{16}$, 675.1901).

***β*-D-Glucosidase Hydrolysis.** The flavonoid glycoside (1 mg) was added to a solution of *β*-D-glucosidase (1 mg) in acetate buffer (1 mL) pH 5, and incubated 15 h at 37 °C. The reaction mixture was analyzed with D-glucose and corresponding flavone by Si gel TLC (CH_2Cl_2−MeOH: 75/25).

Acid Hydrolysis. The flavonoid glycoside (1 mg) was kept at 90 °C, 24 h, in 1N HCl (1 mL). After neutralization by addition of $NaHCO_3$, the reaction mixture was subjected to co-TLC with D-glucose, xylose, and the flavone.

***In Vitro* Cytotoxicity Assays.** Cytotoxicity assays were performed using a modification of the published method.[6] Human nasopharynx carcinoma KB cells were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, sodium bicarbonate (3.7 g/L), 2 mM l-glutamine, 100 units/mL penicillin G, 100 μg/mL streptomycin, and 10 μg/mL gentamycin. The assays were carried out in 96-well microtiter plates in triplicate against KB cell lines (3×10^3 cells/mL). After 72 h of incubation at 37 °C in air−CO_2 (95/5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm on a Titertek Multiskan photometer. The IC_{50} value was defined as the concentration of sample necessary to inhibit cell growth to 50% of the control.

Acknowledgment. We are grateful to Dr. J. P. Brouard (Muséum) for the mass spectra and "Service Central d'Analyze du CNRS" (Lyon) for HRFABMS.

References and Notes

(1) Zahir, A.; Jossang, A.; Bodo, B.; Provost, J.; Cosson, J.-P.; Sévenet, T. *J. Nat. Prod.* **1996**, *59*, 701−703.
(2) Riou, J.-F.; Fossé, P.; Nguyen, C. H.; Kragh Larsen, A.; Bissery, M.-C.; Grondard, L.; Saucier, J.-M.; Bisagni, E.; Lavelle, F. *Cancer Res.* **1993**, *53*, 5987−5993.
(3) Bodo, B.; Zahir, A.; Jossang, A. 20th IUPAC Symposium on the Chemistry of Natural Products. Sept 15−20 **1996**, Chicago, IL.
(4) Nunez-Alancon, J.; Rodriguez, E.; Schmid, R. D.; Mabry, T. J. *Phytochemistry* **1973**, *12*, 1451−1454.
(5) Markham, K. R.; Mabry T. J. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds.; Chapman and Hall: London, 1975, pp 45−77.
(6) Mosmann, T. *J. Immunol. Methods*, **1983**, *65*, 55−63.

NP980284P