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Acuminatanol, the first 2′,2‴-bis-dihydrobiflavonol from the aqueous extract of *Trichoscypha acuminata*

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Abstract—One new 3′,4′,5′,5,7-pentahydroxy-2′,2‴-bis-dihydrobiflavonol, acuminatanol (**1**), was obtained from the aqueous extract of *Trichoscypha acuminata*. The structure elucidation process was performed primarily utilizing a capillary scale NMR probe. Acuminatanol (**1**) is the first example of a bis-dihydroflavonol linked exclusively via the B-rings at C-2′ and C-2‴ positions. To date, it is the first naturally-occurring compound reported from the genus *Trichoscypha*, and the first new natural product published from our compound libraries generated from the aqueous extracts of American and African plants.

Previous publications[1,2] have comprehensively documented our high-throughput natural product chemistry methods as applied to the production and analysis of the compound libraries from organic extracts of plants. In this Letter, we report for the first time our general procedures to generate a compound library from the aqueous extract of the African plant *Trichoscypha acuminata* Engl. (Anacardiaceae). *T. acuminata* is one of the 84 species in the genus.[3] All *Trichoscypha* are small trees found in tropical Africa, and their fruits are commonly consumed by both chimpanzees and lowland gorillas.[4] The stems of *T. acuminata* were collected from Tchimbele state in Gabon in the Spring of 2000. Plant samples were dried on site, and then shipped to Sequoia Sciences. The plant was identified by Gretchen Walters (Missouri Botanical Garden Herbarium, St. Louis, MO). A voucher specimen (No. 933) was deposited at the Herbarium of Missouri Botanical Garden. The dried stems (88 g) were extracted with EtOH/EtOAc (50:50) followed by H_2O/MeOH (30:70) to obtain 4.3 g and 2.1 g of dry organic and aqueous extracts, respectively. Two grams of aqueous extract were loaded onto a 50 g C_{18} column (Phenomenex Strata, 55 μM, 70 A). Two column volumes of H_2O were passed through the column. The column was then rinsed with two column volumes of MeOH after the addition of a polyacrylamide column to the base of the C_{18} column. The eluent was retained, and evaporated in vacuum. Approximately 1 g of the dried eluent was dissolved into 200 mL MeOH and passed through a Centricon™ high molecular weight filter using centrifugation for 16 h at 3000 RPM. The low MW fraction (Flash Fraction 6,[5] approximately 0.6 g) was retained.

A 50 mg aliquot of Flash Fraction 6 was fractionated by preparative C_{18} HPLC from 5% to 85% acetonitrile in H_2O collecting 40, one minute fractions to generate the primary screening library. Compound **1** resided in preparative HPLC fraction 15 of the *T. acuminate* library, which exhibited primary inhibition of HCV replicon in the course of the anti-HCV biological screening.[2d,6] Review of the HPLC–ELSD–MS data acquired on all of the preparative fractions from the Flash Fraction 6 suggested that preparative HPLC fraction 15 contained compounds with molecular weights less than 1000 daltons that could readily be isolated using reversed-phase chromatography. Approximately 300 mg of the remaining Flash Fraction 6 was prepared as described above via preparative HPLC to generate additional material for structure elucidation and additional biological testing. The initial mobile phase gradient applied to isolated compound **1** from HPLC fraction 15 was based

