CHEMICAL COMPONENTS OF THE RHIZOMES OF Drynaria fortunei (KUNZE) J. Sm. (POLYPODIACEAE) IN VIETNAM

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From the ethanol extract of *Drynaria fortunei* (KUNZE) J. Sm., a new phenylpropanoid glycoside, fortunamide (1), was isolated and characterized by spectroscopic methods. Together with a new glycoside, 9 known compounds, including three curcuminoids (2–4), two isoprenylated flavonoids (5, 6), two flavonoids (7, 8), one monoterpenoid (9) and one phenolic acid (10) were isolated and identified by spectral data analysis from the rhizomes of *Drynaria fortunei* (KUNZE) J. Sm. Eight of them were isolated from *Drynaria fortunei* (KUNZE) J. Sm. for the first time.

Keywords: Drynaria fortunei (Kunze) J. Sm.; Flavonoids; Fortunamide.

The rhizomes of *Drynaria fortunei* (KUNZE) J. Sm. (Polypodiaceae) have been used for treatment of osteoporosis, healing bone fractures in traditional Vietnamese and Chinese medicine^{1–5}. Like most ferns, *D. fortunei* is abundant in triterpenoids, most of which are of the hopane and fernane skeletons, and in flavonoids. As a part of our continuing studies on *Drynaria* species in Vietnam^{6–8}, this paper describes the isolation and structural determination of ten secondary metabolites from the ethanolic extract of the rhizomes of *Drynaria fortunei* (KUNZE) J. Sm. One was a new compound and eight were reported for the first time from *D. fortunei*.

EXPERIMENTAL

Abbreviations Used

ODS, octadecylsilyl silica gel; SPE, solid phase extraction.

Plant Material

Rhizomes of *D. fortunei* were collected in Vietnam in December 2007 and authenticated by the centre of medicinal plants and ginseng in Ho Chi Minh city (National Institute of Medicinal Material – Vietnam).

Equipment

NMR spectra (δ , ppm; *J*, Hz) were recorded on a Bruker Avance 500 MHz using TMS as internal standard, ESI-MS and HR-ESI-MS data were recorded on a Agilent 1100 LC-MSD Trap of Institute of Chemistry (Vietnamese Academy of Science and Technology). TLC were performed on silica gel 60 F₂₅₄ (Merck 1,05715), RP₁₈ F_{254s} (Merck). The zones were detected using UV at 254 or 365 nm or solution of FeCl₃–EtOH or H₂SO₄–EtOH. Column chromatography was performed on silica gel (240–430 mesh) or ODS or Sephadex LH20. The purifications of some compounds (1–6, 9, 10) were carried out on preparative HPLC Agilent 1100 Series DAD with UV detector.

Extraction and Isolation

Dried rhizomes of *D. fortunei* (2 kg) were extracted with EtOH 96%. The extract was evaporated to dryness (240 g) under reduced pressure. The ethanol extract was eluted on a silica gel column sequentially with petrol ether, chloroform, ethyl acetate and methanol.

The petrol ether extract (50 g) was fractionated by column chromatography on a silica gel column using a petroleum ether–chloroform gradient to afford four fractions. Fraction 1 was further passed over sephadex LH-20 column using MeOH–CHCl₃ (50:50) as the eluent to give three subfractions, referred to as subfractions 1-1 to 1-3. Subfraction 1-2 was further purified by ODS preparative HPLC (MeCN–H₂O 70:30) to yield 9 (22.5 mg). Fraction 3 was subjected to column chromatography on silica gel with elution by hexane–chloroform gradients (4:1 to 0:100) to afford three subfractions, referred to as 3-1 to 3-3. Subfraction 3-3 was also passed over sephadex LH-20 column and eluted with CHCl₃–MeOH (50:50) to give three subfractions (3-3a to 3-3c). Subfraction 3-3c was further purified by ODS preparative HPLC (MeOH–H₂O 70:30) to afford 5 (25.5 mg).

