Hydrachine A, a Novel Alkaloid from the Roots of *Hydrangea chinensis*

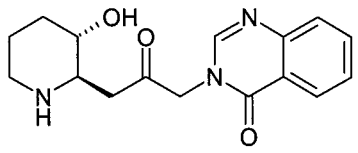
Ramesh Patnam, Fang-Rong Chang, Chung-Yi Chen, Reen-Yen Kuo, Yue-Han Lee, and Yang-Chang Wu*

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan

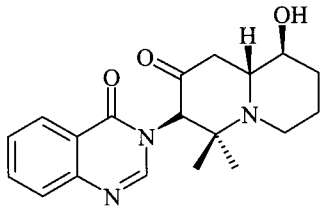
Received February 22, 2001

A novel alkaloid, hydrachine A (**3**), has been isolated, along with 15 known compounds, from the roots of *Hydrangea chinensis*. The structure and stereochemistry of the new alkaloid **3** was determined using extensive 2D NMR data.

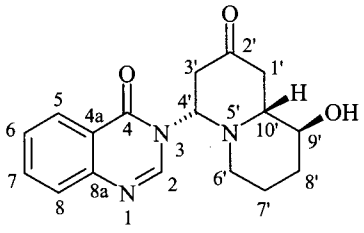
Hydrangea chinensis is a Chinese medicinal plant used in treatment of malaria and cadiovascular diseases.[1] Febrifugine (**1**) was isolated as an active principle against malaria from the roots of *Hydrangea arten*,[2] and the acetone adduct (**2**) of febrifugine was found to be equally effective against *Plasmodium berghei in vivo* as the clinically used drug chloroquine.[3] Prior studies have shown that members of the genus *Hydrangea* produce alkaloids,[2] steroids,[4] triterpenes,[5] isocoumarins,[5] secoiridoid glycosides,[6] and flavanoids.[7] As part of our research on biologically active compounds from Chinese medicinal plants,[8] we investigated the Formosan plant *H. chinenses*. We now report the isolation and structural elucidation of the novel alkaloid hydrachine A (**3**), along with 15 known compounds from the roots of this species.



1



2



3

The crude methanol extract of the roots of this plant showed more than 90% inhibition against HONE-1 (human nasopharyngeal carcinoma) and NUGC (human gastric cancer) cancer cell lines at concentration of 20 μg/mL. This extract was partitioned between *n*-hexane−H_2O, EtOAc−H_2O, and *n*-BuOH−H_2O. Bioactive EtOAc and *n*-BuOH fractions were chromatographed separately on Sephadex LH-20, followed by silica gel column chromatography and preparative TLC (PTLC), which afforded a novel alkaloid (**3**) and 15 known compounds.

Compound **3** was obtained as a semisolid, $[\alpha]^{25}_{D}$ +25.32° (*c* 0.2, $CHCl_3$), and analyzed for $C_{17}H_{19}N_3O_3$ (HREIMS). The UV spectrum of **3** showed absorptions at λ_{max} 224, 230, 233, 265, and 302, indicating the presence of a 4-quinozolone moiety.[9] The IR spectrum of **3** indicated the presence of a trans-fused quinolizidine ring system, including strong Bohlmann bands at 2800−2700 cm^{-1} and an amide (1670 cm^{-1}), a hydroxyl (3600 cm^{-1}), and a carbonyl group (1730 cm^{-1}).[9]

The ^{1}H NMR spectrum of **3** showed signals similar to that of **2** except for the presence of a pair of methylene protons at δ 3.34 (dd) and 2.84 (dd), instead of two methyl groups attached at C-3′, and in the chemical shift of H-4′. The ^{13}C NMR spectrum of **3** showed signals similar to that of **2**, except for the absence of two methyl groups. Comparison of the ^{1}H and ^{13}C spectral data of **2** and **3** suggested that the 4-quinazolinone should be substituted at the C-3′ or C-4′ position of the quinolizidine ring in **3**. The atomic connectivity was further confirmed by the HMBC spectrum. In the HMBC spectrum of **3**, the signal at δ 5.70 showed correlations with signals at δ 145.2, 58.9, 201.3, 160.5, 67.0, and 54.1; this clearly indicated that the 4-quinazolinone was substituted at the C-4′ position of the quinolizidine, not the C-3′ position as in **2**. The COSY spectrum supported the assignment of two fragments between H-3′/H-4′, as well as between H-6′/H-7′/H-8′/H-9′/H-10′/H-1′, which further confirmed the quinolizidine moiety.

