A NEW NEURITOGENETIC COMPOUND BU-4514N PRODUCED BY *Microtetraspora* sp.

SOICHIRO TODA, SATOSHI YAMAMOTO, OSAMU TENMYO, TAKASHI TSUNO, TOSHIFUMI HASEGAWA, MARY ROSSER, MASAHISA OKA, YOSUKE SAWADA, MASATAKA KONISHI and TOSHIKAZU OKI

Bristol-Myers Squibb Research Institute, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

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A novel neuritogenetic compound BU-4514N was isolated from the fermentation broth of *Microtetraspora* sp. T689-92. It showed significant NGF-mimic activity and antibacterial activity against Gram-positive bacteria. Structural studies revealed that BU-4514N was a new chemotype antibiotic related to lydicamycin.

Nerve growth factor (NGF) is a protein known to be essential for development and maintenance of certain sympathetic and sensory neurons in the peripheral nervous system[1]. Recent studies also suggested significant functions of NGF in the cholinergic neurons of the basal forebrain in the central nervous system[2]. PC 12 pheochromocytoma cell has been shown to be a useful model cell for the study of adrenergic neuronal differentiation[3]. It sensitively responds to NGF to differentiate into sympathetic neuron-like cells. In search for potential factors which modulate NGF synthesis or to mimic NGF (stimulate cell differentiation), staurosporine[4] and lactacystin[5] have been isolated from microbial metabolites.

During the course of our screening for microbial metabolites which induce differentiation of PC 12R cells, a clone of the rat pheochromocytoma cell line PC 12, a novel compound designated as BU-4514N was isolated from the culture broth of *Microtetraspora* sp. T689-92. When PC 12R cells were cultured in the presence of BU-4514N, neurites were generated from the PC 12R cells. In addition, BU-4514N was found to have *in vitro* antibacterial activity against Gram-positive bacteria. This paper describes the taxonomy of the producing strain, fermentation, isolation, physico-chemical properties, structure and biological activity of BU-4514N.

Materials and Methods

Taxonomy

The producing strain was isolated from a soil sample collected in Andhra Pradesh, India. Morphological and cultural characteristics were observed after incubation at 37°C for 2 to 4 weeks according to the procedures recommended by SHIRLING and GOTTLIEB[6]. Color names and hue numbers are given according to the Manual of Color Names (Japan Color Enterprise Co., Ltd., 1987). Utilization of carbohydrates and other physiological tests were carried out by the methods described by SHIRLING, GOTTLIEB[6] and WAKSMAN[7]. Temperature range for growth was determined on yeast starch agar[8] using a temperature gradient incubator TN-3 (Tokyo Kagaku Sangyo Co., Ltd.).

Biomass for the chemotaxonomic analysis were prepared by using lyophilized whole cells grown at 32°C for 4 days with a rotary shaker in a liquid medium (glucose 1% and yeast extract 1%, pH 7.0). Cell

Correspondence should be addressed to JUN OKUMURA, Bristol-Myers Squibb Research Institute, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan.

wall analysis was performed by the methods of LECHEVALIER and LECHEVALIER[10] as modified by STANECK and ROBERTS[11]. Phospholipid and mycolate compositions were determined by the methods of LECHEVALIER[12] and MINNIKIN *et al.*[13]. Menaquinone was analyzed by the procedure of COLLINS *et al.*[14]. Fatty acid was determined by the method of SUZUKI and KOMAGATA[15].

Fermentation

A portion of the slant culture of strain T689-92 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of vegetative medium composed of soluble starch 2%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, fish meal D30X (Banyu Nutrient) 0.5% and $CaCO_3$ 0.3%, and incubated for 4 days at 28°C and 200 rpm on rotary shaker. A portion (0.3 ml) of vegetative mycelia obtained were inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a seed medium composed of sucrose 3%, fish meal (Hokuyo Suisan Co.) 0.2%, soybean meal (Nikko Seiyu Co.) 0.5%, peptone (Nihon Seiyaku Co.) 0.2% and $CaCO_3$ 0.6%. The pH of the medium was adjusted to 7.0 before autoclaving. The seed was incubated at 28°C for 4 days on a rotary shaker operating at 200 rpm. A 5-ml portion of the seed culture was transferred into a 500-ml Erlenmeyer flask containing 100 ml of a production medium having the same composition as the seed medium. The fermentation was carried out for 6 days under the same conditions as those for the seed culture. The production of the antibiotic in fermentation broth was monitored by its NGF mimic activity.

