Novel Flavonoids of *Thelypteris torresiana*

An-Shen Lin,[a] Fang-Rong Chang,[a] Hsin-Fu Yen,[b] HannaMaria Björkeborn,[c] Per Norlén,[c] and Yang-Chang Wu*,[a]

[a] *Graduate Institute of Natural Products, Kaohsiung Medical University; Kaohsiung 807, Taiwan:* [b] *National Museum of Natural Science; Taichung 404, Taiwan: and* [c] *Unit of Clinical Pharmacology, Department of Laboratory Medicine, Lund University; S-22185 Lund, Sweden.* Received August 29, 2006; accepted December 13, 2006

In our continuing research on cytotoxic components from the Formosan pteridophyte *Thelypteris torresiana* (Gaud.) Alstonone, two new compounds, a novel flavonoid, flavotorresin (1), and a flavonoid diglycoside, multiflorin C (2), along with five known compounds, were isolated. The structural elucidation was established on the basis of spectroscopic data analysis. The possible biosynthetic pathway of the flavonoids from this fern is summarized.

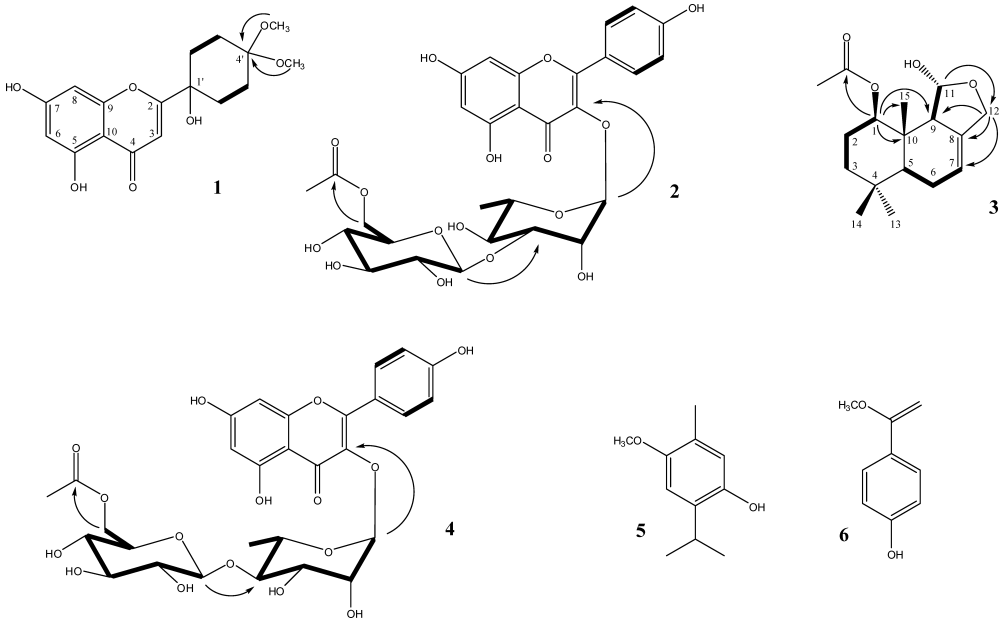
Key words *Thelypteris torresiana*; flavonoid; flavotorresin; multiflorin C

Ferns are widely distributed in humid and warm places. There are 677 species (37 families, 159 genera) found in Taiwan and 50 of them are endemic.[1)] Many species have been used as foods or folk medicine.[2)] In our previous study,[2)] several cytotoxic flavonoids and flavonoid glycosides were found from the species *Thelypteris torresiana* (Gaud.) Alston. In the current investigation, two new compounds, including a novel flavonoid, flavotorresin (**1**), and a flavonoid diglycoside, multiflorin C (**2**), along with five known compounds, a drimane sesquiterpene, thelypterene (**3**), multiflorin A (**4**), two phenols (**5**, **6**), and one steroid (**7**), were isolated. All of the compounds were isolated from Thelypteridaceae for the first time. A possible biosynthetic pathway of the isolated flavonoids is proposed.

Results and Discussion

The wild species of *T. torresiana*, collected in Nantou County, Taiwan, in August 2003, were extracted with MeOH. The combined extracts were chromatographed on Celite 545, silica gel, and Sephadex LH-20 to give compounds **1**—**7**.

