BE-32030 A, B, C, D and E, New Antitumor Substances Produced by *Nocardia* sp. A32030

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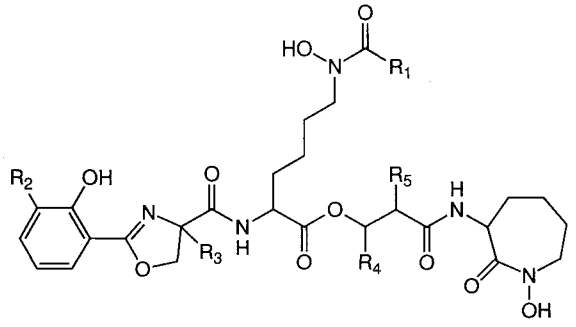
New antitumor substances, designated BE-32030A, B, C, D and E, were isolated from the culture broth of *Nocardia* sp. A32030. The active principles were extracted from the mycelium by methanol and purified by Sephadex LH-20 and reversed-phase column chromatographies and finally by reversed-phase HPLC. BE-32030A, B, C, D and E inhibited the growth of P388 murine leukemia, DLD-1 human colon cancer, PC-13 human lung cancer and MKN-45 human stomach cancer cell lines.

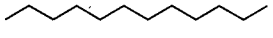
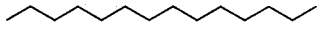
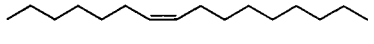
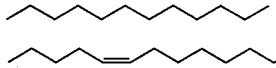
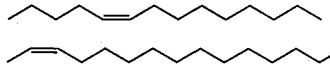
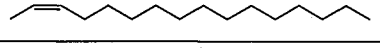
In the course of our screening program for new antitumor substances, a strain A32030 isolated from a soil sample collected in Ishigaki island, Okinawa prefecture, Japan, was found to produce active principles. Bioassay guided fractionation was carried out using the P388 murine leukemia cell line. After extraction of the mycelium of the producing organism, these antitumor substances were purified by Sephadex LH-20 and reversed-phase column chromatographies and finally by reversed-phase HPLC. In this paper, the producing organism, isolation, physico-chemical properties, structure determination and biological properties of BE-32030A, B, C, D and E are described. The structures of BE-32030 A, B, C, D and E are shown in Fig. 1.

Microorganism

BE-32030A, B, C, D and E were produced by a *Nocardia* strain A32030. Characterization of the genus followed the method adopted by the International Streptomyces Project (ISP)[1]. The strain A32030 formed

Fig. 1. Structures of BE-32030A ~ E.



	R_1	R_2	R_3	R_4	R_5
A		H	CH_3	CH_3	H
B		H	CH_3	CH_3	H
C		H	CH_3	CH_3	H
D		OH	CH_3	CH_3	H
E		OH	CH_3	CH_3	H
Mycobactin J		H	H	$CH(CH_3)_2$	CH_3

branching substrate mycelia and aerial mycelia, and fragmentation of the substrate mycelia and the spore chains was observed. The spore surface was smooth. Soluble pigments were absent. Utilization of carbon sources was examined according to the method of PRIDHAM and GOTTLIEB[2] on the agar medium culture at 28°C for 14 days. D-glucose, D-xylose, L-rhamnose, D-galactose, D-mannose were utilized for growth. Utilization of L-arabinose, D-fructose was questionable and raffinose, D-mannitol, inositol, salicin, sucrose were not utilized by the strain. The above-mentioned combination of characteristics indicated that strain A32030 belonged to *Nocardia* sp. It has been deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan (No. FERM P-13395).

Fermentation

The *Nocardia* sp. A32030 was inoculated into 110 ml of a seed medium consisting of 0.5% glucose, 3.0% dextrin, 0.2% sodium succinate, 1.0% sodium glutamate, 0.1% dry yeast, 0.05% ammonium sulfate, 0.1% sodium nitrate, 0.05% magnesium sulfate, 0.05% potassium chloride, 0.1% potassium hydrogen phosphate, 0.3% calcium carbonate, 0.0002% calcium chloride, 0.00002% ferrous sulfate, 0.000004% cupric chloride, 0.000004% manganese chloride, 0.000004% cobalt chloride, 0.000008% zinc sulfate and 0.000024% ammonium molybdate (pH 7.2). The seed culture was incubated for 72 hours at 28°C on a rotary shaker (180 rpm). Two ml each of the culture broth was inoculated into 100 500-ml Erlenmeyer flasks containing 110 ml of medium and cultured on a rotary shaker (180 rpm) at 28°C for 120 hours.

