KS-504 COMPOUNDS, NOVEL INHIBITORS OF Ca^{2+} AND CALMODULIN-DEPENDENT CYCLIC NUCLEOTIDE PHOSPHODIESTERASE FROM *MOLLISIA VENTOSA*

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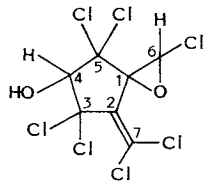
Novel inhibitors of Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase were isolated from the culture broth of a fungus, *Mollisia ventosa* KAC-1148. They were designated as KS-504a, KS-504b and KS-504d. A degradative product of KS-504a, called KS-504e, was also isolated and found to inhibit the enzyme. The 50% inhibitory concentration (IC_{50}) values of KS-504a, KS-504b, KS-504d and KS-504e for bovine brain enzyme were 122, 109, >500 and 139 μM, respectively.

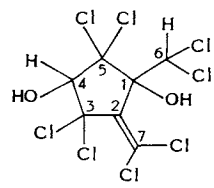
The role of Ca^{2+} is widely accepted as a second messenger in various cell responses such as contraction of muscle, release of neurotransmitters and chemical mediators, secretion of hormones, cell division and proliferation, and cell motility[1]. Several lines of evidence have been accumulating to suggest that many of the Ca^{2+} signals are mediated through calmodulin, a ubiquitous Ca^{2+}-binding protein. Calmodulin has an ability to activate a number of enzymes in a Ca^{2+}-dependent manner[2,3]. Among them is Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase (CaM-PDE)[4,5].

During the course of our screening work to obtain calmodulin inhibitors from microorganisms, we found that novel metabolites of *Mollisia ventosa* KAC-1148 inhibited CaM-PDE. The compounds, designated as KS-504a, KS-504b and KS-504d[6], were isolated from the cultured broth, and their structures were determined (Fig. 1). A degradative product of KS-504a, named KS-504e[6] (Fig. 1), was isolated and found to inhibit CaM-PDE. In this article, we describe taxonomic studies of the producing organism, production, isolation and some physico-chemical and biological properties of KS-504a, KS-504b, KS-504d and KS-504e. Structural elucidation studies will be reported in a separate paper[7].

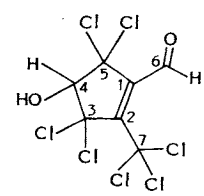
Fig. 1. Structures of KS-504 compounds.
KS-504a and KS-504b were diastereomers.



KS-504a, KS-504b



KS-504d



KS-504e

Materials and Methods

General

^{1}H and ^{13}C NMR spectra were recorded on Jeol JNM FX100 and Bruker AM400 spectrometers with TMS (0 ppm) as an internal standard. IR spectra were obtained using a Shimadzu IR-27G IR Spectrometer. UV spectra were taken with a Hitachi 200-20 Spectrophotometer. MS were measured on a Hitachi M-80B mass spectrometer. Optical rotation was measured with a Perkin-Elmer 141 Polarimeter. MP's were taken with a Yanagimoto melting point apparatus and were uncorrected.

Microorganisms and Taxonomy

The fungus strain KAC-1148 was originally isolated from an ascospore of an apothecium on a fallen twig collected at Teshio-cho, Hokkaido, Japan.

The apothecia are turbinate or cupulate, 1~2 mm diameter, and grayish yellow or cream color with grayish brown margins. The ectal excipulum consists of textura globulosa, whose cell walls are dark brown. The medullary excipulum consists of textura intricata. Asci arrange on the subhymenium, are clavate, the pores are made blue by iodine, 80~90 μm, 8-spored. Paraphyses are hyaline and are observed between the asci. Ascospores are oblong or cylindrical, hyaline, smooth, slightly curved, 16.5~22 μm long, 2~2.5 μm wide, 3-septate.

From the characteristics mentioned above, the fungus was identified as *M. ventosa* P. Karsten[8]. Therefore, we named the strain KAC-1148 as *M. ventosa* KAC-1148, and deposited it at the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, as FERM BP-1333.

Culture and Medium Conditions

A seed medium was composed of glucose 1.0%, peptone 0.5%, dry yeast (Ebios, Asahi Brewery) 0.5%, vegetable juice 20%, and $CaCO_3$ 0.3% (pH 6.0 before sterilization), and a fermentation medium for a tank fermenter of sucrose 5.0%, soybean meal 2.0%, corn steep liquor 1.0%, KCl 0.8% and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05% (pH 6.5 before sterilization).

