Dioflorin, a Minor Flavonoid from *Dioclea grandiflora*

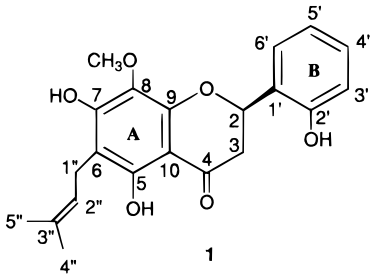
Jnanabrata Bhattacharyya,*,† George Majetich,*,† Tammy M. Jenkins,† and Reinaldo N. Almeida‡

Department of Chemistry, University of Georgia, Athens, Georgia 30602, and Laboratorio de Tecnologia Farmacêutica, Universidade Federal da Paraiba, 58.059-João Pessoa, PB, Brazil

Received September 19, 1997

Dioflorin (**1**) was isolated as a minor constituent from the rootbark of *Dioclea grandiflora*, the crude extract of which demonstrated analgesic activity. The structure of **1** has been determined to be 5,7,2′-trihydroxy-8-methoxy-6-(3-methyl-2-butenyl)flavanone on the basis of spectral analysis.

In view of the interesting biological activity demonstrated by the crude ethanolic extract of the rootbark of *Dioclea grandiflora* Mart. (Leguminosae), a vine that grows in the coastal plain of northeastern Brazil, we began a phytochemical investigation that resulted in the isolation of a new flavanone dioclein as the major component, having significant analgesic activity,[1] and a flavanonol dioclenol as a minor constituent.[2,3] The earlier chromatographic fractions obtained during the isolation of dioclein showed the presence of several other minor constituents, and further column chromatography on silica gel allowed us to isolate a new metabolite, dioflorin, $C_{21}H_{22}O_6$ (M^+ at *m/z* 370), mp 219−221 °C. Herein, we report the structure of dioflorin as a new flavanone (**1**) on the basis of its spectral characteristics.



1

A flavanone skeleton with a OH at C-5 was indicated in the ^{1}H NMR spectrum of **1** by the presence of a characteristic doublet of doublets at 5.72 ppm (1H, J = 12.6, 3.2 Hz) for H-2 along with a broad signal at 12.0 ppm (OH bonded to the carbonyl at C-4) and by a downfield signal at 197.7 ppm for a carbonyl (C-4) in the ^{13}C NMR spectrum.[4] The UV spectrum of **1** in CH_3-OH showed absorption maxima at 296 and 339 nm that shifted to 339 nm in NaOMe. The major absorption band (band II) also shifted to 339 nm in NaOAc. These spectral properties are characteristic of a 5,7-dihydroxyflavanone.[5]

The EIMS of **1** showed a strong M^+ at *m/z* 370 (75) and significant peaks at *m/z* 352 (M − 18), 337 (M − 18 − 15), 315 (M − 55), 309 (*m/z* 337 − 28), 297 (M − 18 − 55 or M − 55 − 18), 285, 250, 235, 222, 207, 195, 149, 121, 120, 91, 69, and 55. The ions at *m/z* 352, 337, and 309 are characteristic of a flavanone. The peaks at *m/z* 250 and 120 resulted from a retro-Diels−Alder (RDA) fragmentation (pathway I) of the heterocyclic ring of a flavanone skeleton.[5] The ion at *m/z* 120 was indicative of the presence of a OH group on the B-ring. The ions at *m/z* 315 (M − 55), 69, and 55 suggested a $(CH_3)_2$-C=CHCH$_2$− (prenyl) substituent.[5] Two 3H singlets at δ 1.60 and 1.58 (two CH_3), a 2H doublet at 3.25 (J = 7.3 Hz, −CH$_2$−), and a 1H triplet at 5.20 ppm (J = 7.3 Hz, −CH=) in the ^{1}H NMR spectrum and two CH_3 quartets at 25.7 and 17.7 ppm, a quaternary carbon resonance at 131.1 ppm, and signals for the −CH$_2$− and the −CH= at 22.0 and 122.7 ppm, respectively, in the ^{13}C NMR spectrum (DEPT) confirmed the presence of a prenyl group.[6]

The presence of an ArOCH$_3$ substituent in **1** was confirmed by the signal at 60.6 ppm in the ^{13}C NMR (DEPT) spectrum and by the 3H singlet at 3.80 ppm in the ^{1}H NMR spectrum. The positions ortho to the ArOCH$_3$ are both substituted[7] as demonstrated by its relatively lowfield shift (60.6).