Assay of Cytotoxic Activity

P388 Assay:

In the *in vitro* cytotoxic assay using the P388 tumor cells, BE-32030A~E were first dissolved in dimethyl sulfoxide (DMSO). The solution was serially diluted with a cell culture medium containing 20% DMSO (20% DMSO-RPMI-1640 medium) with 2.5×10^4 tumor cells per 50 μl and the mixture was incubated under 5% CO_2 at 37°C for 72 hours. The viable cells were then counted with a Coulter counter.

DLD-1, PC-13 and MKN-45 Assay:

BE-32030A~E were first dissolved in dimethyl sulfoxide (DMSO), and serially diluted with phosphate-buffered saline (PBS). The media used for the culture of cancer cells was RPMI-1640 medium containing 10% fetal bovine serum (FBS). The cell line was cultured in 96-well microplates (3×10^3 cells/well) with or without a test sample under 5% CO_2 at 37°C for 72 hours. After fixing with 50% trichloroacetic acid, tumor cells were stained by 0.4% sulforhodamine B and the dye was extracted from the stained cells with 10 mM Tris(hydroxymethyl)aminomethane solution. Absorbance of the extract was read at 540 nm.

Isolation and Chemical Derivatization

The mycelium was obtained by filtration of the whole broth (*ca.* 10 liters). Extraction was carried out twice with 2 liters of methanol and the combined extract was concentrated to about 500 ml. The concentrated solution was extracted with 1 liter of ethyl acetate and the extract was evaporated under reduced pressure. The residue was extracted with about 10 ml of methanol. The methanol solubles were applied to a column of Sephadex LH-20 (2.5×89 cm) and eluted with methanol. The fractions containing active materials BE-32030 A, B, C, D and E were concentrated *in vacuo* to yield 2.48 g of the mixture. Further separation by a reversed-phase chromatography step (Cosmosil $75C_{18}$-OPN, 3.0×29 cm) with 80%, 85% and 90% MeOH yielded BE-32030A~E. BE-32030A~E were finally purified by HPLC using a reversed-phase silica gel column (Chromatorex-ODS, 20×250 mm) with a solvent system of 95% MeOH. Thus, 130 mg of BE-32030A, 381 mg of BE-32030B, 126 mg of BE-32030C, 91 mg of BE-32030D and 82 mg of BE-32030E were obtained. For the purpose of structure determination, hydrolysis of BE-32030B to BE-32030B-1 and acetylation of BE-32030B were performed.

A solution of BE-32030B (41.4 mg) in MeOH (10 ml) was mixed with 500 μl of 1 N NaOH-MeOH. The mixture was stirred at room temperature for 2 hours, and then the mixture was neutralized with 1 N HCl and MeOH was evaporated. The residual mixture was extracted twice with 100 ml of ethyl acetate. The extracts were evaporated and the residue was subjected to silica gel column chromatography. The column was eluted with $CHCl_3$-MeOH (30:1). The fractions containing BE-32030B-1 were concentrated *in vacuo* to yield 30.0 mg residue. The residue was then purified on a Sephadex LH-20 column using MeOH as eluting solvent, which resulted in 19.7 mg of BE-32030B-1.

A solution of BE-32030B (4.4 mg) in pyridine (500 μl) was mixed with 500 μl of acetic anhydride. The mixture was stirred at room temperature for 20 hours, and then 30 ml of H_2O was added and the mixture was extracted twice with 30 ml of ethyl acetate. The pooled extract was

evaporated and the residue was subjected to gel filtration on Sephadex LH-20. The column was eluted with MeOH, which resulted in 4.7 mg of triacetyl-BE-32030B.