A 50-ml culture tube containing 10 ml of a seed medium was inoculated with the mycelia of the organism grown on an agar slant. The inoculated tube was incubated for 5 to 7 days on a reciprocating shaker (300 rpm) at 25°C. A 4-ml aliquot of the culture was transferred into a 300-ml Erlenmeyer flask containing 40 ml of the seed medium and the flask was incubated for 3 days on a rotary shaker (200 rpm) at 25°C. A 4-ml aliquot of the second culture was transferred into a 300-ml Erlenmeyer flask containing 40 ml of the fermentation medium. The flask was then incubated for 6 to 10 days on a rotary shaker (200 rpm) at 25°C. For the production in a 200-liter tank fermenter, 25 ml of second seed culture was transferred into a 2-liter Erlenmeyer flask containing 250 ml of the seed medium and the flask was incubated for 36 hours on a rotary shaker (200 rpm) at 25°C. A flask of the third culture was transferred into a 5-liter jar fermenter containing 2.5 liters of the seed medium and the fermenter was operated for 22 to 30 hours at 25°C with agitation at 300 rpm and aeration of 2.5 liters/minute. The seed culture in four 5-liter jar fermenters was combined and transferred into a 200-liter tank fermenter containing 120 liters of a fermentation medium composed of sucrose 1.0%, soybean meal 2.0%, $MgCl_2 \cdot 6H_2O$ 0.8% and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05% (pH 6.5 before sterilization). Fermentation was carried out for 9 days at 25°C with agitation at 220 rpm, aeration of 180 liters/ minute, and single feeding of 4.8 kg sucrose 3 days after inoculation. For the experiments described in Tables 1~3 fermentation was carried out in 300-ml Erlenmeyer flasks containing 40 ml of a fermentation medium; the control medium was composed of sucrose 5.0%, soybean meal 2.0%, corn steep liquor 1.0%, KCl 0.8%, and $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05% (pH 6.5 before sterilization). The growth was monitored by measuring absorbance at 260 nm of crude nucleic acid extracts. Crude nucleic acid was extracted from mycelia by boiling in 0.5 M perchloric acid for 15 minutes. Production of KS-504 compounds was determined by HPLC after extraction of the compounds from the culture broth as follows. To the culture broth was added the same volume of MeOH. After centrifugal separation, the MeOH extract was adsorbed on a SEP-PAK C18 cartridge (Waters Associates), which was then washed with 50% MeOH and eluted with MeOH. The eluate was injected to a Unisil Q C8 column (4.6×250 mm, 5 μm, Gasukuro Kogyo Co., Ltd.) developed at 40°C with 60% acetonitril at a flow rate of 1.0 ml/minute monitoring absorbance at 254 nm.

Enzyme Assay

Bovine brain CaM-PDE activity was measured as described in a previous paper[9] with indicated concentrations of KS-504 compounds. The reaction mixture contained in a final volume of 0.5 ml, imidazole-HCl buffer 80 mM (pH 6.9), $MgSO_4$ 3 mM, dithiothreitol 0.3 mM, NaCl 100 mM, cAMP 1.2 mM, $CaCl_2$ 50 μM, bovine brain CaM-PDE 26 mU/ml, and calmodulin 4 U/ml. The basal activity was determined using a large amount of the enzyme (18.6 mU/ml) to magnify the activity in the presence of 3 mM ethylene bis(oxyethylenenitrilo)tetraacetic acid (EGTA) instead of Ca^{2+}/calmodulin. Bovine heart CaM-PDE activity in the presence of Ca^{2+}/calmodulin was assayed at the enzyme concentration of 40 mU/ml. The activity of bovine heart calmodulin-independent cyclic nucleotide phosphodiesterase (CaM-independent PDE) was determined in the presence of EGTA at enzyme concentration of 25 mU/ml.

Alkaline Treatment of KS-504d

To a solution of KS-504d (14 mg) in MeOH (5 ml), 1% sodium methoxide MeOH solution was added and stirred for 2 hours at room temperature. The reaction mixture was neutralized with 2 N HCl and evaporated *in vacuo*. The residue was purified by HPLC to give KS-504a (5 mg) and KS-504d (5 mg).

Materials

Bovine brain CaM-PDE amd calmodulin were prepared according to the method of KAKIUCHI *et al.*[10] with some modifications[9]. Bovine heart CaM-PDE and calmodulin-independent PDE, cAMP and 5′-nucleotidase (*Crotalus atrox* venom) were purchased from Sigma Chemical Company. All other reagents were commercially available and of reagent grade.