The chloroform fraction (24.2 g) was rechromatographed on a silica gel column using a chloroform-methanol gradient to yield 2 fractions (C1 and C2). Fraction C2 was filtered on a sephadex LH20 column (CHCl₃–MeOH 50:50 as eluent) to give three subfractions (C2-1 to C2-3). Subfraction C2-3 was purified by ODS preparative HPLC (MeOH–H₂O 70:30) to give 6 (15 mg). Subfraction C2-2 was rechromatographed on a silica gel column and eluted with CHCl₃–MeOH gradient (100:1) to yield eight subfractions, named subfraction C2-2A to C2-2H, which were further purified by ODS preparative HPLC (MeCN–H₂O 45:55) to give 2 (137 mg), 3 (12.4 mg), 4 (12.5 mg).

The ethyl acetate fraction (20 g) was chromatographed on a silica gel column using a chloroform–methanol gradient to yield seven fractions (A1 to A7). Fraction A2 was filtered

through sephadex LH 20 and purified by preparative HPLC (MeOH– H_2O 30:70) to give 10 (60 mg).

The methanolic fraction (143 g) was chromatographed on a standard silica gel column with chloroform–methanol gradient to give seven fractions. Fraction 4 was repeatedly chromatographed (CHCl₃–MeOH 0–100%) on sephadex LH 20 with MeOH–CHCl₃ (50:50) and by ODS column chromatography using MeOH–H₂O (30:70–50:50) to give 7 (60.5 mg) and 8 (7.5 mg). Fraction 5 was chromatographed on silica gel with chloroform–methanol (0–100%), followed by purification on sephadex LH 20 with MeOH, SPE with ACN–MeOH–H₂O (1:1:20) and then subjected to final purification by ODS preparative HPLC with MeCN–MeOH–H₂O (1:1:40), to give 1 (60 mg).

Fortunamide 1: white powder. IR (KBr, cm⁻¹): 3447 (–OH), 1654 (C=O), 1503 (C=C), 1026 (C–O–). ESI-MS, *m/z*: 365.0 [M + Na]⁺. HR-ESI-MS, *m/z*: 341.21877 [M]⁺ (C₁₅H₁₉NO₈). ¹H NMR (500 MHz, DMSO- d_6): 7.11 s, 1 H (H-2); 7.10 d, 1 H, *J* = 8.5 (H-5); 7.04 d, 1 H, *J* = 8.5 (H-6); 7.41 d, 1 H, *J* = 15.5 (H-7); 6.29 d, 1 H, *J* = 15.5 (H-8); 4.75 d, 1 H, *J* = 7 (H-1'); 3.70 m, 1 H (H-6'a); 3.45 m, 1 H (H-6'b); 3.32 m, 1 H (H-3'); 3.25 m, 1 H (H-5'); 3.29 m, 1 H (H-2'); 3.15 m, 1 H (H-4'). ¹³C NMR (125 MHz, DMSO- d_6): 129.0 (C-1); 114.7 (C-2); 146.8 (C-3); 147.1 (C-4); 116.1 (C-5); 120.4 (C-6); 143.3 (C-7); 117.8 (C-8); 167.9 (C-9).

Curcumine (2): yellow powder. ESI-MS, *m/z*: 369.1 [M + H]⁺; 367.1 [M - H]⁻. ¹H NMR (500 MHz, acetone- d_6): 5.97 s, 1 H (H-1); 6.68 d, 1 H, *J* = 15.5 (H-3); 7.58 d, 1 H, *J* = 15.5 (H-4); 7.16 dd, 1 H, *J* = 1.5 and 8 (H-6); 6.87 d, 1 H, *J* = 8 (H-7); 7.32 d, 1 H, *J* = 1.5 (H-10); 6.68 d, 1 H, *J* = 15.5 (H-3'); 7.58 d, 1 H, *J* = 15.5 (H4'); 7.16 dd, 1 H, *J* = 1.5 (H-6'); 6.87 d, 1 H, *J* = 15.5 (H-3'); 7.32 d, 1 H, *J* = 15.5 (H4'); 7.16 dd, 1 H, *J* = 1.5 and 8 (H-6'); 6.87 d, 1 H, *J* = 8 (H-7'); 7.32 d, 1 H, *J* = 1.5 (H4'); 7.16 dd, 1 H, *J* = 1.5 and 8 (H-6'); 6.87 d, 1 H, *J* = 8 (H-7'); 7.32 d, 1 H, *J* = 1.5 (H-10'); 3.90 s, 3 H (9-OMe); 3.91 s, 3 H (9'-OMe). ¹³C NMR (125 MHz, acetone- d_6): 101.6 (C-1); 184.4 (C-2); 122.3 (C-3); 141.3 (C-4); 128.1 (C-5); 123.7 (C-6); 116.2 (C-7); 150.0 (C-8); 148.7 (C-9'); 111.6 (C-10); 184.4 (C-2'); 122.3 (C-3'); 141.3 (C-4'); 128.1 (C-5'); 123.7 (C-6'); 116.2 (C-7'); 150.0 (C-8'); 148.7 (C-9'); 111.6 (C-10'); 56.3 (9/9'-OMe).

