JOURNAL OF NATURAL PRODUCTS

NOTE

pubs.acs.org/jnp

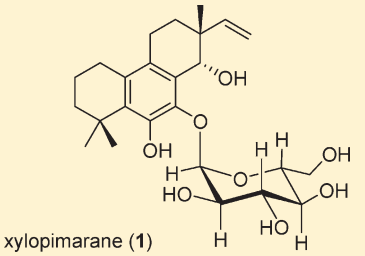
Ring B Aromatic Norpimarane Glucoside from a *Xylaria* sp.

Masahiko Isaka,* Arunrat Yangchum, Patchanee Auncharoen, Kitlada Srichomthong, and Prasert Srikitikulchai

National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phaholyothin Road, Klong Luang, Pathumthani 12120, Thailand

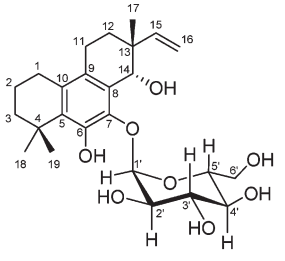
Supporting Information

ABSTRACT: A novel 20-norpimarane glucoside, xylopimarane (**1**), together with the known sphaeropsidin C (**2**) and clonostachydiol (**3**), was isolated from the fungus *Xylaria* sp. BCC 4297. Compound **1** exhibited cytotoxicity to cancer cell lines KB, MCF-7, and NCI-H187 with respective IC_{50} values of 1.0, 13, and 65 μM.

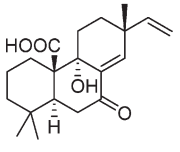


xylopimarane (**1**)

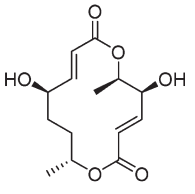
Fungi belonging to the genus *Xylaria* have been the source of bioactive compounds such as xyloketals and xyloallenolide from *Xylaria* sp. (No. 2508),[1,2] multiplolides from *X. multiplex* BCC 1111,[3] xylactam from *X. euglossa*,[4] integric acid from a *Xylaria* sp.,[5] xylarenals from *X. persicaria*,[6] and eremophilanolides from *Xylaria* sp. BCC 21097.[7] As part of our research program on the utilization of fungal sources in Thailand, we investigated the *Xylaria* sp. strain BCC 4297, as an extract of this fungus showed moderate cytotoxic activity against several cancer cell lines. Scale-up fermentation and chemical studies resulted in the isolation of a novel 20-norpimarane glucoside, xylopimarane (**1**), along with the known sphaeropsidin C (**2**)[8] and clonostachydiol (**3**).[9,10]



1



2



3

The molecular formula of **1** was determined by HRESIMS as $C_{25}H_{36}O_8$. The IR spectrum exhibited absorption bands at ν_{max} 3426 and 1636 cm^{-1}, which suggested the presence of OH groups and an aromatic chromophore. The ^1H and ^{13}C NMR, DEPT135, and HMQC spectroscopic data demonstrated that **1** was composed of a sugar unit (C_6) and aglycone (C_{19}). The pyranose ring (C-1′−C-6′) was addressed by COSY data. The coupling constants observed for H-3′ (t, J = 9.3 Hz) and H-4″ (t, J = 9.4 Hz) indicated that methine protons H-2′, H-3′, H-4′, and H-5′ all occupied axial positions. The small coupling constant (J = 3.8 Hz) of H-1′/H-2′ revealed an equatorial orientation of the anomeric proton H-1′. Therefore, the sugar unit was assigned as α-glucopyranose. The aglycone (C_{19}) contained six aromatic quaternary carbons, a vinyl group (δ_C 147.6/δ_H 6.25; δ_C 110.2/δ_H 5.02 and 4.96), a hydroxylated methine (δ_C 68.1, δ_H 4.49, d, J = 4.7 Hz; OH, δ_H 4.27, d, J = 4.7 Hz), two quaternary carbons, five methylenes, and three methyl groups. The planar structure of the aglycone was deduced from COSY and HMBC (Table 1) correlations. Two methyl groups at δ_H 1.42 (δ_C 27.9, CH_3-18) and 1.38 (δ_C 27.2, CH_3-19) showed HMBC correlations to each other and also exhibited correlations to a quaternary carbon at δ_C 34.1 (C-4), an aromatic quaternary carbon at δ_C 131.7 (C-5), and a methylene carbon at δ_C 42.0 (C-3), which indicated the connections of these methyl groups to the same quaternary carbon (C-4). HMBC data also demonstrated that the quaternary carbon at δ_C 38.6 (C-13) was connected to a vinyl group (C-15/C-16), a methyl group at δ_C 19.8 (δ_H 0.79, CH_3-17), a hydroxylated methine (CH-14), and a methylene carbon at δ_C 25.8 (CH_2-12). The presence of a fully substituted benzene (ring B) was indicated by HMBC correlations to the aromatic carbons: from H_2-1, H_2-2, and H_α-11 (δ_H 2.60) to C-10 (δ_C 132.6), from H_2-1, H_2-3, H_3-18, and H_3-19 to C-5 (δ_C 131.7), from H_2-1, H_2-11, and H_β-12 (δ_H 1.50) to C-9 (δ_C 123.2), from H_2-11, H-14, and 14-OH to C-8 (δ_C 129.6), and from H-14 to C-7 (δ_C 143.2). The quaternary carbon at δ_C 147.4, which lacked a HMBC correlation, was assigned

