NEW ANTITUMOR SUBSTANCES, BE-12406A AND BE-12406B, PRODUCED BY A STREPTOMYCETE

II. STRUCTURE DETERMINATION

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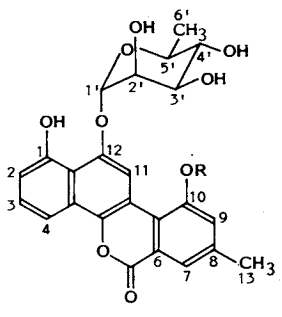
The structure of BE-12406A and BE-12406B, which were isolated from the culture broth of a streptomycete as antitumor substances, were determined by means of spectral analyses and chemical studies. The structure of BE-12406A is 1-hydroxy-10-methoxy-8-methyl-12-α-L-rhamnopyranosyloxy-6*H*-benzo[*d*]naphtho[1,2-*b*]pyran-6-one, and that of BE-12406B is 1,10-dihydroxy-8-methyl-12-α-L-rhamnopyranosyloxy-6*H*-benzo[*d*]naphtho[1,2-*b*]pyran-6-one.

BE-12406A (**1**) and BE-12406B (**2**) are new antitumor substances produced by a streptomycete, strain BA12406, as reported in a previous paper[1]. The structure determination studies of BE-12406A and BE-12406B are described in this paper.

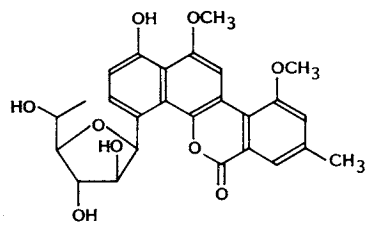
Structure of BE-12406A

The physico-chemical data of **1** and **2** were described in the previous paper[1]. The molecular formula of **1** was established as $C_{25}H_{24}O_9$ from the results of HRFAB-MS (Calcd: *m/z* 469.1499, Found: *m/z* 469.1471 $(M+H)^+$) and elemental analysis (Calcd: C 61.72, H 5.39 for $C_{25}H_{24}O_9 \cdot H_2O$, Found: C 62.26, H 5.31). The UV spectrum of **1** is quite similar to that of gilvocarcin M[2,3], which is known as toromycin M[4,5], therefore the existence of the benzonaphthopyranone skeleton was supposed. Comparison of the ^1H NMR data of **1** with that of gilovocarcin M indicated that **1** has a 1,2,3-substituted aromatic ring system as observed in defucogilvocarcin V, however, the chemical shifts of sugar moiety of **1** differ from

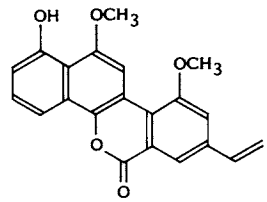
Fig. 1. Structures of BE-12406A, BE-12406B, gilvocarcin M and defucogilvocarcin V.



BE-12406A (**1**) R=CH_3
BE-12406B (**2**) R=H



Gilvocarcin M



Defucogilvocarcin V

Table 1. ^{1}H NMR data for **1**, **2**, **3**, **4** and defucogilvocarcin (300 MHz).

Proton	**1**[a]	**2**[a]	**3**[a]	**4**[b]	Defucogilvocarcin[c]
2	7.00 (br d, 7.6)[d]	6.98 (dd, 1.0, 7.7)	6.92 (dd, 0.8, 7.6)	7.23 (dd, 1.0, 7.6)	7.01 (dd, 0.8, 7.7)
3	7.49 (t, 7.9)	7.49 (t, 7.9)	7.48 (t, 8.1)	7.60 (t, 7.9)	7.54 (t, 8.0)
4	7.85 (br d, 8.2)	7.85 (br d, 8.2)	7.80 (dd, 0.8, 7.6)	8.56 (dd, 1.0, 7.6)	7.87 (dd, 0.8, 7.7)
7	7.78 (br s)	7.69 (br s)	7.79 (br s)	7.98 (br s)	8.03 (d, 1.5)
9	7.48 (br s)	7.29 (br s)	7.48 (s)	7.20 (br s)	7.84 (d, 1.3)
11	8.70 (s)	8.86 (s)	8.30 (s)	8.99 (s)	8.41 (s)
8-CH_3	2.50 (s)	2.42 (s)	2.50 (s)	2.52 (s)	—
10-OCH_3	4.06 (s)	—	4.08 (s)	4.08 (s)	4.21 (s)
10-OH	—	11.07 (s)	—	—	—
Ar-OH	9.75 (br s)	9.75 (br s)	10.95 (br s)	—	—
1′	5.39 (d, 1.2)	5.39 (d, 1.6)	—	5.48 (d, 1.6)	—
2′	4.10 (m)	4.10 (m)	—	5.62 (dd, 1.6, 3.8)	—
3′	3.78 (m)	3.78 (m)	—	5.59 (dd, 3.8, 9.8)	—
4′	3.39 (m)	3.39 (m)	—	5.21 (t, 9.8)	—
5′	3.80 (m)	3.80 (m)	—	4.18 (dq, 9.8, 6.3)	—
6′	1.25 (d, 6.2)	1.23 (d, 6.3)	—	1.27 (d, 6.3)	—
OH	5.13 (br s)	5.08 (br d, 4.3)	—	—	—
OH	4.95 (br d, 5.6)	4.94 (br d, 5.6)	—	—	—
OH	4.85 (br d, 5.8)	4.81 (br d, 5.8)	—	—	—
CH_3CO	—	—	—	2.52 (s)	—
	—	—	—	2.22 (s)	—
	—	—	—	2.09 (s)	—
	—	—	—	2.06 (s)	—

