Secoiridoid Glycoside and Alkaloid Constituents of *Hydrangea chinensis*

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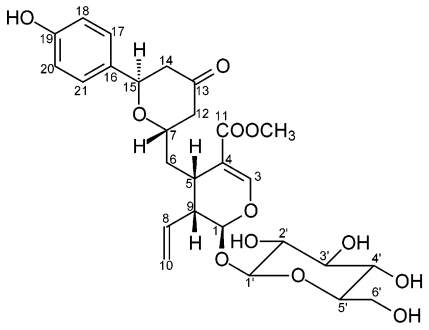
Received May 22, 2003

A new secoiridoid glycoside, hydrachoside A (**1**), along with 14 known compounds, was isolated from the leaves of *Hydrangea chinensis*. The absolute stereochemistry of the side chain attached to C-15 on the secoiridoid glycoside hydrangenoside E (**2**) was determined by NMR spectral analysis. The structures of compounds **1** and **2** were elucidated on the basis of spectral data. The previously reported structure, hydrachine A (**3**), was revised as its epimer, (−)-neodichroine (**4**), a new compound.

Hydrangea chinensis Shimizu & Kao (Saxifragaceae) is distributed at low altitudes in Taiwan and southern mainland China. Its roots are used in traditional Chinese medicine for headache, as a diuretic, and as an antimalarial agent.[1] As part of our research on biologically active compounds from Chinese medicinal plants, we have investigated the secondary metabolites of *H. chinensis* growing in Taiwan. Recently, 16 compounds were reported by our group from the roots of this plant.[2] We report herein the isolation and structural elucidation of 15 compounds, including four secoiridoid glycosides [hydrachoside A (**1**), hydrangenoside E (**2**),[3,4] 7-*epi*-vogeloside,[5] and sweroside[6]], four terpenoids (friedelin,[7] 3β-friedelanol,[8] squalene,[9] and phytol[10]), five simple aromatics (salidroside,[11] *p*-hydroxybenzoic acid,[12] *p*-anisaldehyde,[12] vanillic acid,[13] and *p*-hydroxybenzaldehyde[12]), one organic acid (shikimic acid[14]), and one fatty acid (palmitic acid[15]), from the leaves of *H. chinensis*. Among them, compound **1** bears a novel skeleton. The stereochemistry of the known compound **2** was confirmed from its NOESY spectra. Furthermore, the previously reported structure, hydrachine A (**3**),[2] was revised as the new epimeric structure (−)-neodichroine (**4**).[16,17]

Compound **1** was isolated as a colorless syrup with a molecular formula of $C_{27}H_{34}O_{12}$, as established by HR-FABMS. The optical rotation value $[\alpha]^{25}_D$ was −31.2° (*c* 0.02, methanol). The IR spectrum of **1** revealed the presence of carbonyls at 1694 cm^{-1}, hydroxyls at 3396 cm^{-1}, and a benzene ring at 1621, 1519 cm^{-1}. In the ^{1}H NMR spectrum, a typical downfield olefinic proton of a secoiridoid at δ 7.44 (s, H-3), a semiacetalic proton signal at δ 5.51 (d, J = 5.5 Hz, H-1), an anomeric proton signal at δ 4.68 (d, J = 7.7 Hz, H-1′), a monosubstituted vinyl group at δ 5.71 (1H, dd, J = 17.0, 10.2 Hz, H-8), 5.27 (1H, d, J = 17.0 Hz, H-10 *trans*), and 5.24 (1H, d, J = 10.2 Hz, H-10 *cis*), and a secoiridoidal methyl ester signal at δ 3.69 (3H, s) indicated the presence of a 1-*O*-glucosyl secoiridoid moiety. Furthermore, an acetal carbon at δ 97.9 and an anomeric carbon at δ 100.2, together with the other secoiridoidal and glucosyl carbons in the ^{13}C NMR spectrum (Table 1), confirmed these assignments. In addition, an A_2B_2 system at δ 7.04 (2H, d, J = 8.5 Hz, H-17, 21) and 6.70 (2H, d, J = 8.5 Hz, H-18, 20) in the ^{1}H NMR spectrum suggested the presence of a *p*-disubstituted benzene.[3,4] The *p*-substituted benzene moiety was further confirmed by the downfield carbon signals at δ 156.5 and 133.6 and two signals with double intensities at δ 130.5 and 116.2. On the basis of the previous literature[3,4] and from the rest of the ^{13}C NMR data, the remaining part of the molecule of **1** between