Received: December 1, 2010
Published: January 12, 2011

dx.doi.org/10.1021/np100873t | *J. Nat. Prod.* 2011, 74, 300–302

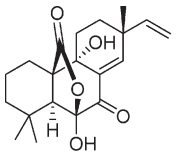
Table 1. NMR Spectroscopic Data for 1 in Acetone-d_6 (500 MHz for ^{1}H, and 125 MHz for ^{13}C)

position	δ_C, mult.	δ_H, mult. (J in Hz)	HMBC
1	27.8, CH_2	2.48−2.46,m	2, 3, 5, 10
2	19.4, CH_2	1.74−1.69,m	1, 4, 10
3	42.0, CH_2	1.60−1.57,m	1, 2, 4, 5, 18, 19
4	34.1, qC		
5	131.7, qC		
6	147.4, qC		
7	143.2, qC		
8	129.6, qC		
9	123.2, qC		
10	132.6, qC		
11	23.2, CH_2	α 2.60, dd (17.3, 6.8)	8, 9, 10, 12, 13
		β 2.43, m	8, 9, 10, 12
12	25.8, CH_2	α 2.21, m	11, 13, 14, 17
		β 1.50, dd (12.9, 7.0)	9, 11, 13, 14, 15, 17
13	38.6, qC		
14	68.1, CH	4.49, d (4.7)	7, 8, 9, 12, 13, 15, 17
15	147.6, CH	6.25, dd (17.7, 10.9)	12, 13, 14, 17
16	110.2, CH_2	5.02, dd (17.7, 1.4)	13, 15
		4.96, dd (10.9, 1.4)	13, 15
17	19.8, CH_3	0.79, s	12, 13, 14, 15
18[a]	27.9, CH_3	1.42, s	3, 4, 5, 19
19[a]	27.2, CH_3	1.38, s	3, 4, 5, 18
6-O*H*		9.47, br s	
14-O*H*		4.20, d (4.7)	8, 13, 14
1′	103.6, CH	5.01, d (3.8)	7, 3′, 5′
2′	72.2, CH	3.75, m	3′
3′	73.3, CH	3.91, t (9.3)	2′, 4′
4′	70.2, CH	3.45, t (9.4)	3′, 5′, 6′
5′	75.6, CH	4.13, m	4′
6′	61.4, CH_2	3.98, br d (11.6); 3.74, m	5′

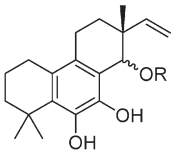
[a] The assignment of CH_3-18 and CH_3-19 can be interchanged.

to C-6 to constitute a benzene ring. The chemical shifts of C-6 and C-7 suggested that these carbons were attached with an oxygen atom. Therefore, the planar structure of the aglycone was assigned as 20-norpimara-5,7,9-triene-6,7,14-triol, wherein either 6-OH or 7-OH should form a glucoside. HMBC correlation from the anomeric proton (H-1′) to C-7 indicated the location of the glucose unit. The co-occurrence of **1** with the known sphaeropsidine C (**2**) and the plausible biosynthetic relation as described below strongly suggested that these compounds should have the same sense of C-13 absolute configuration. Intense NOESY correlations of H-14 and H_3-17 and the absence of the cross-peak for H-14 and H-15 indicated the β-face orientation of H-14. Assignment of the protons of two methylene groups (H_2-11 and H_2-12) was addressed on the basis of the NOESY correlations from H_3-17 to H_β-11 (δ_H 2.43) and H_β-12 (δ_H 1.50). Conformation of ring C was uncertain from the available NMR data. Finally, the D-glucose unit was confirmed by acid hydrolysis of **1**.