Physico-chemical Properties

BE-32030A ~ E are soluble in common organic solvents such as $CHCl_3$, MeOH and DMSO, but insoluble in water. BE-32030A ($C_{39}H_{61}O_{10}N_5$): $[\alpha]_D^{20}$ $-40.3°$ (*c* 1.00, $CHCl_3$); HR FAB-MS *m/z* 760.4498 $(M+H)^+$; UV λ_{max}^{MeOH} nm (ε) 242 (11,000), 248 (11,000), 258 (sh, 6,300), 305 (4,300); IR ν_{max} (KBr) cm^{-1} 3303, 3197, 2927, 2856, 1735, 1641, 1525, 1492, 1456, 1365, 1307, 1257, 1224, 1064, 968, 756. BE-32030B ($C_{41}H_{65}O_{10}N_5$): $[\alpha]_D^{20}$ $-37.4°$ (*c* 1.00, $CHCl_3$); HR FAB-MS *m/z* 788.4807 $(M+H)^+$; UV λ_{max}^{MeOH} nm (ε) 242 (9,700), 248 (10,000), 258 (sh, 5,400), 305 (3,800); IR ν_{max} (KBr) cm^{-1} 3303, 3180, 2925, 2856, 1735, 1641, 1525, 1492, 1456, 1365, 1307, 1259, 1224, 1066, 968, 756. BE-32030C ($C_{43}H_{67}O_{10}N_5$): $[\alpha]_D^{20}$ $-38.9°$ (*c* 1.00, $CHCl_3$); HR FAB-MS *m/z* 814.4986 $(M+H)^+$; UV λ_{max}^{MeOH} nm (ε) 242 (10,700), 248 (11,000), 258 (sh, 6,200), 305 (4,300); IR ν_{max} (KBr) cm^{-1} 3311, 2927, 2858, 1737, 1641, 1529, 1496, 1452, 1363, 1307, 1257, 1220, 1128, 1064, 971, 754. BE-32030D ($C_{39}H_{61}O_{11}N_5$): $[\alpha]_D^{20}$ $-21.6°$ (*c* 1.00, $CHCl_3$); HR FAB-MS *m/z* 776.4437 $(M+H)^+$; UV λ_{max}^{MeOH} nm (ε) 250 (10,000), 256 (10,000), 315 (2,600); IR ν_{max} (KBr) cm^{-1} 3301, 2927, 2856, 1733, 1639, 1525, 1473, 1378, 1357, 1317, 1263, 1216, 1128, 1066, 993, 744. BE-32030E ($C_{41}H_{63}O_{11}N_5$): $[\alpha]_D^{20}$ $-21.0°$ (*c* 1.00, $CHCl_3$); HR FAB-MS *m/z* 802.4598 $(M+H)^+$; UV λ_{max}^{MeOH} nm (ε) 250 (9,800), 256 (9,300), 315 (2,400); IR ν_{max} (KBr) cm^{-1} 3301, 2927, 2856, 1735, 1639, 1529, 1483, 1378, 1357, 1317, 1259, 1216, 1128, 1066, 991, 742.

Table 1. ^{13}C-NMR for BE-32030A, B, C, D, E and B-1 in DMSO-d_6.