Results

Production of KS-504a, KS-504b and KS-504d by Fermentation

M. ventosa KAC-1148 required potassium chloride to produce KS-504a (Table 1A). The production of KS-504a was repressed by addition of ammonium chloride or sodium chloride in place of potassium chloride, but stimulated by magnesium chloride (Table 1). The optimum concentration of $MgCl_2 \cdot 6H_2O$ for the production was 0.8% as shown in Table 1B. Tables 2 and 3 showed the effect of carbon sources and nitrogen sources, respectively, on the production of KS-504a. Sucrose was the best carbon source tested (Table 2A). The production of KS-504a depended on the concentration of sucrose, and increased up to 6% of sucrose (Table 2B). Nitrogen source markedly affected the production of KS-504a and viscosity of the fermentation broth. In a control medium containing soybean meal and corn steep liquor as nitrogen source, the production of KS-504a was 76 μg/ml (Table 3), and the viscosity of the fermentation broth

Table 1. Effects of chloride salts on the production of KS-504a.

(A)

Chloride salt	(%)	KS-504a produced (μg/ml)
KCl	0.0	0.0
	0.4	22.2
	0.8 (control)	37.5
	1.6	0.3
NH_4Cl	0.4	0.0
	0.8	0.0
	1.6	0.0
NaCl	0.8	0.0
	1.6	0.0
$MgCl_2 \cdot 6H_2O$	0.4	57.7
	0.8	67.9

(B)

$MgCl_2 \cdot 6H_2O$ (%)	KS-504a produced (μg/ml)
0.4	117
0.6	178
0.8	189
1.0	118
1.2	123
1.4	102
1.6	44

Table 2. Effects of carbon sources on the production of KS-504a.

(A)

Carbon sources	KS-504a produced (μg/ml)
Glucose	153
Galactose	8
Mannose	154
Fructose	66
Mannitol	30
Sorbitol	9
Lactose	0
Maltose	22
Soluble starch	10
Dextrin	10
Glycerol	27
Sucrose (control)	202

(B)

Sucrose (%)	KS-504a produced (μg/ml)
1	5
2	112
3	136
4	203
5	202
6	225

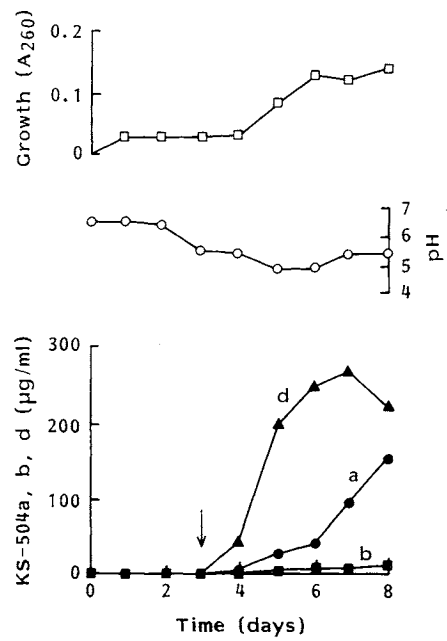
Table 3. Effects of nitrogen sources on the production of KS-504a.

Nitrogen sources	KS-504a produced (μg/ml)
Corn steep liquor	53
Soybean meal	218
Yeast extract	7
Dry yeast	6
Beef extract	2
Malt extract	5
Pharmamedia	4
Peptone	3
$NaNO_3$	49
Control	76

The concentration of $NaNO_3$ was 0.2%, and that of the other nitrogen sources listed was 2.0%.

Fig. 2. Time course of KS-504a, KS-504b and KS-504d production in a 200-liter tank fermenter.

● KS-504a, ■ KS-504b, ▲ KS-504d, ○ pH, □ growth (A_{260}).



Fermentation conditions were described in Materials and Methods. Sucrose was feeded at the time indicated by an arrow.

