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Chemical Constituents from the *Hydrangea chinensis*

Ashraf Taha Khalil, Fang-Rong Chang, Yue-Han Lee, Chung-Yi Chen[1], Chih-Chuang Liaw, Patnam Ramesh, Shyng-Shiou F. Yuan[2], and Yang-Chang Wu

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, [1]Basic Medical Science Education Center, Fooyin University, Kaohsiung county 831, Taiwan, and [2]Department of Obstetrics and Gynecology, Kaohsiung Medical University, Kaohsiung 807, Taiwan

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Two quinazolone alkaloids, (+)-febrifugine (**1**) and isofebrifugine (**2**), along with three coumarin derivatives, 6-hydroxy coumarin (**3**), skimmin (**5**), and umbelliferone-7-O-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranoside (**6**), were isolated from the roots of *Hydrangea chinensis*. Compound **6** is a new compound. In addition, umbelliferone **(4)**, linoleic acid **(7),** two steroidal glycosides **(8, 9)**, three furfural derivatives **(10-12)**, and butyl-β-D-fructofuranoside **(13)** were isolated from the leaves of the same plant. The structures of all isolates were elucidated by spectral methods.

Key words: *Hydrangea chinensis*, (+)-Febrifugine, Umbelliferone-7-O-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranoside, 5-methoxy-2-furfuraldehyde, NMR.

INTRODUCTION

In line with our efforts to search for bioactive compounds from Chinese medicinal plants, we continued our investigation of the Formosan *Hydrangea chinensis* (Saxifragaceae), which is traditionally used for treatment of malaria and heart diseases (Chiang-Su, 1978). In a previous study (Patnam *et al*, 2001), we reported the isolation and characterization of a novel alkaloid, hydrachine A, along with fifteen known compounds from the roots of this species. This paper deals with the isolation and characterization of two quinazolone alkaloidal derivatives, (+)-febrifugine (**1**) and isofebrifugine (**2**), together with three coumarin derivatives; 6-hydroxy coumarin (**3**), skimmin (**5**), and the new coumarin bioside; umbelliferone-7-O-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside (**6**) from the roots of *H. chinensis*. In addition, we report here the isolation and characterization of eight compounds from the leaves of the same species, which are: umbelliferone (**4**), linoleic acid (**7**), β-sitosteryl-3-O-β-D-(6'-hexadecanoyl)-glucopyranoside (**8**), β-sitosterol-3-O-β-D-glucoside (**9**), 5-(acetoxymethyl)-2-furfuraldehyde (**10**), 5-(hydroxymethyl)-2-furfuraldehyde (**11**), 5-methoxy-2-furfuraldehyde (**12**), and butyl-β-D-fructofuranoside (**13**). Compound **1** was previously isolated from *Dichroa febrifuga* Lour. (Saxifragaceae) (Koepfli *et al.*, 1947) and later from the roots of *Hydrangea arten* (Ablondi *et al.*, 1952), and it attracted considerable attention due to its powerful antimalarial activity (Jang *et al.*, 1946). Compound **6** is a new compound.

MATERIALS AND METHODS

Instruments and reagents

All Melting points were determined on a Laboratory Devices Mel-Temp II and were uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were run on a Hitachi 220-20 spectrophotometer. IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 400 MHz spectrometer using TMS as an internal standard. Chemical shifts are reported in parts per million (δ), and coupling constants (J) are expressed in hertz. HREIMS were measured on JEOL JMS-HX 110 mass spectrometer. EIMS was recorded on JEOL JMS-SX/SX 102A mass spectrometer. Silica gel 60 (Merck, 230-400 mesh) was used for column chromatography. Pre-coated silica gel plates (Merck, Kieselgel 60 F-254, 0.5 mm) were used for preparative TLC. Spots were visualized with Dragendorffs reagent or 50% H_2SO_4 and heating. Sephadex

Correspondence to: Yang-Chang Wu, Ph. D., Professor and Director, Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan
E-Mail: yachwu@kmu.edu.tw

LH-20 (Amersham Pharmacia Biotech AB) was used for separation and/or purification.