The stereochemistry of **3** was determined by analysis of the ^{1}H NMR coupling constants and the NOESY correlations (Figure 1). The spin−spin analysis of H-4′/H-3′, H-10′/H-1′, H-10′/H-9′, and H-9′/H-8′ provided the information needed for the assignment of the H-4′, H-10′, and H-9′ configurations.[10,11] The coupling constants $^3J_{H-4'/H-3'pseudoeq}$ (6.4 Hz) and $^3J_{H-4'/H-3'pseudoax}$ (11.2 Hz) suggested that H-4′ is in the axial orientation. This confirms that the 4-quinozolone at the C-4′ position is equatorial. The coupling constants $^3J_{H-10'/H-1'pseudoeq}$ (3.2 Hz) and $^3J_{H-10'/H-1'pseudoax}$ (11.2 Hz) suggested that H-10′ is in the axial orientation. The coupling constant $^3J_{H-9'/H-10'ax}$ (9.0 Hz) suggested that H-9′ is axial, which was further confirmed by the coupling constants $^3J_{H-9'/H-8'eq}$ (5.0 Hz) and $^3J_{H-9'/H-8'ax}$ (11.0 Hz). Therefore, the hydroxyl at the C-9′ is in the equatorial orientation. These assignments were further confirmed by NOESY correlations. In the NOESY spectrum of **3**, the signal at δ 3.46 (1H, ddd, H-9′) showed correlations with the signals at δ 2.50 (1H, dd, H-1′$_{pseudoax}$) and 1.80 (1H, m, H-7′$_{ax}$), which confirms that H-9′ is axial. The signal at δ 2.28 (1H, ddd, H-10′) showed correlations with the signals at δ 2.93 (1H, dd, H-6′$_{ax}$), 1.29 (1H, m, H-8′$_{ax}$), and 5.70 (1H, dd, H-4′), further confirming the stereochemistry of

* To whom correspondence should be addressed. Tel: +886-7-3121101, ext. 2197. Fax: +886-7-3114773. E-mail: yachwu@cc.kmu.edu.tw.

10.1021/np010091t CCC: $20.00
Published on Web 06/16/2001

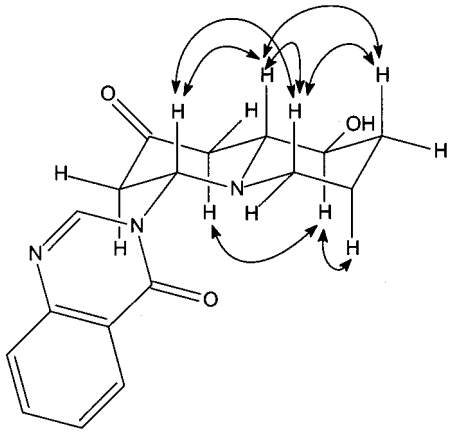


Figure 1. Key NOESY correlations of **3**.

3. Thus, the structure of **3** was established as 3-[9′β-hydroxy-2′-oxo-4′α-quinolizidyl]-4-quinazolinone, which we named hydrachine A. To the best of our knowledge, this is the first report of this class of alkaloid from natural sources.

A number of known compounds (see Experimental Section) were characterized by comparing their spectral data with the literature.[4,5,12−19] None of the compounds isolated from *H. chinensis* showed any significant activity against HONE-1 and NUGC cancer cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined on a Laboratory Devices Mel-Temp II 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 Unity 400 MHz or Varian Unity 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. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.5 mm) were used for PTLC. Spots were detected by spraying with Dragendroff's reagent or 50% H_2SO_4 and heating.