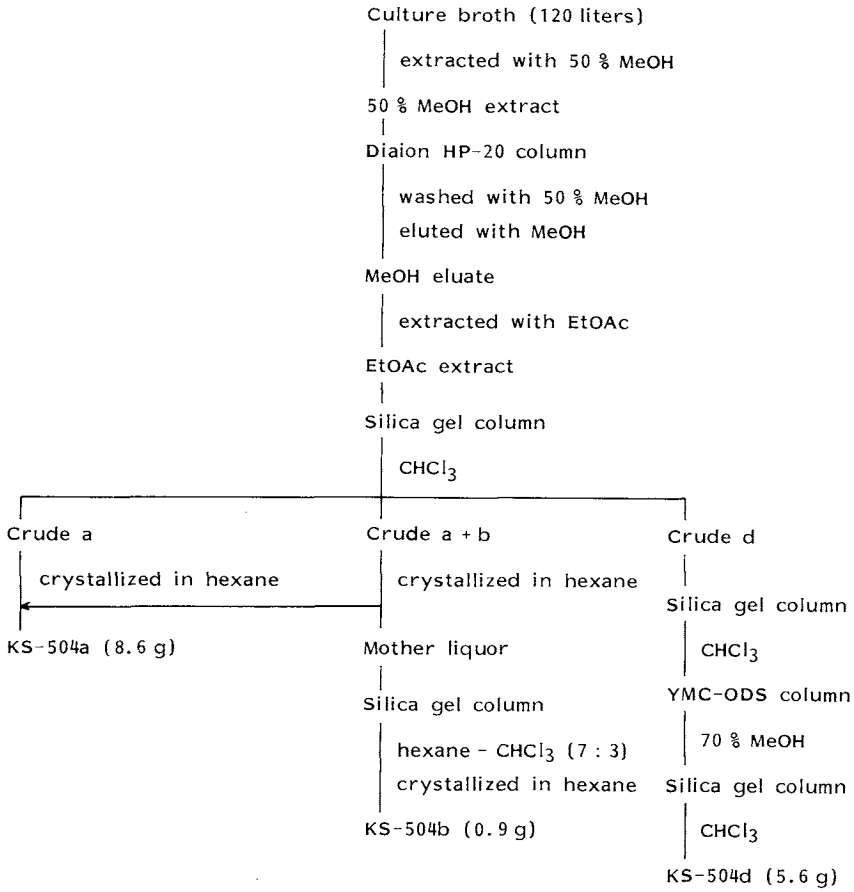
was rather high. High viscosity caused low dissolved oxygen in fermentation broth, which was crucial in low productivity of KS-504a in a jar or a tank fermenter. Soybean meal used as the only nitrogen source increased the production of KS-504a (218 μg/ml) and lowered the viscosity. The others tested decreased the productivity. From these investigations, the modified fermentation medium described in Materials and Methods was used for the production in a 200-liter tank. *M. ventosa* KAC-1148 grew and produced the compound (max 49 μg/ml) in a completely synthetic medium containing sucrose, $NaNO_3$, $MgCl_2 \cdot 6H_2O$, and $Mg_3(PO_4)_2 \cdot 8H_2O$ (Table 3).

Fig. 2 represented a time course of KS-504 compounds production by *M. ventosa* KAC-1148. The amount of KS-504d increased during logarithmic phase of the cell growth, reached maximum (271 μg/ml) after 7-day cultivation and decreased with concomitant lysis of cells. The production of KS-504a initiated later than that of KS-504d, increased rapidly after a plateau of KS-504d production and reached 149 μg/ml in 8-day. The results suggested that KS-504d might be a precursor of KS-504a. The level of KS-504b was max 9 μg/ml after 8-day cultivation.

Purification and Isolation of KS-504a, KS-504b and KS-504d

The purification procedures were outlined in Fig. 3. Fifty %-methanol extracts from whole broths

Fig. 3. Purification of KS-504a, KS-504b and KS-504d.



was passed through a Diaion HP-20 column. After washing with 50% methanol, the column was eluted with methanol. Methanol was removed, *in vacuo*, from the eluate. The resulting aqueous solution was extracted by ethyl acetate. The extracted materials were analyzed by HPLC as shown in Fig. 4. KS-504 compounds were detected and determined by HPLC. The materials were subjected to silica gel column chromatography using chloroform as elution solvent. KS-504a-containing fractions (crude a), KS-504a and KS-504b-containing fractions (crude a + b), and KS-504d-containing fractions (crude d) were combined separately, and concentrated *in vacuo*. The crude a and crude a + b fractions were dissolved in hexane and crystallized to obtain colorless crystals of KS-504a. The mother liquor of crude a + b fraction was concentrated and subjected to silica gel column chromatography. KS-504b-rich fractions were eluted with hexane - chloroform (7 : 3). KS-504b was crystallized from hexane solution and obtained as colorless needles. Crude d fraction was subjected to silica gel column chromatography (chloroform), YMC-ODS column chromatography (70% aqueous methanol), and again silica gel column chromatography (chroloform). KS-504d was obtained as colorless powder.