Plant material

The roots and leaves of *H. chinensis* were collected from Pintong County, Taiwan, 1999. A voucher specimen (Saxifra-1-1) is deposited in the Graduate Institute of Natural Products, Kaohsiung, Taiwan.

Extraction and isolation

The concentrated methanolic extract of the roots (2.0 kg) of *Hydrangea chinensis* (Saxifragaceae) was successively extracted with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. The ethyl acetate extract was subjected to column chromatography (CC) on Si-gel and gradiently eluted with ethyl acetate/MeOH mixture. Compounds, which were not isolated before (Patnam *et al.*, 2001), were purified on Si-gel column using $CHCl_3$/MeOH mixture for gradient elution. Crystallization of each compound afforded **1** (colorless crystals, 18 mg), **2** (white powder, 13 mg), and **3** (colorless needles, 32 mg). The *n*-BuOH extract of the root was fractionated using Sephadex LH-20 and elution was effected using H_2O, H_2O/MeOH (1:1), and then MeOH. The third fraction eluted with pure MeOH was subjected to CC on Si-gel using EtOAc/MeOH for gradient elution to yield **5** (colorless needles, 20 mg) and **6** (colorless needles, 10 mg). The leaves (1.0 kg) were extracted with MeOH and the extract was concentrated *in vacuo* to a syrupy liquid and then successively extracted with *n*-hexane, EtOAc, and finally with *n*-BuOH. CC of the EtOAc extract on Si-gel, eluting with $CHCl_3$/MeOH gradient, afforded **4** (18 mg), **7** (14 mg), **8** (6 mg), and **9** (23 mg). Purification and subsequent fractionation of the *n*-BuOH extract was carried out as mentioned above to give **10** (31 mg), **11** (32 mg), **12** (19 mg), and **13** (24 mg).

(+)-Febrifugine (1)

Colorless prisms; mp 135-138 °C; $[\alpha]_D^{25}$ + 17.0° (*c* 0.77, $CHCl_3$); UV λ_{max}^{MeOH} nm 224, 265, 313; IR (KBr) ν_{max} cm^{-1}: 3270 (bonded O-H st), 1720 (C=O st), 1680 (conj.C=O st), 1611; HREIMS *m/z* : 301.1433 $[M]^+$, calcd for $C_{16}H_{19}N_3O_3$, 301.1426; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.33 (1H, ddt, *J* = 5, 11, 13 Hz, H-4"), 1.55 (1H, dt, *J* = 4, 13 Hz, H-5"), 1.68 (1H, dt, *J* = 4, 13 Hz, H-5"), 2.02 (1H, ddd, *J* = 5, 11, 13 Hz, H-4"), 2.47 (1H, dt, *J* = 4, 13 Hz, H-6"), 2.60 (1H, dd, *J* = 7.5, 16 Hz, H-3'), 2.75 (1H, ddd, *J* = 5, 7.5, 9 Hz, H-2"), 2.96 (1H, dd, *J* = 4, 13 Hz, H-6"), 3.10 (1H, dd, *J* = 5, 16 Hz, H-3'), 3.28 (1H, ddd, *J* = 5, 9, 11 Hz, H-3"), 4.68 (1H, d, *J* = 17 Hz, H-1'), 4.84 (1H, d, *J* = 17 Hz, H-1'), 7.48 (1H, ddd, *J* = 1.2, 8, 8.5 Hz, H-6), 7.67 (1H, dd, *J* = 1.2, 8 Hz, H-8), 7.73 (1H, ddd, *J* = 1.3, 8, 8.5 Hz, H-7), 8.10 (1H, s, H-2), 8.25 (1H, dd, *J* = 1.2, 8 Hz, H-5); ^{13}C-NMR, Table I.