Chemicals and Analytical Instruments

TLC was performed on precoated silica gel plates (Kieselgel $60F_{254}$, Merck). MP's were determined on a Shibayama micro melting point apparatus and were not corrected. IR and UV spectra were recorded on a JASCO IR-810 spectrophotometer and a IDEC-610C spectrometer, respectively. ^1H and ^{13}C NMR spectra were measured on a JEOL JNM-GX 400 spectrometer. Mass spectral data was obtained by a JEOL JMS-AX 505H mass spectrometer. The optical rotation was determined with a JASCO DIP-140.

Extraction and Purification

The harvested whole broth (9.0 liters) was stirred with BuOH (4.5 liters). The organic layer was separated by the aid of a Sharples centrifuge (Kokusan No. 4A) and evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of ethyl acetate and water (1 : 1, 1.5 liters). After stirring 15 minutes, the aqueous layer was separated and re-stirred with BuOH (750 ml). The BuOH extract was evaporated under reduced pressure to give 2.38 g of crude product which was applied on a column of silica gel (Wakogel C-200, 600 ml). The column was developed with CH_2Cl_2 - EtOH (1 : 1, 1 liter) and CH_2Cl_2 - EtOH - conc NH_4OH - H_2O (10 : 10 : 1 : 1, 2.2 liters), successively. The eluate was collected in 20-ml fractions and each fraction was monitored by TLC (SiO_2, CH_2Cl_2 - EtOH - conc NH_4OH - H_2O (4 : 7 : 1 : 1)) and NGF mimic activity. The active fractions were combined and evaporated to dryness to give 617 mg of light brown powder. This sample was chromatographed on a reversed phase silica gel column (YMC GEL ODS A60; Yamamura Chemical Lab., 700 ml) using aqueous acetonitrile (40% ~ 80%) containing 0.1% of trifluoroacetic acid as an eluant. The active fractions were combined and evaporated under reduced pressure to afford 603 mg of white amorphous powder, which showed single peak in HPLC analysis (column: YMC-Pack (A-301-3) 4.6 i.d. × 100 mm, Yamamura Chemical Lab., mobil phase: CH_3CN - H_2O - trifluoroacetic acid (50 : 50 : 0.1), flow rate: 1 ml/minute, detection: UV absorption at 254 nm, Rt: 8.6 minutes). The sample was crystallized from aqueous methanol to give colorless fine needles (530 mg).

Acetylation of BU-4514N

To a solution of BU-4514N (161 mg) and triethylamine (35 mg) in methanol (20 ml) was added acetic anhydride (95 mg) and the mixture was stirred at room temperature for 5 hours. The reaction mixture was diluted with water (20 ml) and was applied on a column of Diaion HP-20 (Mitsubishi Chemical Industries Ltd., 20 i.d. × 250 mm), and the column was eluted with 50% aqueous methanol (200 ml), 50% aqueous acetonitrile (200 ml) and 80% aqueous acetonitrile (400 ml), successively. The fractions containing the acetate were pooled and concentrated to dryness to give 149 mg of white amorphous powder. MP 139 ~ 141°C. UV λ_{max} (MeOH) nm (ε) 286 (8,930), 248 (6,450), 204 (11,500). IR (KBr) cm^{-1} 3440, 2830, 1660, 1545, 1455, 1380, 1115, 1020, 975. ^1H NMR (DMSO-d_6) δ 0.77 (1H, q, J = 12.0 Hz), 0.87 (3H, d,

J=6.5 Hz), 0.88 (3H, d, J=6.5 Hz), 0.97 (3H, d, J=5.8 Hz), 1.65 (3H, s), 1.87 (3H, s), 3.62 (1H, br), 3.75 (1H, br d, J=9.1 Hz), 3.85 (1H, d, J=19.1 Hz), 3.96 (1H, dq, J=6.5 and 1.9 Hz), 4.58 (1H, br), 5.03 (1H, br s), 7.78 (1H, d, J=9.0 Hz), 9.29 (1H, br). FAB-MS (m/z) 519 $(M+H)^+$, 344, 156, 126.