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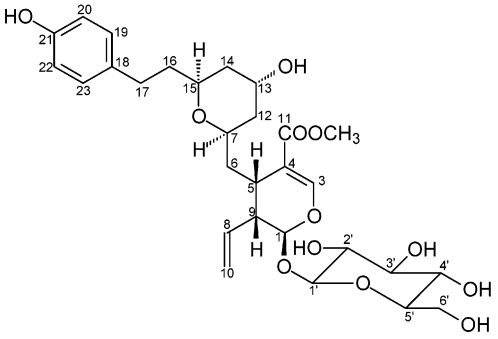

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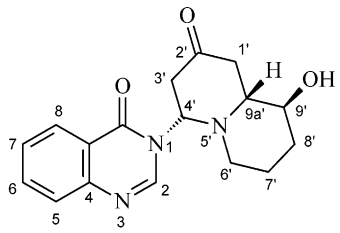

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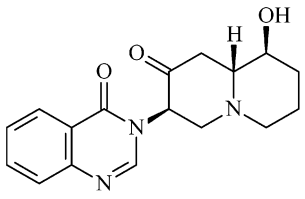

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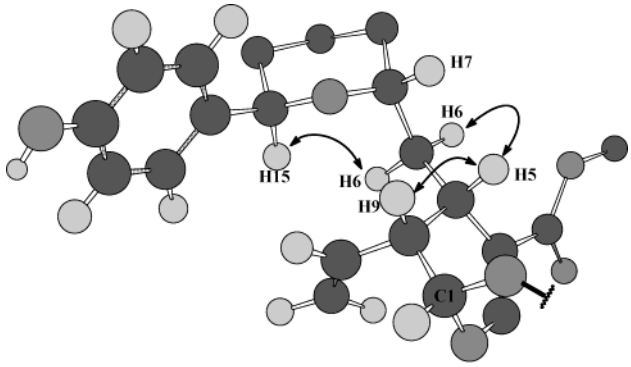
10.1021/np0302394 CCC: $25.00
Published on Web 08/20/2003

Table 1. ^{1}H and ^{13}C NMR Spectral Data of **1**[a,b]

position	δ_C	δ_H (mult.; *J* in Hz)	HMBC (^{1}H → ^{13}C)
1	97.9	5.51 (1H, d, 5.5) pseudoequatorial	C-1′, C-3, C-8
3	153.5	7.44 (1H, s)	C-1, C-4, C-11
4	112.0		
5	29.9	2.94 (1H, dd, 9.0, 6.0) pseudoaxial	C-3, C-4, C-6, C-8, C-9, C-11
6	36.0	1.78 (2H, dd, 9.0, 6.0)	C-4, C-7, C-9
7	71.6	3.25 (1H, m) equatorial	C-13
8	135.7	5.71 (1H, dd, 17.0, 10.2)	C-9
9	45.9	2.67 (1H, m) pseudoequatorial	C-1, C-4
10	120.0	5.27 (1H, d, 17.0) *trans* 5.24 (1H, d, 10.2) *cis*	C-8, C-9
11	169.2		
12	31.7	2.57 (1H, dd, 14.0, 6.0) axial 2.66 (1H, m) equatorial	C-6, C-7
13	210.3		
14	37.1	1.67 (1H, m) axial 1.91 (1H, m) equatorial	C-12, C-15, C-16
15	72.7	4.01 (1H, dd, 9.3, 4.8) axial	C-13, C-14, C-17
16	133.6		
17	130.5	7.04 (1H, d, 8.5)	C-18, C-19
18	116.2	6.70 (1H, d, 8.5)	C-16, C-19
19	156.5		
20	116.2	6.70 (1H, d, 8.5)	C-16, C-19
21	130.5	7.04 (1H, d, 8.5)	C-19, C-20
OCH_3	51.7	3.69 (3H, s)	C-11
1′	100.2	4.68 (1H, d, 7.5)	C-1
2′	74.7	3.18 (1H, dd, 9.0, 7.5)	C-1′
3′	78.5	3.30 (1H, m)	C-2′
4′	72.1	4.30 (1H, dd, 7.5, 6.5)	
5′	78.0	3.34 (1H, d, 7.5)	C-4′, C-6′
6′	62.8	3.65 (1H, dd, 12.0, 7.5) 3.90 (1H, dd, 12.0, 2.0)	

[a] At 500 MHz. [b] In CD_3OD.



