Isoflavones from *Psorothamnus arborescens* via high-throughput natural product chemistry methods

Jin-Feng Hu*, Eliane Garo, Grayson W. Hough, Matt G. Goering, Hye-Dong Yoo, Mark O'Neil-Johnson and Gary R. Eldridge

Lead Discovery and Rapid Structure Elucidation Group, Sequoia Sciences, Inc., 1912 Innerbelt Business Centre Drive, St. Louis, Missouri 63114, USA

One new isoflavone, arborestin (**2**), together with the known fremontin (**1**), were obtained from *Psorothamnus arborescens* via high-throughput natural product chemistry methods. The isoflavones were isolated and the structure elucidation was performed using a capillary scale NMR probe and HR-/LR-ESIMS data.

Keywords: *Psorothamnus arborescens*, high-throughput natural product chemistry, CapNMR probe, miniaturisation, arborestin

Previous publications have comprehensively documented our high-throughput natural product chemistry methods and the advanced capillary NMR techniques applied to the isolation and elucidation of a number of novel and mass-limited bioactive components from North American and African plants.[1,2] In a continuation of our projects directed toward the discovery of novel biofilm inhibitors from plants,[3] the natural products library constructed of the organic extract obtained from the aerial parts of *Psorothamnus arborescens* (Torrey ex A. Gray) (Fabaceae) displayed potent inhibition of the formation of the bacterial biofilm *Pseudomonas aeruginosa* PA01. *P. arborescens* (Mojave indigobush), one of nine species in the genus, is a perennial shrub less than 1m tall with a violet purple corolla. It is found mostly on desert mountains, slopes, canyons and dry riverbeds from an elevation of 100–1900m in the southwestern USA and Mexico.[4-7] It is closely related to *P. fremontii* which was found only in the Mojave Desert (California) and has been used by the indigenous tribes to stop internal hemorrhage and for the treatment of stomach sickness.[4,8] Another species, *P. polyadenius*, is reported to have been used by Native American tribes to treat several ailments, including smallpox, pneumonia, tuberculosis and influenza.[8,9] Simple pigments, potential protein kinase C (PKC) inhibitors, were found from *P. junceus* collected in the Gulf Coast desert between San Ignacio and Santa Rosalia, Baja California Sur, Mexico.[10,11] The essential oil from *P. scoparius* has been investigated.[12] Most recently, a few isoflavones and chalcones with antiprotozoal activities have been isolated either from the root of *P. arborescens*[13] collected from E. Tulare County, California, or from the twigs with leaves and flowers of *P. polyadenius*[14] collected from Washoe County, Nevada, USA.

The *Psorothamnus* library was generated and analysed as previously described.[1-3] The isoflavones were located in the preparative HPLC fraction 16 which was found to exhibit potential inhibition of the formation of the bacterial biofilm *Pseudomonas aeruginosa* PA01.[3] The compounds in fraction 16 were purified through semi-preparative HPLC systems. The structures of these two mass-limited isoflavones (**1** and **2**) were elucidated or dereplicated using the mass spectra and capillary NMR probe techniques.

The ESI mass spectra of **1** showed clear $[M-H]^-$ and $[M + H]^+$ peaks at *m*/*z* 353 and 355, respectively. The NMR spectral data of **1** were in full agreement with the known isoflavone, fremontin. The structure of fremontin (**1**), previously isolated from *P. fremontii*,[15] has been recently revised by a re-isolation from *P. arborescens* in 2006.[13] In fact, **1** was also isolated and its structure determined by 2002 by our group. The results of our 2D NMR experiments were identical with the revised structure containing two *ortho*-OH groups at C-3' and C-4' positions and an isoprenyl group at C-6' position in the B-ring.[13] The molecular weight of compound **2** and its chemical formula of $C_{21}H_{20}O_6$ were deduced from the positive mode high-resolution ESI mass spectrum (HR-ESIMS), which showed the $[M + Na]^+$ ion peak at *m*/*z* 391.1159 ($C_{21}H_{20}O_6Na$ requires 391.1157). The ^1H NMR spectral data (Table 1) of **2** showed similar general features to those of **1**, except for a methoxyl group which resonated at δ 3.91 (3H, s). The OMe group was assigned at C-4' position according to a clear NOE correlation between δ 3.91 and δ 7.10 (H-5') in the NOESY spectrum. This was also confirmed by the HMBC experiment which revealed an OH group at C-3', an OMe group at C-4' and an isoprenyl group at C-6' positions in the B-ring.

The purified isoflavones **1** and **2** were tested for their inhibition of the formation of the bacterial biofilm *Pseudomonas aeruginosa* PA01,[3] but were found to be inactive.