Acid Methanolysis

A solution of acetyl-BU-4514N (653 mg) in 0.05 N HCl - MeOH (20 ml) was kept at room temperature for 2 days. After adding 1 ml of conc NH_4OH, the reaction mixture was evaporated *in vacuo*. The residue was purified by reversed phase silica gel column chromatography (YMC GEL ODS A60; 21 i.d. × 250 mm) with CH_3CN - H_2O (from 20 : 80 to 80 : 20) as eluant and then with preparative HPLC (column; YMC D-ODS-5; 20 i.d. × 250 mm, mobile phase; CH_3CN - H_2O (6.5 : 93.5)) to yield pure **1a** (47 mg) and **1b** (16 mg). **1a**: MP 103~104°C, $[\alpha]_D$ −115° (c 0.6, EtOH), HRFAB-MS m/z 188.1291 $(M+H)^+$, calcd for $C_9H_{18}NO_3$: 188.1287, ^1H NMR ($CDCl_3$) δ 1.08 (3H, d, J=6.5 Hz), 1.58 (1H, m), 1.70 (1H, m), 1.72 (1H, m), 1.97 (1H, m), 2.03 (3H, s), 3.33 (3H, s), 3.93 (1H, br dd, J=9.0 and 1.7 Hz), 4.06 (1H, dq, J=6.5 and 1.7 Hz), 4.66 (1H, d, J=2.7 Hz), 5.93 (1H, d, J=9.0 Hz). **1b**: MP 104~105°C, $[\alpha]_D$ +108° (c 0.45, EtOH), HRFAB-MS m/z 188.1314 $(M+H)^+$, ^1H NMR ($CDCl_3$) δ 1.18 (3H, d, J=6.4 Hz), 1.46 (1H, m), 1.68 (1H, m), 1.75 (1H, m), 1.94 (1H, m), 2.01 (3H, s), 3.50 (3H, s), 3.68 (1H, dq, J=6.4 and 1.7 Hz), 3.92 (1H, br dd, J=9.7 and 1.7 Hz), 4.36 (1H, dd, J=8.1 and 2.1 Hz), 5.98 (1H, d, J=9.0 Hz).

Cells and Cultivation

PC 12R cells derived from PC 12 cells were cultured in Opti-MEM medium (GIBCO) containing 5% heat-inactivated fetal bovine serum (GIBCO) and 50 μg/ml of amikacin (Bristol-Myers Squibb), in a humidified atmosphere of 5% CO_2 and 95% air at 37°C to a cell density of approximately 1×10^8 cells per 175 cm^2 tissue culture flask (Lux). The PC 12R cell density was adjusted to 1×10^5 cells/ml and 180 ml aliquot of the cell suspension was added to assay wells in a 96-well microtiter plate (Sumitomo). Cultures were pre-incubated for 3 hours at 37°C in a 5% CO_2 - 95% air environment.

Assay for Neurite Outgrowth

The test sample in 20 μl of water at varied concentrations was added to wells containing PC 12R cells pre-treated with 0.1 ng/ml NGF. After 24 hours' incubation, the medium was removed and the cells were fixed with 0.3% glutaraldehyde (Tokyo Kasei) in DULBECCO's phosphate buffered saline and stained with Harris hematoxylin solution (Merck). The stained cells were observed under a phase-contrast microscope connected with a video camera-CRT system. PC 12R cells were magnified 500 times on the CRT monitor. The number of spikes of the negative (without NGF, 6~7 mm) and positive control cells (with NGF, >10 mm) were measured in order to establish the standard length of a spike of the PC 12R cells. The wells which contain the same or a higher number than the positive cells in a well were determined as active.

Assay for Antibacterial Activity

The antimicrobial activity of BU-4514N was determined by the serial 2-fold dilution method after overnight incubation at 32°C. Nutrient agar medium (Difco) was used for Gram-positive and Gram-negative bacteria.

