Schweinfurthins I and J from *Macaranga schweinfurthii*[⊥]

Paul Klausmeyer,[†] Que N. Van,[‡] Johnson Jato,[§] Thomas G. McCloud,[†] and John A. Beutler*[,∥]

Natural Products Support Group, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland 21702, Laboratory of Proteomics and Analytical Technologies, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, Maryland 21702, Molecular Targets Laboratory, Center for Cancer Research, NCI-Frederick, Frederick, Maryland 21702, and Bioresources Development and Conservation Programme-Cameroon, Yaounde, Cameroon

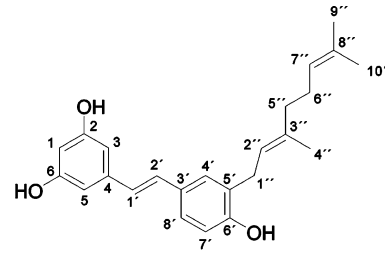
Received October 7, 2009

Demand for the experimental antineoplastic agent schweinfurthin A, for developmental testing, prompted a re-collection of leaf material of *Macaranga schweinfurthii* from the original collection site in Cameroon. During chromatographic purification of the organic solvent extract, analytical UPLC-PDA-TOFMS of stilbene-enriched fractions revealed the presence of six known schweinfurthins and two previously unknown stilbenes. The structures of these new compounds, schweinfurthins I and J (**1** and **2**), were elucidated by 1D- and 2D-NMR techniques.

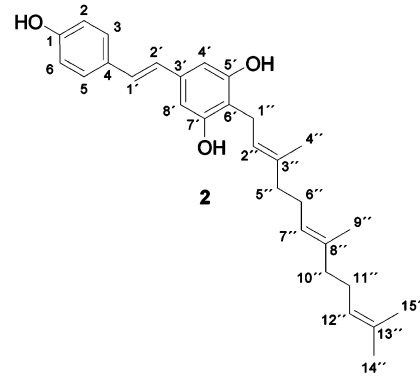
The schweinfurthins are a promising group of anticancer prenyl stilbenes obtained from several species of the plant genus *Macaranga* (Euphorbiaceae).[1−3] Various schweinfurthins display selectivity for CNS tumor and leukemia cell lines in the NCI 60-cell assay, and they appear to act by a novel mechanism of action.[4] In the course of scaled-up isolation of schweinfurthin A[1] from *Macaranga schweinfurthii* (Euphorbiaceae) as required for *in vivo* animal studies, two additional compounds exhibiting the characteristic UV absorbance spectra of a stilbene, yet possessing unique molecular weights within this stilbene class, were detected by UPLC/PDA/MS analysis. These unknown compounds, with molecular weights of 364 Da (**1**) and 432 Da (**2**), were well separated from other constituents during preparative C_8 HPLC.

High-resolution mass spectrometric analysis of **1** provided a molecular formula of $C_{24}H_{28}O_3$, corresponding to an unsaturation number of 11 and consistent with a stilbene with two additional degrees of unsaturation. This was supported by ^{13}C NMR analysis. The ^1H NMR spectrum of **1** showed two olefinic signals [δ 5.12 (m) and 5.35 (tq, J = 7.3, 1.3 Hz)], three methylene multiplets (δ 2.07, 2.14, 3.30), and three methyl doublets (δ 1.59, 1.64, 1.73, J = 1.3 Hz, each). Overall, these signals corresponded very closely to those published for the geranyl group of the known schweinfurthins A−C.[1] Analysis of the aromatic region of the ^1H NMR spectrum revealed two ortho-coupled doublets (δ 6.72, 7.18, J = 8.2 Hz each) and five meta-coupled doublets (δ 6.14, 6.42, 6.42, 7.18, 7.20, J = 2.1, 2.1, 2.1, 2.3, 2.3 Hz, respectively), representing two distinct long-range spin systems. The possibility of substituents bound symmetrically about an aromatic ring was suggested by the presence of a peak at δ 6.42 (H-3 and H-5) that integrated for two protons, each of these bearing an HSQC correlation to δ 105.8 (C-3/5). The signal at δ 6.14 was assigned to the H-1 position on the basis of its HMBC correlations with C-3/5 (δ 105.8). Attachment of OH groups to the C-2 and C-6 positions, resulting in a symmetrical orientation of like substituents, was reflected by the large chemical shift of C-2/6 (δ 159.8). To maintain symmetry, the attachment of the terminal ring to the *trans*-stilbene olefinic group was assigned to the C-4 position. No HMBC correlations were observed from the protons in the terminal ring to the geranyl group carbons, supporting attachment to the other ring.