Table I. ^{13}C-NMR (100 MHz) data of **1** and **2** in $CDCl_3$

C	δ_c	δ_c
	1	**2**
2	145.7	148.5
4	161.8	161.7
4a	123.2	122.1
5	127.8	127.7
6	127.5	127.3
7	134.7	135.8
8	126.8	127.1
8a	148.9	148.5
1'	55.4	51.2
2'	203.1	105.0
3'	46.1	44.6
2"	61.2	56.8
3"	72.4	77.2
4"	42.0	43.4
5"	26.5	20.1
6"	35.2	26.8

Isofebrifugine (2)

White powder; $[\alpha]_D^{25}$ + 25.0° (*c* 0.60, MeOH); UV λ_{max}^{MeOH} nm 225, 265, 305, 313; IR (KBr) ν_{max} cm^{-1}: 3400 (O-H st.), 1678 (conj.C=O st), 1607; HREIMS *m/z* : 301.1439 $[M]^+$, calcd for $C_{16}H_{19}N_3O_3$, 301.1426; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 1.50 (1H, m, H-5"), 1.57 (1H, ddd,, *J* = 4, 5, 11 Hz, H-4"), 1.78 (1H, m, H-5"), 1.88 (1H, m, H-3'), 2.08 (1H, dd, *J* = 4, 13 Hz, H-3'), 2.12 (1H, m, H-4"), 2.54 (1H, dt, *J* = 2, 11 Hz, H-6"), 3.02 (1H, m, H-6"), 3.37 (1H, dd, *J* = 3, 5 Hz, H-2"), 3.93 (1H, m, H-3"), 4.35 (1H, d, *J* = 14 Hz, H-1'), 4.74 (1H, d, *J* = 14 Hz, H-1'), 7.50 (1H, ddd, *J* = 1.5, 8, 8.5 Hz, H-6), 7.70 (1H, dd, *J* = 1.5, 8 Hz, H-8), 7.76 (1H, ddd, *J* = 1.5, 8, 8.5 Hz, H-7), 8.15 (1H, s, H-2), 8.35 (1H, dd, *J* = 1.5, 8 Hz, H-5); ^{13}C-NMR, Table I.

6-Hydroxy coumarin (3)

Colorless prisms, mp 230-231 °C; UV λ_{max}^{MeOH} nm (log ε): 220 (3.11), 258 (2.01), 330 (4.75); IR (KBr) ν_{max} cm^{-1}: 3370, 1678, 1610 ; EIMS *m/z* 162 $[M]^+$, $C_9H_6O_3$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 6.38 (1H, d, *J* = 9.6 Hz, H-3), 7.07 (1H, dd, *J* = 8.4, 2.0 Hz, H-7), 7.13 (1H, d, *J* = 2 Hz, H-5), 7.47 (1H, d, *J* = 8.4 Hz, H-8), 7.68 (1H, d, *J* = 9.6 Hz, H-4); ^{13}C-NMR, Table II.

Umbelliferone (4)

Colorless prisms, mp 223-225 °C; UV λ_{max}^{MeOH} nm (log ε): 216 (2.71), 245 (3.55), 258 (4.10), 279 (4.17), 322 (3.78); IR (KBr) ν_{max} cm^{-1}: 3411, 1680, 1610; EIMS *m/z* 162 $[M]^+$, $C_9H_6O_3$; ^{1}H-NMR (CD_3OD, 400 MHz) δ_H: 6.19 (1H, d, *J* =

Table II. ^{13}C-NMR (100 MHz) data of compounds **3**, **5**, **6** in pyridines-d_5

C	δ_C		
	3	5	6
2	160.6	162.6	160.9
3	115.4	112.8	113.7
4	144.2	144.5	143.7
5	112.3	129.3	129.5
6	154.5	113.7	113.9
7	119.8	161.4	160.7
8	117.2	103.0	103.9
4a	120.2	111.9	113.8
8a	147.8	155.9	155.8
1'		99.6	99.7
2'		73.5	73.9
3'		77.4	77.6
4'		69.6	79.0
5'		76.7	78.8
6'		60.8	62.1
1"			102.3
2"			72.6
3"			72.3
4"			73.9
5"			71.1
6"			18.7

9.5 Hz, H-3), 6.78 (1H, d, J = 2.3 Hz, H-8), 6.87 (1H, dd, J = 8.5, 2.3 Hz, H-6), 7.46 (1H, d, J = 8.5 Hz, H-5), 7.86 (1H, d, J = 9.5 Hz, H-4); ^{13}C-NMR, Table II.