Results

Taxonomy of the Producing Strain

The substrate mycelia were well developed, extensively branched and did not fragment into short elements. Rudimentary and retarded aerial mycelia were only produced on both yeast starch agar, GAUZE's I agar and maltose - BENNETT's agar[9], but no sporulating aerial mycelium was observed by scanning electron microscope. They were rudiment without sporulation and were powdery with pale reddish yellow to grayish yellow. The color of vegetative mycelia and reverse side of colony ranged from pale yellow to reddish brown or dull yellow. No diffusible pigment was produced. The macroscopic properties of strain T689-92 on various agar media are summarized in Table 1.

Table 1. Cultural characteristics of strain T689-92.

Medium	Vegetative mycelium	Reverse side	Aerial mycelium	Diffusible pigment
Sucrose nitrate agar (Waksman med. 1)	Pale yellow (128)	Soft reddish yellow (146)	None	None
Glycerol nitrate agar	Pale yellow (128)	Soft yellow (147)	None	None
Yeast extract-malt extract agar (ISP med. 2)	Grayish brown (118) ~dark yellowish brown (105)	Yellowish brown (99)	None	None
Oatmeal agar (ISP med. 3)	Soft reddish yellow (146)	Soft reddish yellow (146)	None	None
Inorganic salts-starch agar (ISP med. 4)	Gold (162)	Soft reddish yellow (146)	None	None
Glycerol asparagine agar (ISP med. 5)	Dull yellow (150)	Dull yellow (150)	None	None
Tyrosine agar (ISP med. 7)	Dull yellow (150)	Dull yellow (150)	None	None
Nutrient agar (Waksman med. 14)	Gold (161)	Gold (161)	None	None
Yeast starch agar	Yellowish brown (98)	Grayish brown (118)	Pale reddish yellow (130), powdery, thin	None
GAUZE's I agar	Yellowish brown (99)	Yellowish brown (99)	Grayish yellow (155), powdery, thin	None
Oatmeal-yeast extract agar	Soft reddish yellow (146)	Soft reddish yellow (146)	None	None
BENNETT's agar (Waksman med. 30)	Yellowish brown (99)	Yellowish brown (99)	None	None
Maltose-BENNETT's agar	Soft reddish yellow (146)	Brownish gold (160)	Pale reddish yellow (130), powdery, thin	None

Observation after incubation at 28°C for 3 weeks.

Table 2. Physiological characteristics of strain T689-92.

Test	Results
Starch hydrolysis (on ISP med. 4)	Positive
Nitrate reduction (Difco, nitrate broth)	Positive
Milk (Difco, 10% skimmed milk)	
Coagulation	Positive
Peptonization	Positive
Gelatin liquefaction	
On plain gelatin	Negative
On glucose peptone gelatin	Negative
Melanin formation (on ISP med. 7)	Negative
Temperature range for growth (°C)	18~44
Optimum temperature (°C) (on yeast starch agar)	31~42
pH range for growth	6~8
Optimum pH (on trypticase soy broth, BBL)	7

Table 3. Utilization of carbon sources by strain T689-92.

Carbon source	Growth
D-Glucose	++
L-Arabinose	±
D-Xylose	+
Inositol	++
Mannitol	+
D-Fructose	+
L-Rhamnose	+
Sucrose	+
Raffinose	−

−, Negative; ±, doubtful; +, weak positive; ++, strong positive. (ISP med. 9, 37°C for 21 days).

The physiological characteristics and the utilization of carbon sources are shown in the Tables 2 and 3, respectively. Analysis of hydrolyzed whole cells indicated the presence of *meso*-diaminopimelic acid and the absence of LL isomer. Sugar analysis of hydrolyzed whole cells indicated the presence of glucose, galactose, mannose, madurose and ribose. This represents a type III and a type B sugar pattern. Mycolic acids were not detected. By phospholipids analysis, the wall had a type IV containing phosphatidylinositol,

diphosphatidilglycerol and unknown glucosamine-containing phospholipid. Analysis of the menaquinone composition revealed 69% MK-9 (H_4), 15% MK-9 (H_2), 10% MK-9 (H_6), 3% MK-9 (H_8) and 3% MK-9 (H_0). Analysis of the fatty acids exhibited high amounts of 14-methylpentadecanoic acid (26%), 10-methylheptadecanoic acid (21%) and α-hydroxy-14-methylpentadecanoic acid (10%).