Compound **1** was obtained as a pale yellow amorphous solid. HR-ESI-MS yielded a pseudomolecular ion peak at *m/z* 359.1106 $[M+Na]^+$, which was consistent with the molecular formula $C_{17}H_{20}O_7$. The UV and IR spectra showed a typical chromen-4-one core.[3)] In the ^{1}H-NMR spectrum, a chelated hydroxy proton signal at δ 13.57 and two meta-coupled aromatic proton signals at δ 6.59 and 6.72 (each 1H, d, J=2.2 Hz) indicated a 5,7-dihydroxychromenone moiety. Symmetrical proton signals at δ 1.9—2.4 (8H) (Fig. 1) corresponding to carbon signals at δ 32.5 (2C) and 28.0 (2C) showed the typical shape for a chair-formed cyclohexane ring, thus indicating that the ring B is in a chair conformation with a 1,1,4,4-tetra-substitution.[3)] Two highfield-shifted methoxyl signals at δ 3.22 and 3.19 (each 3H, s) suggested geminal attachments to the same carbon C-4′ based on HMBC correlations. Furthermore, in the HMBC spectrum, a proton signal at δ 3.64 (D_2O shifted) was correlated with an oxygenated carbon signal at δ 70.8, which was assigned to C-1′. The molecule consists of fragment ions at *m/z* $[M-184+H]^+$ and $[M-152-OH]^+$ observed in the EI-MS



Main HMBC (→) and Selected ^{1}H–^{1}H COSY (——) Correlations for **1**—**6.**

* To whom correspondence should be addressed. e-mail: yachwu@kmu.edu.tw

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resulting from a retro-Diels-Alder (RDA) cleavage of ring C in good agreement with the presence of a 5,7-dihydroxychromenone and a 1′-hydroxy-4′,4′-dimethoxycyclohexane moiety.[4] Therefore compound **1** was assigned to be 5,7-dihydroxy-2-(1-hydroxy-4,4-dimethoxycyclohexyl)-chromen-4-one and named flavotorresin.

Compound **2** was isolated as a yellow amorphous solid. HR-ESI-MS yielded a pseudomolecular ion peak at *m/z* 637.1773 $[M+H]^+$, which was consistent with the molecular formula $C_{29}H_{32}O_{16}$. The UV and IR spectra indicated a typical flavonoid skeleton.[3] Compounds **2** and **4** were purified by using reverse-phase HPLC (C-18, 250 mm×4.6 mm) and had long retention times (t_R=48.0, 61.5 min, respectively) with tailing peaks. In the ^{1}H-NMR spectrum of **2**, two anomeric proton signals at δ 6.05 and 5.30, 10 oxygenated protons at δ 3.97—4.56, and a methyl at δ 1.49, along with the analysis of their coupling constants, indicated the existence of glucosyl and rhamnosyl moieties. Comparably, one broad proton signal at δ 13.18 (1H, br s) and two meta-coupled proton signals at δ 6.71 and 6.73 (each 1H, d, J=2.0 Hz), together with an AA′XX′ system at δ 7.31 and 8.48 (each 2H, d, J=8.8 Hz), indicated that the aglycone was kaempferol.[5] In the HMBC spectra, the correlation from the proton signal at δ 5.30 (1H, d) was to the carbon signal at δ 69.7. By comparing their 1D and 2D NMR data, **2** and **4** were found to be almost identical. Compound **4** showed a typical 1,4-linkage between two glycosyl moieties, while the HMBC data demonstrated that **2** had a 3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside substitution. The acetyl groups in both compounds were assigned to C-6‴ based on the HMBC spectrum. By comparing the spectral data and the previous reports in the literature,[6,7] the differences between **2** and **4** were the 1,3- and 1,4-glycosyl linkages, respectively. Compound **2** was assigned to be kaempferol 3-*O*-(6‴-*O*-acetyl-3″-β-D-glucopyranosyl)-α-L-rhamnopyranoside and named multiflorin C.

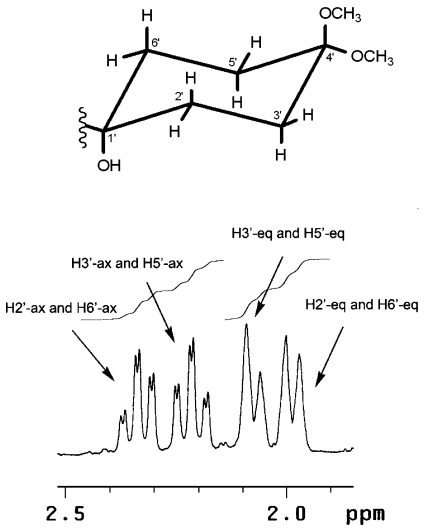

Fig. 1. The Chair-Formed Symmetrical Cyclohexane Ring Signals of Compound **1**

Using the comparison of their 1D and 2D NMR spectroscopic and physical data with those from previous reports in the literature, compounds **3**—**7** were assigned to be 1β-acetoxy-11,12-epoxy-7-drimen-11-ol,[8,9] multiflorin A,[6,7] 2-isopropyl-4-methoxy-5-methyl-phenol,[10] methylparaben,[11] and β-sitosterol-3-*O*-β-D-glucopyranoside,[12] respectively.