Plant Material. The roots 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 Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The roots of *H. chinensis* (2 kg) were extracted with MeOH (3 × 5 L) at room temperature. The combined MeOH extracts were filtered, and the solvent was removed under reduced pressure to yield a brownish viscous residue (100.5 g). The crude MeOH extract was partitioned between *n*-hexane−H_2O, EtOAc−H_2O, and *n*-BuOH−H_2O. The organic layers were concentrated under reduced pressure to yield an *n*-hexane extract (19.5 g), an EtOAc extract (20 g), and an *n*-BuOH extract (50.5 g). The EtOAc and *n*-BuOH extracts showed significant cytotoxicity toward HONE-1 and NUGC cancer cell lines. The EtOAc extract was subjected to gel filtration chromatography [Sephadex LH-20, MeOH] followed by silica gel chromatography eluting with *n*-hexane, *n*-hexanes−EtOAc mixtures, and finally with EtOAc to afford umbelliferone (200 mg),[5] 7-methoxycoumarin (10 mg),[12] 5-hydroxycoumarin (5 mg),[13] 7-hydroxy-8-methoxycoumarin (5 mg),[14] isoarborinol (100 mg),[5] rubiarbonol B (10 mg),[5] hydrangenol (2 mg),[5] hydrangenoside A (3 mg),[6] β-sitosterol (1.5 g),[4] β-sitosterol-β-D-glucopyranoside (50 mg),[15] syringaldehyde (5 mg),[16] 4-hydroxy-*trans*-cinnamic acid methyl ester (3 mg),[17] *p*-coumaric acid methyl ester (2 mg),[18] and *p*-hydroxybenzaldehyde (10 mg). The *n*-BuOH extract was subjected to gel filtration chromatography [Sephaex LH-20, MeOH], reversed-phase Diaion HP-20 column chromatography eluting with H_2O, H_2O−MeOH mixtures, and finally with MeOH, silica gel column chromatography eluting with $CHCl_3$, $CHCl_3$−MeOH mixtures, and finally with MeOH, and preparative TLC to afford 4-quinazolone (20 mg)[19] and hydrachine A (**3**, 10 mg).

Hydrachine A (3) (3-[9β-hydroxy-2-oxo-4α-quinolizidyl]-4-quinazolinone): semisolid; $[\alpha]^{25}_D$ +25.32° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 224 (1.47), 230 (1.35), 233 (1.30), 265 (0.59), and 302 (0.28) nm; IR (KBr) ν_{max} 3600, 2700−2800, 1730, and 1670 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.28 (1H, dd, J = 8.0, 1.6 Hz, H-5), 7.88 (1H, s, H-2), 7.80 (1H, ddd, J = 8.4, 7.2, 1.6 Hz, H-7), 7.71 (1H, dd, J = 8.4, 1.6 Hz, H-8), 7.50 (1H, ddd, J = 8.0, 7.2, 1.6 Hz, H-6), 5.70 (1H, dd, J = 11.2, 6.40 Hz, H-4′), 3.46 (1H, ddd, J = 11.0, 9.0, 5.0 Hz, H-9′), 3.34 (1H, dd, J = 10.4, 6.4 Hz, H-3′$_{pseudoeq}$), 3.18 (1H, dd, J = 15.2, 3.2 Hz, H-1′$_{pseudoeq}$), 2.93 (1H, br. dd, J = 13.0, 4.0 Hz, H-6′$_{ax}$), 2.84 (1H, dd, J = 11.2, 10.4 Hz, H-3′$_{pseudoax}$), 2.50 (1H, dd, J = 15.2, 11.2 Hz, H-1′$_{pseudoax}$), 2.28 (1H, ddd, J = 11.2, 9.0, 3.2 Hz, H-10′), 2.18 (1H, br. dt, J = 13.0, 4.0 Hz, H-6′$_{eq}$), 2.07 (1H, m, H-8′$_{eq}$), 1.80 (1H, m, H-7′$_{ax}$), 1.74 (1H, m, H-7′$_{eq}$), and 1.29 (1H, m, H-8′$_{ax}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.3 (s, C-17), 160.5 (s, C-4), 147.5 (s, C-8a), 147.5 (s, C-8a), 145.2 (d, C-2), 134.6 (d, C-7), 127.4 (d, C-6), 127.3 (d, C-8), 126.9 (d, C-5), 121.7 (s, C-4a), 72.8 (d, C-9′), 67.0 (d, C-10′), 59.4 (d, C-4′), 58.9 (t, C-5′), 54.1 (t, C-6′), 43.7 (t, C-1′), 32.8 (t, C-8′), and 23.0 (t, C-7′); EIMS *m*/*z* 314 $[M+1]^+$ (7), 199 (3), 171 (31), 168 (31), 167 (96), 166 (84), 149 (42), 139 (25), 122 (25), 111 (24), 110 (100), 103 (26), 96 (52), 82 (39), 76 (31), 55 (48), and 43 (40); FABMS *m*/*z* 314 $[M + 1]^+$ (39), 312 (17), 304 (27), 284 (35), 282 (58), 256 (13), 168 (47), 167 (28), 147 (28), 133 (49), 95 (31), 83 (38), 81 (50), 69 (100), 57 (66), 55 (76), and 43 (40); HREIMS *m*/*z* 313.1420 (calcd for $C_{17}H_{19}N_3O_3$, 313.1426).