The above characteristics of T689-92, especially the chemotaxonomic properties were in good agreement with those of the genus *Microtetraspora*[16], although its morphological properties did not allow specific characterization. It was concluded that T689-92 was a strain of *Microtetraspora*.

Strain T689-92 was deposited with the American Type Culture Collection (ATCC), 12301 Parklaw Drive, Rockville, Md. 20852 U.S.A. under the provisions of the Budapest Treaty, under Accession Number ATCC 55291.

Physico-Chemical Properties

The physico-chemical properties of BU-4514N are summarized in Table 4. The antibiotic was practically insoluble in water, chloroform, ethyl acetate and benzene, but soluble in acidic water, dimethylsulfoxide and methanol. It showed positive responses to iodine, ferric chloride and ninhydrin reagents and negative to Sakaguchi reaction on a TLC plate. The UV spectrum of BU-4514N in methanol exhibited maxima at 249 and 286 nm. The ^{13}C NMR spectrum of BU-4514N revealed 27 carbon signals (Table 5). Its molecular formula was established to be $C_{27}H_{42}N_2O_5$ by FAB-MS spectrum and elemental analysis.

Table 4. Physico-chemical properties of BU-4514N.

Nature	Colorless fine needles
MP	185~186°C
Optical rotation	$[\alpha]_D^{25}$ $-140\pm1°$ (c 0.5, 0.1 N HCl)
Mass (FAB-MS) (m/z)	475 $(M+H)^+$
Molecular formula	$C_{27}H_{42}N_2O_5$
Elemental analysis	
Calcd for $C_{27}H_{42}N_2O_5\cdot\frac{3}{4}H_2O$:	C 66.43, H 8.98, N 5.74
Found:	C 66.21, H 8.94, N 5.66
UV λ_{max} nm (ε)	
MeOH	286 (8,530), 251 (4,360), 204 (6,300)
0.01 N HCl-MeOH	286 (9,000), 254 (sh, 4,320), 204 (6,540)
0.01 N NaOH-MeOH	287 (8,200), 248 (9,200), 205 (8,440)
IR ν_{max} (KBr) cm^{-1}	3350, 2970, 2930, 1650, 1565, 1560, 1445, 1240, 1110, 1020, 995

Table 5. ^{13}C and ^1H NMR spectral data[a] for BU-4514N.

Carbon No.	δ_C (multiplicity)	δ_H (multiplicity)	Carbon No.	δ_C (multiplicity)	δ_H (multiplicity)
1	179.10 (s)		15	78.71 (d)	3.35 (m)
2	104.20 (s)		16	23.78 (q)	1.04 (d, J=6.0 Hz)
3	204.13 (s)		17	193.21 (s)	
4	52.34 (s)		18	51.71 (t)	3.88 (d, J=18.8 Hz),
5	41.92 (d)	1.55 (m)			3.92 (d, J=18.8 Hz)
6	29.62 (t)	1.01 (m), 1.84 (m)	19	15.65 (q)	13.4 (s)
7	37.72 (t)	1.06 (m), 1.74 (m)	20	24.01 (q)	0.89 (d, J=6.8 Hz)
8	35.34 (d)	1.46 (m)	21	23.70 (q)	1.69 (s)
9	44.27 (t)	0.76 (q, J=12.4 Hz), 1.76 (m)	1′	100.49 (d)	4.79 (br s)
10	41.36 (d)	1.78 (m)	2′	24.93 (t)	1.66 (m), 1.82 (m)
11	126.10 (d)	5.07 (br s)	3′	23.70 (t)	1.86 (m), 2.17 (m)
12	137.56 (s)		4′	51.04 (d)	3.32 (m)
13	43.64 (d)	2.57 (br)	5′	65.34 (d)	4.21 (dq, J=6.8, 1.3 Hz)
14	42.90 (t)	1.32 (m), 1.53 (m)	6′	18.14 (q)	1.13 (d, J=6.8 Hz)

[a] In a mixture of CD_3OD-D_2O-4 N DCl (1 : 1 : 0.08).