Umbelliferone-7-*O*-β-D-glucopyranoside (skimmin) (5)

Colorless needles, mp 203-204 °C; UV λ_{max}^{MeOH} nm (log ε): 2[illegible]5 (2.51), 316 (3.76); IR (KBr) ν_{max} cm^{-1}: 3400, 1697, 1620; HREIMS m/z: 324.0243 [M]$^+$, calcd for $C_{15}H_{16}O_8$; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ_H: 5.69 (1H, d, J = 7.6 Hz, H-1), 6.30 (1H, d, J = 9.6 Hz, H-3), 7.17 (1H, dd, J = 8.4, 2.4 Hz, H-6), 7.23 (1H, d, J = 2.4 Hz, H-8), 7.37 (1H, d, J = 8.8 Hz, H-5), 7.62 (1H, d, J = 9.6 Hz, H-4); ^{13}C-NMR, Table II.

Umbelliferone-7-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranoside (6)

Colorless needles, m.p. 165-170 °C; UV λ_{max}^{MeOH} nm (log ε): 2[illegible]8 (2.50), 320 (3.30); IR (KBr) ν_{max} cm^{-1}: 3452 (OH), 3050 (C-H st. ar.), 1695 (C=O st.), 1610; HRFABMS m/z: 471.1504 [M+1]$^+$, calc. for $C_{21}H_{26}O_{12}$, 471.4413; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ_H: 1.79 (3H, d, J = 6 Hz, H-6"), 5.62 (1H, d, J = 7.6 Hz, H-1'), 6.31 (1H, d, J = 9.6 Hz, H-3), 6.40 (1H, d, J = 1.2 Hz, H-1"), 7.26 (1H, dd, J = 8, 1.2 Hz, H-6), 7.29 (1H, d, J = 1.2 Hz, H-8), 7.30 (1H, d, J = 8 Hz, H-5), 7.62 (1H, d, J = 9.6 Hz, H-4); ^{13}C-NMR, Table II.

Linoleic acid (7)

Colorless oil, EIMS m/z 280 [M]$^+$, $C_{18}H_{32}O_2$; IR (Neat) ν_{max} cm^{-1}: 1714, 1630; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H: 0.89 (3H, t, J = 7.2 Hz, C<u>H</u>$_3$), 1.25 (14H, br.s., [C<u>H</u>$_2$]$_7$), 1.59 (2H, m, H-3), 2.34 (2H, t, J = 7.2 Hz, C<u>H</u>$_2$COOH), 2.80 (2H, t, J = 6.4 Hz, H-11), 5.34 (4H, m, H-9,10,12,13).

β-Sitosterol-3-*O*-β-D-(6'-hexadecanoyl)-glucopyranoside (8)

White waxy powder, m.p. >300 °C; FABMS m/z (rel. int.), 837 [M+Na]$^+$ (0.5), 184, 239, $C_{51}H_{90}O_7$; IR (KBr) ν_{max} cm^{-1}: 3450 (O-H), 1735 (C=O), 1255, 1100, 720; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 0.68 (3H, s, CH_3-18), 0.81 (3H, t, J = 7.2 Hz, CH_3-16"), 0.82, 0.83 (each 3H, d, J = 7.1 Hz, CH_3-26, 27), 0.85 (3H, t, J = 7.4 Hz, CH_3-29), 0.93 (3H, d, J = 6.4 Hz, CH_3-21), 1.00 (3H, s, CH_3-19), 1.25 (br.s, [CH_2]$_n$), 2.23 (2H, t, H-2"), 3.46 (1H, ddd, J = 8.5, 5, 2.5 Hz, H-5'), 3.52 (1H, dd, J = 8.5,7.8 Hz, H-2'), 3.57 (1H, t, J = 8.5 Hz, H-4'), 3.87 (1H, t, J = 8.5, H-3'), 4.12 (1H, dd, J = 13, 5 Hz, H$_a$-6'), 4.33 (1H, dd, J = 12, 5 Hz, H-3), 4.38 (1H, dd, J = 13, 2.5 Hz, H$_b$-6'), 4.47 (1H, d, J = 7.8 Hz, H-1'), 5.49 (1H, br.d, J = 4.4 Hz, H-6).