Demethoxycurcumine (3): yellow powder. ESI-MS, *m/z*: 361.1 [M + Na]⁺. ¹H NMR (500 MHz, acetone- d_6): 5.97 s, 1 H (H-1); 6.63 d, 1 H, *J* = 15.5 (H-3); 7.58 d, 1 H, *J* = 15.5 (H-4); 7.33 s, 1 H (H-6); 6.87 d, 1 H, *J* = 8.5 (H-9); 7.16 d, 1 H, *J* = 8.5 (H-10); 6.69 d, 1 H, *J* = 15.5 (H-3'); 7.59 d, 1 H, *J* = 15.5 (H-4'); 7.54 d, 1 H, *J* = 8.5 (H-6'); 6.89 d, 1 H, *J* = 8.5 (H-7'); 6.89 d, 1 H, *J* = 9 (H-9'); 7.54 d, 1 H, *J* = 9 (H-10'); 3.92 s, 3 H (9-OMe). ¹³C NMR (125 MHz, acetone- d_6): 101.6 (C-1); 184.4 (C-2); 121.9 (C-3); 141.3 (C-4); 127.6 (C-5); 123.8 (C-6); 148.8 (C-7); 150.1 (C-8); 116.8 (C-9); 111.5 (C-10); 184.5 (C-2'); 122.2 (C-3'); 141.0 (C-4'); 128.1 (C-5'); 130.9 (C-6'); 116.2 (C-7'); 160.6 (C-8'); 116.2 (C-9'); 130.9 (C-10'); 56.3 (9-OMe).

Bisdemethoxycurcumine (4): yellow powder. ESI-MS, m/z: 309.1 [M + H]⁺; 307.1 [M - H]⁻. ¹H NMR (500 MHz, acetone- d_6): 5.98 s, 1 H (H-1); 6.65 d, 2 H, J = 15.5 (H-3/H-3'); 7.59 d, 2 H, J = 15.5 (H-4/H-4'); 7.55 d, 2 H, J = 9 (H-6/H-6'); 6.89 d, 2 H, J = 8.5 (H-7/H-7'); 6.89 d, 2 H, J = 8.5 (H-9/H-9'); 7.55 d, 2 H, J = 8.5 (H-10/H-10'). ¹³C NMR (125 MHz, acetone- d_6): 101.6 (C-1); 184.5 (C-2/C-2'); 122.0 (C-3/C-3'); 141.5 (C-4/C-4'); 127.7 (C-5/C-5'); 130.9 (C-6/C-6'); 116.7 (C-7/C-7'); 160.5 (C-8/C-8'); 116.7 (C-9/C-9'); 130.9 (C-10/C-10').