Structural Studies

The UV absorption maxima of BU-4514N in acidic and basic solutions were quite similar to those of lydicamycin[17,18] which has an α-acyltetramic acid in the molecule. In addition, the FAB-MS spectrum showed a fragment ion peak at *m/z* 126, supporting the presence of a tetramoyl function. The ^{13}C NMR spectrum demonstrated 27 carbons which were analyzed as five methyl, seven methylene, nine methine and six quaternary carbons by the DEPT technique. Among them, the signals at δ 204.13, 193.21, 179.10, 104.20 and 51.71 were in good agreement with those of α-acyltetramic acid moiety in lydicamycin[17,18]. The correlation of the protons and carbons was established as shown in the Table

Fig. 1. Structure of BU-4514N.

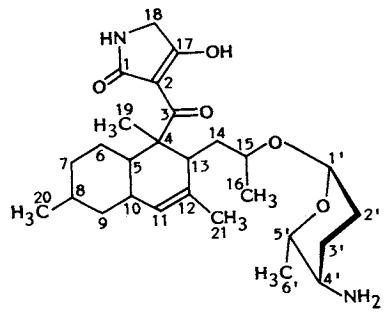

Fig. 2. Partial structures of BU-4514N.

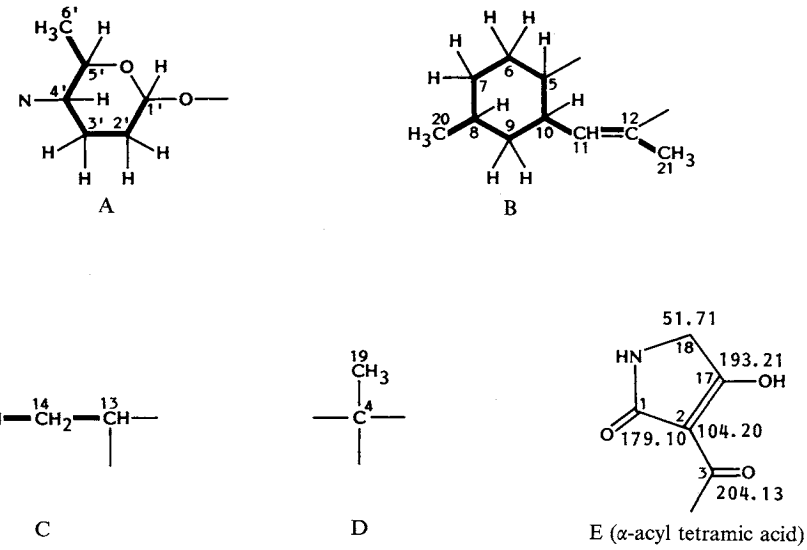

Fig. 3. FAB-MS fragmentation of BU-4514N and its acetate.

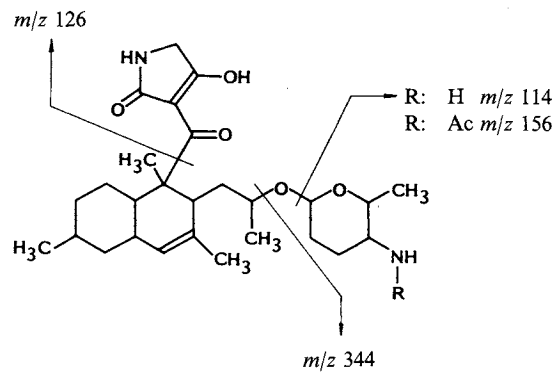

Table 6. Neurite outgrowth activity of BU-4514N and NGF.

Compound	Concentration	No. of neurites[a]/ No. of cells[b]	Ratio (%)
BU-4514N	12.5 μg/ml	244/1,123	21.7
	6.3 μg/ml	149/1,570	9.5
	3.1 μg/ml	25/1,848	1.4
	1.6 μg/ml	2/1,611	0.12
NGF	0.1 ng/ml (running control)	2/1,565	0.13
	5 ng/ml (positive control)	72/1,053	6.8
	0 ng/ml (negative control)	0/1,298	0

[a] Total number of neurites over 10 mm long (observed on the monitor; sum of 5 fields/well and 3 wells/concentration).
[b] Total number of cells (sum of 5 fields/well and 3 wells/concentration).