Three protons, exhibiting an ortho- and a meta-coupling, were assigned to a second aromatic ring. The attachment of this ring structure to the trans-olefinic group (C-1′ and C-2′) was suggested by an HMBC resonance from H-2′ (δ 6.92, J = 16.2 Hz) to the quaternary carbon at C-3′ (δ 130.4). Long-range coupling of H-4′ to H-8′ (J = 2.3 Hz) established the relative positions of these two protons as meta, and a 1,1-ADEQUATE correlation showed that H-4′/8′ was adjacent to C-3′. The H-1″ proton of the geranyl group (δ 3.30) was correlated to C-5′ (δ 129.4) and to C-6′ (δ 156.4) by HMBC resonances, suggesting the proximity of a phenolic carbon to the site of attachment with the geranyl group. Quaternary and phenolic carbons were definitively assigned by ω_1-refocused 1,1-ADEQUATE[5] correlations (H-1 to C2/6, H-3/5 to C4, H-4′/8′ to C-3′, H-7′ to C-6′, H-1″ to C-5′, H-4″ and H-5″ to C-3″, and H-9″ and H-10″ to C-8″) (see Supporting Information). These experiments led to the structure of **1**.



1



2

HRMS analysis of **2** provided a molecular formula of $C_{29}H_{36}O_3$, (unsaturation number 12, one more than for **1**), which was supported by ^{13}C NMR analysis. The ^1H NMR spectrum of **2** revealed three

⊥ Dedicated to the late Dr. John W. Daly of NIDDK, NIH, Bethesda, Maryland, and to the late Dr. Richard E. Moore of the University of Hawaii at Manoa for their pioneering work on bioactive natural products.

* To whom correspondence should be addressed. Tel: (301) 846-1942. Fax: (301) 846-6177. E-mail: beutlerj@mail.nih.gov.

† Natural Products Support Group, SAIC-Frederick, Inc.

‡ Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc.

∥ Molecular Targets Laboratory, Center for Cancer Research, NCI-Frederick.

§ Bioresources Development and Conservation Programme-Cameroon.

10.1021/np9006348
Published on Web 12/10/2009

Table 1. NMR Spectroscopic Data (500 MHz, d_4-MeOH) for Schweinfurthins I (**1**) and J (**2**)

