BEQUINOSTATINS C AND D, NEW INHIBITORS OF GLUTATHIONE *S*-TRANSFERASE, PRODUCED BY *Streptomyces* sp. MI384-DF12

Sir:

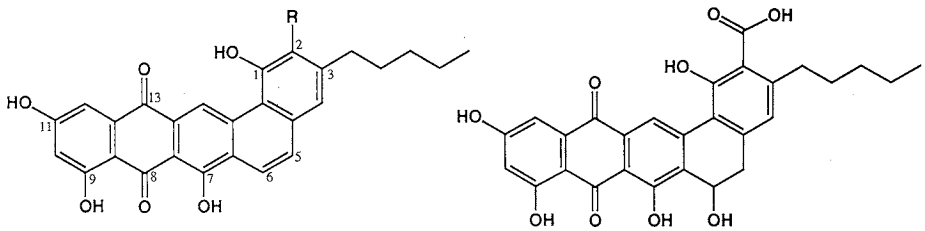
We have previously reported that *Streptomyces* sp. MI384-DF12 produces benastatins A, B and C, and bequinostatins A (**1**) and B as novel inhibitors of glutathione *S*-transferase (GST, EC 2.5.1.18)[1~4]. Our efforts to isolate other congeners from the strain resulted in the discovery of two minor components designated bequinostatins C (**2**) and D (**3**) as shown in Fig. 1. In this communication, the production, isolation, physico-chemical properties, structures and biological properties of **2** and **3** are reported.

A loopful of slant culture of *Streptomyces* sp. MI384-DF12 (FERM P-11270) was inoculated into 110 ml of a seed medium consisting of galactose 2.0%, dextrin 2.0%, Bacto Soytone 1.0%, corn steep liquor 0.5%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (pH 7.4) in a 500-ml Erlenmeyer flask, and cultured at 30°C for 3 days on a rotary shaker (180 rpm). Two ml of this seed culture were inoculated into 110 ml of the production medium consisting of glycerol 2.0%, soy bean meal (Ajinomoto Co., Inc.) 1.5%, K_2HPO_4 0.1% and $CoCl_2 \cdot 6H_2O$ 0.0005% (pH 6.2 adjusted with 1 M KH_2PO_4 before sterilization) in a 500-ml Erlenmeyer flask and cultured at 27°C for 4 days on a rotary shaker (180 rpm).

The isolation procedure is shown in Fig. 2. The culture broth (30 liters) was filtered at pH 4 and separated into the mycelial cake and the culture filtrate. The mycelial cake was extracted with 75% aq Me_2CO; the extract was filtered and concentrated *in vacuo* to an aqueous solution. The solution was extracted with EtOAc and the extract was concentrated to dryness under reduced pressure. The dried material was chromatographed on a silica gel column. After washing the column with $CHCl_3$-EtOAc (4:1), the active substances were eluted with EtOAc. The eluate was evaporated to dryness and applied on a second silica gel column. The fractions eluted with $CHCl_3$-MeOH-28% NH_4OH (95:5:1) are composed of **3** and the fractions eluted with MeOH-28% NH_4OH (100:1) comprise **2**. Each eluate was evaporated to dryness to obtain a reddish brown powder. Then each of them was loaded onto a column of YMC GEL ODS (Yamamura Chemical Industries Co., Ltd., Japan), and eluted with MeOH-28% NH_4OH (100:1) and MeOH-H_2O-28% NH_4OH (80:20:1), respectively. The fractions containing **3** were collected and evaporated to dryness to give a reddish powder. The powder was suspended in water and extracted with an equal volume of EtOAc. The extract was concentrated to dryness under reduced pressure. The dried reddish powder was dissolved in a small amount of DMF, and then MeOH was added to give pure **3** as a red powder. The other fractions containing **2** were also collected and concentrated to an aqueous solution, and adjusted to pH 2 to give pure **2** as a red powder. The total yields of **3** and **2** were 9.6 mg and 80.6 mg, respectively.

The physico-chemical properties of **2** and **3** are summarized in Table 1. **2** has a low solubility of 0.2 mg/ml in DMSO at 25°C. **3** is soluble in DMSO, and sparingly soluble in MeOH. The molecular weight of **2** was determined to be 486 from the FAB-MS peaks at *m/z* 487 $(M+H)^+$ and *m/z* 485 $(M-H)^-$. Since the molecular weight of **1** was 504, it suggested that **2** was the dehydrated product of **1**. To verify this, 5,6-anhydrobequinostatin A was prepared from **1**. 1 N HCl (0.5 ml) was added to a suspension of **1** (8.7 mg) and MeOH (5 ml), and the resulting suspension was stirred under reflux for 3.5 hours. After the reaction, the mixture was evaporated to dryness under reduced pressure. The residue was suspended in MeOH, and filtered, and

Fig. 1. Structures of bequinostatins A, C and D.



