STRUCTURES OF OA-6129A, B_1, B_2 AND C, NEW CARBAPENEM ANTIBIOTICS PRODUCED BY *STREPTOMYCES* SP. OA-6129

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The chemical structures of OA-6129A, B_1, B_2 and C, new carbapenem antibiotics having a pantetheinyl group at C-3 were elucidated by spectroscopic analysis and chemical transformation as presented in Fig. 1.

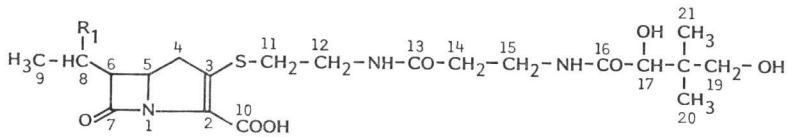
New members of the carbapenem family having a pantetheinyl side chain at C-3*, OA-6129A (**1**), B_1 (**2**), B_2 (**3**) and C (**4**) were isolated from the fermentation broth of *Streptomyces* sp. OA-6129[1]. These carbapenem compounds showed a wide spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria[2] and potent inhibitory effect on β-lactamases.

The present paper describes the structure determination of carbapenem compounds, OA-6129A (**1**), B_1 (**2**), B_2 (**3**) and C (**4**) including their stereochemistry.

Structure and Absolute Configuration of OA-6129A

OA-6129A (**1**), B_1 (**2**), B_2 (**3**) and C (**4**) sodium salts were isolated and purified as freeze-dried preparations. Although they gave negative ninhydrin tests, amino acid analysis indicated the presence of β-alanine in their acid hydrolysates. The four compounds shared a UV maximum at 300 nm; and IR absorption bands at 1750~1760 cm^{-1} (β-lactam), 1660 (amide) and 1600 (carboxylate). Proton NMR analysis of these carbapenems revealed two singlets at δ 0.86~0.92 attributed to geminal dimethyls, a singlet at δ 3.93~3.95 based on methine of α-hydroxylic acid and a triplet at δ 2.45~2.48 assigned to α-methylene attached to carbonyl, which were identified with $-\underset{|}{C}(CH_3)_2$, $-\underset{|}{C}-CH(OH)-CO$ and $HN-CH_2-$

Fig. 1. Structures and stereochemistries of the OA-6129 group of carbapenem compounds.



	R_1	C-5	C-6	C-8	Pantoyl
OA-6129A (**1**)	H	*R*	*R*	—	*R*
OA-6129B_1 (**2**)	OH	*R*	*R*	N.D.	*R*
OA-6129B_2 (**3**)	OH	*R*	*S*	*S*	*R*
OA-6129C (**4**)	OSO_3H	*R*	*R*	N.D.	*R*

N.D.: Not determined.

* In this paper we employ the numbering system specified in Fig. 1.

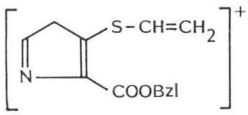
Table 1. ^{1}H NMR data for the OA-6129 group of carbapenem compounds (sodium salts).

Proton No.	OA-6129A		OA-6129B$_1$		OA-6129B$_2$		OA-6129C	
	(ppm)	(Hz)	(ppm)	(Hz)	(ppm)	(Hz)	(ppm)	(Hz)
21	0.89	s	0.86	s	0.87	s	0.86	s
20	0.92	s	0.89	s	0.92	s	0.89	s
9	1.00	t; 7.5	1.33	d; 6.0	1.28	d; 7.0	1.49	d; 6.5
8	1.60~2.00	m	3.95~4.40	m	3.95~4.35	m	4.78	dq; 9.5 & 6.5
14	2.48	t; 6.5	2.47	t; 6.5	2.45	t; 6.5	2.47	t; 7.0
19, 15, 12, 11, 4	2.80~3.65	m	2.75~3.55	m	2.75~3.60	m	2.70~3.60	m
6	2.80~3.65	m	3.60	dd; 10.0 & 5.0	2.75~3.60	m	3.83	dd; 5.5 & 9.5
17	3.95	s	3.93	s	3.94	s	3.94	s
5	3.95	m	3.95~4.40	m	3.95~4.35	m	4.10~4.43	m

Spectra were recorded at 90 MHz in D_2O. Chemical shifts are given in ppm downfield from internal DSS.

CH_2–CO, respectively, in comparison with the ^{1}H NMR spectrum of calcium pantothenate in D_2O* (Table 1). The above-described findings suggested that the four antibiotics were congeneric compounds possessing a carbapenem skeleton and a pantothenyl group.

The molecular formula of **1**, $C_{20}H_{31}N_3O_7S$ (MW 457) was deduced from the field-desorption mass spectra of *p*-nitrobenzyl ester **6** [*m/z* 615 (M+Na)] and diacetate **7** [*m/z* 677 (M+1)] (Fig. 2). Benzyl ester **5** gave a maximum UV absorption at 318 nm and a strong fragment ion at *m/z* 259 attributable to



supporting that **1** had the same chromophore as PS-5. Proton NMR spectroscopic comparison revealed that all the signals of PS-5 *p*-nitrobenzyl ester[8] except acetyl were located in **6**, indicating that **1** possessed an ethyl group at the C-6 position. In addition, the ^{1}H NMR spectrum of **6** showed two characteristic singlet signals at δ 0.87 and 0.95; and a methylene triplet at δ 2.39 convertible to a singlet by irradiation of methylene at δ 3.46, which were assigned to the vicinal dimethyls of pantoyl and the α-methylene of β-alanine, respectively. A multiplet (1H) and a singlet were also observed at δ 3.93, the latter being assigned to the methine of pantoyl. The low-field shift of the methine singlet by acetylation in **7** (δ 4.80) allowed to distinguish the me-