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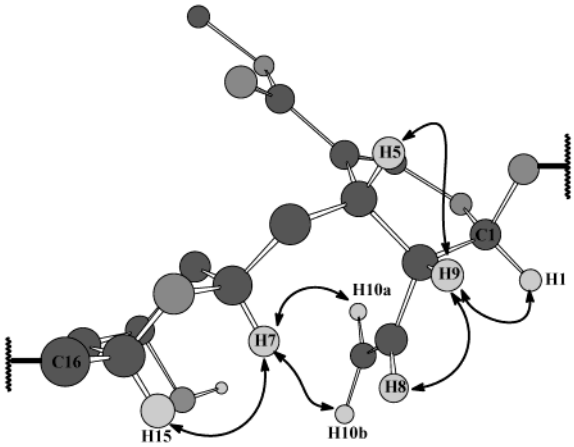

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Figure 1. Key NOESY correlations of **1** and **2**.

the phenyl and the 1-*O*-glucosyl secoiridoid moieties was assigned as a 2,6-disubstituted tetrahydropyran-4-one moiety, with one carbonyl signal at δ 210.3, two oxymethine carbon signals at δ 72.7 and 71.6, and two methylene carbon signals at δ 37.1 and 31.7.

The stereochemistry of C-1, C-5, and C-9 in **1** was determined by analysis of the ^{1}H and ^{13} C NMR data, the NOESY correlations (Figure 1), and inspection of a computer-generated model. On a biosynthetic basis, H-5 was assigned with a pseudo-β-axial configuration.[20,21] The coupling constant of H-1 (5.5 Hz) showed that it is α-oriented and the glucosyl moiety is in the β-position, similar to those recorded in the previous literature.[18,19] Subsequently, the β-orientation of H-9 was confirmed by the NOE correlation and coupling constants.

The stereochemistry of C-15 was determined by a CD method. Compound **1** possesses two chromophores: one appears for the phenyltetrahydropyranone moiety and the other is an α,β-unsaturated carboxylic ester of a secoiridoid. In 1982, Haslegrave et al. reported the CD data of (−)-2-phenyltetrahydropyran-4-one, which possesses a 2*S* configuration and showed a negative Cotton effect at 286 nm.[22] The CD spectrum of **1** exhibited a positive Cotton effect at 286 nm, which indicated that the configuration of C-15 should be *R* (Figure S1, Supporting Information). On the basis of Woodward's law and published UV data for secoiridoids,[23] the latter chromophore should have a UV absorption maximum at 230−235 nm, which will not interfere with the observation of the CD absorption for the stereochemistry at C-15. The vicinal *J* values between H-14 and H-15 were 9.3 and 4.8 Hz, indicating that H-15 is in an α-axial orientation. Furthermore, an NOE correlation between H-15 and H-6 and the absence of any NOE correlation between H-15 and H-7 suggested that H-7 is β-equatorial. Accordingly, the structure and absolute stereochemistry of **1** was proved for hydrachoside A, which was supported from its COSY, HMQC, and HMBC NMR data (Table 1).

In determining the stereochemistry of hydrangenoside E (**2**), Inouye and co-workers used some innovative synthetic methods to prove the stereochemistry of H-7 and H-15 in a series of *Hydrangea* secoiridoid glucosides.[3,4] However, they did not record the NMR data fully, which can now be easily applied for the determination and comparison of these compounds. Using modern NMR techniques, the ^{1}H and ^{13}C NMR data are detailed for **2** in Table 2. In the NOESY spectrum (Figure 1), H-7 and H-15 showed a significant correlation, proving that they have a 1,3-diaxial configuration. Important correlations between H-7 and H-10a/H-10b gave evidence that H-7 is in the α-orientation. Therefore, both H-7 and H-15 can be assigned as α-axial, and C-7 and C-15 possess the *R* and *S* configuration, respectively. The orientation of H-15 is the same as that of **1**. The absolute configuration of **2** was coincident with the study by Inouye et al.[3,4] Because compound **2** is without the chromophore, 2-phenyltetrahydropyran-4-one, as found in **1**, the Cotton effect at 286 nm disappeared (Figure S1) in its CD spectrum.