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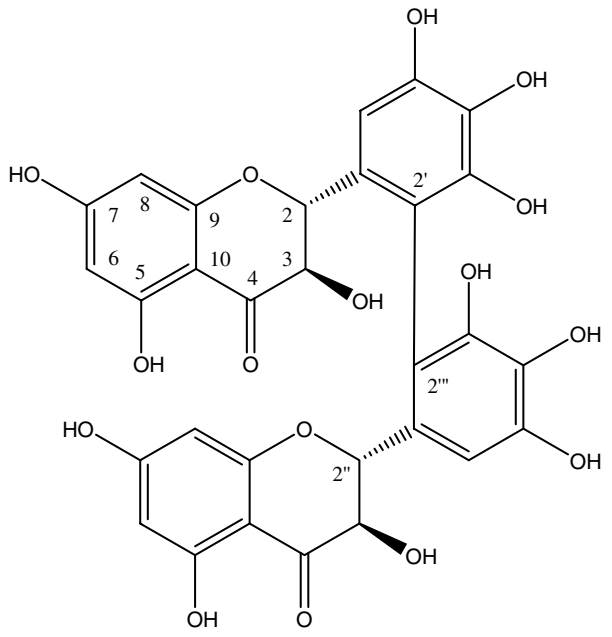
on the elution profile observed during the preparative HPLC separation that created the fraction. The isolation of the bis-dihydroflavonol from preparative HPLC fraction 15 was performed using a semipreparative Keystone BetaMax Neutral C_{18} (8×250 mm I.D., 5 mm) column. A semipreparative HPLC method was developed which resulted in an isocratic gradient of 16% acetonitrile in H_2O acidified with 0.05% TFA for 30 min, then followed by a linear gradient of acetonitrile from 16% to 25% over 20.0 min, and finally followed by an isocratic gradient of 95% acetonitrile for 5.0 min to afford compound **1** (160 μg, $t_R = 20.3$ min). The quantity was estimated based upon methods using HPLC/ELSD described previously.[2a] Based upon our experiences from participating in various discovery projects with academic and private laboratories, we have determined that approximately 100 μg of compound is required to confirm biological activity and propose a chemical structure from spectra generated using a capillary scale NMR probe.[2a] Using these data, each team collaboratively deliberates to prioritize each of the proposed compounds identified during primary and confirmatory screening and also considers structural novelty and synthetic accessibility. Resources are then appropriately allocated to pursue the compounds of interest.



1

NMR data for the structure elucidation were acquired on a Bruker Avance 600 MHz NMR system (Bruker Instruments, Rheinstetten, Germany) equipped with a 5 μL capillary scale NMR probe, CapNMR™ probe (MRM/Protasis, Savoy, IL), having a 1.5 μL active volume. Purified 2′,2‴-bis-dihydroflavonol was dissolved in 6.5 μL CD_3OD and loaded manually into the probe. For compound **1**, 130 μg was diluted with 6.5 μL CD_3OD. Injection: 5 μL from which 30 μg was in the active volume (1.5 μL). Data acquisition for ^1H NMR: Number of scans (NS) = 64, 5 min.; for ^1H–^1H COSY: NS = 4, 32 min; for HSQC: NS = 128, 128 increments, 5 h; for HMBC: NS = 200, 128 increments, 8 h acquisition time, HMBC long-range coupling delay optimized at 63 ms.

The molecular weight of compound **1** and its elemental formula of $C_{30}H_{22}O_{16}$ were deduced from the positive-mode HRESIMS, which showed a $[M+H]^+$ ion peak at m/z 639.0985. The ^1H, COSY, and HSQC NMR (CD_3OD) spectra of **1** revealed the structure to be a typical 5,7-dihydroxy-dihydroflavonol [δ 5.89 (d, $J = 2.0$ Hz, H-8, δc: 97.2), 5.72 (d, $J = 2.0$ Hz, H-6, δc: 96.6), 4.78 (d, $J = 11.4$ Hz, H-2, δc: 81.5), and 4.54 (d, $J = 11.4$ Hz, H-3, δc: 73.6)]. In the ^1H NMR spectrum (CD_3OD), one additional proton signal resonated at δ 6.79 (s). Based on the HMBC NMR experiments (Fig. 1), this proton was assigned to H-6′ in the B-ring. The δc values of all carbons and their assignments could be made by a combination of 2D NMR techniques (^1H–^1H COSY, HSQC, and HMBC). According to the molecular formula and the observed NMR signals (CD_3OD), the structure is a symmetrical bis-dihydroflavonol. The linkage position at C-2′ and C-2‴ (δ 116.2, s, C-2′ and C-2‴) was deduced by the key HMBC correlations (Fig. 1). Therefore, compound **1** was elucidated to be 3′,4′,5′,5,7-pentahydroxy-2′,2‴-bis-dihyroflavonol.[7] The relative stereochemistry at C-2 and C-3 in the C-ring was determined by the big coupling constant (11.4 Hz) between H-2 and H-3, which requires both protons to be at the opposite axial positions. Unfortunately, the crude materials of *T. acuminata* collected from Gabon in 2000 and the remaining quantity[8] of the Flash Fraction 6 proved to be insufficient for generating additional material. Therefore, we were unable to obtain an optical rotation value or a circular dichroic spectrum to establish the absolute configuration of **1**.