Bequinostatin C (**2**) R=COOH
Bequinostatin D (**3**) R=H

Bequinostatin A (**1**)

Fig. 2. Isolation procedure of bequinostatins C and D.

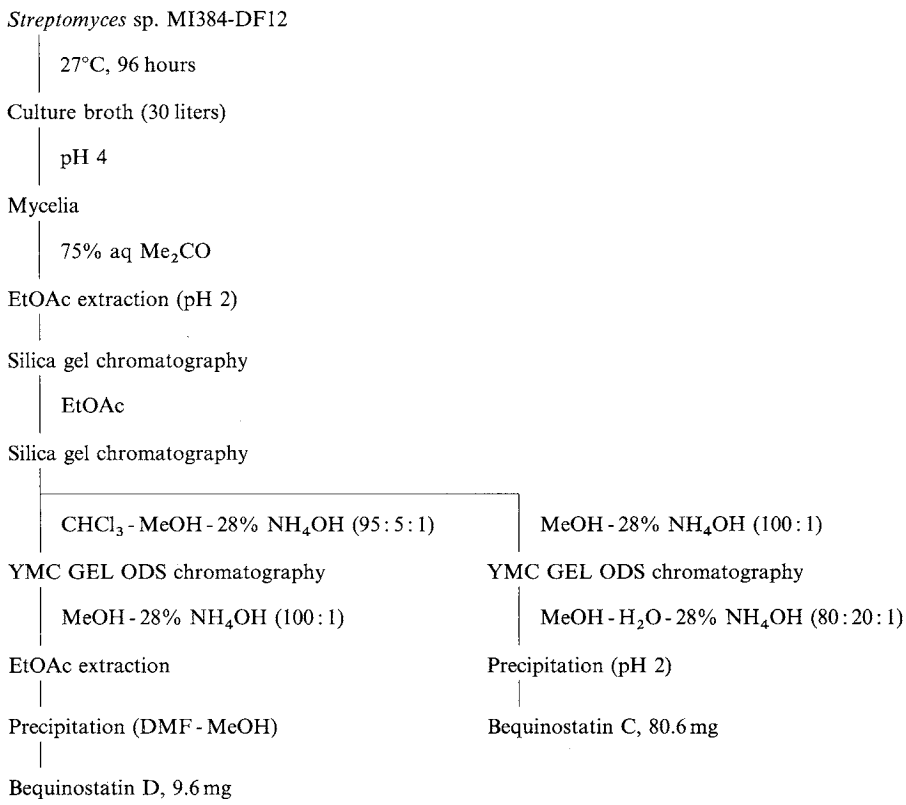


Table 1. Physico-chemical properties of bequinostatins C and D.

	Bequinostatin C	Bequinostatin D
Appearance	Red powder	Red powder
MP	306~308°C (dec)	310~312°C (dec)
Molecular formula	$C_{28}H_{22}O_8$	$C_{27}H_{22}O_6$
FAB-MS (*m/z*), Positive	487 $(M+H)^+$	443 $(M+H)^+$
FAB-MS (*m/z*), Negative	485 $(M-H)^-$	441 $(M-H)^-$
UV λ_{max}^{MeOH} nm (log ε)	260 (sh, 4.57), 281 (4.64), 324 (4.32), 370 (4.48), 474 (4.32)	271 (4.62), 285 (4.56), 336 (sh, 4.44), 354 (4.52), 474 (4.29)
$\lambda_{max}^{MeOH-HCl}$ nm (log ε)	257 (4.60), 292 (4.66), 362 (4.51), 380 (sh, 4.40), 465 (4.33)	272 (4.62), 286 (4.59), 335 (4.40), 355 (4.45), 470 (4.34)
$\lambda_{max}^{MeOH-NaOH}$ nm (log ε)	269 (4.54), 307 (sh, 4.54), 316 (4.54), 364 (4.59), 531 (4.22)	260 (sh, 4.58), 312 (4.46), 350 (sh, 4.38), 385 (4.47), 508 (4.30)
IR ν_{max}^{KBr} cm^{-1}	3275, 2925, 1620, 1452, 1405, 1334, 1290, 1249, 1118, 1039, 862, 769	3386, 2932, 1627, 1468, 1400, 1348, 1273, 1182, 1110, 1017, 863, 780
Rf values on TLC	0.12 ($CHCl_3$-MeOH, 4:1, silica gel)	0.84 ($CHCl_3$-MeOH, 4:1, silica gel)
Color reaction	Phosphomolybdate-H_2SO_4, $FeCl_3$	Phosphomolybdate-H_2SO_4, $FeCl_3$
Solubility Soluble:	DMSO	DMSO, MeOH
Insoluble:	H_2O	H_2O