To our knowledge, this is the first report of a natural ring B aromatic 20-norpimarane skeleton. In 1972, Ellestad and co-workers reported the isolation of LL-S491β (**4**) and LL-S491γ (7β-hydroxy analogue of **4**) from the fungus *Aspergillus chevalieri*.[11,12] The paper also describes that ethanolic hydrochloric acid treatment of **4** gave decarboxylation/aromatization products **5a** and **5b**.[11] Since **5a** is identical to the aglycone of **1**, we assume that the same transformation occurred during fermentation of BCC 4297. Thus, compound **4**, produced by oxidation of sphaeropsidin C (**2**), is the possible biosynthetic precursor of **1**. Glucoside formation should be the last biosynthetic step.



4



5a R = H
5b R = CH_3

Xylopimarane (**1**) exhibited cytotoxic activity against the human cancer cell lines KB (oral carcinoma), MCF-7 (breast cancer), and NCI-H187 (small-cell lung cancer), with IC_{50} values of 1.0, 13, and 65 μM, respectively. It also showed cytotoxicity (IC_{50} 41 μM) to nonmalignant Vero cells (African green monkey kidney fibroblasts).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a GBC Cintra 404 spectrophotometer. FTIR spectra were taken on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers. ESITOF mass spectra were measured with a Bruker micrOTOF mass spectrometer.

Fungal Material. The fungus used in this study was isolated from an unidentified dead wood in Hala Bala Wildlife Sanctuary, Narathiwat Province, Thailand, and it was deposited in the BIOTEC Culture Collection on February 24, 2000, as BCC 4297. On the basis of the characteristics of fruiting bodies and morphology, this fungus was assigned to the genus *Xylaria* (family *Xylariaceae*) by one of the authors (P.S.).

Fermentation and Isolation. The fungus BCC 4297 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 2 × 250 mL Erlenmeyer flasks containing 25 mL of potato dextrose broth (PDB; potato starch 4.0 g/L, dextrose 20.0 g/L). After incubation at 25 °C for 8 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1 L Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated at 25 °C for 8 days on a rotary shaker (200 rpm). These secondary cultures were pooled, each 25 mL portion was transferred into 20 × 1 L Erlenmeyer flasks containing 250 mL of malt extract broth (MEB; malt extract 6.0 g/L, yeast extract 1.2 g/L, maltose 1.8 g/L, dextrose 6.0 g/L), and final fermentation was carried out at 25 °C for 83 days under static conditions. The culture was filtered to separate the residue (mycelia) and the filtrate (broth). The broth was extracted with EtOAc (3 × 5 L), and the combined organic layer was concentrated to obtain a brown gum (834 mg; extract A). The mycelium was macerated in MeOH (1 L, rt, 2 days) and filtered. H_2O (150 mL) and hexanes (1 L) were added to the filtrate, and the layers were separated. The aqueous MeOH phase was partially evaporated, and the residue was extracted with EtOAc (2 × 600 mL). The combined EtOAc solution was washed with H_2O (100 mL) and concentrated under reduced pressure to obtain a brown gum (183 mg, extract B). Extract A was subjected to Sephadex LH-20 column chromatography (3.0 × 50 cm, MeOH) to obtain six pooled fractions, A1−A6. Fraction A3 (545 mg) was chromatographed on silica gel (3.0 × 15 cm, MeOH/CH_2Cl_2, step gradient elution from 2:98 to 10:90) to obtain 11 fractions, A3-1−A3-11. Fraction A3-4 (58 mg) was purified by preparative HPLC using a reversed-phase column (SunFire Prep C_{18} OBD, 19 × 150 mm, 5 μm; mobile phase MeCN/H_2O, gradient from 30:70 to

dx.doi.org/10.1021/np100873t |J. Nat. Prod. 2011, 74, 300–302

80:20, flow rate 10 mL/min) to furnish **2** (7.7 mg). Fraction A3-6 (91 mg) was also further fractionated by preparative HPLC ($MeCN/H_2O$, gradient from 20:80 to 40:60) to afford **3** (20.0 mg). Fraction A4 (97 mg) was subjected to silica gel column chromatography (2.0 × 15 cm, $MeOH/CH_2Cl_2$, step gradient elution from 2:98 to 11:89) to give **1** (13.2 mg). Extract B was chromatographed on Sephadex LH-20 (2.0 × 60 cm, MeOH) and preparative HPLC ($MeCN/H_2O$) to furnish **3** (1.1 mg) and **1** (6.0 mg).