Experimental

General experimental procedures

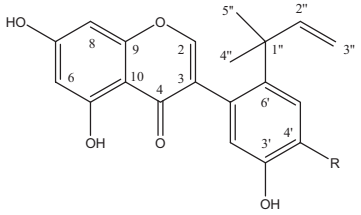
For instrumentation and general procedures, see the preceding papers.[2,16]

Plant material

The aerial parts of *P. arborescens* were collected from Riverside County, California, USA in the Spring of 2000. Plant samples were dried on site and then shipped to Sequoia Sciences, where they were lyophilised. The plant was identified by John Stone (Missouri Botanical Garden Herbarium, St. Louis, MO). A voucher specimen (No.0029) was deposited at the Herbarium of Missouri Botanical Garden.

Extraction and isolation

Dried aerial parts (182 g) were extracted with EtOH/EtOAc (50 : 50) followed by H_2O/MeOH (30: 70) to obtain 9.6 g and 11.1 g dry organic and aqueous extracts, respectively. One gram of the organic extract was loaded on the Flash Master II automated chromatographic system using our standard elution gradient to generate the Flash Fractions.[2,16] The Flash Fraction 3 (EtOAc, neat) totaled 303 mg; a 50 mg aliquot was fractionated by preparative C_{18} HPLC from 30% to 70% acetonitrile in H_2O collecting 40 1-min fractions. Compounds **1** and **2** resided in preparative HPLC fraction 16, which primarily exhibited biofilm inhibition vs *Pseudomonas aeruginosa* (PA01). Review of the HPLC-ELSD-MS data acquired on all of the preparative fractions from the Flash Fraction 3 suggested that preparative HPLC fraction 16 contained compounds with molecular weights less than 600 daltons that could readily be isolated using reversed-phase chromatography.



Fremontin (**1**): R = OH
Arborestin (**2**): R = OMe

* Correspondent. E-mail: jhu@sequoiasciences.com

Table 1 ^{1}H and ^{13}C NMR Data (in CD_3OD) for **1** and **2**

	δ_H/mult	*J*/Hz	δc
No.	**1**[a]	**2**[a]	**2**[a,b]
2	7.73 (1H, s)	7.77 (1H, s)	155.5
3			126.9
4			183.2
5			163.4
6	6.22 (1H, d, *J* = 1.9)	6.24 (1H, d, *J* = 1.9)	99.7
7			164.8
8	6.33 (1H, d, *J* = 1.9)	6.40 (1H, d, *J* = 1.9)	95.3
9			159.7
10			106.1
1'			122.4
2'	6.46 (1H, s)	6.53 (1H, s)	121.5
3'			144.6
4'			147.2
5'	6.99 (1H, s)	7.10 (1H, s)	112.7
6'			140.1
1"			42.1
2"	5.99 (1H, dd, *J* = 17.6, 10.5)	6.04 (1H, dd, *J* = 17.6, 10.5)	150.1
3"	4.75 (1H, brd, *J* = 17.6)	4.63 (1H, brd, *J* = 10.6)	
	4.80 (1H, brd, *J* = 17.6)	4.68 (1H, brd, *J* = 10.5)	109.8
5"	1.34 (3H, s)	1.41 (3H, s)	29.1
6"	1.30 (3H, s)	1.37 (3H, s)	29.6
4'-OMe		3.91 (3H, s)	56.6

[a]Recorded using a CapNMR™ probe. Sample: *ca* 90 µg in 6.5 µl CD_3OD. Injection: *ca* 70 µg in 5 µl, and *ca* 20 µg in active volume (1.5 µl). Data acquisition for ^{1}H: Number of scans (NS) = 64, 5 min; for ^{1}H-^{1}H COSY: NS = 4, 32 min; for NOESY: NS = 16, mixing time of 300 ms, 120 min; for HSQC: NS = 128, 128 increments, 5 h; for HMBC: NS = 200, 128 increments, 8 h acquisition time, HMBC long-range coupling delay optimised at 63 ms.
[b]Assignments were made by a combination of 1D and 2D NMR (^{1}H-^{1}H COSY, HSQC, and HMBC) experiments.

The initial mobile phase gradient applied to isolating compounds **1** and **2** from HPLC fraction 16 was based on the elution profile observed during the preparative HPLC separation that created the fraction. A semi-preparative HPLC [Keystone BetaMax Neutral C18 (8 × 250 mm I.D., 5 mm)] method was then developed which resulted in a linear gradient of acetonitrile in H_2O from 40% to 50% over 22.0 min, and followed by an isocratic gradient of 95% B for 5.0 min, to afford compounds **1** (170 µg, t_R = 16.5 min) and **2** (140 µg, t_R = 17.7 min). The quantities were estimated based upon methods using HPLC/ELSD previously described.[16]

Arborestin (**2**). ^{1}H and ^{13}C NMR data see Table 1. ESIMS *m*/*z* 367 $[M-H]^-$, 369 $[M + H]^+$, 391 $[M + Na]^+$. HR-ESIMS *m*/*z* 391.1159 $[M + Na]^+$ (calcd for $C_{21}H_{20}O_6Na$, 391.1157).