	A	B	C	D	E	B-1
1	110.0	110.0	110.0	110.3	110.3	110.1
2	159.0	159.0	159.0	148.1	148.1	159.0
3	116.6	116.6	116.6	145.7	145.7	116.7
4	133.9	133.9	133.9	119.3	119.4	134.0
5	118.9	118.9	118.9	118.6	118.6	118.9
6	128.0	128.0	128.0	117.9	117.9	128.0
8	164.0	164.0	164.0	164.6	164.6	164.1
10	73.7	73.8	73.8	73.6	73.7	73.8
11	74.6	74.6	74.6	74.6	74.7	74.6
12	25.8	25.8	25.8	25.8	25.8	25.8
13	172.7	172.7	172.8	172.8	172.8	172.9
Lys 1						
C=O	170.9	170.9	170.9	170.9	170.9	172.3
α	52.3	52.3	52.3	52.2	52.3	52.1
β	29.8	29.8	29.8	29.8	29.9	29.8
γ	22.5	22.6	22.6	22.5	22.6	22.7
δ	25.8	25.8	25.8	25.8	25.8	25.8
ε	46.8	46.8	46.8	46.8	46.8	46.7
OCH_3						51.8
Lys 2						
C=O	168.7	168.7	168.7	168.7	168.7	
α	50.7	50.7	50.7	50.7	50.7	
β	30.5	30.5	30.5	30.5	30.5	
γ	26.9	27.0	27.0	26.9	26.9	
δ	25.5	25.5	25.5	25.5	25.5	
ε	52.3	52.3	52.3	52.2	52.3	
3-Hydroxybutanoic acid						
C=O	167.9	168.0	168.0	167.9	168.0	
α	41.2	41.3	41.3	41.2	41.2	
β	68.6	68.6	68.6	68.6	68.6	
γ	19.3	19.3	19.3	19.3	19.3	
Fatty acid						
1′	172.6	172.6	172.6	172.6	172.6	172.7
2′	31.6	31.6	31.6	31.6	31.6	31.6
3′	24.2	24.2	24.2	24.2	24.2	24.2
4′	[a]	[a]	28.4	[a]	26.3	[a]
5′	[a]	[a]	[a]	[a]	129.1	[a]
6′	[a]	[a]	26.5[b]	[a]	130.1	[a]
7′	[a]	[a]	129.5[c]	[a]	26.6	[a]
8′	[a]	[a]	129.6[c]	[a]	[a]	[a]
9′	[a]	[a]	26.6[b]	[a]	[a]	[a]
10′	31.2	[a]	[a]	31.2	[a]	[a]
11′	22.0	[a]	[a]	22.0	[a]	[a]
12′	13.9	31.2	[a]	13.9	31.2	31.2
13′		22.0	[a]		22.0	22.1
14′		13.9	31.2		13.9	13.9
15′			22.0			
16′			13.9			

[a] 28.6 ~ 29.1 overlapped signals.
[b,c] May be interchanged.

Structure Determination

Firstly, the structure of BE-32030B was determined. The molecular formula of BE-32030B was established as $C_{41}H_{65}O_{10}N_5$ from the result of HRFAB-MS and ^{13}C-NMR spectral analysis. The ^1H-^1H COSY, HOHAHA and HSQC spectra of BE-32030B indicated the presence of a 1,2-disubstituted benzene, a 3-hydroxybutanoic acid, a myristic acid and two lysine moieties. Based on the HMBC spectrum, the main part of the structure of BE-32030B was deduced (Fig. 2). In this spectrum, the ε-methylene proton of Lys-2 (δ_H 3.47) was coupled to its own carbonyl carbon at δ_C 168.7, implying a cyclic structure for Lys-2. Acetylation of BE-32030B with acetic anhydride in pyridine gave a triacetyl derivative (FAB-MS *m/z* 914 $(M+H)^+$). The D_2O-exchangeable protons of BE-32030B at δ_H 11.5 ppm, 9.68 ppm and 9.46 ppm were replaced by 9 protons at δ_H 2.33 ppm (3H, s) and δ_H 2.12 ppm (6H, s). These fact and the partial structures assembled from HMBC (Fig. 2) suggested that the structure of BE-32030B was quite similar to that of mycobactines[3~11]. The two Lys ε-*N*-hydroxyl groups in the mycobactines are known to chelate ferric ions[10]. Hydrolysis of BE-32030B with 1 N NaOH/MeOH at room temperature for 60 minutes afforded BE-32030B-1 that was a methyl ester core of

Table 2-1. ^{1}H NMR data for BE-32030A, B and C in DMSO-d_6.