β-Sitosterol-3-*O*-β-D-glucoside (9)

White powder, m.p. >300 °C; $C_{35}H_{60}O_6$; IR (KBr) ν_{max} cm^{-1}: 3405, 1632, 1100; ^{1}H-NMR (pyridine-d_5, 200 MHz) δ_H: 0.69 (3H, s, CH_3-18), 0.79, 0.81 (each 3H, d, J = 6.4 Hz, CH_3-26, 27), 0.82 (3H, t, J = 7.1 Hz, CH_3-29), 0.90 (3H, d, J = 6.4 Hz, CH_3-21), 1.01 (3H, s, CH_3-19), 3.94 (1H, m, H-4'), 4.06 (1H, dd, J = 8, 9 Hz, H-2'), 4.29 (1H, t, J = 9 Hz, H-3'), 4.42 (1H, m, H-5'), 4.57 (1H, br.d, J = 12 Hz, H$_a$-6'), 5.07 (1H, d, J=8 Hz, H-1'), 5.17 (1H, dd, J = 12, 5 Hz, H$_b$-6'), 5.33 (1 H, dd, J = 12, 5 Hz, H-3), 5.48 (1H, br.d, J = 5 Hz, H-6).

5-(Acetoxymethyl)furfural (10)

Colorless oil, EIMS m/z, 168 [M]$^+$, $C_8H_8O_4$; UV λ_{max}^{MeOH} nm (log ε): 204 (4.91), 280 (3.11), 291sh (2.92); IR (Neat) ν_{max} cm^{-1}: 1736 (C=O ester), 1673 (C=O, conjugated), 1248 (C-O, ester); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H: 2.10 (3H, s, C<u>H</u>$_3$-CO-), 5.11 (2H, s, C<u>H</u>$_2$-O), 6.58 (1H, d, J = 3.4 Hz, H-4), 7.20 (1H, d, J = 3.4 Hz, H-3), 9.63 (1H,s, C<u>H</u>O); ^{13}C-NMR (50 MHz, $CDCl_3$) δ_C: 177.7 (<u>C</u>HO), 170.2 (CH_3-<u>C</u>=O), 155.3 (C-5), 152.8 (C-2), 121.5 (C-4), 112.5 (C-3), 57.7 (<u>C</u>H$_2$-O), 20.6 (<u>C</u>H$_3$-CO-).

5-(Hydroxymethyl)furfural (11)

Colorless oil, EIMS m/z, 126 [M]$^+$, $C_6H_6O_3$; UV λ_{max}^{MeOH} nm (log ε): 222 (3.95), 276 (4.05); IR (Neat) ν_{max} cm^{-1}:

3420 (OH), 2852 (C-H), 1674 (C=O, conjugated), 1590, 1517, 1192, 1024; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H : 4.72 (2H, s, C$\underline{H_2}$OH), 6.52 (1H, d, J = 3.4 Hz, H-4), 7.21 (1H, d, J = 3.4 Hz, H-3), 9.58 (1H,s, C$\underline{H}$O); ^{13}C-NMR (50 MHz, $CDCl_3$) δ_c: 177.6 (C$\underline{H}$O), 160.5 (C-5), 152.4 (C-2), 122.7 (C-4), 110.0 (C-3), 57.6 ($\underline{C}H_2$-O).

5-(Methoxyfurfural) (12)

Colorless oil, EIMS *m/z*, 126 $[M]^+$, $C_6H_6O_3$; UV λ_{max}^{MeOH} nm (log ε): 280 (3.91); IR (Neat) ν_{max} cm^{-1}: 1675 (C=O, conjugated), 1522, 1190, 1050; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ_H: 4.62 (3H, s, OC$\underline{H_3}$), 6.59 (1H, d, J = 3.4 Hz, H-4), 7.39 (1H, d, J = 3.4 Hz, H-3), 9.53 (1H,s, C$\underline{H}$O).

