

COMMUNICATION TO THE EDITOR

GT32-B New 20-Membered Macrocyclic Lactam Antibiotic

Sir:

From a culture broth of *Streptomyces* sp., we have isolated GT32-A which is identical with the 20-membered macrocyclic lactam antibiotic BE-14106 isolated by KOJIRI *et al.* in 1992.¹⁾ and a new compound GT32-B. GT32-B is structurally related to BE-14106 and exhibited antiproliferative activity. In this communication, we report the production, isolation and structural determination of GT32-B.

The producing organism was isolated from soil and taxonomically classified as a *Streptomyces* sp. A seed broth was prepared by inoculating spores of the producing strain into a medium consisting of glucose 1.0%, soluble starch 1.0%, bacto tryptone 0.5%, yeast extract 0.5%, beef extract 0.3% and CaCO₃ 0.2% (pH 7.2). After inoculation at 28°C for 48 hours, a 5%-vegetative seed culture was inoculated into a fermentation medium consisting of soluble starch 4.0%, soy bean meal 1.0%, dry yeast 5.0%, corn steep liquor 0.5%, KH₂PO₄ 0.5%, ZnSO₄ 10 mg/liter, CoCl₂ 10 mg/liter, NiSO₄ 10 mg/liter, Mg₃(PO₄)·8H₂O 0.05% (pH 7.0). The peak titers were usually reached after 4 days incubation at 28°C in jar fermenter culture.

GT32-A and -B were accumulated in the mycelium. Therefore the culture broth (30 liters) was filtered, isopropanol was added into cake and it was then filtered. After the filtrate was diluted with deionized water, the filtrate was applied to a column of Diaion HP-20 (2 liters) (Mitsubishi Chemical Industries Limited, Japan). The column was washed with deionized water (6 liters) and 60% methanol (6 liters) and then eluted with methanol (4 liters). After concentration, the residue was

subjected to silica gel (Merck Art. No. 7734) column chromatography using CHCl₃-MeOH-EtOAc (60:1:6) as the eluant. The active fractions were further purified by HPLC using a packed column (YMC-ODS SH-363-5, 80% MeOH) to yield 30 mg of GT32-A and 10 mg of GT32-B.

GT32-A was identified as the 20-membered macrocyclic lactam antibiotic²⁾ BE-14106¹⁾ by spectral analyses (UV, IR, NMR, and HRFAB-MS).

The structure of GT32-B was determined by the spectral analyses in addition to comparison of its spectra with those of GT32-A. The molecular formula of GT32-B was established as C₂₇H₃₇NO₂ from the results of the HRFAB-MS and NMR spectral analyses. In the comparison of the ¹³C NMR spectra of GT32-A and GT32-B, a clear difference was observed; one of the two -CH(OH)- signals at 69.7 and 72.1 ppm observed in GT32-A was lacking and one -CH₂- signal at 35.9 ppm was now present in GT32-B. These MS and NMR data suggested that GT32-B is a deoxy derivative at the position 8 or 9 of GT32-A. From the observed ¹H-¹H COSY connectivity, the structure of GT32-B was determined as shown in Fig. 1.

Fig. 1. Structure of GT32-A and GT32-B.