[a] In DMSO-d_6.
[b] In $CDCl_3$.
[c] Data in ref 6.
[d] Multiplicity, J in Hz.

Table 2. ^{13}C NMR data for **1**, **2**, **3** and gilvocaracin M (75 MHz).

Carbon	**1**[a]	**2**[a]	**3**[a]	Gilvocarcin M[b]	Carbon	**1**[a]	**2**[a]	**3**[a]	Gilvocarcin M[b]
1	153.4	153.7	154.0	152.5	10b	112.6	113.5	113.6	112.7
2	111.8	111.7	110.5	111.4	11	109.5	110.1	105.3	101.1
3	127.7	127.8	128.1	128.8	12	148.4	148.4	149.6	151.3
4	112.0	112.0	112.4	125.5	12a	115.8	115.9	114.1	114.5
4a	125.5	125.8	125.3	123.4	8-CH_3	21.0	20.8	21.0	21.0
4b	140.6	140.5	138.8	141.3	10-OCH_3	56.1	—	56.3	56.0
6	159.7	160.2	160.1	159.4	1′	101.5	101.2	—	80.9
6a	122.0	122.3	122.9	121.1	2′	70.0	70.1	—	79.0
7	121.5	122.9	121.7	120.7	3′	70.5	70.7	—	78.7
8	140.0	139.8	140.2	139.7	4′	71.7	71.8	—	86.1
9	118.8	120.7	119.0	118.0	5′	69.8	70.0	—	66.6
10	156.6	155.6	157.0	156.4	6′	17.7	17.8	—	20.1
10a	120.4	118.9	120.8	120.5	12-OCH_3	—	—	—	55.7

[a] In DMSO-d_6.
[b] Data in ref 3.

Fig. 2. Difference NOE experiments of **4**.

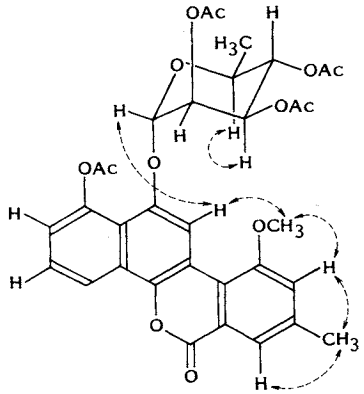
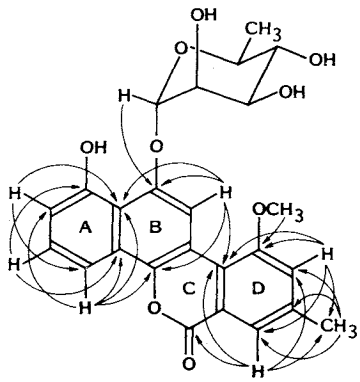


Fig. 3. LSPD experiments of **1**.



those of gilvocarcins[3], chrysomycins[7] or ravidomycins[8]. The ^1H and ^{13}C NMR data of **1** are shown in Tables 1 and 2, respectively. Hydrolysis of **1** with 1 N HCl-MeOH at 60°C for 90 minutes afforded the chromophore part (**3**). This observation and the chemical shift of an anomeric carbon in the ^{13}C NMR of **1** suggested that the sugar moiety in **1** should be a *O*-sugar. The acetylation of **1** with acetic anhydride in pyridine gave a tetra-acetyl derivative (**4**) (FAB-MS *m/z* 619 $(M+H)^+$). The ^1H NMR data of **4** are listed in Table 1. From the ^1H-^1H COSY spectrum of **4**, the assignments of protons in the sugar moiety could be deduced. By comparison of the ^1H NMR spectrum of **4** with that of **1**, the sugar moiety in **1** was indicated to be a rhamnose. NOEs were observed between the methyl proton (δ 2.52) and 7-H (δ 7.98) and 9-H (δ 7.20) protons, the methoxy proton (δ 4.08) and 9-H and 11-H (δ 8.99) protons, the anomeric-H (δ 5.48) and 11-H protons (Fig. 2). These data suggested that the location of the methyl and methoxy groups were identical with those of gilvocarcin M, and the sugar moiety was located at C-12 position. This presumption was confirmed by the long-range selective proton decoupling (LSPD) experiments of **1** (Fig. 3). From the observation of ^1H-^{13}C long range coupling between the anomeric proton and the C-12 carbon (δ 148.4), the rhamnose moiety should be connected to C-12. The location of OH group in A-ring was determined by the LSPD experiments. The signal for C-4b (δ 140.6) was collapsed by irradiation of the signal at 7.85 ppm, therefore, C-4 carbon should bear a proton, and the signal for the C-1 carbon (δ 153.4) was collapsed by irradiation of the signal at 3-H (Fig. 3). These data suggested that OH group in A-ring was located at C-1 position.