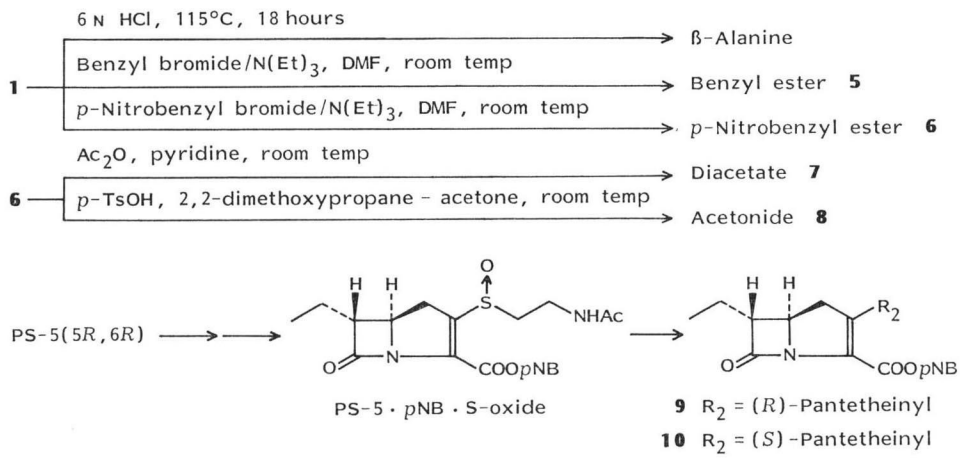
Table 2. ^{13}C NMR data for OA-6129A (sodium salt).

	Position	Chemical shift
$-CH_3$	9	13.1 (q)
	20, 21	21.8 (q), 23.1 (q)
$-CH_2-$	8	24.1 (t)
	4	32.9 (t)
	11, 12, 14, 15	39.9 (t), 40.0 (t), 41.6 (t), 41.9 (t)
	19	71.0 (t)
–CH–	5, 6	59.4 (d), 62.0 (d)
	17	78.4 (d)
$-\overset{\vert}{\underset{\vert}{C}}-$	18	41.2 (s)
$>C=$	2, 3	130.1 (s), 141.7 (s)
–CO–	7, 13, 16	176.4 (s), 177.5 (s)×2
	10	185.7 (s)

^{13}C NMR spectrum was recorded at 25.2 MHz in D_2O. Chemical shifts are given in ppm relative to internal TSP.

* ^{1}H and ^{13}C NMR data for calcium pantothenate in D_2O using 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt and TSP as internal standards, respectively. ^{1}H NMR (δ) 0.90 (3H, s, CH_3), 0.92 (3H, s, CH_3), 2.39 (2H, t, J=7.0 Hz, $-CH_2$–CO), 3.27~3.56 (4H, m, NCH_2, CH_2O), 3.94 (1H, s, CH): ^{13}C NMR 22.1 (q), 23.2 (q), 38.8 (t), 39.4 (t), 41.3 (s), 71.1 (t), 78.5 (d), 177.4 (s), 183.1 (s).

Fig. 2.



thylene of AB-type quartet (J=11.0 Hz) at δ 3.90 from the signal in the region of δ 2.65~3.70, resulting in the assignment of CH_2OAc. Remaining two protons of amide (NH) were found at δ 6.37 and 6.80. The ^{13}C NMR signals of **1** were the combinations of those of deacetyl-PS-5[3] and pantothenic acid* (Table 2). Accordingly we concluded that **1** was pantoyl-β-alanyldeacetyl-PS-5.

The relative configuration of **1** between H-5 and H-6 was clearly demonstrated to be *trans* on the basis of the coupling constant ($J_{5,6}$=3.0 Hz) in the lanthanide-induced ^1H NMR spectrum[Eu(FOD)$_3$] of the acetonide **8**. For determination of the absolute configuration of the pantoyl group, PS-5 was converted to **1**[4] (Fig. 2). S-Oxide of PS-5 *p*-nitrobenzyl ester was treated with (*R*)- and (*S*)-pantetheines at a temperature of -50~-30°C to provide **9** and **10** in DMF, respectively. Product **9** was spectroscopically identical with **6**. Finally the structure of **1** was concluded to be (5*R*,6*R*)-6-ethyl-7-oxo-3[(*R*)-pantetheinyl]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

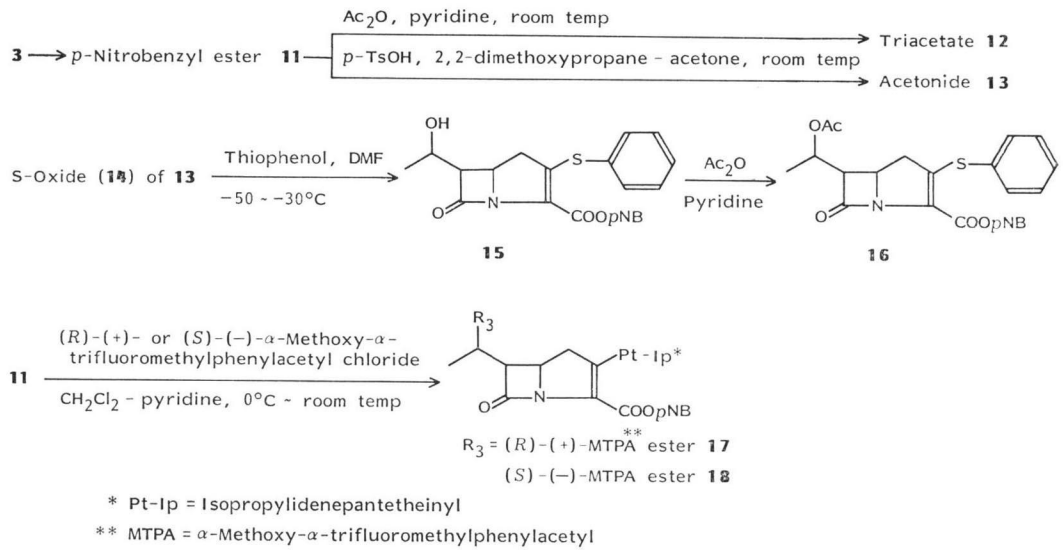
Structures and Absolute Configurations of OA-6129B$_1$ and B$_2$

The molecular formula of **3** was deduced to be $C_{20}H_{31}N_3O_5S$ from the field-desorption mass spectra of the triacetate **12** [*m/z* 735 (M+1)] and the acetonide **13** [*m/z* 649 (M+1)]. In the ^1H NMR spectrum of **3**, instead of the ethyl group in **1**, a multiplet and a methyl doublet were seen at δ 3.95~4.35 and 1.28, respectively. The latter signal changed to a singlet by irradiation of the multiplet at δ 4.10. The remaining signals were very similar to those of **1**, indicating that **3** possessed a hydroxyethyl group at C-6 and a pantetheinyl group at C-3. The relative configuration of **3** between H-5 and H-6 was determined as outlined in Fig. 3.