	A	B	C
2-OH	11.5 (1H, s)	11.5 (1H, s)	11.5 (1H, s)
3-OH	—	—	—
H-3	6.99 (1H, d, J=7.8)	6.99 (1H, d, J=7.8)	6.99 (1H, d, J=7.8)
H-4	7.45 (1H, t, J=7.8)	7.45 (1H, t, J=7.8)	7.44 (1H, t, J=7.8)
H-5	6.92 (1H, t, J=7.8)	6.92 (1H, t, J=7.8)	6.92 (1H, t, J=7.8)
H-6	7.62 (1H, d, J=7.8)	7.62 (1H, d, J=7.8)	7.62 (1H, d, J=7.8)
H-11	4.64 (1H, d, J=8.5)	4.64 (1H, d, J=8.6)	4.64 (1H, d, J=8.5)
	4.27 (1H, d, J=8.5)	4.27 (1H, d, J=8.6)	4.26 (1H, d, J=8.5)
H-12	1.52 (3H, s)	1.52 (3H, s)	1.52 (3H, s)
Lys 1			
NH	8.29 (1H, d, J=6.4)	8.29 (1H, d, J=7.3)	8.29 (1H, d, J=7.7)
α	4.14 (1H, m)	4.14 (1H, m)	4.14 (1H, m)
β	1.60~1.76[a]	1.60~1.76[a]	1.60~1.76[a]
γ	1.22[b]	1.22[b]	1.22[b]
δ	1.30~1.52[c]	1.30~1.52[c]	1.30~1.52[c]
ε	3.40 (2H, m)	3.40 (2H, m)	3.41 (2H, m)
OH	9.46 (1H, s)	9.46 (1H, s)	9.46 (1H, s)
OCH_3	—	—	—
3-Hydroxybutanoic acid			
α	2.49[d]	2.49[d]	2.49[d]
	2.43 (1H, dd, J=14.3, 7.3)	2.43 (1H, dd, J=14.3, 7.3)	2.42 (1H, dd, J=14.3, 7.3)
β	5.11 (1H, m)	5.11 (1H, m)	5.12 (1H, m)
γ	1.16 (3H, d, J=6.1)	1.16 (3H, d, J=6.1)	1.15 (3H, d, J=6.4)
Lys 2			
NH	8.08 (1H, d, J=7.0)	8.08 (1H, d, J=7.3)	8.08 (1H, d, J=7.4)
α	4.46 (1H, m)	4.46 (1H, m)	4.46 (1H, m)
β	1.60~1.76[a], 1.30~1.52[c]	1.60~1.76[a], 1.30~1.52[c]	1.60~1.76[a], 1.30~1.52[c]
γ	1.80 (1H, m), 1.60~1.76[a]	1.80 (1H, m), 1.60~1.76[a]	1.80 (1H, m), 1.60~1.76[a]
δ	1.60~1.76[a], 1.30~1.52[c]	1.60~1.76[a], 1.30~1.52[c]	1.60~1.76[a], 1.30~1.52[c]
ε	3.85 (1H, dd, J=15.6, 10.6)	3.85 (1H, dd, J=15.6, 10.6)	3.85 (1H, dd, J=15.6, 10.6)
	3.47 (1H, dd, J=15.6, 4.5)	3.47 (1H, dd, J=15.6, 4.5)	3.47 (1H, dd, J=15.9, 4.5)
OH	9.68 (1H, s)	9.68 (1H, s)	9.68 (1H, s)
Fatty acid			
H-2′	2.26 (2H, m)	2.26 (2H, m)	2.26 (2H, m)
H-3′	1.30~1.52[c]	1.30~1.52[c]	1.30~1.52[c]
H-4′	1.22[b]	1.22[b]	1.22[b]
H-5′,6′	—	—	—
H-6′,9′	—	—	1.95 (4H, m)
H-7′	—	—	—
H-7′,8′	—	—	5.30 (2H, m)
CH_2	1.22[b]	1.22[b]	1.22[b]
CH_3	0.83 (3H, t, J=6.8)	0.84 (3H, t, J=6.8)	0.83 (3H, t, J=6.8)

[a,b,c] Overlapped signals.
[d] Overlapped with solvent.

Fig. 2. Partial structures of BE-32030B from HMBC spectrum.