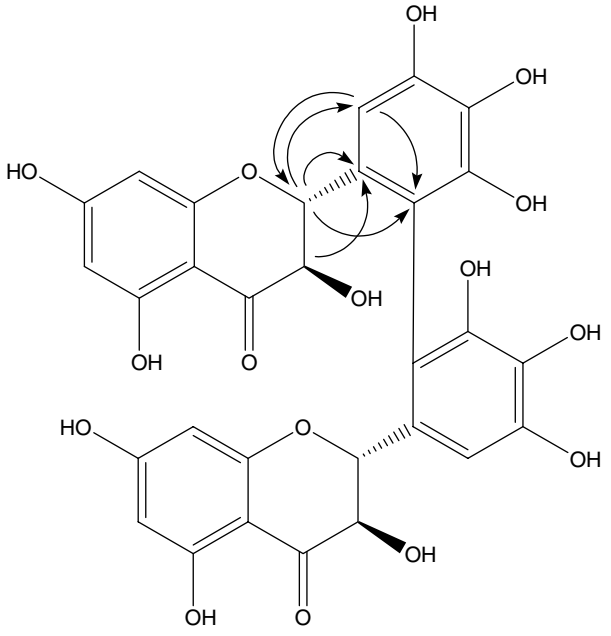


Figure 1. Key correlations in the HMBC spectrum.

A few bis-flavonols with a C–C linkage via the B-rings have been previously isolated from the plants such as *Hypnum cupressiforme*,[9] *Pseudotsuga menziesii*,[10] *Pilotrichella flexilis*,[11] and Douglas-fir sapwood.[12] The first natural biflavonoid with flavanol (catechin) and dihydroflavonol (taxifolin) constituent units coupled at the B-ring through C-2′ and C-2‴ was isolated from commercial willow bark (Salix spp.) three decades ago.[13] Compound **1** is the first example of a bis-dihydroflavonol linked exclusively at C-2′ and C-2‴ positions. We were unable to locate any references to compounds isolated from *Trichoscypha*. Acuminatanol (**1**) is the first new representative in our compound libraries from the aqueous extracts. The purified 2′,2‴-bis-dihydroflavonol (**1**) was tested for its anti-HCV bioactivity in an HCV subgenomic replicon system,[2d] but was found inactive.