Isolation of KS-504e

Crystals of KS-504a decomposed at room temperature in summer. A new compound, designated as KS-504e, was found to be produced in the decomposed crystals and to have inhibitory activity for

Table 4. Physico-chemical properties of KS-504 compounds.

	a	b	d	e
Molecular formula	$C_7H_3O_2Cl_7$	$C_7H_3O_2Cl_7$	$C_7H_4O_2Cl_8$	$C_7H_3O_2Cl_7$
Appearance	Colorless prism	Colorless needle	Colorless powder	Colorless prism
MP (°C)	102.0~102.5	93.0~93.5	78.0~79.0	89.0~89.5
$[\alpha]_D$	−113° (*c* 0.36, MeOH)	−35° (*c* 0.32, $CHCl_3$)	+4.8° (*c* 0.32, $CHCl_3$)	+9.0° (*c* 0.34, $CHCl_3$)
TLC, Rf				
$CHCl_3$[a]	0.48	0.39	0.14	0.35
90% MeOH[b]	0.57	0.57	0.71	0.75
IR[c]	3490, 3100, 2960, 1585, 1400, 1366, 1304, 1288, 1175, 1155, 1135, 1080, 1039, 999, 945, 930, 895, 807, 759, 710, 695, 637, 613, 586	3550, 3260, 3030, 2960, 2320, 1615, 1605, 1395, 1372, 1287, 1275, 1200, 1178, 1156, 1112, 1080, 994, 955, 938, 900, 830, 815, 715, 662, 630, 610, 498, 455	3540, 3300, 3050, 1592, 1395, 1315, 1278, 1150, 1108, 1060, 960, 927, 888, 815, 682, 648, 605, 530	3450, 1748, 1723, 1695, 1620, 1393, 1288, 1260, 1200, 1160, 875, 765, 609
CI-MS				
Positive	364 (M^+)	364 (M^+)	383 $(M-H_2O+H)^+$, 365 $(M-HCl+H)^+$	365 $(M+H)^+$
Negative	399 $(M+Cl)^-$	399 $(M+Cl)^-$	434 $(M-H+Cl)^-$, 399 $(M-H)^-$	399 $(M+Cl)^-$
UV λ_{max}^{MeOH} (ε)	243 (9,100)	241 (10,000)	237 (2,700)	221 (5,200)

[a] Silica gel $60F_{254}$ plate.
[b] RP-$18F_{254}$S plate.
[c] a: KBr, b, d and e: $CHCl_3$.
CI: Chemical ionization.

Table 5. ^1H and ^{13}C NMR spectral data of KS-504 compounds.

	a	b	d	e
^1H NMR	100 MHz, $CDCl_3$	400 MHz, $CDCl_3$	100 MHz, $CDCl_3$	400 MHz, $CDCl_3$
6-H	6.55 (1H, s)	5.70 (1H, s)	6.80 (1H, s)	10.46 (1H, s)
4-H	4.94 (1H, br s)	4.79 (1H, br s)	4.91 (1H, br d, J=12.0 Hz)	4.97 (1H, br s)
1-OH			3.87 (1H, br s)	
4-OH	3.45 (1H, br s)	3.46 (1H, br s)	3.50 (1H, br d, J=12.0 Hz)	3.57 (1H, br s)
^{13}C NMR	25 MHz, $CDCl_3$+CD_3OD	100 MHz, $CDCl_3$	25 MHz, $CDCl_3$	25 MHz, $CDCl_3$
	136.9 (s)	136.1 (s)	137.9 (s)	184.3 (d, J=198 Hz)
	130.8 (s)	127.6 (s)	134.7 (s)	148.0 (s)
	87.7 (s)	86.4 (s)	93.2 (s)	139.0 (s)
	87.7 (d, J=153 Hz)	86.4 (d, J=156 Hz)	86.3 (dd, J=156, 3.1 Hz)	89.0 (d, J=159 Hz)
	85.0 (d, J=2.3 Hz)	84.6 (d, J=3.1 Hz)	85.5 (d, J=10 Hz)	88.9 (s)
	69.3 (d, J=224 Hz)	73.0 (d, J=227 Hz)	85.2 (s)	86.4 (s)
	66.7 (s)	70.6 (s)	73.1 (d, J=181 Hz)	83.9 (s)