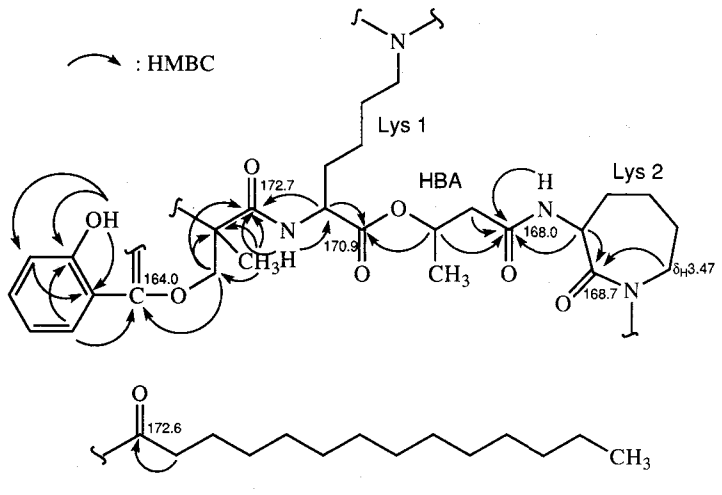


Table 2-2. ^{1}H NMR data for BE-32030D, E and B-1 in DMSO-d_6.

	D	E	B-1
2-OH	11.5 (1H, s)	11.5 (1H, s)	11.5 (1H, s)
3-OH	9.17 (1H, s)	9.17 (1H, s)	—
H-3	—	—	6.99 (1H, d, J=7.8)
H-4	6.96 (1H, dd, J=7.8, 1.5)	6.95 (1H, dd, J=7.8, 1.2)	7.44 (1H, t, J=7.8)
H-5	6.72 (1H, t, J=7.8)	6.72 (1H, t, J=7.8)	6.92 (1H, t, J=7.8)
H-6	7.07 (1H, dd, J=7.8, 1.5)	7.07 (1H, dd, J=7.8, 1.2)	7.62 (1H, d, J=7.8)
H-11	4.61 (1H, d, J=8.8)	4.61 (1H, d, J=8.8)	4.62 (1H, d, J=8.6)
	4.26 (1H, d, J=8.8)	4.26 (1H, d, J=8.8)	4.26 (1H, d, J=8.6)
H-12	1.51 (3H, s)	1.51 (3H, s)	1.52 (3H, s)
Lys 1			
NH	8.27 (1H, d, J=7.6)	8.27 (1H, d, J=7.3)	8.34 (1H, d, J=7.6)
α	4.14 (1H, m)	4.14 (1H, m)	4.21 (1H, m)
β	1.60~1.76[a]	1.60~1.76[a]	1.60~1.76[a]
γ	1.22[b]	1.22[b]	1.22 (2H, m)
δ	1.30~1.51[c]	1.30~1.51[c]	1.40~1.52[c]
ε	3.42 (2H, m)	3.41 (2H, m)	3.41 (2H, m)
OH	9.47 (1H, s)	9.48 (1H, s)	9.46 (1H, s)
OCH_3	—	—	3.62 (3H, s)
3-Hydroxybutanoic acid			
α	2.49[d]	2.49[d]	—
	2.43 (1H, dd, J=14.0, 7.3)	2.43 (1H, dd, J=14.3, 7.6)	—
β	5.11 (1H, m)	5.11 (1H, m)	—
γ	1.16 (3H, d, J=6.1)	1.16 (3H, d, J=6.1)	—
Lys 2			
NH	8.08 (1H, d, J=7.3)	8.08 (1H, d, J=7.3)	—
α	4.45 (1H, m)	4.46 (1H, m)	—
β	1.60~1.76[a], 1.30~1.52[c]	1.60~1.76[a], 1.30~1.52[c]	—
γ	1.80 (1H, m), 1.60~1.76[a]	1.80 (1H, m), 1.60~1.76[a]	—
δ	1.60~1.76[a], 1.30~1.52[c]	1.60~1.76[a], 1.30~1.52[c]	—
ε	3.85 (1H, dd, J=15.6, 10.6)	3.85 (1H, dd, J=15.6, 10.6)	—
	3.47 (1H, dd, J=15.5, 4.5)	3.47 (1H, dd, J=15.8, 4.5)	—
OH	9.68 (1H, s)	9.68 (1H, s)	—
Fatty acid			
H-2′	2.26 (2H, m)	2.26 (2H, m)	2.26 (2H, m)
H-3′	1.30~1.52[c]	1.30~1.52[c]	1.40~1.52[c]
H-4′	1.22[b]	1.96 (2H, m)	—
H-5′,6′	—	5.31 (2H, m)	—
H-6′,9′	—	—	—
H-7′	—	1.96 (2H, m)	—
H-7′,8′	—	—	—
CH_2	1.22[b]	1.22[b]	1.22[b]
CH_3	0.84 (3H, t, J=6.8)	0.83 (3H, t, J=6.8)	0.83 (3H, t, J=6.8)

[a,b,c] Overlapped signals.
[d] Overlapped with solvent.