A biogenesis-like transformation to form 1,4-dioxygenated cyclohexane-related compounds has already been established

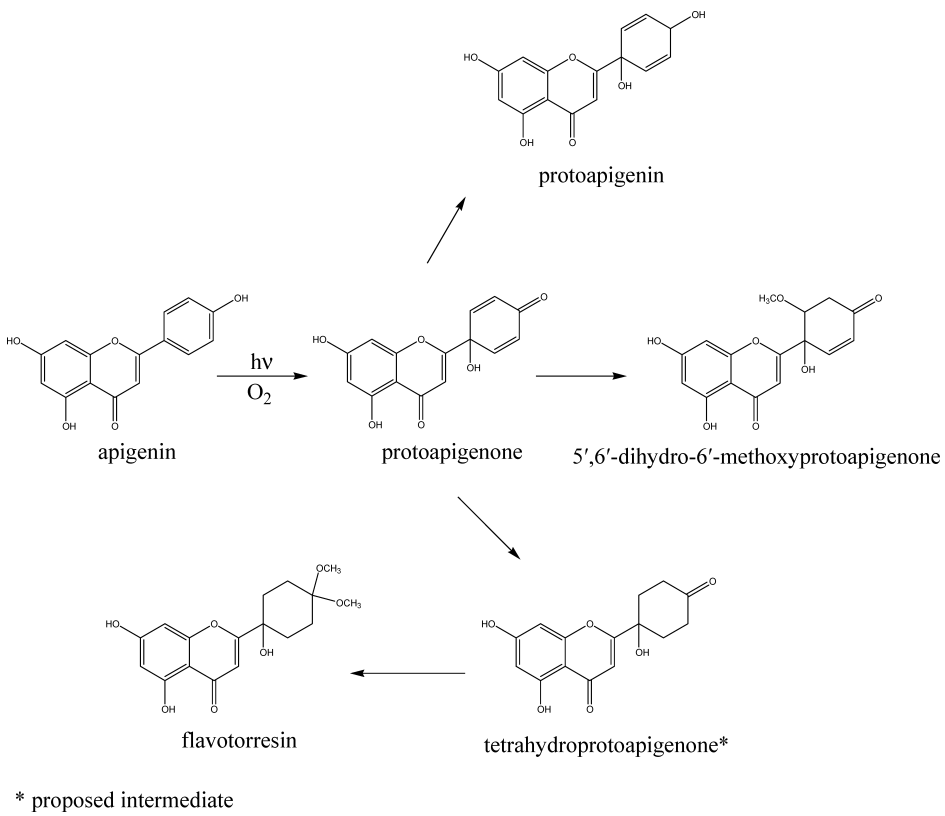

Chart 1. Possible Biosynthesis Pathway of Flavonoids from *T. torresia*

by Hauteville *et al.*[13] and Endo *et al.*[14] On the basis of their reports, a possible biosynthetic pathway for compounds isolated from *T. torresiana*[2] has been proposed (Chart 1). The cytotoxic compound protoapigenone could be obtained chemically from apigenin with the help of hν/O_2, but might also be obtained through biological oxidation procedures.[13,14] Accordingly, protoapigenone is theoretically the precursor of the other isolated compounds flavotorresin, protoapigenin, and 5′,6′-dihydro-6′-methoxyprotoapigenone. Flavotorresin, however, could be an artifact if tetrahydroprotoapigenone reacted with MeOH during the separation and purification.

Experimental

General Procedures Optical rotations were measured with a Jasco P-1020 polarimeter. UV spectra were obtained on a Hitachi 200-20 spectrophotometer. IR spectra were measured on a Mattson Genesis II spectrophotometer. ^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H COSY, HMBC, HMQC, and NOESY spectra were obtained on a Varian NMR (Unity Plus 400). Low-resolution EIMS were recorded on a Bruker APEX II mass spectrometer or a Quattro GC/MS spectrometer equipped with a direct inlet system. High-resolution ESI-MS was collected on a Bruker APEX II mass spectrometer or Quattro GC/MS spectrometer. Silica gel 60 (Merck, 230—400 mesh) and Celite 545 (Merck, 0.02—0.1 nm) were used for column chromatography. Shimadzu LC-10AT pumps, a SPD-10A UV–vis detector, Hypersil ODS 5 μm (250×4.6 mm i.d.), and preparative ODS 5 μm (250×21.2 mm i.d.) columns were employed for the HPLC analysis. The TLC spots were detected by spraying with 50% H_2SO_4 and then heating on a hotplate.

