J. Antibiot. 60(12): 748–751, 2007

NOTE

THE JOURNAL OF ANTIBIOTICS

Menisporopsin B, a New Polyester from the Seed Fungus *Menisporopsis theobromae* BCC 4162

Vasimon Ruanglek, Supichar Chokpaiboon, Nakul Rattanaphan, Siribhorn Madla, Patchanee Auncharoen, Taridaporn Bunyapaiboonsri, Masahiko Isaka

Received: September 17, 2007 / Accepted: December 10, 2007

Abstract A new linear polyester, menisporopsin B, along with the known macrocyclic polyester, menisporopsin A, was isolated from the seed fungus *Menisporopsis theobromae* BCC 4162. The structure of menisposopsin B was addressed primarily by spectroscopic analyses, and the stereochemistry was established by chemical correlation. Menisporopsin B exhibited antimalarial activity with an IC_{50} value of 1.0 μg/ml.

Keywords *Menisporopsis theobromae*, seed fungi, menisporopsin B, polyester

As part of the research program on novel bioactive compounds from local fungi in Thailand [1, 2], we previously reported the isolation of an antimalarial and cytotoxic macrocyclic polyester, menisporopsin A (**1**), from the seed fungus *Menisporopsis theobromae* BCC 4162, which was fermented in peptone - yeast extract - glucose medium (PYGM) under static condition [3]. Recent studies on optimization of culture conditions led to the conclusion that fructose, instead of glucose in PYGM, is more suitable as carbon source [4]. Shaking not only enhanced the production of menisporopsin A, but also reduced the incubation time [4]. Taken these two factors together, fermentation of BCC 4162 in peptone - yeast extract - fructose medium (see Experimental) under shaking condition was further studied. In addition to menisporopsin A, a new metabolite was discovered in the time profile studies. Therefore, we have undertaken the large scale fermentation, isolation and structure elucidation of the new analogue, menisporopsin B (**2**).

T. Bunyapaiboonsri (Corresponding author), **V. Ruanglek, S. Chokpaiboon, N. Rattanaphan, S. Madla, P. Auncharoen, M. Isaka:** National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, 113 Phaholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand,
E-mail: taridaporn@biotec.or.th.

Results and Discussion

2 was isolated as a white solid with the same molecular formula as **1** ($C_{40}H_{46}O_{17}$; *m/z* 821.2626 for $[M+Na]^+$, $\Delta = -0.7$ mmu). 2D NMR data (^{1}H-NMR, ^{13}C-NMR, COSY, NOESY, HMQC, and HMBC) suggested that this compound possessed a unit of 3,4-dihydro-6,8-dihydroxy-3-(2-hydroxypropyl)isocoumarin, two units of 2,4-dihydroxy-6-(2-hydroxypropyl)benzoic acid and two units of 3-hydroxybutyric acid. ^{1}H- and ^{13}C-NMR spectra of **2** revealed resonances close to those of **1** for both two units of dihydroxybenzoic acid and a unit of 3-hydroxybutyric acid. Notable NMR differences between these two compounds were the downfield resonances of H-9/C-9 (δ_H 4.77/δ_C 76.1), and upfield shifts of H-39/C-39 (δ_H 4.03/δ_C 63.8) of **2** when compared to those of **1** (H-9/C-9, δ_H 3.95/δ_C 69.1; and H-39/C-39, δ_H 5.56 or 5.54/δ_C 69.3 or 69.8). On the basis of these data, an ester linkage at C-9 (instead of a secondary alcohol), and a terminal hydroxyl group at C-39 (instead of an ester linkage) were proposed for **2**. The dihydroisocoumarin unit and the terminal 3-hydroxybutyric acid unit of **2** were assigned based on COSY cross signals and HMBC data. From ^{13}C-NMR/DEPT spectra, an ester carbonyl (δ_C 170.3), four aromatic quaternary carbons (δ_C 165.1, 164.2, 141.8 and 100.4), two aromatic methines (δ_C 107.0 and 101.2), two oxygenated methines (δ_C 76.1 and

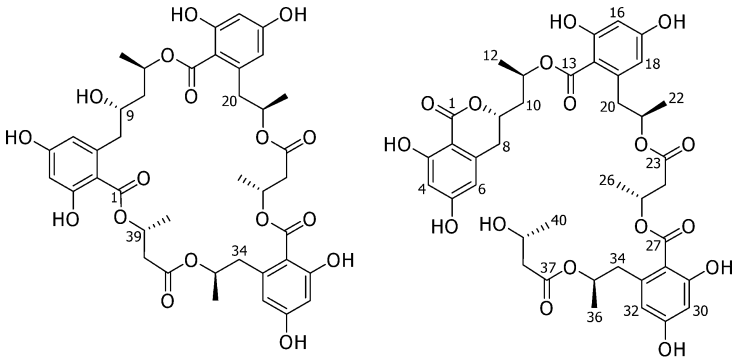

Fig. 1 Structures of menisporopsins A (**1**) and B (**2**).