S-Oxide (**14**) of the acetonide **13** was treated with thiophenol to yield a 6-hydroxyethyl-3-phenylthio derivative **15** which was led to a 6-acetoxyethyl-3-phenylthio derivative **16** by acetylation. The observed coupling constant of **16** ($J_{5,6}$=3.0) revealed that the two β-lactam hydrogen atoms had a *trans* orientation, as was the case in **1**. The absolute configuration of **3** at C-8 was determined to be *S* by NMR spectrometric comparison of the (*R*)-(+)-MTPA and (*S*)-(−)-MTPA derivatives **17** and **18** of the acetonide **13** (chemical shift of the methyl at C-9 δ 1.52 for the former and 1.42 for the latter)[5]. Thus **3** is (5,6-*trans*)-6-[(*S*)-1-hydroxyethyl]-7-oxo-3-pantetheinyl-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

* See footnote on p. 1474.

Fig. 3.



* Pt-Ip = Isopropylidenepantetheinyl

** MTPA = α-Methoxy-α-trifluoromethylphenylacetyl

In the ^{1}H NMR compound **2**, a minor component of the OA-6129 group of carbapenems, closely resembled **3** except for a double doublet at δ 3.60. As this signal (J=5.0 and 10.0 Hz) showed the same chemical shift and coupling constants as H-6 of epithienamycin A[6], **2** was considered to be an epimer of **3** at C-6.

Structure and Relative Configuration of OA-6129C

Elemental analysis established the molecular formula of **4** ($C_{20}H_{31}N_3O_{11}S_2$). The existence of hydroxysulfonyloxyethyl in **4** was indicated by an IR absorption band at 1220~1250 cm^{-1}; and supported by its higher relative mobility on high voltage paper electrophoresis at pH 8.4 in comparison with **1**, **2** and **3** (Rm PS-5 1.69 for **4**; and 0.67 for **1**, **2** and **3**). When the ^{1}H NMR spectra of **3** and **4** were compared, the signals of the C-9 methyl doublet and the C-8 multiplet of **4** were located at δ 1.51 and 4.78, respectively, which were 0.23 and 0.60 ppm lower than the corresponding signals of **3**. The *cis* configuration of the β-lactam of **4** was apparent from the coupling constant ($J_{5,6}$=5.5 Hz). Therefore the structure of **4** is (5,6-*cis*)-6-(1-hydroxysulfonyloxyethyl)-7-oxo-3-pantetheinyl-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.

Experimental

General Methodology

Ultraviolet (UV) and IR spectra were recorded in a Hitachi 200-20 spectrometer and a Hitachi 260-30 spectrometer, respectively. ^{1}H NMR spectra were obtained with a Varian EM 390 spectrometer with tetramethylsilane in $CDCl_3$, CD_2Cl_2 and C_5D_5N or with DSS (2,2-dimethyl-2-silapentane-5-sulfonate) in D_2O as internal standard. Chemical shifts are given in parts per million downfield from the standard. ^{13}C NMR spectrometry was performed with a JOEL FX-100 spectrometer using TSP (sodium 2,2,3,3-tetradeutero-3-trimethylsilylpropionate) as internal standard. Hitachi RMU-7 mass spectrometer was used for field desorption mass spectrometry. Optical rotations were measured in a Jasco DIP-181 polarimeter. Amino acids were analyzed with a Hitachi 835 amino acid analyzer. Pre-coated silica gel plates F254 and silica gel 60 (70~230 mesh, E. Merck, Darmstadt) were employed for thin-layer chromatography (TLC) and column chromatography, respectively. Organic solutions were dried

over anhydrous sodium sulfate and, if necessary, were concentrated to dryness *in vacuo* at a temperature below 45°C with a rotary evaporator.

OA-6129A (**1**) Sodium Salt

$C_{20}H_{30}N_3O_7SNa$ (MW 479): $[\alpha]_D^{24}$ +11.6° (*c* 1.0, 0.01 M phosphate buffer, pH 8.4): $\lambda_{max}^{H_2O}$ nm (ε) 300 (5,600): ν_{max}^{KBr} cm^{-1} 1760 (β-lactam), 1660 (amide), 1600 (carboxylate): ^1H and ^{13}C NMR (see Tables 1 and 2).

OA-6129B$_1$ (**2**) Sodium Salt

$C_{20}H_{30}N_3O_8SNa$ (MW 495): $[\alpha]_D^{24}$ +24.2° (*c* 0.5, H_2O): $\lambda_{max}^{H_2O}$ nm (ε) 300 (6,400): ν_{max}^{KBr} cm^{-1} 1750 (β-lactam), 1650 (amide), 1560 (carboxylate): ^1H NMR (see Table 1).

OA-6129B$_2$ (**3**) Sodium Salt

$C_{20}H_{30}N_3O_8SNa$ (MW 495): $[\alpha]_D^{24}$ +14.7° (*c* 1.0, 0.01 M phosphate buffer, pH 8.4): $\lambda_{max}^{H_2O}$ nm (ε) 300 (5,400): ν_{max}^{KBr} cm^{-1} 1760 (β-lactam), 1660 (amide), 1600 (carboxylate): ^1H NMR (see Table 1).

OA-6129C (**4**) Sodium Salt

$C_{20}H_{29}N_3O_{11}S_2Na_2$ (MW 597): $[\alpha]_D^{24}$ +17.4° (*c* 0.55, 0.01 M phosphate buffer, pH 8.2): Elemental analysis (for $C_{20}H_{29}N_3O_{11}S_2Na_2 \cdot H_2O$) Found C 39.18, H 4.91, N 6.86, S 10.08%; Calcd. C 39.02, H 5.08, N 6.83, S 10.42%: $\lambda_{max}^{H_2O}$ nm (ε) 300.5 (7,600): ν_{max}^{KBr} cm^{-1} 1750 (β-lactam), 1660~1595 (amide, carboxylate), 1250~1220 (sulfoxyldioxy): ^1H NMR (see Table 1).