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

References and Notes

(1) Chiang-Su New Medical College. *The Dictionary of Chinese Medicine* (I); Shun-Hai Scientific Technology: 1978; p 45.
(2) Ablondi, F.; Gordon, S.; Morton, J., II; Williams, J. H. *J. Org. Chem.* **1952**, *17*, 14−18.
(3) Takaya, Y.; Tasaka, H.; Chiba, T.; Uwai, K.; Tanitsu, M. A.; Kim, H. S.; Wataya, Y.; Miura, M.; Takeshita, M.; Oshima, Y. *J. Med. Chem.* **1999**, *42*, 3163−3166.
(4) Bani, T.; Debabrata, C.; Swapna, D.; Subhas, M. *J. Indian Chem. Soc.* **1975**, *52*, 1222.
(5) Yoshikawa, M.; Harada, E.; Naitoh, Y.; Inoue, K.; Matsuda, H.; Shimoda, H.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1994**, *42*, 2225−2230.
(6) Inouye, H.; Takeda, Y.; Uesato, S.; Uobe, K.; Hashimoto, T.; Shingu, T. *Tetrahedron Lett.* **1980**, *21*, 1059−1062.
(7) Yoshikawa, M.; Murakami, T.; Ueda, T.; Shimoda, H.; Yamahara, Y.; Matsuda, H. *Heterocycles* **1999**, *50*, 411−418.
(8) Pan, W. B.; Chang, F. R.; Wu, Y. C. *Chem. Pharm. Bull.* **2000**, *48*, 1350−1353.
(9) Battacharyya, J.; Pakrashi, S. C. *Heterocycles* **1979**, *12*, 929−931.
(10) Asres, K.; Gibbons, W. A.; Phillipson, J. D.; Mascagni, P. *J. Nat. Prod.* **1986**, *49*, 117−121.
(11) Maximo, P.; Lourenco, A. *J. Nat. Prod.* **2000**, *63*, 201−204.
(12) Banthorpe, D. V.; Bilyard, H. J.; Watson, D. G. *Phytochemistry* **1985**, *24*, 2677−2680.
(13) Guidugli, F. H.; Pestchanker, M. J.; De Salmeron, M. S. A.; Giordano, O. S. *Phytochemistry* **1986**, *25*, 1923−1926.
(14) Campbell, W. E.; Provan, G. J.; Waterman, P. G. *Phytochemistry* **1982**, *21*, 1457−1458.
(15) Anjaneyulu, A. S. R.; Rao, D. S. *Indian J. Chem.* **1997**, *36*, 424−433.
(16) Wu, Y. C.; Chang, G. Y.; Ko, F. N.; Teng, C. M. *Planta Med.* **1995**, *61*, 146−149.
(17) Chen, C. Y.; Chang, F. R.; Teng, C. M.; Wu, Y. C. *J. Chin. Chem. Soc.* **1999**, *46*, 77−86.
(18) Sanz, J. F.; Rustaiyan, A.; Marco, J. A. *Phytochemistry* **1990**, *29*, 2919−2921.
(19) Murata, K.; Takano, F.; Fushiya, S.; Oshima, Y. *J. Nat. Prod.* **1998**, *61*, 729−733.

NP010091T