69.3), two methylene carbons (δ_C 40.5 and 32.7), and a methyl group (δ_C 19.3) were observed for the dihydroisocoumarin unit. COSY cross signals suggested partial structure from C-8 to C-12, while the remaining part from C-1 to C-8 was assigned based on ^{1}H and ^{13}C chemical shift values, and HMBC correlations from H-8 to C-2, C-6 and C-7, and from H-6 to C-1, C-2, C-4 and C-8. The sequence of all five hydroxy acid units *via* ester linkages was determined by HMBC correlations observed from H-11 to C-13, H-21 to C-23, H-25 to C-27, and H 35 to C-37, therefore, the gross structure of **2** was established.

By structural comparison, it was assumed that **2** derived from **1** *via* intramolecular translactonization. The time profile of metabolite production strongly suggested that this transformation occurred during fermentation period. To correlate the stereochemistry, chemical transformation of **1** to **2** was examined. Treatment of **1** with K_2CO_3 in THF at room temperature for 16 hours gave **2** as a sole product. Accordingly, the absolute configuration of **2** was shown to be the same as **1**.

The antimalarial and cytotoxic activities of **2** were close to those of **1**. **2** exhibited antimalarial activity against *Plasmodium falciparum* K1 with an IC_{50} value of 1.0 μg/ml. It also displayed cytotoxic activities against cancer cell-lines, BC, KB, and NCI-H187 (with respective IC_{50} values of 3.7, 8.9, and 4.8 μg/ml), and non-malignant Vero cells with an IC_{50} value of 16.4 μg/ml.

Macrocyclic or linear polyesters consisting of 2,4-dihydroxy-6-(2-hydroxypropyl)benzoic acid and 3-hydroxybutyric acid residues have been reported as metabolites of *Hypoxylon oceanicum* [5], *Penicillium verruculosum* [6, 7], and *Scedosporium apiospermum* [8], whereas macrocyclic or linear polyesters containing 3,4-dihydro-6,8-dihydroxy-3-(2-hydroxypropyl)isocoumarin residues are unusual.

Experimental

Extraction and Isolation

The fungus BCC 4162 was incubated in 800 ml of peptone - yeast extract - fructose medium (fructose 10 g, yeast extract 20 g, bacto-peptone 5.0 g, ammonium tartrate 0.5 g, KH_2PO_4 1.0 g, Na_2HPO_4 2.25 g, Na_2SO_4 0.28 g, $CaCl_2$ 0.1 g, $MgCl_2$ 0.41 g, $MnSO_4 \cdot 4H_2O$ 44.3 mg, $ZnSO_4 \cdot 7H_2O$ 40.5 mg, ferric citrate 53.1 mg and citric acid 53.1 mg, per liter) on a rotary shaker at 200 rpm for 7 days at 25°C, and then filtered. Mycelia were macerated in MeOH (500 ml, 2 days) and filtered. The filtrate was diluted with H_2O (50 ml) and defatted with hexane (2×300 ml). The aqueous MeOH layer was concentrated under reduced pressure. The residue was dissolved in EtOAc (350 ml), and washed with H_2O (100 ml). The organic layer was concentrated under reduced pressure to provide a yellow gum (672.9 mg). The crude mycelial extract was fractionated using a Sephadex LH-20 column (elution with MeOH) to provide 8 fractions. Fraction 6 was purified by preparative HPLC using a reversed-phase column (Nova-Pak HR C_{18}, 6 μm, 25×100 mm; MeCN : H_2O=45 : 55) to furnish **2** (43.1 mg). Fraction 7 was rechromatographed on a Sephadex LH-20 column (elution with MeOH) to afford **1** (87.9 mg).

Menisporopsin B (2)

White solid; mp 119~120°C; $[\alpha]_D^{26}$ −79.8 (*c* 0.2, MeOH); UV λ_{max}^{MeOH} nm (log ε) 216 (5.04), 266 (4.77), 303 (4.45); IR ν_{max} (KBr) cm^{-1} 3388, 2982, 2935, 1714, 1649, 1620, 1453, 1315, 1260, 1167, 1094, 1049; HR-MS (ESI-TOF): *m/z* 821.2626 $[M+Na]^+$ (calcd for $C_{40}H_{46}O_{17}Na$, 821.2633); ^{1}H (500 MHz)- and ^{13}C (125 MHz)-NMR data, see Table 1.