Acid-catalyzed Hydrolysis of OA-6129A (**1**)

Five milligrams of OA-6129A sodium salt was dissolved in 1 ml of 6 N hydrochloric acid and heated at 115°C for 18 hours in a sealed glass tube. After excess hydrochloric acid was removed by evaporation, the residue was analyzed by high voltage paper electrophoresis in 85% formic acid - acetic acid - water (25: 75: 900) at pH 1.8 and with an automatic amino acid analyzer. The hydrolysate gave a ninhydrin-positive spot at Rm 1.54 (in reference to alanine 1.00) which corresponded to β-alanine. The identity with β-alanine was confirmed from the retention time of the hydrolysate (59 minutes) by automatic amino acid analysis. Analytical conditions: ion exchanger (Hitachi Custom Ion Exchange Resin #2619), column (4.0 mm×150 mm), pre-column (4.0 mm×50 mm), eluent (MCI Buffer 835-pH-kit).

OA-6129A Benzyl Ester (**5**)

To a solution of 44.6 mg of OA-6129A sodium salt in 80 ml of dimethylformamide, 0.25 ml of triethylamine and 0.18 ml of benzyl bromide were added under ice-cooling. The solution was kept for 30 minutes under ice-cooling and then 3 hours at room temperature. The reaction mixture was diluted with 100 ml of ethyl acetate and was washed with 20 ml of 0.01 M phosphate buffer, pH 8.4, saturated with sodium chloride. The aqueous solution was separated and re-extracted with 100 ml of methylene chloride. The methylene chloride extract and the ethyl acetate solution were combined, dried and concentrated to dryness. The residue was dissolved in a small volume of methylene chloride and charged on a column of 12 g of silica gel. The column was successively eluted with 2: 1, 1: 1 and 1: 3 mixtures of benzene - acetone and acetone. All fractions were monitored by silica gel TLC. The acetone fractions which gave a UV-absorbing spot at Rf 0.39 with a solvent system of benzene - acetone (1: 1) were collected and concentrated to dryness to yield 21.4 mg of the benzyl ester **5**.

5: $[\alpha]_D^{24}$ +31.5° (*c* 1.0, CH_2Cl_2): $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 318 (7,400): $\nu_{max}^{CH_2Cl_2}$ cm^{-1} 1772 (β-lactam), 1700 (ester), 1665 (amide): ^1H NMR (CD_2Cl_2) 0.88 (3H, s), 0.97 (3H, s), 1.03 (3H, t, *J*=7.5 Hz), 1.60~2.10 (3H, m), 2.39 (2H, t, *J*=6.5 Hz), 2.85~3.67 (12H, m), 3.93 (1H, m, C-5H), 3.93 (1H, s), 4.17 (1H, br), 5.17 (1H, d, *J*=13.0 Hz), 5.32 (1H, d, *J*=13.0 Hz), 6.73 (1H, br), 7.35 (5H, s): (CD_2Cl_2+D_2O) 0.88 (3H, s), 0.95 (3H, s), 1.02 (3H, t, *J*=7.5 Hz), 1.55~2.00 (2H, m), 2.39 (2H, t, *J*=6.5 Hz), 2.80~3.67 (11H, m), 3.93 (1H, dt, *J*=3.0 & 9.0 Hz), 3.93 (1H, s), 5.13 (1H, d, *J*=13.0 Hz), 5.28 (1H, d, *J*=13.0 Hz), 7.35 (5H, s): MS (*m/z*) 469, 455, 418, 308, 287, 259, 242.

OA-6129A *p*-Nitrobenzyl Ester (**6**)

Triethylamine (0.2 ml) and 285 mg of *p*-nitrobenzyl bromide in 1.5 ml of dimethylformamide were added under ice-cooling to a stirred solution of 63.5 mg of OA-6129A sodium salt in 9.0 ml of dimethylformamide, and then allowed to react under the same conditions as described above. The methylene

chloride extract was concentrated to 2 ml and the concentrate was applied on a column of 12 g of silica gel. The column was successively eluted with 1: 1 and 1: 3 mixtures of benzene - acetone and acetone only. Under silica gel TLC monitoring (benzene - acetone, 1: 1), acetone fractions which contained a UV-absorbing substance at Rf 0.33 were collected. Removal of the solvent by evaporation provided 36.5 mg of the *p*-nitrobenzyl ester **6**.

6: $[\alpha]_D^{24}$ +52.4° (*c* 1.0, CH_2Cl_2): $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 319 (8,400), 270 (10,500): $\nu_{max}^{CH_2Cl_2}$ cm^{-1} 1770, 1700, 1665: ^1H NMR ($CDCl_3$) 0.87 (3H, s), 0.95 (3H, s), 1.04 (3H, t, *J*=7.5 Hz), 1.50~2.20 (3H, m), 2.40 (2H, t, *J*=6.5 Hz), 2.80~3.70 (12H, m), 3.94 (2H, m), 4.17 (1H, br), 5.19 (1H, d, *J*=14.0 Hz), 5.45 (1H, d, *J*=14.0 Hz), 6.74 (1H, br), 7.63 (2H, d, *J*=14.0 Hz), 8.18 (2H, d, *J*=9.0 Hz): FD-MS (*m/z*) 615 (M+Na), 593 (M+1).

Diacetyl-OA-6129A *p*-Nitrobenzyl Ester (**7**)

One hundred milligrams of OA-6129A *p*-nitrobenzyl ester (**6**) in 5 ml of pyridine was acetylated at room temperature for 16 hours in the presence of 0.5 ml of acetic anhydride. The reaction mixture was poured onto 30 g of ice, agitated for 10 minutes, and then extracted twice with 100 ml each of ethyl acetate. The extract was washed twice with 30 ml portions of 0.1 M phosphate buffer, pH 6.8, dried and concentrated to dryness. The evaporation residue was dissolved in a small volume of methylene chloride and charged on a silica gel column (15 g). Fractions eluted with a 1: 2 mixture of benzene - acetone were combined, and evaporated to dryness to yield 63 mg of the diacetate **7** that gave a UV-absorbing spot at Rf 0.38 on a silica gel TLC plate developed with a 1: 1 mixture of benzene - acetone.