Butyl-β-D-fructofuranoside (13)

Colorless needles; m.p. >300 °C; $C_{10}H_{20}O_6$; $[\alpha]_D^{25}$ - 20.7° (*c* 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 3440; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ_H: 0.85 (3H, dd, J = 7.5, 7.0 Hz, H-4'), 1.37 (2H, m, H-3'), 1.56 (2H, m, H-2'), 3.70 (1H, dt, J = 9.2, 7.2 Hz, H_a-1'), 3.76 (1H, dt, J = 9.2, 7.2 Hz, H_b-1'), 4.01 (1H, d, J = 12 Hz, H_a-1), 4.10 (1H, d, J = 12 Hz, H_b-1), 4.23 (1H, d, J = 11.5 Hz, H_a-6), 4.39 (1H, br.d, J = 11.5 Hz, H_b-6), 4.43 (1H, br.d, J = 7.0 Hz, H-3), 4.54 (1H, dd, J = 9.0, 7.0 Hz, H-4), 4.93 (1H, d, J = 9.0 Hz, H-5); ^{13}C-NMR (pyridine-d_5, 100 MHz) δ_c: 14.7 (C-4'), 20.3 (C-3'), 33.1 (C-2'), 61.1 (C-1'), 64.7 (C-6), 65.5 (C-1), 71.1 (C-3), 71.8 (C-4), 72.6 (C-5), 101.7 (C-2).

RESULTS AND DISCUSSION

Chromatographic fractionation of the methanolic extract of the roots of *Hydrangea chinensis* (Saxifragaceae) afforded compounds **1-5**.

Compound **1** gave an orange color with Dragendorffs reagent indicating its alkaloidal nature. In its ^{1}H-NMR spectrum, the coupling constants of four aromatic protons at δ 8.25 (dd, J = 1.2, 8 Hz), 7.48 (ddd, J = 1.2, 8, 8.5 Hz), 7.73 (ddd, J = 1.3, 8, 8.5 Hz), and 7.67 (dd, J = 1.2, 8 Hz), assigned to H-5, 6, 7, and 8, respectively, indicated the presence of an *ortho*-disubstituted benzene ring (H-5,6,7 and 8). Besides, the distinctly downfield shifted singlet at δ 8.10, assigned to H-2, together with the C=O signal at δ 161.8 in the ^{13}C-NMR (Table I) suggested the presence of 4-quinazolone which is substituted at position-3 as indicated by the absence of the downfield proton broad singlet at *ca.* δ 10.0 attributable to N$\underline{H}$ (Spasov *et al.*, 1985, Claudia *et al.*, 1994). In the HMBC spectrum, the carbon signal at δ 203.1 exhibited 3-bond correlation with a proton signal at δ 2.75 and 4-bond correlation with the singlet at 8.10 assigned to H-2" and H-2 respectively. In addition, the ketonic signal at δ 161.8 revealed 3-bond correlation with the proton signal at δ 4.68 assigned to H-1'. The COSY spectrum revealed a correlation peak between proton signals at δ 2.75 and 3.28, which were assigned to H-2" and H-3", respectively. It was assumed that the latter proton was attached to a hydroxylated carbon (C-3"). The IR spectrum showed absorption bands at 1680 (C=O at C-4), 3270 (bonded OH at C-3"), and 1720 (C=O at C-2') cm^{-1}. The HRMS, $[\alpha]_D$, 1 and 2-D NMR data were consistent with those reported for febrifugine (Murata *et al.*, 1998 and Kobayashi *et al.*, 1999). Accordingly, it was concluded that **1** is (+)-febrifugine. Comparing the spectral data of **2** with those of **1**, it was noticed that they are very similar except for the disappearance of C=O signal at δ 203.1 (C-2') in **1** and the emergence of a quaternary oxygenated carbon signal at δ 105.0 ppm in **2**. The latter carbon signal showed 3-bond correlation with the proton signals at δ 3.93 and 3.37, which were assigned to H-3" and H-2", respectively. All spectral data indicated that **2** is identical to isofebrifugine (Murata *et al.*, 1998 and Kobayashi *et al.*, 1999). In acidic medium, compound **1** was converted interchangeably to **2**.