	schweinfurthin I (**1**)			schweinfurthin J (**2**)		
position	δ_C, mult.	δ_H (J In Hz)	HMBC[a]	δ_C, mult.	δ_H (J In Hz)	HMBC[a]
1	102.7, CH	6.14, t (2.1)	3, 5	158.4, qC		2, 3, 5, 6
2	159.8, qC		1, 3	116.5, CH	6.75, d (8.7)	
3	105.8, CH	6.42, d (2.1)	1, 5, 1′, 2′	128.7, CH	7.32, d (8.7)	5, 1′
4	141.6, qC		1′, 2′	131.0, qC		
5	105.8, CH	6.42, d (2.1)	1, 3, 1′, 2′	128.7, CH	7.32, d (8.7)	3, 1′
6	159.8, qC		1, 5	116.5, CH	6.75, d (8.7)	
1′	126.8, CH	6.74, d (16.2)	3, 5	128.3, CH	6.89, d (16.2)	
2′	129.9, CH	6.92, d (16.2)	4′	127.3, CH	6.74, d (16.2)	4′, 8′
3′	130.4, qC		1′, 2′, 7′	137.7, qC		1′, 2′, 4′, 8′
4′	128.9, CH	7.20, d (2.3)	2′, 8′	105.7, CH	6.45, s	2′, 8′
5′	129.4, qC		1″	157.4, qC		4′, 1″
6′	156.4, qC		4′, 7′, 8′, 1″	116.0, qC		4′, 8′, 1″, 2″
7′	116.1, CH	6.72, d (8.1)		157.4, qC		8′, 1″
8′	126.2, CH	7.18, dd (8.2, 2.3)	2′, 4′	105.7, CH	6.45, s	2′, 4′
1″	29.1, CH_2	3.30, obs[b]	4′, 7′, 2″	23.3, CH_2	3.29, obs[b]	4′, 8′, 2″
2″	124.2, CH	5.35, tq (7.3, 1.3)	1″, 4″, 5″	124.8, CH	5.25, tq (7.1, 1.2)	1″, 4″, 5″
3″	136.9, qC		1″, 4″, 5″, 6″	134.9, qC		1″, 4″, 5″
4″	16.2, CH_3	1.73, d (1.3)	2″, 5″	16.3, CH_3	1.77, d (1.3)	2″, 5″
5″	40.9, CH_2	2.07, m	2″, 4″, 6″, 7″	41.0, CH_2	1.96, m	2″, 4″, 6″
6″	27.8, CH_2	2.14, dd (15.0, 7.2)	5″, 7″	27.6, CH_2	2.06, m	5″, 7″
7″	125.5, CH	5.12, m	5″,6″,9″,10″	125.6, CH	5.08, tq (7.0, 1.4)	6″, 9″
8″	132.4, qC		6″, 9″, 10″	135.8, qC		6″, 9″, 10″
9″	25.9, CH_3	1.64, d (1.3)	10″	16.2, CH_3	1.55, d (1.4)	7″, 10″
10″	17.8, CH_3	1.59, d (1.3)	7″, 9″	40.9, CH_2	1.90, m	7″, 9″, 11″
11″				27.9, CH_2	1.99, m	10″
12″				125.7, CH	5.05, t pentet (7.1, 1.4)	10″, 14″
13″				132.0, qC		11″, 14″, 15‴
14″				25.9, CH_3	1.63, d (1.3)	12″, 15″
15″				17.8, CH_3	1.57, d (1.3)	12″, 14″

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon. [b] Signal partially obscured.

nonconjugated olefinic protons (δ 5.05, 5.08, 5.25) and four aliphatic methyl groups (δ 1.55, 1.57, 1.63, 1.77), which were attributed to the presence of a farnesyl residue. In addition to the trans-olefinic protons (δ 6.74, 6.89, J = 16.2 Hz), further analysis of the ^{1}H NMR data revealed only three other signals in the aromatic region (δ 6.45, 6.75, 7.32), each integrating to two protons. The HSQC data confirmed that each proton of a pair was chemically equivalent, giving substantial proof for symmetrical arrangement of substituents in both rings of the *trans*-stilbene substructure. ^{1}H NMR and COSY data showed that δ 6.75 (H-2/6) and δ 7.32 (H-3/5) were coupled (J = 8.7 Hz), and these signals had HMBC correlations to a phenolic carbon (δ 158.4). To maintain the symmetrical placement of substituents in the terminal ring, the trans-olefinic group was assigned para to C-1 (δ 158.4). The ^{1}H NMR singlets at δ 6.45 (H-4′, H-8′) were assigned to the central ring, both ortho in relation to C-3′. HMBC resonances from H-4′/H-8′ (δ 6.45) to C-2′ (δ 127.3) and from H-1″ (δ 3.29) of the farnesyl group to the phenolic carbons (C-5′ and C-7′) provided further evidence for this arrangement. As in **1**, an ω_1 refocused 1,1-ADEQUATE experiment confirmed the assignment of quaternary and phenolic carbon atoms. This led to the structure of **2** as shown.