7: $[\alpha]_D^{24}$ +22.8° (*c* 1.0, CH_2Cl_2): $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 316.5 (7,500), 269 (10,800): $\nu_{max}^{CHCl_3}$ cm^{-1} 1770, 1735, 1670: ^1H NMR ($CDCl_3$) 1.03 (3H, s), 1.06 (6H, m), 1.70~2.10 (2H, m), 2.03 (3H, s, $COCH_3$), 2.12 (3H, s, $COCH_3$), 2.37 (2H, t, *J*=6.5 Hz, N-CH_2-CH_2-CO), 2.65~3.67 (9H, m), 3.79 (1H, d, *J*=11.0 Hz, -C*H*H-O), 3.75~4.10 (1H, m, C-5H), 4.00 (1H, d, *J*=11.0 Hz, -CH*H*-O), 4.80 (1H, s, O-CH-CO), 5.18 (1H, d, *J*=14.0 Hz), 5.48 (1H, d, *J*=14.0 Hz), 6.37 (1H, br, NH), 6.80 (1H, br, NH), 7.60 (2H, d, *J*=9.0 Hz), 8.17 (2H, d, *J*=9.0 Hz): FD-MS (*m/z*) 677 (M+1).

Isopropylidene-OA-6129A *p*-Nitrobenzyl Ester (**8**)

To a solution of 100 mg of OA-6129A *p*-nitrobenzyl ester (**6**) in 10 ml of acetone were added 0.5 ml of 2,2-dimethoxypropane, 20 mg of anhydrous sodium sulfate and 40 mg of anhydrous *p*-toluenesulfonic acid with stirring at room temperature, and the mixture was kept stirred for 3 hours. The reaction mixture was mixed with 0.1 ml of triethylamine and then poured into 50 ml of ethyl acetate. The organic layer was separated and rinsed with 20 ml each of 0.1 M phosphate buffers, pH 8.4 and pH 6.8. The organic extract was dried and then concentrated to dryness. The residue was subjected to silica gel (5 g) column chromatography using 5: 1, 3: 1, 1: 1, 1: 2 and 1: 5 mixtures of benzene - acetone as eluents. Fractions obtained by the 1: 1 mixture of benzene - acetone were combined and the evaporation of the solvent afforded 72 mg of the acetonide **8** which exhibited a UV-absorbing spot at Rf 0.56 on a silica gel TLC plate developed with a 1: 1 mixture of benzene - acetone.

8: $[\alpha]_D^{24}$ +34.9° (*c* 1.0, $CHCl_3$): $\lambda_{max}^{CHCl_3}$ nm (ε) 319 (6,200), 270 (9,800): $\nu_{max}^{CHCl_3}$ cm^{-1} 1770, 1660: ^1H NMR ($CDCl_3$) 0.97 (3H, s), 1.03 (3H, s), 1.07 (3H, t, *J*=7.5 Hz), 1.40 (3H, s), 1.43 (3H, s), 1.70~2.00 (2H, m), 2.43 (2H, t, *J*=6.5 Hz), 2.50~3.80 (11H, m), 3.97 (1H, dt, *J*=3.0 & 9.0 Hz, C-5H), 4.03 (1H, s), 5.20 (1H, d, *J*=14.0 Hz), 5.49 (1H, d, *J*=14.0 Hz), 6.52 (1H, br), 6.93 (1H, br), 7.58 (2H, d, *J*=9.0 Hz), 8.15 (2H, d, *J*=9.0 Hz).

p-Nitrobenzyl 6-Ethyl-7-oxo-3-[(*R*)-pantetheinyl]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**9**)

PS-5 *p*-nitrobenzyl ester S-oxide (112 mg)[3] was dissolved in 10 ml of dimethylformamide and mixed at −30°C with 53 μl of triethylamine. (*R*)-Pantetheine (105 mg) that was prepared from (D)-(+)-pantothenic acid was added to the solution at −30°C under swirling and kept swirled at −30°C for 30 minutes. The reaction was terminated by pouring the reaction mixture into 100 ml of methylene chloride. The methylene chloride layer was recovered; rinsed twice with 30 ml each of 0.1 M phosphate buffer, pH 6.8; dried and concentrated to dryness. The evaporation residue in a small volume of methylene chloride was adsorbed on a silica gel column (7.5 g). Elution with 1: 1 and 1: 2 mixtures of benzene - acetone followed by evaporation provided 34.8 mg of the (*R*)-pantetheinyl derivative **9** which showed a Rf value of 0.38 on a silica gel TLC plate developed with a 1: 2 mixture of benzene - acetone.

9: $[\alpha]_D^{24}$ +51.7° (*c* 1.0, CH_2Cl_2): $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 321 (10,100), 270 (9,700): $\nu_{max}^{CH_2Cl_2}$ cm^{-1} 1775, 1700, 1668: ^1H NMR (CD_2Cl_2) 0.89 (3H, s), 0.97 (3H, s), 1.05 (3H, t, *J*=7.0 Hz), 1.60~2.00 (3H, m), 2.40 (2H, t, *J*=7.0 Hz), 2.75~3.60 (11H, m), 3.80~4.10 (3H, m), 5.20 (2H, d, *J*=14.0 Hz), 5.47 (2H, d, *J*=14.0 Hz), 6.60 (1H, t, *J*=6.0 Hz), 7.28 (1H, br), 7.64 (2H, d, *J*=9.0 Hz), 8.20 (2H, d, *J*=9.0 Hz); ($CD_2Cl_2+D_2O$) 0.86 (3H, s), 0.95 (3H, s), 1.03 (3H, t, *J*=7.0 Hz), 1.60~2.00 (2H, m), 2.40 (2H, t, *J*=7.0 Hz), 2.80~3.60 (11H, m), 3.92 (1H, s), 3.95 (1H, dt, *J*=3.0 & 9.0 Hz), 5.18 (2H, d, *J*=14.0 Hz), 5.47 (2H, d, *J*=14.0 Hz), 6.80 (1H, br), 7.30 (1H, br, NH), 7.63 (2H, d, *J*=9.0 Hz), 8.18 (2H, d, *J*=9.0 Hz): FD-MS (*m/z*) 615 (M+Na), 593 (M+1).

p-Nitrobenzyl 6-Ethyl-7-oxo-3-[(*S*)-pantetheinyl]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**10**)

The same reaction and purification procedure as described for the (*R*)-pantetheinyl isomer **9** was repeated with 206 mg of PS-5 *p*-nitrobenzyl ester S-oxide, 112 μl of triethylamine and 3 ml of dimethylformamide containing 222 mg of (*S*)-(−)-pantetheine which was prepared from L-(+)-pantoyl lactone[7] to give 191 mg of the (*S*)-pantetheinyl derivative **10**.