Table 1 NMR data for menisporopsin B in acetone-d_6

Position	δ_C	δ_H (multiplicity, J in Hz)[a]
3,4-Dihydro-6,8-dihydroxy-3-(2-hydroxypropyl)isocoumarin [1 unit]		
1	170.3	
2	100.4	
3	165.1[b]	
4	101.2	6.23 (1H, d, 2.2)
5	164.2[b]	
6	107.0	6.29 (1H, d, 2.4)
7	141.8	
8	32.7	3.00 (1H, dd, 16.4, 3.7), 2.93 (1H, dd, 16.4, 11.0)
9	76.1	4.77 (1H, d, m)
10	40.5	2.40 (1H, ddd, 14.3, 8.1, 6.9), 2.11 (1H, ddd, 14.3, 6.4, 5.0)
11	69.3	5.55 (1H, m)
12	19.3	1.47 (3H, d, 6.3)
2,4-Dihydroxy-6-(2-hydroxypropyl)benzoic acid [2 units]		
13, 27	170.6; 169.2	
14, 28	104.7; 104.2	
15, 29	165.3[b]; 165.2[b]	
16, 30	101.7; 101.7	6.26 (1H, d, 2.4); 6.24 (1H, d, 2.4)
17, 31	162.3[b]; 162.5[b]	
18, 32	112.7; 112.9	6.33 (1H, d, 2.5); 6.29 (1H, d, 2.4)
19, 33	142.6; 142.9	
20, 34	42.2; 42.3	3.25 (1H, dd, 13.3, 6.6), 3.05 (1H, dd, 13.3, 8.8); 3.24 (1H, dd, 13.3, 5.6), 2.82 (1H, dd, 13.5, 9.2)
21, 35	71.5; 70.8	5.23 (1H, m); 5.15 (1H, m)
22, 36	19.5; 19.7	1.21 (3H, d, 6.2); 1.23 (3H, d, 6.2)
3-Hydroxybutyric acid [1 unit; the middle]		
23	169.1	
24	40.4	2.73 (1H, dd, 15.8, 7.3), 2.67 (1H, dd, 15.8, 5.9)
25	68.8	5.54 (1H, m)
26	19.0	1.36 (3H, d, 6.4)
3-Hydroxybutyric acid [1 unit; the end]		
37	170.7	
38	44.0	2.32 (1H, dd, 14.9, 7.0), 2.25 (1H, dd, 14.9, 6.0)
39	63.8	4.03 (1H, m)
40	22.3	1.04 (3H, d, 6.2)

[a] Broad signals of phenolic protons were observed at δ_H 11.61 and 11.19. [b] Assignment of carbon resonances can be interchanged.

Chemical Transformation of 1 to 2

To a solution of **1** (3.0 mg) in THF (2.5 ml) was added K_2CO_3 (10.0 mg). The mixture was stirred at room temperature for 16 hours, and then evaporated under reduced pressure. The residue was dissolved in EtOAc (8.0 ml) and washed with H_2O (4×3.0 ml). The organic layer was concentrated *in vacuo* to afford a crude reaction mixture, which was subsequently purified by preparative HPLC (MeCN : H_2O = 45 : 55) to yield a white solid (2.5 mg). Its specific rotation value, ^{1}H-NMR and MS data were identical with those of the isolated **2**. The identification was also confirmed using HPLC analysis by co-injection with the isolated **2**.

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

References

1. Isaka M, Kittakoop P, Kirtikara K, Hywel-Jones NL, Thebtaranonth Y. Bioactive substances from insect pathogenic fungi. Acc Chem Res 38: 813–823 (2005)
2. Bunyapaiboonsri T, Yoiprommarat S, Intereya K, Kocharin K. New diphenyl ethers from the insect pathogenic fungus *Cordyceps* sp. BCC 1861. Chem Pharm Bull 55: 304–307 (2007)
3. Chinworrungsee M, Kittakoop P, Isaka M, Maithip P, Supothina S, Thebtaranonth Y. Isolation and structure elucidation of a novel antimalarial macrocyclic polylactone, menisporopsin A, from the fungus *Menisporopsis theobromae*. J Nat Prod 67: 689–692 (2004)
4. Madla S, Kittakoop P, Wongsa P. Optimization of culture conditions for production of antimalarial menisporopsin A by the seed fungus *Menisporopsis theobromae* BCC 4162. Lett Appl Microbiol 43: 548–553 (2006)
5. Schlingmann G, Milne L, Carter GT. Isolation and identification of antifungal polyesters from the marine fungus *Hypoxylon oceanicum* LL-15G256. Tetrahedron 58: 6825–6835 (2002)
6. Ito M, Tsuchida Y, Mizoue K, Hanada K. NG-011 and NG-012, novel potentiators of nerve growth factor. II. The structure determination of NG-011 and NG-012. J Antibiot 45: 1566–1572 (1992)
7. Breinholt J, Jensen GW, Nielsen RI, Olsen CE, Frisvad JC. Antifungal macrocyclic polylactones from *Penicillium verruculosum*. J Antibiot 46: 1101–1108 (1993)
8. Kondo H, Kurama M, Nakajima S, Osada K, Ookura A, Suda H. Estrogenic BE-26263 and its manufacture with *Scedosporium apiospermum*. Jpn Kokai Tokkyo Koho: JP 05032658 (1993)