the filtrate was washed with MeOH to give 5,6-anhydrobequinostatin A (5.4 mg, 65% yield). 5,6-Anhydrobequinostatin A: Rf 0.12 ($CHCl_3$-MeOH, 4:1); mp 305~307°C (dec.); FAB-MS *m/z* 487 $(M+H)^+$, *m/z* 485 $(M-H)^-$; IR (KBr) cm^{-1} 3070, 2935, 1612, 1434, 1385, 1340, 1282, 1253, 1101, 1028, 875, 794. These data supported that the structure of **2** was 8,13-dihydro-1,7,9,11-tetrahydroxy-8,13-dioxo-3-pentylbenzo[*a*]naphthacene-2-carboxylic acid.

The molecular weight and formula of **3** were elucidated as $C_{27}H_{22}O_6$ (MW 442) from the FAB-MS peaks at *m/z* 443 $(M+H)^+$ and *m/z* 441 $(M-H)^-$ and ^1H and ^{13}C NMR spectra of **3**. ^1H NMR (200 MHz, DMSO-d_6): δ 0.89 (3H, t, J=6.6 Hz), 1.34 (4H, m), 1.67 (2H, m), 2.69 (2H, br t, J=7.6), 6.58 (1H, d, J=2.3), 7.07 (1H, br s), 7.18 (1H, d, J=2.4), 7.29 (1H, br s), 7.88 (1H, d, J=9.3), 8.15 (1H, d, J=8.8), 10.02 (1H, s), 10.95 (br s), 12.19 (s), 13.32 (br s) ppm. ^{13}C NMR (50 MHz, DMSO-d_6): δ 13.9, 21.9, 30.1, 31.0, 34.9, 107.9, 108.9, 109.7, 109.9, 114.9, 117.4, 119.6, 120.3, 121.7, 125.7, 128.4, 130.9, 134.9, 136.1, 136.5, 144.5, 157.2, 159.8, 164.9, 166.0, 181.8, 189.9 ppm. Since the molecular weight and formula of **2** were $C_{28}H_{22}O_8$ (MW 486), it suggested that **3** was the decarboxylated product of **2**. To verify this, 2-decarboxybequinostatin C was prepared from **2**. The solution of **2** (50 mg), $CuSO_4$ (1.5 mg), and quinoline (0.5 ml) was stirred at 150°C for 30 minutes under argon. After the reaction, the mixture was cooled and solidified. The solid was dissolved in 0.1 N methanolic NH_4OH (5 ml) and mixed with H_2O (2 ml) and 1 N HCl (4.5 ml) to give a precipitate. The precipitate was filtered, and the filtrate was washed with H_2O and then with MeOH to give 2-decarboxybenquinostatin C (39.3 mg, 86% yield). 2-Decarboxybequinostatin C: Rf 0.84 ($CHCl_3$-MeOH, 4:1); mp 310~312°C (dec.); FAB-MS *m/z* 443 $(M+H)^+$; IR (KBr) cm^{-1} 3376, 2950, 1628, 1474, 1408, 1339, 1277, 1180, 1119, 1044, 870, 761; ^1H NMR (200 MHz, DMSO-d_6) δ 0.89 (3H, t, J=6.4 Hz), 1.34 (4H, m), 1.68 (2H, m), 2.71 (2H, br t, J=7.6), 6.56 (1H, br s), 7.10 (1H, br s), 7.18 (1H, br s), 7.34 (1H, br s), 7.96 (1H, d, J=9.0), 8.20 (1H, d, J=9.0), 10.09 (1H, s), 11.01 (br), 12.23 (br s), 13.44 (br) ppm. These data supported that the structure of **3** was 8,13-dihydro-1,7,9,11-tetrahydroxy-8,13-dioxo-3-pentylbenzo[*a*]naphthacene.

The inhibitory activities of **2** and **3** against human pi class GST (GSTπ) were measured as described previously[3]. Their IC_{50} values were 40.0 and 0.6 μg/ml, respectively. **2** and **3** had no significant antimicrobial activity at 100 μg/ml. They exhibited no toxicity after ip injection in mice at a dose of 100 mg/kg.

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