10: $[\alpha]_D^{24}$ +30.3° (*c* 1.0, CH_2Cl_2): $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 322 (10,400), 270 (9,900): $\nu_{max}^{CHCl_3}$ cm^{-1} 1772, 1700, 1660: ^1H NMR ($CDCl_3$) 0.90 (3H, s), 0.97 (3H, s), 1.05 (3H, t, *J*=7.5 Hz), 1.60~2.10 (2H, m), 2.43 (2H, t, *J*=6.0 Hz), 2.80~3.80 (12H, m), 3.94 (1H, dt, *J*=3.0 & 9.0 Hz), 3.97 (1H, d, *J*=5.0 Hz), 4.25 (1H, d, *J*=5.0 Hz), 5.20 (1H, d, *J*=14.0 Hz), 5.47 (1H, d, *J*=14.0 Hz), 6.88 (1H, t, *J*=6.0 Hz), 7.20~7.40 (1H, br), 7.63 (2H, d, *J*=9.0 Hz), 8.17 (2H, d, *J*=9.0 Hz): ($CDCl_3+D_2O$) 0.89 (3H, s), 0.96 (3H, s), 1.06 (3H, t, *J*=7.5 Hz), 1.60~2.05 (2H, m), 2.42 (2H, t, *J*=6.0 Hz), 2.75~3.70 (11H, m), 3.94 (1H, s), 3.95 (1H, dt, *J*=3.0 & 9.0 Hz), 5.18 (1H, d, *J*=14.0 Hz), 5.47 (1H, d, *J*=14.0 Hz), 7.05 (1H, t, *J*=6.0 Hz), 7.38 (1H, br), 7.63 (2H, d, *J*=9.0 Hz), 8.17 (2H, d, *J*=9.0 Hz): FD-MS (*m/z*) 615 (M+Na), 593 (M+1).

OA-6129B_2 *p*-Nitrobenzyl Ester (**11**)

One hundred and ninety milligrams of OA-6129B_2 in 6.0 ml of dimethylformamide was mixed with 0.2 ml of triethylamine and 210 mg of *p*-nitrobenzyl bromide in 2 ml of dimethylformamide under cooling with ice. The solution was allowed to stand for 5 minutes under ice-cooling and for 3 hours at room temperature. The reaction mixture was diluted with 100 ml of methylene chloride and then washed twice with 20 ml each of 0.1 M phosphate buffer, pH 6.8. The aqueous layer was separated and back-extracted twice with 100 ml each of methylene chloride. The organic layer and the back-extracts were combined, dried, and concentrated to dryness. Silica gel (6 g) column chromatography using 1: 1, 1: 2 and 1: 3 mixtures of benzene - acetone and acetone only as eluents yielded 85 mg of the *p*-nitrobenzyl ester **11** showing a Rf value of 0.15 with a solvent system of benzene - acetone (1: 4).

11: $[\alpha]_D^{24}$ +41.4° (*c* 1.0, dioxane): $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 320 (10,500), 271 (10,500): ν_{max}^{KBr} cm^{-1} 1760, 1695, 1640: ^1H NMR (pyridine-d_5; δ 1.0~5.0) 1.30 (6H, s), 1.55 (3H, d, *J*=7.0 Hz), 2.70 (2H, t, *J*=6.5 Hz), 2.90~4.05 (11H, m), 4.10~4.50 (2H, m), 4.52 (1H, s): FD-MS (*m/z*) 523 [(M+1) − CH_3–CHOH–CH=CO].

Triacetyl-OA-6129B_2 *p*-Nitrobenzyl Ester (**12**)

To a solution of 12 mg of OA-6129B_2 *p*-nitrobenzyl ester (**11**) in 0.5 ml of pyridine, 0.15 ml of acetic anhydride was added with stirring under ice-cooling. The acetylation was carried out for 5 minutes under ice-cooling and for 3 hours at room temperature. Ice water (10 g) was poured into the reaction mixture and stirred for 10 minutes. The solution was shaken with 20 ml of ethyl acetate. The ethyl acetate layer was recovered, washed with 10 ml each of 0.1 M phosphate buffers, pH 6.8, pH 8.4 and pH 6.8, dried, and concentrated to dryness. The residue was dissolved in a small volume of methylene chloride and adsorbed on a column of 2 g of silica gel. The column was developed successively with benzene - acetone mixtures at mixing ratios of 5: 1, 3: 1, 2: 1, 1: 1 and 1: 5. The derivative **12** [7.9 mg; Rf 0.59 with a solvent system of benzene - acetone (1: 3)] was recovered from the benzene - acetone (1: 1) eluate.

12: $[\alpha]_D^{24}$ +23.2° (*c* 0.5, $CHCl_3$): $\lambda_{max}^{CHCl_3}$ nm (ε) 320 (12,000), 270 (12,000): $\nu_{max}^{CHCl_3}$ cm^{-1} 1780, 1735, 1672: ^1H NMR ($CDCl_3$) 1.05 (3H, s), 1.08 (3H, s), 1.43 (3H, d, *J*=7.0 Hz), 2.03 (3H, s), 2.10 (3H, s), 2.13 (3H, s), 2.38 (2H, t, *J*=6.0 Hz), 2.70~3.70 (9H, m), 3.82 (1H, d, *J*=11.5 Hz), 4.02 (1H, d, *J*=11.5 Hz), 3.97~4.27 (1H, m), 4.80 (1H, s), 5.10~5.45 (1H, m), 5.22 (1H, d, *J*=14.5 Hz), 5.50 (1H, d,

J=14.5 Hz), 6.29 (1H, br), 6.75 (1H, br), 7.63 (2H, d, J=9.0 Hz), 8.21 (2H, d, J=9.0 Hz): FD-MS (m/z) 735 (M+1).