The authors acknowledge Dr Jim Miller, John Stone, Adam Bradley, Gretchen Walters from Missouri Botanical Garden for the plant collections and identifications, and Dr. John W. Costerton's research group at Centre for Biofilm Engineering, Montana State University (Bozeman, Montana) for the anti-biofilm *in vitro* assays. We would like to acknowledge Tim Peck, Dean Olson and Jim Norcross from Magnetic Resonance Microsensors (Savoy, IL.) for making the first 5 µl proton indirect carbon gradient CapNMR probe available to Sequoia Sciences. Finally, the authors would like to acknowledge the scientific collaboration with Advanced Chemistry Development, Inc, (ACD Labs) and the use of ACD/SpecManager and ACD/Structure Elucidator.

Received 14 April 2007; accepted 15 May 2007
Paper 07/4599 doi: 10.3184/030823407X215591

References

1 G.R. Eldridge, H.C. Vervoort, C.M. Lee, P.A. Cremin, C.T. Williams, S.M. Hart, M.G. Goering, M. O'Neil-Johnson and L. Zeng, *Anal. Chem.*, 2002, **74**, 3963.
2 J.-F. Hu, H.-D. Yoo, C.T. Williams, E. Garo, P.A. Cremin, L. Zeng, H.C. Vervoort, C.M. Lee, S.M. Hart, M.G. Goering, M. O'Neil-Johnson and G.R. Eldridge, *Planta Med.*, 2005, **71**, 176.
3 J.-F. Hu, E. Garo, M.G. Goering, M. Pasmore, H.-D. Yoo, T. Esser, J. Sestrich, P.A. Cremin, G.W. Hough, P. Perrone, Y.-S. L. Lee, N.-T. Le, M. O'Neil-Johnson, J.W. Costerton and G.R. Eldridge, G.R., *J. Nat. Prod.*, 2006, **69**, 118.
4 D. Isely, *The Jepson Manual: Higher Plants of California*, eds J.C. Hickman, University of California Press. 1993, pp. 642–643.
5 R.C. Barneby, Daleae Imagines: An Illustrated Revision of Errazuriza Philippi, Psorothamnus Rydberg, Marina Leibmann, and Dalea Lucanus emend. Barneby, Including All Species of Leguminosae Tribe Amorpheae Borissova Ever Referred to Dalea; New York Botabical Garden: Bronx, NY, 1977, pp 31-38.
6 A. Cronquist, *Intermountain Flora; Vascular Plants of the Intermountain West, U.S.A*; Published for the New York Bptanical Garden by Hafner Pub. Co.: New York, 1972, pp 29-32.
7 M. DeDecker, *Flora of the Northern Mojave Desert, California*; California Native Plant Society: Berkeley, CA, 1984,p 61.
8 P. Train, W.A. Archer and J.R. Henrichs, *Medicinal Uses of Planta by Indian Tribes of Nevada*; Quarterman Publications: Lawrence, MA, 1982, pp 42-44.
9 E.K. Balls, *Early uses of California Plants*; University of California Press: Berkeley, California, 1962, pp 77-78.
10 X.H. Li, H.B. Zhang, C.L. Ashendel, P. Fanwick and C.-J. Chang, *Tetrahedron Lett*., 1998, **39**, 3417.
11 H.B. Zhang, X.H. Li, C.L. Ashendel and C.-J. Chang, *J. Nat. Prod.*, 2000, **63**, 1244.
12 M.E. Lucero, R.E. Estell and E.L. Fredrickson, *J. Essent. Oil Res.*, 2003, **15**, 108.
13 M.M. Salem and K.A. Werbovetz, *J. Nat. Prod.*, 2006, **69**, 43.
14 M.M. Salem and K.A. Werbovetz, *J. Nat. Prod.*, 2005, **68**, 108.
15 G. Manikumar, K. Gaetano, M.C. Wani, H. Taylor, T.J. Hughes, J. Warner, R. McGivney and M.E. Wall, *J. Nat. Prod.*,1989, **52**, 769.
16 J.-F. Hu, E. Garo, H.-D. Yoo, P.A. Cremin, L. Zeng, M.G. Goering, M. O'Neil-Johnson and G.R. Eldridge, *Phytochemical Anal.*, 2005, **16**, 127.