Bavachinine (5): orange powder. ESI-MS, *m/z*: 361.1 [M + Na]⁺. ¹H NMR (500 MHz, acetone- d_6): 5.43 dd, 1 H, *J* = 13 and 3 (H-2); 2.66 dd, 1 H, *J* = 3 and 16.5 (H-3a); 3.00 dd, 1 H, *J* = 13 and 16.5 (H-3b); 7.57 s, 1 H (H-5); 6.53 s, 1 H (H-8); 7.39 d, 1 H, *J* = 8.5 (H-2'); 6.89 d, 1 H, *J* = 8.5 (H-3'); 6.89 d, 1 H, *J* = 8.5 (H-5'); 7.39 d, 1 H, *J* = 8.5 (H-6'); 3.23 d, 2 H, *J* = 7 (H-11); 5.26 m, 1 H (H-12); 1.70 s, 3 H (H-14); 1.72 s, 3 H (H-15). ¹³C NMR (125 MHz, acetone- d_6): 80.6 (C-2); 44.6 (C-3); 190.6 (C-4); 127.3 (C-5); 124.8 (C-6); 164.6 (C-7); 99.8

1136

(C-8); 163.2 (C-9); 114.9 (C-10); 131.3 (C-1'); 128.9 (C-2'); 116.1 (C-3'); 158.5 (C-4'); 116.1 (C-5'); 128.9 (C-6'); 29.3 (C-11); 123.0 (C-12); 133.0 (C-13); 17.7 (C-14); 25.8 (C-15).

Isobavachalcone (6): orange powder. ESI-MS, *m/z*: 324.2 [M + H]⁺. ¹H NMR (500 MHz, acetone-*d*₆): 6.54 d, 1 H, *J* = 8.5 (H-5); 7.97 d, 1 H, *J* = 9 (H-6); 3.38 d, 2 H, *J* = 7 (H-7); 5.27 m, 1 H (H-8); 1.64 s, 3 H (H-10); 1.77 s, 3 H (H-11); 7.73 d, 2 H, *J* = 8.5 (H-2'/H-6'); 6.93 d, 2 H, *J* = 8.5 (H-3'/H-5'); 7.76 d, 1 H, *J* = 15.5 (H-α); 7.85 d, 1 H, *J* = 15.5 (H-β). ¹³C NMR (125 MHz, acetone-*d*₆): 114.4 (C-1); 165.1 (C-2); 116.1 (C-3); 162.7 (C-4); 108.0 (C-5); 130.2 (C-6); 22.1 (C-7); 123.3 (C-8); 131.4 (C-9); 25.8 (C-10); 17.8 (C-11); 127.6 (C-1'); 131.6 (C-2'/C-6'); 116.7 (C-3'/C-5'); 160.9 (C-4'); 118.5 (C-α); 144.9 (C-β); 193.0 (C=O).

(-)-*Epicatechine* (7): white powder. ESI-MS, m/z: 313.0 [M + Na]⁺. ¹H NMR (500 MHz, DMSO- d_6): 4.73 s, 1 H (H-2); 4.01 s, 1 H (H-3); 2.46 dd, 1 H, J = 3.5 and 16.5 (H-4a); 2.66 dd, 1 H, J = 4 and 16.5 (H-4b); 5.89 d, 1 H, J = 2 (H-6); 5.72 d, 1 H, J = 2 (H-8); 6.89 s, 1 H (H-2'); 6.64 d, 1 H, J = 9 (H-6'); 6.66 d, 1 H, J = 8.5 (H-5'). ¹³C NMR (125 MHz, DMSO- d_6): 78.0 (C-2); 64.9 (C-3); 28.1 (C-4); 156.4 (C-5); 95.1 (C-6); 156.2 (C-7); 94.1 (C-8); 155.7 (C-9); 98.5 (C-10); 130.6 (C-1'); 114.8 (C-2'); 144.4 (C-3'); 144.4 (C-4'); 114.7 (C-5'); 117.9 (C-6').