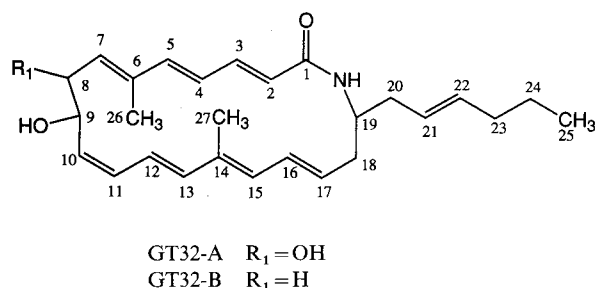
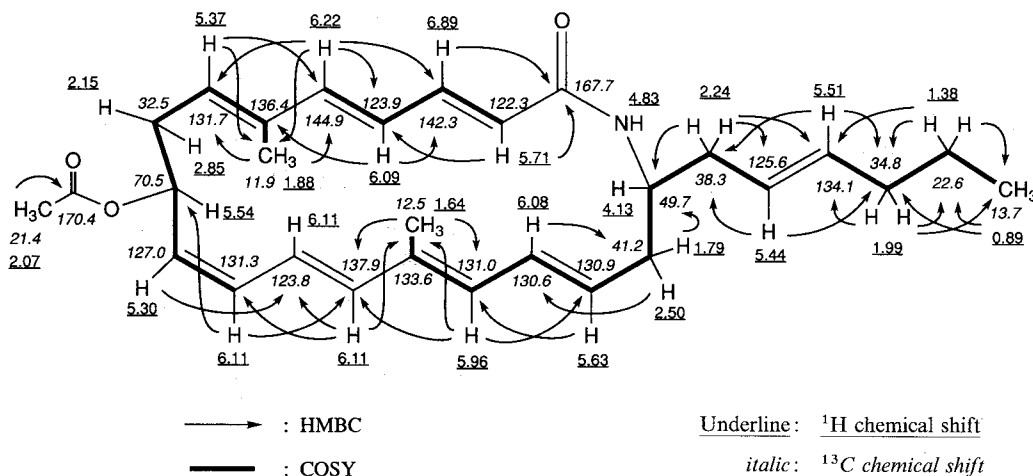


Fig. 2. The 2D NMR analyses of monoacetyl GT32-B.



Acetylation of GT32-B afforded a monoacetyl derivative (HRFAB-MS m/z 450.3011 (M+H)⁺, Δ +0.2 mmu) which exhibited improved solubility. The structure of GT32-B, including the configuration of the double bonds, was confirmed by the 2D NMR analyses (COSY,

NOESY, ¹H-¹³C HSQC, and ¹H-¹³C HMBC experiments) of the acetylated derivative. The results of the 2D NMR analyses are shown in Fig. 2.

Physico-chemical properties of GT32-B are summarized in Table 1. ¹H and ¹³C chemical shifts for GT32-A and GT32-B are shown in Table 2.

Both compounds exhibited weak antimicrobial activities against *Pseudomonas aeruginosa* BinH#1 (MIC: 42 μ g/ml). GT32-A and -B showed antiproliferative activity against H-ras transformed BALB3T3 cell line, having IC₅₀ values of 1.7 μ M and 0.8 μ M, respectively. Additionally, both compounds have inhibitory activity against mixed lymphocyte reaction (MLR) with an IC₅₀ value of 0.4 μ M.

Table 1. Physico-chemical properties of GT32-B.

Appearance	White powder
Molecular formula	C ₂₇ H ₃₇ NO ₂
HR-MS (m/z)	FAB-MS
Obsd:	408.2878 (M+H) ⁺
Calcd:	408.2902 for C ₂₇ H ₃₈ NO ₂
UV $\lambda_{\max}^{\text{MeOH}}$ nm (ε)	280 (74,000), 289 (92,000), 310 (sh, 20,000), 325 (sh, 15,000)
IR (KBr) ν_{\max} cm ⁻¹	3365, 3273, 2954, 2924, 1647, 1608, 1539, 1011, 991, 966

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Table 2. NMR data for GT32's^{a,b}.