The chemical shift of C-2 in C-2′-unsubstituted flavanones usually appears at 79.0 (±1.5 ppm) in the ^{13}C NMR spectrum.[8] In dioflorin (**1**), however, the C-2 signal appeared at 74.9 ppm, which is unusually upfield and indicated the presence of a substituent at C-2′ of the B-ring. The substituent at C-2′ is a hydroxyl group as supported by the presence of the ion at *m/z* 120 in the EIMS. The chemical shifts of the B-ring carbons are consistent with literature values for similarly substituted (C-2′-OH) flavanones.[9] Moreover, the ^{1}H NMR spectrum of **1**, which showed the presence of four adjacent ArH signals, supported this assignment.

On the basis of a 5,7,2′-trihydroxyflavanone partial structure for **1**, the prenyl and a OCH$_3$ group must be at C-6 and C-8 on the A ring. The ^{13}C NMR chemical shift of C-6 at 107.5 ppm is consistent with having the prenyl unit at C-6 on the basis of comparison with the spectra of similar compounds.[10] This dictates that the OCH$_3$ group must be at C-8. These specific assignments have been confirmed by a selective INEPT experiment based on ^{13}C−^{1}H long-range couplings.[11] When the H-1″ was pulsed, NOE enhancement of the signals at 156.7 (C-7), 155.8 (C-5), and 131.1 (C-3″) ppm was observed. This enhancement is only possible if the

* To whom correspondence should be addressed. Fax: (706) 542-9454. E-mail: majetich@sunchem.chem.uga.edu; jbhatt@sunchem.chem.uga.edu.
† University of Georgia.
‡ Universidade Federal da Paraiba.

S0163-3864(97)00431-X CCC: $15.00
Published on Web 02/25/1998

prenyl substituent is located at C-6. Thus, dioflorin is best represented by structure **1**.

Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer operating at 300.13 MHz for ^{1}H NMR and 75.47 MHz for ^{13}C NMR. The pulse sequence for the selective INEPT experiments was obtained by modifying the Bruker standard pulse sequence according to Bax.[11] The long-range coupling value (^{lr}J) for the selective INEPT experiments was 6 Hz. In the selective INEPT experiments, the decoupling power used were $S_1 = 45$ L (for soft pulse) and $S_2 = 0$ L (for decoupling with CPD). The power OL in the Bruker AC 300 spectrometer was approximately 1 W. The following delays were utilized: D1 = 3 s (relaxation delay for ^{1}H, prepare decoupler power for soft pulse); D2 = 1/4 *J* LR-0.15 s (refocusing delay); the D3 = 1/4 *J* LR-0.0075 s (for polarization transfer from a *CH*); D5 = 0.0075 s (for allowing evolution of antiphase magnetization), and D6 = 0.015 s (to refocus shifts and to set the decoupler power).

Plant Material. The plant material for the present work was collected and identified by Professor Maria de Fatima Agra in January 1991 near Joao Pessoa, Brazil. The voucher specimen was prepared and deposited at the LPX Herbarium at the Universidade Federal da Paraiba, Joao Pessoa, Brazil.[2,3]