In the ^1H NMR spectrum of **4**, a NOE was observed between the C-5′ and the C-3′ protons, but not observed between the C-5′ and C-1′ protons. These data suggested the α-linkage of the sugar. The large coupling constant $J_{C\text{-}1'\text{-}1'\text{-}H}$ (169 Hz) of **1** also supported the α-linkage of rhamnose in **1**[9]. Methanolysis of **1** gave 1-*O*-methyl sugar (**5**), which was identical with α-1-*O*-methyl rhamnose by comparison of the ^1H and ^{13}C NMR spectra. The optical rotation value of **5** ($[\alpha]_D^{20}$ −72.9°, *c* 1, MeOH)[9] suggested that the configuration of the rhamnose is L-form. From the data described above, the structure of **1** was determined to be 1-hydroxy-10-methoxy-8-methyl-12-α-L-rhamnopyranosyloxy-6*H*-benzo[*d*]naphtho[1,2-*b*]pyran-6-one, as shown in Fig. 1.

Structure of BE-12406B

The molecular formula of **2** was determined as $C_{24}H_{22}O_9$ by HRFAB-MS (Calcd: *m/z* 455.1342, Found: *m/z* 455.1346 $(M+H)^+$). As the UV spectrum of **2** is similar to the spectrum of **1**, **2** apparently

has the same chromophore as **1**. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2, respectively) are similar to those of **1** except that a methoxy signal in **1** is not observed in **2**, instead, a phenolic proton was found in the ^{1}H NMR spectrum of **2**. By comparison of the NMR data and the molecular formula between **1** and **2**, it was suggested that the methoxy group in **1** is replaced by the hydroxy group in **2**. This was confirmed by comparison between the homogate decoupling spectrum of **1** and **2**. The signal of C-10 carbon was split into a broad singlet in **2**, whereas it was split into a broad quartet in **1**, while the other signals remained essentially identical. From the large coupling constant $J_{C\text{-}1'\text{-}1'\text{-}H}$ (171 Hz) of **2** and the optical rotation value ($[\alpha]_D^{20}$ −82.12°, *c* 1, MeOH), the structure of **2** was determined to be 1,10-dihydroxy-8-methyl-12-α-L-rhamnopyranosyloxy-6*H*-benzo[*d*]naphtho[1,2-*b*]pyran-6-one, as shown in Fig. 1.

Experimental

MS was carried out on a Jeol JMS-DX 300 spectrometer. NMR spectra were recorded on a Varian VXR 300 spectrometer with ^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz. TMS was used as an internal standard. Optical rotations were measured by a Horiba SEPA-200 high-sensitivity polarimeter.

Hydrolysis of BE-12406A

To a suspension of BE-12406A (**1**, 41 mg) in 4.5 ml of MeOH, 10% dry HCl-MeOH (1.5 ml) was added and kept for 90 minutes at 60°C. The reaction mixture was filtered to remove pale yellow crystals (**3**, 29 mg) and the crystals were washed with MeOH. The filtrate and washing were combined and evaporated to give a residue, which was purified on a Sephadex LH-20 column with MeOH as an eluant to give **5** (9.8 mg). FAB-MS (negative): *m/z* 177 $(M-H)^-$; ^{1}H NMR (D_2O) δ 4.70 (1-H), 3.94 (2-H), 3.72 (3-H), 3.64 (5-H), 3.44 (4-H), 3.40 (OCH_3), 1.31 (6-H); ^{13}C NMR (D_2O) δ 102.6 (C-1, J=169 Hz), 73.8 (C-4), 72.1 (C-3), 71.8 (C-2), 70.2 (C-5), 56.5 (OCH_3), 18.5 (C-6).

Tetra-acetyl BE-12406A (**4**)

To a suspension of BE-12406A (**1**, *ca.* 8 mg) in pyridine (2 ml), acetic anhydride (0.2 ml) was added and the suspension was stirred for 15 hours at room temperature. To the reaction mixture, H_2O was added and evaporated twice, the residue was extracted with $CHCl_3$. The extract was evaporated to give 5.2 mg of **4**.

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