Fig. 3. Methanolysis of BE-32030B.

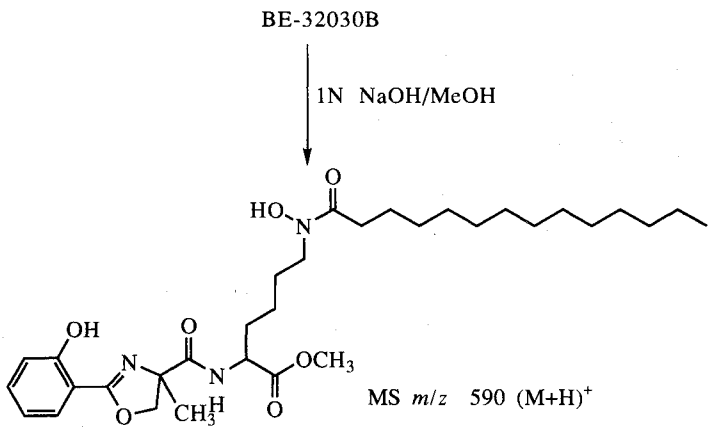
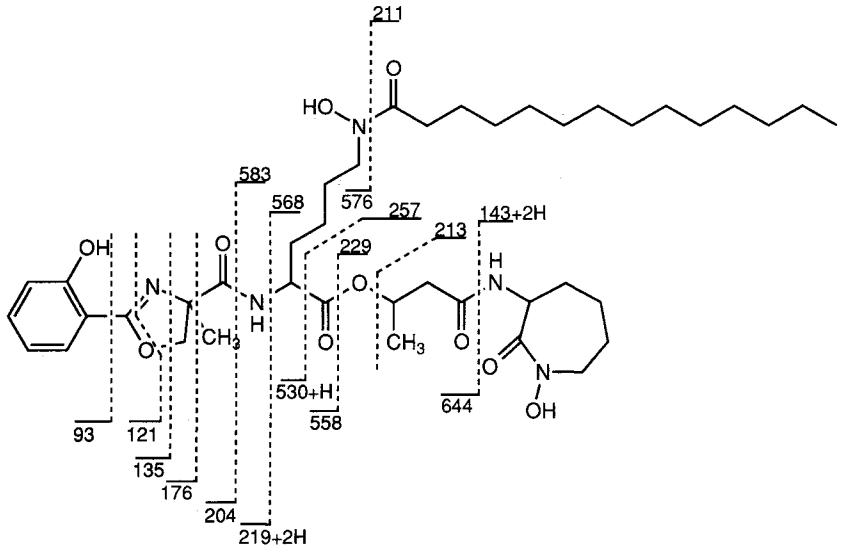


Fig. 4. Mass spectral fragmentation of BE-32030B.

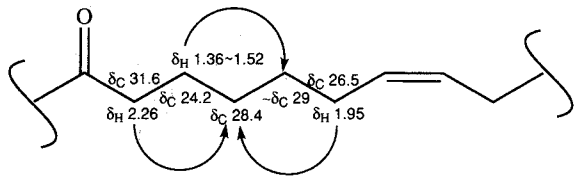


BE-32030 minus Lys-2 and 3-hydroxybutanoic acid (Fig. 3). From the data described above, the structure of BE-32030B was determined as shown in Fig. 1. This structure was supported by linked scan FAB-MS experiments (Fig. 4).

The molecular formula of BE-32030A was established as $C_{39}H_{61}O_{10}N_5$ from the results of HRFAB-MS, ^1H- and ^{13}C-NMR spectral analyses. BE-32030A showed similar ^1H- and ^{13}C-NMR spectra to those of BE-32030B except for the lack of two acyl methylene signals. From the studies of ^1H-^{13}C long-range coupling correlations in the HMBC spectrum, the structure of BE-32030A was determined as shown in Fig. 1.