Compounds **1** and **2** gave GI_{50} values of 10 and 2.8 μM, respectively, when assayed in the NCI 60 human tumor cell line screen (see Supporting Information). These compounds did not show the remarkable selectivity toward CNS, melanoma, non-small cell lung, or renal cell lines that has been demonstrated with schweinfurthins A, B, D, E, F, and G.[1,3,4]

The ongoing interest in schweinfurthins as therapeutic agents has heightened the need for a reliable natural source. UPLC/UV analysis indicated that this Cameroonian leaf collection could potentially yield 200 mg of schweinfurthin A, 410 mg of schweinfurthin B, 360 mg of schweinfurthin C, 50 mg of schweinfurthin D, 20 mg of schweinfurthin F, 200 mg of schweinfurthin I, and 50 mg of schweinfurthin J. Given the widespread occurrence of *M. schweinfurthii* across Africa, this investigation suggests that the species represents an economic source of bioactive prenyl stilbenes. Further studies aimed at assessing the effects of temporal/geographical variations on schweinfurthin content in the plant should be performed in order to optimize the yield of these compounds in future collection efforts.

Experimental Section

General Experimental Procedures. UV spectra were acquired on a Lambda 20 UV/vis spectrophotometer (Perkin-Elmer). NMR spectra were recorded with a Varian 500 MHz INOVA spectrometer equipped with a cryogenic probe in methanol-d_4 with TMS as the internal standard. HRESIMS was performed with a Waters LCT Premier time-of-flight mass spectrometer. UPLC-MS hardware used for analyses and accurate mass determinations included a Waters Acquity chromatographic system and a Waters LCT Premier time-of-flight mass spectrometer. HPLC-MS hardware used for purification consisted of a Waters 600 pump, a Micromass ZQ electrospray mass spectrometer (cone voltage = 30), a Waters 2996 photodiode array spectrometer, and a Sedex 75 evaporative laser light scattering detector. Scale-up purification was accomplished on Matrex C_{18} adsorbent (Varian) and Sephadex LH-20 (Pharmacia) and by C_8 bonded-phase HPLC (Varian).

Plant Material. The leaves of *Macaranga schweinfurthii* were collected near Mundemba, Korup National Forest, Cameroon, by Johnson Jato in September 2008, with identification by Paul Misili of the National Herbarium at Yaounde, Cameroon. After drying in the field, the leaf material was shipped to the NCI at Frederick, MD, where a leaf voucher is maintained in the DTP Repository as V09I1195-J.

Extraction and Isolation. Dried leaves (7.6 kg) were ground in a Holmes Bros. hammer mill fitted with a 4.7 mm mesh screen. The ground material was transferred to several 6 L borosilicate glass percolators, covered with dichloromethane (CH_2Cl_2), and allowed to steep overnight. The extract was drained and the solvent removed by rotary evaporation. A final drying step was performed under high vacuum, resulting in 260 g of CH_2Cl_2-soluble material. The above procedure was repeated with CH_2Cl_2−MeOH (9:1) 165 g, CH_2Cl_2−MeOH (1:9) 199 g, MeOH 549 g, and H_2O 5.4 g. UPLC-PDA-MS analysis showed the presence of schweinfurthin A as well as other stilbenes in the CH_2Cl_2-soluble extract.

The CH_2Cl_2 extract was subjected to a three-step partitioning sequence. First, hexane−MeOH−H_2O (9:1) yielded 104 g of hexane-soluble material and ~ 140 g of MeOH−H_2O (9:1)-soluble material. The water content of the MeOH−H_2O (9:1) fraction was brought to

50% and then partitioned against CH_2Cl_2, resulting in 99 g of CH_2Cl_2 solubles and 42 g of MeOH−H_2O (1:1) solubles. The dried CH_2Cl_2 fraction was resolubilized in MeOH, then partitioned in MeOH−H_2O (9:1) against an equal volume of hexane−methyl *tert*-butyl ether (MTBE) (4:1), yielding a MeOH−H_2O (9:1)-soluble fraction and 11.7 g of hexane−MTBE (4:1) solubles.