The coumarin nature of **6** was deduced from UV absorption at 215 and 316 nm, and IR absorption bands at 1697 (C=O st.), 3050, and 1610 (aromatic C=C st.) cm^{-1}. The MS spectrum of **6** showed molecular ion peak at *m/z* 470 corresponding to the molecular formula $C_{21}H_{26}O_{12}$. The ^{1}H-NMR (400 MHz, pyridine-d_5) revealed two doublets at δ 6.31 and 7.62, each with J = 9.6 Hz, characteristic of an α,β-unsaturated ketone of a coumarin ring, as well as an aromatic proton signals at δ 7.26 (dd, J = 8, 1.2 Hz), 7.29 (d, J = 1.2 Hz), and 7.30 (d, J = 8 Hz) indicating the presence of a trisubstituted aromatic ring. Furthermore, ^{13}C-NMR and DEPT spectra (Table II) revealed the an apparent upfield-shifted coumarin carbon signal at δ 103.9, which could be assigned to C-8. Therefore, C-7 substitution, rather than C-6 substitution, was suggested, because this carbon must be flanked by two oxygenated carbons. The presence of two sugar moieties was indicated by two anomeric proton signals at δ 5.62 (d, J = 7.6 Hz) and 6.40 (d, J = 1.1 Hz), and two anomeric carbon signals at δ 99.7 and 102.3. The NMR data of the first sugar suggested that it is glucose with substitution at C-4 as indicated by the relative downfield shift of C-4 to δ 79.0. The large J value (7.6 Hz) of the first anomeric proton indicated the β-glycosidic linkage of glucose. The presence of α-L-rhamnose as the second sugar was deduced from the small J value of its anomeric proton (1.1 Hz) together with the upfield methyl signal at δ 1.79 (3H, d, J = 6 Hz, H-6) correlated to a carbon signal at δ 18.7. The HMBC spectrum confirmed the sugar linkage by a cross peak between C-7 (δ 160.7) and the anomeric proton of D-glucose (δ 5.62), and a cross peak between C-4' of D-glucose (δ 79.0) and the anomeric proton of L-rhamnose (δ 6.40). Thus, the structure of **6** was established as umbelliferone-7-*O*-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside. It is noteworthy that coumarin biosides