In 2002, Michael published an updated review of quinoline, quinazoline, and acridone alkaloids.[16] He mentioned that hydrachine A (**3**)[2] and (+)-neodichroine (**4**)[16,17] might be the same compound. After checking the NMR data of hydrachine A (**3**) using both deuterated chloroform and pyridine as solvents, the ^{1}H and ^{13}C NMR data obtained are summarized in Table S1 (Supporting Information). In our past assignments, the structure of **3** is incorrect

Table 2. ^{1}H and ^{13}C NMR Spectral Data of **2**[a,b]

position	δ_C	δ_H (mult.; *J* Hz)	HMBC (^{1}H → ^{13}C)
1	97.9	5.55 (1H, d, 4.4) pseudoequatorial	C-1′, C-8, C-5
3	152.9	7.42 (1H, d, 1.6)	C-1, C-5, C-11
4	112.0		
5	28.3	3.17 (1H, m) pseudoaxial	C-7, C-3, C-11
6	35.7	1.35 (1H, m)	C-4
		2.15 (1H, m)	C-12
7	69.9	3.89 (1H, dd, 11.8, 2.2) axial	C-13
8	135.4	5.68 (1H, ddd, 17.0, 10.2, 2.4)	C-5
9	44.6	2.79 (1H, m) pseudo-equatorial	C-1, C-4, C-10
10	120.0	5.23 (1H, dd, 10.2, 1.4) *cis*	C-9
		5.28 (1H, dd, 17.0, 1.4) *trans*	
11	169.5		
12	39.1	1.50 (1H, m) axial	C-14
		1.63 (1H, m) equatorial	
13	65.3	4.11 (1H, br. t, 2.6) equatorial	C-15
14	40.2	1.39 (1H, m) axial	C-12
		1.59(1H, m) equatorial	
15	71.7	3.65 (1H, m) axial	C-17, C-13
16	39.6	1.52 (1H, m)	C-15
		1.63 (1H, m)	C-18
17	31.6	2.64 (2H, m)	C-15
18	134.4		
19	130.6	7.06 (1H, d, 8.6)	C-17
20	116.2	6.75 (1H, d, 8.6)	C-21
21	156.1		
22	116.2	6.75 (1H, d, 8.6)	C-18
23	130.6	7.06 (1H, d, 8.6)	C17, C19
OCH_3	51.7	3.69 (3H, s)	C-11
1′	99.9	4.69 (1H, d, 7.6)	C-1
2′	74.6	3.23 (1H, dd, 9.0, 7.6)	C-3′
3′	78.3	3.30 (1H, m)	C-5′
4′	71.5	3.17 (1H, t, 8.0)	C-5′
5′	77.9	3.35 (1H, d, 8.0)	C-6′
6′	62.9	3.67 (1H, dd, 12.0, 5.0)	C-4′
		3.90 (1H, dd, 12.0, 2.4)	

[a] At 400 MHz. [b] In CD_3OD.

because of an error in the linkage of the quinazolinone unit to C-4′. Deng et al. are correct in their assignment of the structural skeleton but possibly wrong in the stereochemistry determination of (+)-neodichroine, which has a positive optical rotation value {$[\alpha]_D$ +198.8° (*c* 0.17, MeOH)}.[17] Michael also suggested the stereochemistry of (+)-neodichroine.[16] When compound **3** was subjected to an optical rotation measurement, it was interesting to find that it possesses a negative optical rotation value {$[\alpha]_D$ −25.3° (*c* 0.20, MeOH)}, which is different from (+)-neodichroine. There is no available CD model which can be used to determine the stereochemistry of neodichroine. Furthermore, (+)-febrifugine and (+)-isofebrifugine were also isolated from this species, and insufficient samples were available to determine their stereochemistry. The structure and stereochemistry of (±)-neodichroine (**4**) were assigned as Michael has predicted.

The biosynthetic pathways of compounds **1** and **2** may be through an aldol-type condensation of secologanin with [(C_6−C_3) + (1 × acetyl)], in the case of compound **1**, or with [(C_6−C_3) + (2 × acetyl)], in the case of compound **2**, formed by the shikimate−malonate route, followed by decarboxylation and ether-ring formation.[24]

Experimental Section

General Experimental Procedures. Melting points were determined on a Laboratory Devices Mel-Temp II apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained 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 Varian Inova 500, Varian Unity Plus 400 MHz, or Varian Gemini 200 MHz spectrometers using TMS as internal standard. Chemical shifts are reported in parts per million (δ), and coupling constants (*J*) are expressed in hertz. LREIMS were recorded on a JEOL JMS-SX/SX 102A mass spectrometer or Quattro GC−MS spectrometer having a direct inlet system. HREIMS were measured on a JEOL JMS-HX 110 mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography. Sephadex LH-20 (Amersham Pharmacia Biotech AB) was used for separation and/or purification. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for preparative TLC. Spots were detected by spraying with Dragendroff's reagent or 50% H_2SO_4 and heating. The molecular models were generated with the ChemBats3D Ultra 7.0 program, and with energy minimizations by MM2 Software.