The molecular formula of BE-32030C was established as $C_{43}H_{67}O_{10}N_5$ from the results of HRFAB-MS, ^1H- and ^{13}C-NMR spectral analyses. In the ^1H-NMR spectrum of BE-32030C, six protons at δ_H 5.30 ppm (2H, m) and δ_H 1.95 ppm (4H, m) were newly observed comparing to BE-32030B and all other signals of BE-32030C were quite similar to that of BE-32030B. These data suggested the presence of an unsaturated fatty acid in BE-32030C. In the HMBC spectrum of BE-32030C, the carbon signal at δ_C 28.4 ppm was correlated with 2′-H (δ_H 2.26) proton and the proton next to olefinic proton (δ_H 1.95). The methylene carbon signal at about 29 ppm was correlated with 3′-H. These data suggested that C-2′ to C-6′ were sp^3 carbons, and that the unsaturation was between C-7′ and C-8′ (Fig. 5). The *cis*-configuration of the C-7′ and C-8′ double bond was confirmed by the coupling constant ($J_{7',8'} = 11.0$ Hz). From the above results, the structure of BE-32030C was determined as shown in Fig. 1.

Fig. 5. Part of HMBC spectrum of BE-32030C.



The molecular formula of BE-32030D was established as $C_{39}H_{61}O_{11}N_5$ from the result of HRFAB-MS, ^1H- and ^{13}C-NMR spectral analyses. In the ^1H-NMR data of BE-32030D, the presence of a 1,2,3-trisubstituted benzene group was revealed and the phenolic proton at δ_H 9.17 was newly observed comparing to BE-32030B. In the HMBC spectrum of BE-32030D, the proton at δ_H 9.17 ppm was correlated to C-2 (δ_C 148.1 ppm), C-3 (δ_C 145.7) and C-4 (δ_C 119.3 ppm). From the studies described above, the structure of BE-32030D was determined as shown in Fig. 1.

The molecular formula of BE-32030E was established as $C_{41}H_{63}O_{11}N_5$ from the results of HRFAB-MS, ^1H- and ^{13}C-NMR spectral analyses. In the ^1H-NMR spectrum of BE-32030E, the presence of a 1,2,3-trisubstituted benzene ring was suggested and the phenolic proton at δ_H 9.17, olefinic protons at δ_H 5.31 (2H, m) and methylene protons at δ_H 1.96 (4H, m) were newly observed comparing to BE-32030B. The above data suggested the presence of an unsaturated fatty acid in its

Table 3. Growth inhibition of tumor cell lines by BE-32030A ~ E.

	IC_{50} (μg/ml)			
	P388	DLD-1	PC-13	MKN-45
A	0.36	0.49	0.30	0.19
B	0.16	0.25	0.88	0.05
C	0.19	0.22	0.25	0.06
D	0.39	0.59	0.26	0.19
E	0.18	0.28	0.22	0.19

structure. In the HMBC spectrum of BE-32030E, the proton at δ_H 2.26 ppm (2'-H) was correlated to the carbon at δ_C 26.3 ppm next to olefinic carbon (C-4'). This data suggested that the double bond was present between C-5' and C-6'. The *cis*-configuration of the C-5' and C-6' double bond was confirmed by the coupling constant ($J_{5',6'}$ = 11.0 Hz). From the results described above, the structure of BE-32030E was determined as shown in Fig. 1.

Bioactivity

BE-32030A ~ E showed antiproliferative effects *in vitro* against P388 murine leukemia, DLD-1 human colon cancer, PC-13 human lung cancer and MKN-45 human stomach cancer cell lines. The IC_{50} values against these cell lines of BE-32030A, B, C, D and E were shown in Table 3. Acute toxicity of BE-32030B was tested at 3, 10, 30, 100 and 200 mg/kg (female CDF_1 mouse) and no death was found on day 5 after a single i.p. injection at all doses. Antitumor activity of BE-32030B is under evaluation. Recently, it was reported that formobactin, the structure of which is related to the group of mycobactins produced by *Nocardia* sp., had strong inhibitory activity against lipid peroxidation in rat brain homogenate[12]. Accordingly, it may be interesting to test the inhibitory activity against lipid peroxidation of BE-32030 compounds.

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