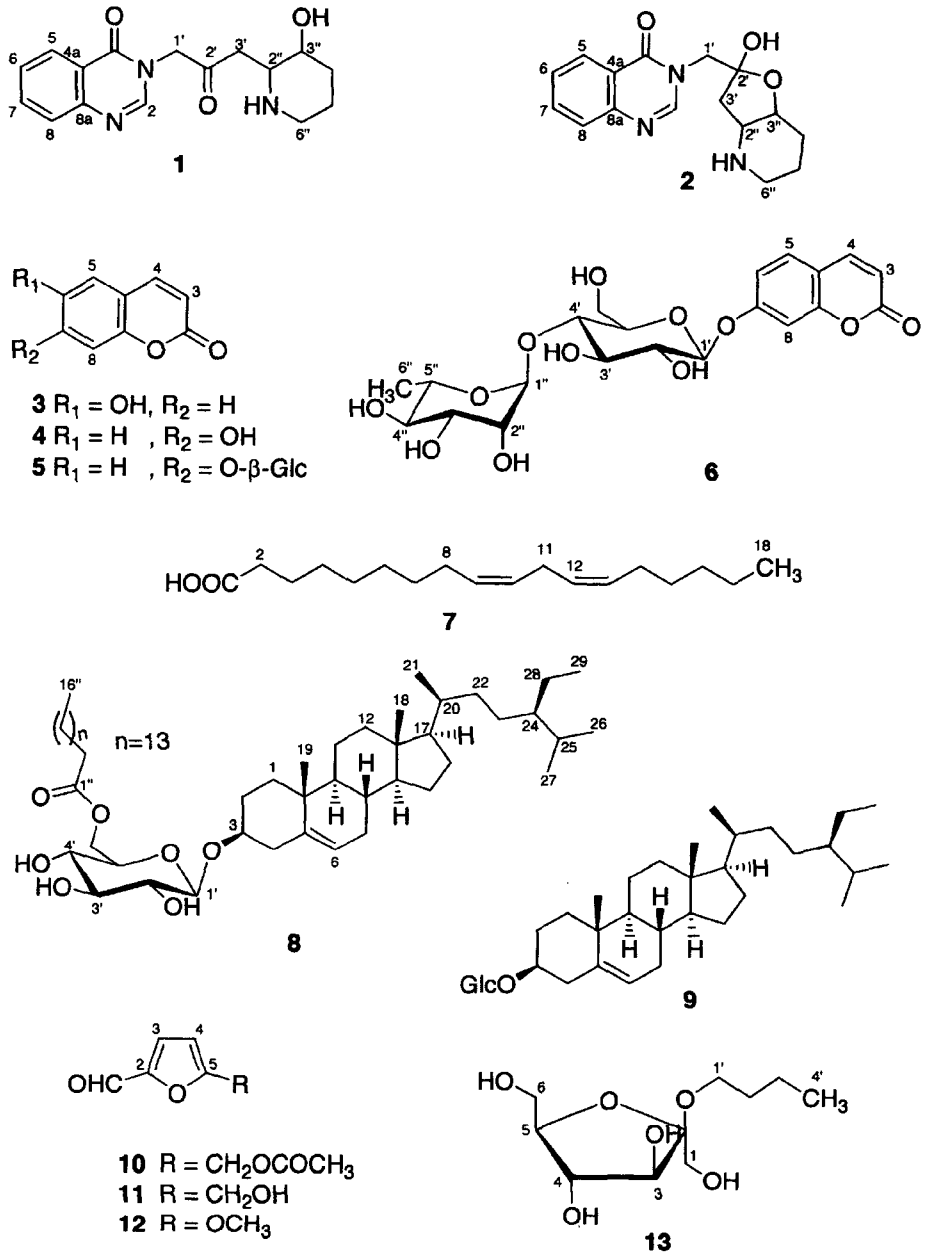

Fig. 1. The structures of compounds **1-13**

are very rare in nature. To the best of our knowledge, this is the first report of the isolation of **6** from a natural source.

Compound **3** was identified as 6-hydroxy coumarin through comparison of its spectral data (Table II and experimental section) with the previously published ones (Guidugli *et al.*, 1986). In the same manner, the structure of compound **5** was deduced to be umbelliferone-7-O-β-D-glucopyranoside (skimmin) (Strack *et al.*, 1989 and Reisch and Achenbach, 1992).

On the other hand, compounds isolated from the leaves were identified as: umbelliferone (**4**) (Reisch *et al.*, 1992), linoleic acid (**7**) (Fauhl *et al.*, 2000), β-sitosteryl-3-O-β-D-(6'-hexadecanoyl)-glucopyranoside (**8**) (Ulubelen *et al.*, 1988), β-sitosterol-3-O-β-D-glucoside (**9**) (Kojima *et al.*, 1990), 5-(acetoxymethyl)-2-furfuraldehyde (**10**) (Hearn, 1996 and Numata *et al.*, 1990), 5-(hydroxymethyl)-2-furfuraldehyde (**11**) (Nishibe *et al.*, 1973), 5-methoxy-2-furfuraldehyde (**12**), butyl-β-D-fructofuranoside (**13**) (Zhang *et al.*, 1996). The characterization was based on study of their physical, chemical, and spectral data as well as comparison with those in the literature. Compound **13** is most probably an artifact due to prolonged contact of fructose with *n*-BuOH. 5-(Hydroxymethyl)-2-furfuraldehyde (**11**) has been previously isolated from the fruits of *Prunus mum* and from

several other natural sources and proved to possess calcium antagonist as well as aldose reductase inhibitory activities (Ichikawa *et al.*, 1989 and Shimizu *et al.*, 1993). However, we could not trace any previous isolation of 5-methoxy-2-furfuraldehyde (**12**) from a natural source.

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