Acknowledgments

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References and notes

1. Eldridge, G. R.; Vervoort, H. C.; Lee, C. M.; Cremin, P. A.; Williams, C. T.; Hart, S. M.; Goering, M. G.; O'Neil-Johnson, M.; Zeng, L. *Anal. Chem.* **2002**, *74*, 3963–3971.
2. (a) Hu, J.-F.; Garo, E.; Yoo, H.-D.; Cremin, P. A.; Zeng, L.; Goering, M. G.; O'Neil-Johnson, M.; Eldridge, G. R. *Phytochem. Anal.* **2005**, *16*, 127–133; (b) Hu, J.-F.; Yoo, H.-D.; Williams, C. T.; Garo, E.; Cremin, P. A.; Zeng, L.; Vervoot, H. C.; Lee, C. M.; Hart, S. M.; Goering, M. G.; O'Neil-Johnson, M.; Eldridge, G. R. *Planta Med.* **2005**, *71*, 176–180; (c) Yoo, H.-D.; Cremin, P. A.; Zeng, L.; Garo, E.; Williams, C. T.; Lee, C. M.; Goering, M. G.; O'Neil-Johnson, M.; Eldridge, G. R.; Hu, J.-F. *J. Nat. Prod.* **2005**, *68*, 122–124; (d) Hu, J.-F.; Patel, R.; Li, B.; Garo, E.; Hough, G. W.; Goering, M. G.; Yoo, H.-D.; O'Neil-Johnson, M.; Eldridge, G. R. *J. Nat. Prod.* **2007**, *70*, 604–607.
3. Breteler, F. J. *Adansonia* **2001**, *23*, 247–264.
4. (a) Tutin, C. E. G.; Fernandez, M. *Am. J Primatol.* **1993**, *30*, 195–211; (b) Denouter, R. W.; Vanveenendaal, W. L. H. *Acta Bot. Neerl.* **1986**, *35*, 425–435.
5. The number (6) of this Flash Fraction was subsequently assigned from the Flash Fractions (1–5) obtained from the organic extracts (see Refs. 2a,b).
6. More than 20,000 preparative HPLC fractions generated from different plants by Sequoia Sciences were screened during this HCV research project.
7. Acuminatanol (**1**): ^{1}H NMR (in CD_3OD, 600 MHz) δ 6.79 (2H, s, H-6′ and H-6‴), 5.89 (2H, d, $J = 2.0$ Hz, H-8 and H-8″), 5.72 (2H, d, $J = 2.0$ Hz, H-6 and H-6″), 4.78 (2H, d, $J = 11.4$ Hz, H-2 and H-2″), and 4.54 (2H, d, $J = 11.4$ Hz, H-3 and H-3″); ^{13}C NMR (in CD_3OD, 150 MHz) δ 81.5 (d, C-2 and C-2″), 73.6 (d, C-3 and C-3″), 196.2 (s, C-4 and C-4″), 163.3 (s, C-5 and C-5″), 96.6 (d, C-6 and C-6″), 167.4 (s, C-7 and C-7″), 97.2 (d, C-8 and C-8″), 165.3 (s, C-9 and C-9″), 101.1 (s, C-10 and C-10″), 127.3 (s, C-1′ and C-1‴), 116.2 (s, C-2′ and C-2‴), 146.4 (s, C-3′ and C-3‴), 135.3 (s, C-4′ and C-4‴), 146.5 (s, C-5′ and C-5‴), and 107.6 (s, C-6′ and C-6‴); ESIMS m/z 637 $[M-H]^-$, 1275 $[2M-H]^-$, 639 $[M+H]^+$; HRESIMS m/z 639.0985 $[M+H]^+$ ($C_{30}H_{23}O_{16}$ requires 639.0986).
8. The total amount of the Flash Fraction 6 was originally estimated to be 0.6 g, but our records may suggest it was slightly less than this quantity.
9. Sievers, H.; Burkhardt, G.; Becker, H.; Zinsmeister, H. D. *Phytochemistry* **1992**, *31*, 3233–3237.
10. Foo, L. Y.; Helm, R.; Karchesy, J. *Phytochemistry* **1992**, *31*, 1444–1445.
11. Brinkmeier, E.; Geiger, H.; Zinsmeister, H. D. *Z. Naturforch.* **2000**, *55c*, 866–869.
12. Dellus, V.; Mila, I.; Scalbert, A.; Menard, C.; Michon, V.; Du Penhoat, C. L. M. H. *Phytochemistry* **1997**, *45*, 1573–1578.
13. Kolodziej, H. *J. Chem. Soc., Chem. Commun.* **1987**, 205–206.