The water content of the MeOH−H_2O (9:1) fraction was increased to 40% and then carefully poured into a 4 L percolator packed with 800 g of pre-equilibrated Matrex bonded-phase C_{18} silica (Varian). This column was eluted stepwise with increasing percentages of MeOH in H_2O. The majority of schweinfurthins and other stilbenes were eluted from the column with MeOH−H_2O (8:2), yielding 2.7 g of enriched material. This fraction was loaded onto a 120 cm long by 5 cm inner diameter glass column filled with 1 kg of Sephadex LH-20 and eluted over 20 h with CH_2Cl_2−MeOH (1:3) at a flow rate of 2.0 mL/min. After analysis, pooling resulted in 18 chemically distinct fractions, the 11th (185 mg) containing 78% schweinfurthin A, the 13th (54 mg) containing 11% of a MW 432 stilbene, and the 14th (226 mg) containing 87% of a MW 364 stilbene. These three fractions were further chromatographed to >95% purity by C_{18} HPLC, producing 95 mg of schweinfurthin A, 20 mg of **1**, and 3.5 mg of **2** (% wt/wt recovered from 7.6 kg of dried leaves = 0.0012%, 0.00026%, and 0.000046%, respectively).

Schweinfurthin I (1): yellow oil; UV (MeOH) λ_{max} (log ϵ) 305 (4.50), 320 (4.50) nm; IR (NaCl) ν_{max} 3420 (br), 1600, 1504, 1444, 1343, 1256, 1151, 1105, 1008, 992, 959 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 365.2121 $[M + H]^+$ (calcd for $C_{24}H_{29}O_3$, 365.2117).

Schweinfurthin J (2): yellow oil; UV (MeOH) λ_{max} (log ϵ) 309 (4.35), 325 (4.36) nm; IR (NaCl) ν_{max} 3415 (br), 1605, 1582, 1510, 1426, 1383, 1354, 1239, 1167, 1032, 966 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 433.274 $[M + H]^+$ (calcd for $C_{29}H_{37}O_3$, 433.2743).

NCI 60 Human Tumor Cell Line Screen. Details of the assay may be found in refs 6 and 7.

Acknowledgment. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. This research was supported [in part] by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. This research was supported [in part] by the Developmental Therapeutics Program in the Division of Cancer Treatment and Diagnosis of the National Cancer Institute. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. We gratefully acknowledge J. Britt, S. Staffone, and C. Manning for technical assistance.

Supporting Information Available: 1D and 2D NMR data of compounds **1** and **2** and NCI 60 human tumor cell line screen dose−response curves are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Beutler, J. A.; Shoemaker, R. H.; Johnson, T.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 1509–1512.
(2) Thoison, O.; Hnawia, E.; Guéritte-Voegelein, F.; Sévenet, T. *Phytochemistry* **1992**, *31*, 1439–1442.
(3) Yoder, B.; Norris, A.; Miller, J. S.; Ratovoson, F.; Razafitsalama, J.; Adriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. *J. Nat. Prod.* **2006**, *70*, 342–346.
(4) Beutler, J. A.; Jato, J.; Cragg, G. M.; Wiemer, D. F.; Neighbors, J. D.; Salnikova, M. S.; Hollingshead, M. G.; Scudiero, D.; McCloud, T. G. The schweinfurthins: Issues in development of a plant-based anticancer lead. In *Medicinal and Aromatic Plants. Agricultural, Commercial, Ecological, Legal, Pharmacological and Social Aspects*; Bogers, R. J., Craker, L., Lange, D., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp 301−309.
(5) Köck, M.; Reif, B.; Fenical, W.; Griesinger, C. *Tetrahedron Lett.* **1996**, *37*, 363–366.
(6) Scudiero, D. A.; Shoemaker, R. H.; Paul, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827–4833.
(7) Shoemaker, R. H. *Nat. Rev.-Cancer* **2006**, *6*, 813–823.

NP9006348