Pos. No.	GT32-A in DMSO- <i>d</i> ₆		GT32-B in DMSO- <i>d</i> ₆		GT32-B monoacetate in CDCl ₃	
	¹³ C (100 MHz)	¹ H (400 MHz)	¹³ C (100 MHz)	¹ H (500 MHz)	¹³ C (100 MHz)	¹ H (400 MHz)
1	166.6 s		166.6 s		167.7 s	
2	123.8 d	5.90 (d, 14.9)	123.9 d	5.87 (d, 15.0)	122.3 d	5.71 (d, 14.9)
3	139.5 d	6.65 (ddd, 3.3, 7.0, 15.0)	139.7 d	6.63 (ddd, 2.6, 7.6, 15.0)	142.3 d	6.89 (dd, 10.7, 14.9)
4	124.9 d	6.14 (m)	124.0 d	6.12 (m)	123.9 d	6.09 (m)
5	142.5 d	6.14 (m)	143.1 d	6.13 (m)	144.9 d	6.22 (m)
6	131.5 s		134.8 s		136.4 s	
7	138.7 d	5.18 (d, 8.0)	134.1 d	5.31 (dd, 7.7, 9.2)	131.7 d	5.37 (m)
8	72.1 d	4.56 (br d, 8.0)	35.9 t	1.98 (m) 2.69 (ddd, 5.1, 5.9, 7.4)	32.5 d	2.15 (m) 2.85 (m)
9	69.7 d	4.30 (br d, 8.3)	66.7 d	4.43 (m)	70.5 d	5.54 (m)
10	131.3 d	5.40 (m)	132.3 d	5.27 (m)	127.0 d	5.30 (m)
11	128.5 d	6.00 (d, 10.8, 11.2)	128.0 d	5.97 (m)	131.3 d	6.11 (m)
12	123.8 d	5.90 (d, 11.2, 14.9)	123.6 d	5.96 (m)	123.8 d	6.11 (m)
13	136.3 d	6.08 (d, 14.9)	136.0 d	6.06 (d, 14.7)	137.9 d	6.11 (m)
14	132.9 s		132.9 s		133.6 s	
15	130.3 d	5.83 (d, 11.2)	130.3 d	5.83 (d, 10.9)	131.0 d	5.96 (d, 11.0)
16	130.7 d	6.17 (dd, 11.2, 14.9)	130.6 d	6.19 (dd, 10.9, 15.1)	130.6 d	6.08 (m)
17	129.9 d	5.42 (m)	130.0 d	5.41 (m)	130.9 d	5.63 (m)
18	39.8 t	1.76 (m) 2.32 (m)	39.6 t	1.80 (m) 2.32 (m)	41.2 t	1.79 (m) 2.50 (m)
19	49.3 d	3.81 (m)	49.3 d	3.82 (m)	49.7 d	4.13 (m)
20	38.0 t	2.14 (dd, 6.3, 6.3)	38.0 t	2.14 (dd, 6.6, 6.6)	38.3 t	2.24 (dd, 6.8, 11.6)
21	127.1 d	5.43 (m)	127.1 d	5.39 (m)	125.6 d	5.44 (m)
22	131.7 d	5.43 (m)	131.7 d	5.43 (m)	134.1 d	5.51 (m)
23	34.1 t	1.95 (dt, 6.3, 6.9)	34.0 t	1.94 (m)	34.8 t	1.99 (dt, 6.8, 7.3)
24	22.0 t	1.32 (tq, 7.3, 7.3)	22.0 t	1.33 (tq, 7.3, 7.3)	22.6 t	1.38 (tq, 7.3, 7.3)
25	13.4 q	0.84 (t, 7.3)	13.3 q	0.85 (t, 7.3)	13.7 q	0.89 (t, 7.3)
26	12.4 q	1.81 (br s)	11.9 q	1.82 (br s)	11.9 q	1.88 (br s)
27	11.9 q	1.58 (br s)	11.5 q	1.60 (br s)	12.5 q	1.64 (br s)
NH		7.23 (d, 10.3)		7.23 (d, 10.1)		4.83 (d, 10.3)
8-OH		4.84 (br s)				
9-OH		4.98 (br s)				
OCOCH ₃		-		-	21.4 q	2.07 (s)
OC ₂ CH ₃		-		-	170.4 s	-

a) Measured on JEOL JMN- α 400 and Bruker AM500 spectrometers.

b) δ ppm from TMS as an internal standard (multiplicity, J in Hz).

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