Liquiritine (8): yellow powder. ESI-MS, m/z: 441.1 [M + Na]⁺. ¹H NMR (500 MHz, DMSO- d_6): 5.48 dd, 1 H, J = 2.5 and 12 (H-2); 2.64 dd, 1 H, J = 2.5 and 17 (H-3a); 3.05 dd, 1 H, J = 12.5 and 17 (H-3b); 7.61 d, 1 H, J = 8.5 (H-5); 6.45 d, 1 H, J = 8.5 (H-6); 6.28 s, 1 H (H-8); 7.06 d, 2 H, J = 8.5 (H-3'/H-5'); 7.43 d, 2 H, J = 8.5 (H-2'/H-6'); 4.87 d, 1 H, J = 7.5 (H-1"); 3.15 m, 1 H (H-2"); 3.23 m, 1 H (H-3"); 3.05 m, 1 H (H-4"); 3.26 m, 1 H (H-5"); 3.31 m, 1 H (H-6a); 3.69 m, 1 H (H-6b). ¹³C NMR (125 MHz, DMSO- d_6): 78.4 (C-2); 43.1 (C-3); 189.3 (C-4); 128.1 (C-5); 111.1 (C-6); 163.8 (C-7); 102.5 (C-8); 163.0 (C-9); 112.5 (C-10); 132.4 (C-1'); 116.1 (C-3'/C-5'); 127.7 (C-2'/C-6'); 157.3 (C-4'); 100.3 (C-1"); 73.1 (C-2"); 76.5 (C-3"); 69.7 (C-4"); 76.9 (C-5"); 60.6 (C-6").

Bakuchiol (9): yellow oil. ESI-MS, *m/z*: 279.3 [M + Na]⁺. ¹H NMR (500 MHz, acetone- d_6): 7.25 d, 1 H, *J* = 8.5 (H-2); 6.77 d, 1 H, *J* = 9 (H-3); 6.77 d, 1 H, *J* = 9 (H-5); 7.25 d, 1 H, *J* = 8.5 (H-6); 6.27 d, 1 H, *J* = 16.5 (H-7); 6.08 d, 1 H, *J* = 16 (H-8); 1.48 m, 2 H (H-10); 1.95 m, 2 H (H-11); 5.11 m, 1 H (H-12); 1.65 s, 3 H (H-14); 1.57 s, 3 H (H-15); 1.19 s, 3 H (H-16); 5.89 m, 1 H (H-17); 4.99 m, 2 H (H-18). ¹³C NMR (125 MHz, acetone- d_6): 130.3 (C-1); 128.0 (C-2); 116.1 (C-3); 157.5 (C-4); 116.1 (C-5); 128.0 (C-6); 127.8 (C-7); 135.3 (C-8); 43.1 (C-9); 42.1 (C-10); 23.9 (C-11); 125.7 (C-12); 131.4 (C-13); 25.7 (C-14); 17.6 (C-15); 23.7 (C-16); 147.0 (C-17); 112.0 (C-18).

Protocatechuic acid (**10**): white powder. ESI-MS, *m/z*: 177.1 [M + Na]⁺; 153.0 [M – H]⁻. ¹H NMR (500 MHz, acetone- d_6): 7.54 s, 1 H (H-2); 6.89 d, 1 H, *J* = 7.5 (H-5); 7.48 d, 1 H, *J* = 7.5 (H-6). ¹³C NMR (125 MHz, acetone- d_6): 168.1 (COOH); 122.9 (C-1); 117.4 (C-2); 145.5 (C-3); 150.7 (C-4); 115.6 (C-5); 123.7 (C-6).

RESULTS AND DISCUSSION

The ethanol extract of *D. fortunei* was partitioned successively with petroleum ether, chloroform, ethyl acetate and methanol. After column chromatography on silica gel (normal and reverse phase), sephadex LH-20 as adsorbent, the ethyl acetate, petroleum ether and chloroform fractions yielded 1, 2 and 4 known compounds, respectively. Separation of the methanol extract by repeated chromatography on silica gel and sephadex LH-20, followed by purification on a preparative HPLC afforded 3 compounds, one of which is a new glycoside (1), together with two known compounds. The spectral data of the above nine known compounds (2–10) were in good agreement with those published in the literatures. The compounds were thus identified as curcumine⁹, demethoxycurcumine⁹, bisdemethoxycurcumine⁹, bisdemethoxycurcumine⁹, bavachinine¹⁰, isobavachalcone¹⁰, (–)epicatechine¹¹, liquiritine¹², bakuchiol¹³, protocatechuic acid¹⁴.