Plant Material Plant materials of *T. torresiana* were collected from Nantou County, Taiwan, in August 2003 and identified by botanist Dr. Hsin-Fu Yen. A voucher specimen (YCWF-001) was deposited at the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation Fraction T3 (25.1 g) from the MeOH extract of *T. torresiana*[2] was separated by column chromatography on a silica gel column (1.2 kg) with gradient systems of *n*-hexane/EtOAc (3 : 1, 2 : 1, 1 : 1, 1.2 l each) and EtOAc (1.5 l) to give 30 subfractions (T3.1—30). Fraction T3.15 (30 mg, *n*-hexane/EtOAc 2 : 1, 500—700 ml) was further separated by using preparative reverse-phase HPLC (MeOH/H_2O 50 : 50, flow rate=3.5 ml/min) to yield **1** (2 mg, t_R=35.5 min). Fraction T3.28 (1.65 g, EtOAc 300—500 ml) was separated on a Sephadex LH-20 gel column (100 g) with MeOH (0.5 ml/min) to yield nine fractions (T3.28.1—9). Fraction T3.28.9 was further separated using preparative reverse-phase HPLC (MeOH/H_2O 50 : 50, flow rate=3.5 ml/min) to give **2** (4 mg, t_R=48.0 min) and **4** (7 mg, t_R=61.5 min). Fraction T3.10 (115 mg, *n*-hexane/EtOAc 3 : 1, 1000—1200 ml) was chromatographed over a silica gel column (5.8 g) using $CHCl_3$ alone (1500 ml) to yield **3** (15.0 mg, 550—650 ml).

Flavotorresin (**1**): Pale yellow amorphous solid; $[\alpha]_D^{22}$ −3.5° (c=0.20, MeOH); UV (MeOH) λ_{max} (log ε): 228 (3.28), 248 (3.21), 256 (3.17), 294 (2.89), 318 (2.76) nm; IR (neat) ν_{max} 3382, 2955, 2923, 2853, 1654, 1620, 1259 cm^{-1}; ^{1}H- and ^{13}C-NMR: Table 1. EI-MS *m*/*z*: 167, 153, 152, 136, 105; ESI-MS *m*/*z*: 359 $[M+Na]^+$; HR-ESI-MS *m*/*z*: 359.1106 (Calcd for $C_{17}H_{20}O_7Na$, 359.1107).

Multiflorin C (**2**): Yellow amorphous solid; $[\alpha]_D^{22}$ −1.4° (c=0.50, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.32), 262 (4.21), 298 (3.93), 348 (4.10) nm; IR (neat) ν_{max} 3389, 2922, 1649, 1606, 1360, 1180 cm^{-1}; ^{1}H- and ^{13}C-NMR: Table 1. ESI-MS *m*/*z*: 637 $[M+H]^+$; HR-ESI-MS *m*/*z*: 637.1773 (Calcd for $C_{29}H_{33}O_{16}$, 637.1769).

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Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1** and **2** in Pyridine-d_5 (400 MHz)

No.	**1**		**2**	
	δ_C	δ_H (mult; *J*, Hz)	δ_C	δ_H (mult; *J*, Hz)
2	176.2		158.1	
3	105.5	6.97 (s)	135.1	
4	183.3		178.6	
5	163.1		162.8	
6	99.9	6.68 (d, 2.0)	99.9	6.73 (d, 2.0)
7	166.0		166.0	
8	94.9	6.74 (d, 2.0)	94.7	6.71 (d, 2.0)
9	158.8		157.8	
10	105.5		105.3	
1′	70.8		122.0	
2′, 6′	32.5	ax. 2.34 (td, 13.2, 3.6) eq. 1.99 (br d, 13.2)	132.0	7.31 (d, 8.8)
3′, 5′	28.1	ax. 2.22 (td, 13.2, 3.6) eq. 2.08 (br d, 13.2)	116.1	8.48 (d, 8.8)
4′	99.5		161.7	
OMe	47.4	3.22 (s)		
	47.5	3.19 (s)		
5-OH		13.57 (br s)		13.18 (br s)
1′-OH		3.64 (s)		
1″			104.1	6.05 (d, 7.2)
2″			76.1	4.32 (m)
3″			69.7	4.56 (m)
4″			69.8	4.26 (m)
5″			76.0	4.32 (m)
6″			18.4	1.49 (d, 6.0)
1‴			102.5	5.30 (d, 1.2)
2‴			71.6	3.97 (m)
3‴			78.6	4.32 (m)
4‴			70.8	4.34 (m)
5‴			77.5	4.14 (m)
6‴			68.8	4.02 (m), 4.49 (m)
OAc			170.5	
			20.9	1.81 (s)

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