5 by ^{13}C-^{1}H COSY spectrum. The ^{1}H-^{1}H COSY spectral analysis indicated the five partial structures, including a tetramic acid moiety, shown in Fig. 2. The chemical shifts at C-1′ (δ 100.49 (d)) and C-5′ (δ 65.34 (d)) indicated a sugar structure for A. The chemical shifts of 4′-H (δ 3.32 (m)) and C-4′ (δ 51.04 (d)) suggested a 4-amino structure for the sugar. Acetylation of BU-4514N with acetic anhydride in methanol gave a monoacetate (δ 7.78, d, J=9.0 Hz, -NH and 1.87, s, CH_3CO). In the FAB-MS spectrum of the acetate, the fragment ion peak of m/z 114 observed for the parent compound disappeared and a new signal at m/z 156 was observed (Fig. 3).

Table 7. Antibacterial activity of BU-4514N.

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P JC-1	1.6
S. aureus Smith	3.1
S. aureus A15036 (MRSA)	3.1
Micrococcus luteus PCI 1001	1.6
Bacillus subtilis PCI 219	0.8
Escherichia coli Juhl A15119	>100
E. coli K12	>100
E. coli NIHJ JC-2	>100
Klebsiella pneumoniae PCI302	>100
Citrobacter freundii GN 7391	>100
Salmonella typhi 901	>100
Pseudomonas aeruginosa A9843A	>100

Medium: Nutrient agar pH 7.0.
Inoculum size: 10^5 cells/ml.
Incubation conditions: 32°C, 18 hours.

Methanolysis of the acetate gave a mixture of methyl glycosides of *N*-acetylaminosugars (**1a** and **1b**). **1a** and **1b** gave the same pseudomolecular ion peak (m/z 188.1291 for **1a**, 188.1314 for **1b** and 188.1287 calcd for $C_9H_{18}NO_3$: 188.1287). **1a** and **1b** were determined as methyl α- and β-4-amino-2,3,6-trideoxy-*threo*-hexopyranoside by ^{1}H NMR. Absolute structure of the sugar was estimated as L form by the optical rotational value of **1a** ($[\alpha]_D$ −115° (*c* 0.6, EtOH), lit.[19] $[\alpha]_D$ −114.3° (*c* 1.02, EtOH)).

α-Pyranoside configuration of the aminosugar in BU-4514N was evidenced from the coupling constant of its anomeric proton (δ 4.79, br s) compared with those of **1a** (δ 4.66, br d, J=2.7 Hz) and **1b** (4.36, dd, J=8.1 and 2.1 Hz).

The ^{13}C-^{1}H long rang COSY experiment on BU-4514N established the connectivities of the partial structures. The correlations 19-CH_3 with C-3, C-4 and C-5, 5-H with C-19, 13-H with C-4, C-12, C-19 and C-21 and 1′-H with C-15 were observed. Thus, the total structure of BU-4514N was determined as shown in Fig. 1. The stereochemistry of the aglycone moiety has not yet been clarified.

Biological Activities

NGF Mimic Activity

BU-4514N significantly induced the neurite outgrowth both in number and length at the concentration

range of 12.5~6.3 μg/ml, in comparison to the negative control (Table 6).

Antibacterial Activity

As shown in Table 7, BU-4514N showed fairly potent *in vitro* antibacterial activity against Gram-positive bacteria.

Discussion

BU-4514N was isolated from the culture broth of *Microtetraspora* sp. T689-92 showed NGF mimic activity inducing neurite outgrowth. The compound might be useful for treatment of various nerve diseases including senile dementia such as ALZHEIMER's disease. The structure of this antibiotic is similar to lydicamycin[17,18], vermisporin[20] and MBP049-13[21] in terms of the presence of an α-acyl tetramic acid and decalin skeleton in the molecule. Lydicamycin and vermisporin were reported to have inhibitory activity against Gram-positive bacteria and anaerobic bacteria, respectively, while MBP049-13 was described to have enzyme inhibitory activity. Although, the stereochemistry of BU-4514N is not determined, the structure-activity relationships of these antibiotics may prove to be interesting in terms of mechanism of action study of this type of molecules.

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