Plant Material. The leaves of *H. chinensis* Shimizu & Kao were collected from Pintong County, Taiwan, in April 2000. A voucher specimen (Saxifra-1-1), identified by Dr. Hsin-Fu Yen (Associate Researcher of Plant Taxonomy, National Museum of Natural Science, Taichung, Taiwan), is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. Fresh leaves of *H. chinensis* (1.5 kg) were cut into small pieces and then extracted with MeOH (5 L × 6) at room temperature. The combined MeOH extracts were filtered, and the solvent was removed under reduced pressure to yield a brownish viscous residue (200 g). The residue was dissolved in a water/methanol mixture (95:5) and then successively extracted with *n*-hexane, EtOAc, and finally *n*-BuOH. Each organic layer was separately concentrated under reduced pressure to yield the *n*-hexane (52 g), EtOAc (70 g), and *n*-BuOH (60 g) extracts. The EtOAc and *n*-BuOH extracts showed significant cytotoxic activity toward the HONE-1 and NUGC cancer cell lines. The *n*-hexane extract was chromatographed over silica gel (1500 g) and eluted in a gradient fashion with *n*-hexane/$CHCl_3$ mixtures. Purification of the eluted fractions afforded squalene (23 mg) (*n*-hexane/EtOAc, 10:1, R_f 0.71), friedelin (46 mg) (*n*-hexane/EtOAc, 10:1, R_f 0.25), 3β-friedelanol (2 mg) (*n*-hexane/EtOAc, 10:1, R_f 0.27), phytol (23 mg) ($CHCl_3$/MeOH, 10:1, R_f 0.85), and palmitic acid (18 mg) (*n*-hexane/EtOAc, 15:1, R_f 0.71). The ethyl acetate extract was chromatographed over a column containing silica gel (2100 g) and using $CHCl_3$/MeOH mixtures of increasing polarity for elution. The eluted fractions afforded *p*-anisaldehyde (3 mg) ($CHCl_3$/acetone, 5:1, R_f 0.72), *p*-hydroxybenzaldehyde (7 mg) ($CHCl_3$/acetone, 5:1, R_f 0.55), **2** (11 mg) ($CHCl_3$/MeOH, 10:1, R_f 0.56), **1** (1.4 mg) ($CHCl_3$/MeOH, 10:1, R_f 0.60), *p*-hydroxybenzoic acid (5 mg) ($CHCl_3$/MeOH, 10:1, R_f 0.63), salidroside (8 mg) ($CHCl_3$/MeOH, 10:1, R_f 0.80), shikimic acid (18 mg) ($CHCl_3$/acetone, 5:1, R_f 0.71), 7-*epi*-vogeloside (6 mg) ($CHCl_3$/MeOH, 10:1, R_f 0.59), sweroside (53 mg) ($CHCl_3$/MeOH, 10:1, R_f 0.61), and vanillic acid (4 mg) ($CHCl_3$/MeOH, 10:1, R_f 0.91).

Hydrachoside A (1): colorless syrup; $[\alpha]_D^{25}$ −31.2° (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (4.34), 279 (3.24) nm; IR (neat) ν_{max} 3396, 1694, 1710 br, 1621, 1519 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; FABMS *m/z* 551 $[M + H]^+$ (0.1), 207 (2.1), 176 (2.5), 136 (30), 107 (16), 73 (5), 57 (16); HRFABMS *m/z* 551.2147 $[M + H]^+$ (calcd for $C_{27}H_{34}O_{12}$, 551.2129).

Hydrangenoside E (2): colorless oily liquid; $[\alpha]_D^{25}$ −40.9° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (4.62), 279 (3.73) nm; IR (neat) ν_{max} 3430, 1713, 1611, 1514 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 2; FABMS *m/z* 581 $[M + H]^+$ (0.21), 413 (3), 329 (3), 289 (2.9), 176 (58), 154 (13), 136 (47); HRFABMS *m/z* 581.2606 $[M + H]^+$ (calcd for $C_{29}H_{40}O_{12}$, 581.2598).

Acknowledgment. This investigation was supported by a grant from the National Science Council and the National Health Research Institute of the Republic of China (Grant No. NSC-90-2323-B-037-003).

Supporting Information Available: Table of NMR spectral data for compound **3** and CD spectra of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP0302394