Extraction and Isolation. The plant material was extracted as described previously.[3] The $CHCl_3$-soluble portion of the crude ethanolic extract was chromatrographed on silica gel (E. Merck, 230−400 mesh) and eluted with $CHCl_3$, and 75 mL fractions were collected. Fractional crystallization of fractions 21−24 from benzene−hexane gave a greenish-yellow powder. Recrystallizations from benzene gave pure dioflorin (**1**) as light-cream crystals: mp 219−221 °C (uncorrrected); $[\alpha]^{29.5}_D$ −106.4° (CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 296 (4.32) and 339 (3.78) nm; 339 nm in NaOMe, 280 ν_{max} and 319 nm in $AlCl_3$ (with or without HCl), and 297 and 339 nm in NaOAc; IR (film) 3380 (br H-bonded OH), 3240 (OH), 1644 (conj. C═O), 1597 (Ar), 1444 (Ar), 1361, 1272, 1239, 1173, 1027, 745 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.00 (1H, s, OH-5), 9.00 (1H, br s, OH), 8.80 (1H, br s, OH), 7.53 (1H, m, ArH), 7.12 (1H, m, ArH), 6.87 (2H, m, ArH), 5.72 (1H, dd, J = 12.6, 3.2 Hz, H-2), 5.20 (1H, t, J = 6.6 Hz, CH-2″), 3.79 (3H, s, $ArOCH_3$), 3.25 (2H, d, J = 6.6 Hz, CH_2-1″), 3.02−2.80 (2H, m, H-3), 1.60 (3H, s, CH_3-4″), 1.58 (3H, s, CH_3-5″); ^{13}C NMR ($CDCl_3$, 74.9 MHz) δ 197.8 (s, C-4), 156.7 (s, C-7), 155.8 (s, C-5), 153.8 (s, C-2′), 153.0 (s, C-9), 131.1 (s, C-3″), 129.2 (d, C-4′), 128.7 (s, C-8), 126.6 (d, C-6′), 126.0 (s, C-1′), 122.7 (d, C-2″), 120.0 (d, C-5′), 115.6 (s, C-3′), 107.5 (s, C-6), 102.6 (s, C-10), 74.9 (d, C-2), 61.0 (q, CH_3O), 42.4 (t, C-3), 25.7 (q, C-4"), 22.0 (t, C-1″), 17.6 (q, C-5″); EIMS *m/z* 370 (M^+), 352, 337, 309, 297, 281, 250, 249, 235, 222, 207, 195 (55), 149, 121, 120, 91, 69, 55, 44 (100); HRFABMS *m/z* 371.1521 $[M + H]^+$ (calcd for $C_{21}H_{22}O_6$, 371.1495).

Acknowledgment. The authors thank Professor Maria de Fatima Agra for the collection and identification of the plant material. Appreciation is extended to Dr. Robert S. Phillips for UV spectra and Mr. Paul Spearing for his help in carrying out the NMR experiments.

References and Notes

(1) Batista, J. S.; Almeida, R. N.; Bhattacharyya, J. *J. Ethnopharmacol.* **1995**, *45*, 207−210.
(2) Bhattacharyya, J.; Batista, J. S.; Almeida, R. N. *Phytochemistry* **1995**, *38*, 277−278.
(3) Bhattacharyya, J.; Majetich, G.; Spearing, P.; Almeida, R. N. *Phytochemistry* **1997**, *46*, 385−387.
(4) Agrawal, P. K.; Thakur, R. S.; Mansal, M. C. In *C-13 NMR of Flavonoids*; Agrawal, P. K., Ed.; Elsevier: Amsterdam, 1989; pp 95−182.
(5) Mabry, T. J., Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970; pp 165−174, 253−273.
(6) Mabry, T. J.; Markham, K. R. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds; Chapman and Hall: London, 1975; pp 78−126.
(7) Jain, A. C.; Zutshi, M. K. *Tetrahedron* **1973**, *29*, 3347−3350.
(8) Shirataki, Y.; Komatsu, M.; Yokoe, I.; Manaka, A. *Chem. Pharm. Bull.* **1981**, *29*, 3033−3036.
(9) Agrawal, P. K. In *C-13 NMR of Flavonoids*; Agrawal, P. K., Ed.; Elsevier: Amsterdam, 1989; pp 41−94.
(10) Mitscher, L. A.; Gollapudi, S. R.; Khanna, I. K.; Drake, S. D.; Hanumaiah, T.; Ramaswamy, T.; Jagannada Rao, K. V. *Phytochemistry* **1985**, *24*, 2885−2887.
(11) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314−318.

NP9704314