Isopropylidene-OA-6129B$_2$ *p*-Nitrobenzyl Ester (**13**)

Twenty milligrams of OA-6129B$_2$ *p*-nitrobenzyl ester (**11**) was dissolved in a mixture of 5 ml of acetone and 2 ml of 2,2-dimethoxypropane; and 100 mg of anhydrous sodium sulfate and 0.5 mg of *p*-toluenesulfonic acid were added to the solution with stirring at room temperature. Thirty minutes later, 6 μl of triethylamine was fed to the reaction mixture and then concentrated to about 0.5 ml. The concentrate was diluted with 30 ml of methylene chloride and washed with 20 ml of 0.1 M phosphate buffer, pH 6.8. The organic layer was recovered, dried and concentrated to dryness. The solid was subjected to silica gel (2 g) column chromatography using 2: 1, 1: 1 and 1: 2 mixtures of benzene - acetone. Fractions eluted with the 1: 1 and 1: 2 mixtures of benzene - acetone were collected and concentrated to dryness to afford 6.6 mg of the acetonide **13** which gave a UV-absorbing spot of Rf 0.62 on a silica gel TLC plate developed with a 1: 4 mixture of benzene - acetone.

13: $[\alpha]_D^{24}$ +55.1° (c 0.5, CH_2Cl_2): $\lambda_{max}^{CHCl_3}$ nm (ε) 319 (9,700), 270 (11,900): $\nu_{max}^{CHCl_3}$ cm^{-1} 1778, 1700, 1668: ^{1}H NMR ($CDCl_3$) 0.95 (3H, s), 1.02 (3H, s), 1.37 (3H, d, J=7.0 Hz), 1.40 (3H, s), 1.43 (3H, s), 2.41 (2H, t, J=6.5 Hz), 2.75~3.80 (11H, m), 4.02 (1H, s), 4.00~4.30 (2H, m), 5.16 (1H, d, J=14.5 Hz), 5.44 (1H, d, J=14.5 Hz), 6.56 (1H, br), 6.92 (1H, br), 7.55 (2H, d, J=8.0 Hz), 8.12 (2H, d, J=8.0 Hz): FD-MS (m/z) 563 [(M+1) − $CH_3CHOHCH{=}C{=}O$], 562 (M − $CH_3CHOHCH{=}C{=}O$).

Isopropylidene-OA-6129B$_2$ *p*-Nitrobenzyl Ester S-Oxide (**14**)

m-Chloroperbenzoic acid (42.9 mg, 0.187 mmol) in 1 ml of methylene chloride was dropwise added at −30°C to a solution of 110 mg (0.17 mmol) of isopropylidene-OA-6129B$_2$ *p*-nitrobenzyl ester in 7.7 ml of methylene chloride; and allowed to stand at −30°C for 30 minutes. The reaction mixture was diluted with a solution of 0.027 ml (0.163 mmol) of triethylamine in 100 ml of methylene chloride and then rinsed with 20 ml of saturated aqueous solution of sodium bicarbonate and with 20 ml of 0.1 M phosphate buffer, pH 6.8. The organic layer was recovered, dried, and concentrated to dryness. The evaporation residue in a small amount of methylene chloride was charged on a silica gel (5 g) column and developed with 1: 1, 1: 3 and 1: 10 mixtures of benzene - acetone. Fractions containing a UV-absorbing substance of Rf 0.24 [silica gel TLC with a solvent system of benzene - acetone (1: 3)] were combined and concentrated to dryness, providing 81.8 mg of isopropylidene-OA-6129B$_2$ *p*-nitrobenzyl ester S-oxide (**14**).

14: $[\alpha]_D^{24}$ +18.5° (c 1.0, $CHCl_3$): $\lambda_{max}^{CHCl_3}$ nm (ε) 314 (7,400), 268 (12,100): $\nu_{max}^{CHCl_3}$ cm^{-1} 1780, 1705, 1660: ^{1}H NMR ($CDCl_3$) 0.97 (3H, s), 1.04 (3H, s), 1.37 (3H, d, J=7.0 Hz), 1.43 (6H, m), 2.41 (2H, t, J=6.5 Hz), 2.80~3.80 (11H, m), 4.03 (1H, s), 4.00~4.50 (2H, m), 5.19 (1H, d, J=14.5 Hz), 5.46 (1H, d, J=14.5 Hz), 6.17 (1H, br), 6.95 (1H, br), 7.67 (2H, m), 8.17 (2H, m): FD-MS (m/z) 687 (M+Na), 665 (M+1).

p-Nitrobenzyl 6-(1-Hydroxyethyl)-7-oxo-3-phenylthio-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**15**)

Forty-four milligrams (0.066 mmol) of isopropylidene-OA-6129B$_2$ *p*-nitrobenzyl ester S-oxide (**14**) was dissolved in 8 ml of dimethylformamide and mixed at −50°C with 28 μl (0.198 mmol) of triethylamine. After 20 μl (0.198 mmol) of thiophenol was added, the mixture was stirred for 30 minutes at −50°C. The reaction mixture was poured into 100 ml of methylene chloride and washed three times with 20 ml each of 0.1 M phosphate buffer, pH 6.8. The organic solution was separated, dried, and concentrated to dryness. The evaporation residue was dissolved in a small volume of methylene chloride and adsorbed onto a silica gel (5 g) column. After rinsing with benzene, the column was eluted with a 10: 1 mixture of benzene - acetone. Under monitoring by silica gel TLC (solvent system; benzene - acetone, 3: 1), fractions containing a substance of Rf 0.86 were collected and evaporated to dryness to provide 15 mg of the 3-phenylthio derivative **15**.