Xylopimarane (***1***)*:* colorless solid; mp 174−175 °C; $[\alpha]^{26}_D$ +75 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.66), 228 sh (3.95), 288 (3.44) nm; IR (KBr) ν_{max} 3420, 2928, 1636, 1429, 1029, 1008 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) and ^{13}C NMR (125 MHz, acetone-d_6) data, see Table 1; HRMS (ESI-TOF) *m/z* 487.2301 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_8Na$, 487.2302).

Hydrolysis of 1. Compound **1** (1.9 mg) was hydrolyzed in 5% aqueous hydrochloric acid (0.5 mL) at 90 °C for 2 h. The mixture was washed with Et_2O (2 × 1 mL), and the aqueous layer was concentrated under reduced pressure to obtain D-glucose (0.7 mg, ^1H NMR in D_2O/CD_3OD); $[\alpha]^{27}_D$ +85 (*c* 0.035, H_2O)).

Biological Assays. Anticancer activities against KB cells (oral human epidermoid carcinoma), MCF-7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer) were evaluated using the resazurin microplate assay.[13] The IC_{50} values of the standard compound doxorubicin hydrochloride were 0.27 μM for KB cells, 4.9 μM for MCF-7 cells, and 0.13 μM for NCI-H187 cells. Cytotoxicity to Vero cells (African green monkey kidney fibroblasts) were performed using the green fluorescent protein microplate assay (GFPMA).[14] Ellipticine was used as the standard of cytotoxicity (IC_{50} 7.3 μM).

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +66-25646700, ext 3554. Fax: +66-25646707. E-mail: isaka@biotec.or.th.

■ ACKNOWLEDGMENT

Financial support from the Bioresources Research Network, National Center for Genetic Engineering and Biotechnology (BIOTEC), is gratefully acknowledged.

■ REFERENCES

(1) Lin, Y.; Wu, X.; Feng, S.; Jiang, G.; Luo, J.; Zhou, S.; Vrijmoed, L. L. P.; Jones, E. B. G.; Krohn, K.; Steingröver, K.; Zsila, F. *J. Org. Chem.* **2001**, *66*, 6252–6256.

(2) Lin, Y.; Wu, X.; Feng, S.; Jiang, G.; Zhou, S.; Vrijmoed, L. L. P.; Jones, E. B. G. *Tetrahedron Lett.* **2001**, *42*, 449–451.

(3) Boonphong, S.; Kittakoop, P.; Isaka, M.; Pittayakhajonwut, D.; Tanticharoen, M.; Thebtaranonth, Y. *J. Nat. Prod.* **2001**, *66*, 6252–6256.

(4) Wang, X.-N.; Tan, R.-X.; Liu, J.-K. *J. Antibiot.* **2005**, *58*, 268–270.

(5) Singh, S. B.; Zink, D.; Polishook, J.; Valentino, D.; Shafiee, A.; Silverman, K.; Felock, P.; Teran, A.; Vilella, D.; Hazuda, D. J.; Lingham, R. B. *Tetrahedron Lett.* **1999**, *40*, 8775–8779.

(6) Smith, C. J.; Morin, N. R.; Bills, G. F.; Dombrowski, A. W.; Salituro, G. M.; Smith, S. K.; Zhao, A.; MacNeil, D. J. *J. Org. Chem.* **2002**, *67*, 5001–5004.

(7) Isaka, M.; Chinthanom, P.; Boonruangprapa, T.; Rungjindamai, N.; Pinruan, U. *J. Nat. Prod.* **2010**, *73*, 683–687.

(8) Evidente, A.; Sparapano, L.; Fierro, O.; Bruno, G.; Giordano, F.; Motta, A. *Phytochemistry* **1997**, *45*, 705–713.

(9) Grabley, S.; Hammann, P.; Thiericke, R.; Wink, J. *J. Antibiot.* **1993**, *46*, 343–345.

(10) Rao, A. V. R.; Murthy, V. S.; Sharma, G. V. M. *Tetrahedron Lett.* **1995**, *36*, 143–146.

(11) Ellestad, G. A.; Kunstmann, M. P.; Mirando, P.; Morton, G. O. *J. Am. Chem. Soc.* **1972**, *94*, 6206–6208.

(12) The same compound (**4**) was later isolated from the fungus *Sphaeropsis sapinea* f. sp. *cupressi* and named sphaeropsidin A: Evidente, A.; Sparapano, L.; Motta, A.; Giordano, F.; Fierro, O.; Frisullo, F. *Phytochemistry* **1996**, *42*, 1541–1546.

(13) O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421–5426.

(14) Changsen, C.; Franzblau, S. G.; Palittapongarnpim, P. *Antimicrob. Agents Chemother.* **2003**, *47*, 3682–3687.