Compound 1 was obtained as a white powder, which gave a positive reaction with $FeCl_3$ -EtOH indicating a phenolic compound. The IR spectrum of 1 showed the corresponding carbonyl absorption (v 1654 cm⁻¹), hydroxyl



Collect. Czech. Chem. Commun. 2011, Vol. 76, No. 9, pp. 1133-1139

(v 3447 cm⁻¹), and aromatic ring (1503 cm⁻¹). The ¹H NMR (500 MHz, DMSO-*d*₆) spectrum of 1 showed signals of one trisubstituted aromatic $\delta_{\rm H}$ [7.04 (1 H, d, *J* = 8.5 Hz, H-6); 7.10 (1 H, d, *J* = 8 Hz, H-5) and 7.11 (1 H, s, H-2)], two *trans* olefinic protons $\delta_{\rm H}$ [6.29 (1 H, d, *J* = 16 Hz, H-8); 7.41 (1 H, d, *J* = 16 Hz, H-7)] and a β-glucose moiety $\delta_{\rm H}$ [4.75 (1 H, d, *J* = 7 Hz, H-1'); 3.70 (1 H, H-6'a); 3.45 (1 H, m, H-6'b); 3.32 (1 H, H-3'); 3.25 (1 H, H-5'); 3.29 (1 H, H-2') and 3.15 (1 H, H-4')]. The ¹³C NMR and DEPT spectra indicated that 1 possessed 15 carbons, which were sorted out into one carbonyl $\delta_{\rm C}$ 167.9, two olefinic carbons $\delta_{\rm C}$ [117.8; 143.3], six aromatic carbons including two oxygenated ones $\delta_{\rm C}$ [114.7; 116.1; 120.4; 129.0; 146.8; 147.1] and a β-D-glucose moiety $\delta_{\rm C}$ [60.7; 69.7; 73.2; 75.8; 77.3; 101.6].

In the HMBC spectrum of 1, two signals of olefinic protons showed long range correlations to the carbonyl and a quaternary aromatic carbon indicating that the double bond was attached to the aromatic ring at C-1 ($\delta_{\rm C}$ 129.0). Because of the resonance effect of the carbonyl group, the chemical shifts of H-7 and H-8 were $\delta_{\rm H}$ 7.41 and 6.29, respectively. Two hydroxy groups could have been attached to the aromatic ring at C-3/C-4 or C-2/C-4.

In addition, H-7 showed correlations with two aromatic carbons $\delta_{\rm C}$ [114.7; 120.4], which confirmed that these two carbons were C-2 and C-6, respectively. Consequently, C-3 and C-4 bear the two hydroxy groups. Proton at $\delta_{\rm H}$ 7.04 (1 H, d, J = 8.5 Hz) gave HMBC correlations with C-7 and an oxygenated aromatic carbon. This result suggests that the proton was H-6. Consequently, two protons H-5 and H-2 had chemical shifts at $\delta_{\rm H}$ [7.10 (1 H, d, J = 8 Hz)] and [7.11 (1 H, s)], respectively. The HMBC spectrum showed that the signal of the oxygenated carbon at $\delta_{\rm C}$ 147.1 gave correlations just with H-6 and the anomeric proton proving that the signal at $\delta_{\rm C}$ 147.1 was C-4 and suggesting C-4 as the location of the glucose moiety. The coupling constant of the anomeric proton J = 7 Hz attested to the β -conformation of the glucose unit.

The signal of the carbonyl at $\delta_{\rm C}$ 167.9 indicated that 1 could have a carboxy or amide group. However, the IR spectrum of 1 did not reveal the presence of the carboxy group, and ESI-MS gave molecular peak of 342 amu consistent with the molecular formula of C₁₅H₁₉NO₈. The HR-ESI-MS gave the molecular peak at *m*/*z* 341.21877 amu and the result of element analysis confirmed the above molecular formula of C₁₅H₁₉NO₈.

Based on these spectral features of 1, we have assigned the structure of $4-O-\beta$ -D-glucopyranosyl caffeic acid amide to this natural compound. Amide 1 appears to be a new natural product, for which we suggested the trivial name fortunamide.

Except for (-)-epicatechine (7), all compounds were isolated from *Drynaria fortunei* for the first time.

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