15: $[\alpha]_D^{24}$ +8.9° (c 1.0, $CHCl_3$): $\nu_{max}^{CHCl_3}$ cm^{-1} 1772, 1700, 1660: ^{1}H NMR ($CDCl_3$) 1.30 (3H, d, J=6.5 Hz), 1.94 (1H, br), 2.65 (2H, d, J=9.0 Hz), 3.17 (1H, dd, J=3.0 & 5.5 Hz), 3.80~4.20 (2H, m), 5.14 (1H, d, J=14.0 Hz), 5.43 (1H, d, J=14.0 Hz), 7.20~7.50 (5H, m), 7.53 (2H, d, J=9.0 Hz), 8.08 (2H, d, J=9.0 Hz): EI-MS (m/z) 440 (M).

p-Nitrobenzyl 6-(1-Acetoxyethyl)-7-oxo-3-phenylthio-1-azabicyclo[3. 2. 0]hept-2-ene-2-carboxylate (**16**)

One-tenth milliliter of acetic anhydride was added under stirring with ice-cooling to a solution of 21.5 mg of ester **15** in 1 ml of pyridine. After 5 minutes, the solution was warmed to room temperature and allowed to stand for 18 hours. The reaction mixture was poured on 5 g of ice, stirred for 10 minutes, and extracted with 30 ml of ethyl acetate. The organic phase was recovered and rinsed successively with 10 ml each of phosphate buffers, pH 6.8, pH 8.4 and pH 6.8. The solvent was dried and concentrated to dryness. Purification by silica gel (2 g) column chromatography (eluents; benzene - acetone, 100: 1, 50: 1, 30: 1 and 20: 1) resulted in the acetate **16** which showed an Rf value of 0.27 on a silica gel TLC plate developed with a 10: 1 mixture of benzene - acetone.

16: $[\alpha]_D^{24}$ +10.9° (*c* 1.0, $CHCl_3$): $\lambda_{max}^{CHCl_3}$ nm (ε) 320 (11,100), 271 (9,800): $\nu_{max}^{CHCl_3}$ cm^{-1} 1780, 1740, 1705: ^1H NMR ($CDCl_3$) 1.36 (3H, d, *J*=6.5 Hz, CH–CH_3), 2.03 (3H, s, $COCH_3$), 2.66 (2H, d, *J*=9.0 Hz, C–4H_2), 3.28 (1H, dd, *J*=3.0 & 4.5 Hz, C–6H), 3.92 (1H, dt, *J*=3.0 & 9.0 Hz, C–5H), 5.15 (1H, dq, *J*=4.5 & 6.5 Hz, CH–C*H*OAc), 5.23 (1H, d, *J*=14.0 Hz, C*H*H–Ar), 5.50 (1H, d, *J*=14.0 Hz, CH*H*–Ar), 7.20~7.57 (5H, m, S–Ar·H), 7.60 (2H, d, *J*=9.0 Hz, Ar·H), 8.15 (2H, d, *J*=9.0 Hz, Ar·H): EI-MS (*m/z*) 482 (M), 440 (M−CH_2CO), 354 [M−($CH_3CHOAcCHCO$)].

(*R*)-(+)-α-Methoxy-α-trifluoromethylphenylacetylisopropylidene-OA-6129B_2 *p*-Nitrobenzyl Ester (**17**)

To a solution of 19 mg of isopropylidene-OA-6129B_2 *p*-nitrobenzyl ester (**13**) in 10 ml of pyridine was added 1 ml of methylene chloride containing 60 μl of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride under cooling with ice water. The solution was stirred for 10 minutes under ice-cooling and then for a further 3 hours at room temperature. The reaction mixture was diluted with 50 ml of methylene chloride and washed successively with 20 ml each of water and 0.1 M phosphate buffers, pH 8.4 and pH 6.8. The organic layer was collected, dried and concentrated to dryness. The evaporation residue was purified by silica gel (3 g) column chromatography using 10: 0, 10: 1, 5: 1, 3: 1, 1: 1 and 1: 3 mixtures of benzene - acetone as eluents. Fractions containing a UV-absorbing material of Rf 0.59 (silica gel TLC; benzene - acetone, 1: 1) were combined and concentrated to dryness, yielding 7.0 mg of the (*R*)-(+)-MTPA ester (**17**).

17: $\lambda_{max}^{CHCl_3}$ nm (ε) 319 (8,300), 269 (9,900): $\nu_{max}^{CHCl_3}$ cm^{-1} 1780, 1750, 1700, 1665: ^1H NMR ($CDCl_3$) 0.94 (3H, s), 1.00 (3H, s), 1.39 (3H, s), 1.43 (3H, s), 1.52 (3H, d, *J*=7.5 Hz, CH–CH_3), 2.43 (2H, t, *J*=6.5 Hz), 2.80~3.75 (14H, m), 3.96 (1H, m), 4.03 (1H, s), 5.22 (1H, d, *J*=14.0 Hz), 5.40 (1H, m), 5.41 (1H, d, *J*=14.0 Hz), 6.48 (1H, br), 6.87 (1H, br), 7.25~7.64 (7H, m), 8.12 (2H, d, *J*=8.0 Hz): FD-MS (*m/z*) 865 (M+1).

(*S*)-(−)-α-Methoxy-α-trifluoromethylphenylacetylisopropylidene-OA-6129B_2 *p*-Nitrobenzyl Ester (**18**)

(*S*)-(−)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (0.036 ml) in 0.5 ml of methylene chloride was treated with 8 mg of isopropylidene-OA-6129B_2 *p*-nitrobenzyl ester (**13**) in 0.5 ml of pyridine, and worked up as described for the (*R*)-(+)-isomer. The evaporation residue was subjected to silica gel (2 g) column chromatography to afford 3.1 mg of the (*S*)-(−)-MTPA ester **18** (Rf 0.59 on a silica gel TLC plate developed with a 1: 1 mixture of benzene - acetone).

18: $\lambda_{max}^{CH_2Cl_2}$ nm (ε) 318 (8,400), 269 (13,700): $\nu_{max}^{CHCl_3}$ cm^{-1} 1778, 1740, 1695, 1660: ^1H NMR ($CDCl_3$) 0.93 (3H, s), 1.00 (3H, s), 1.39 (3H, s), 1.42 (3H, s), 1.42 (3H, d, *J*=7.5 Hz, CH–CH_3), 2.43 (2H, t, *J*=6.5 Hz), 2.68~3.85 (14H, m), 4.03 (1H, s), 4.05 (1H, m), 5.22 (1H, d, *J*=14.0 Hz), 5.43 (1H, d, *J*=14.0 Hz), 5.45 (1H, m), 6.47 (1H, br), 6.90 (1H, br), 7.22~7.70 (7H, m), 8.12 (2H, d, *J*=8.0 Hz): FD-MS (*m/z*) 865 (M+1).

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