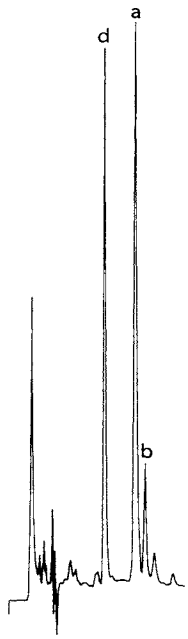
CaM-PDE. KS-504e was also synthesized by heating KS-504a in dry ethyl acetate containing 1.7 N HCl, for 20 hours at 60°C. For isolation of KS-504e, the solution was washed with water and the ethyl acetate layer was applied to a silica gel column. The column was eluted with hexane-chloroform (7:3). The

fractions containing KS-504e were combined and dried *in vacuo*. KS-504e was crystallized from hexane solution and yielded as colorless prisms.

Physico-chemical Properties

Physico-chemical properties of KS-504 compounds were summarized in Table 4. ^{1}H and ^{13}C NMR spectral data were described in Table 5. KS-504 compounds were visualized with I_2 vapor or by heating after sprayed with 50% H_2SO_4. The compounds did not give the following color reactions: Rydon-Smith reaction and the reactions with anisaldehyde, with aniline-phthalate, with ninhydrin, and with $FeCl_3$. The compounds were soluble in various organic solvents such as methanol, chloroform, acetone, ethyl acetate, acetonitrile, hexane, benzene, and dimethyl sulfoxide. KS-504d is slightly soluble in water, but other KS-504 compounds were not. KS-504d was rapidly degraded in alkaline-methanol at room temperature. The major degradation product was shown to be KS-504a by HPLC, ^{1}H NMR, and $[\alpha]_D$; KS-504b was not detected in the products. KS-504a and KS-504b were also degraded under alkaline conditions; KS-504a was more labile than KS-504b.

Fig. 4. HPLC analysis of ethyl acetate extracts.



Conditions were described in Materials and Methods. The retention time of KS-504a, KS-504b and KS-504d was 9.34, 10.09 and 7.04 minutes, respectively.

Table 6. Effects of KS-504 compounds on various cyclic nucleotide phosphodiesterase.

Enzyme	IC_{50} (μM)			
	a	b	d	e
Bovine brain CaM-PDE				
CaM-dependent	122	109	>500	139
Basal	>250	>250	NT	>250
Bovine heart CaM-PDE				
CaM-dependent	226	207	NT	169
Basal	>250	>250	NT	>250
Bovine heart				
CaM-independent PDE	>250	>250	NT	57

NT: Not tested.

Table 7. Antibacterial activities of KS-504 compounds.

Test organisms	MIC (μg/ml)			
	a	b	d	e
Staphylococcus aureus ATCC 6538P	25	25	50	<3.13
Streptococcus faecium ATCC 10541	50	50	>100	12.5
Bacillus subtilis No. 10707	12.5	25	12.5	<3.13
Escherichia coli ATCC 26	>100	>100	>100	100
Klebsiella pneumoniae ATCC 10031	>100	>100	>100	50
Proteus vulgaris ATCC 6897	50	>100	>100	<3.13
Shigella sonnei ATCC 9290	>100	>100	>100	100
Salmonella typhi ATCC 9992	>100	>100	>100	12.5
Pseudomonas aeruginosa BMH No. 1	>100	>100	>100	>100
Candida albicans ATCC 10231	50	50	100	12.5

In acidic methanol solution, KS-504 compounds were stable at room temperature. KS-504a was converted to KS-504e in dry ethyl acetate containing HCl at 60°C (see above). KS-504b was, however, stable under the same conditions.

The structures of KS-504a, KS-504b and KS-504e (Fig. 1) have been determined by HIRAYAMA and SHIMIZU using single crystal X-ray analyses[7]. Because KS-504d could not be crystallized, the structure was elucidated by the comparison of spectral data with that of KS-504a and KS-504b, and chemical conversion experiments.

Negative chemical ionization MS of KS-504d exhibited a molecular ion cluster of $(M-H)^-$ which was identical with the pattern calculated as $C_7H_3O_2Cl_8$. The molecular formula is greater by HCl than KS-504a and KS-504b. The ^1H NMR spectrum of KS-504d showed two methine protons and two hydroxy protons; that is, KS-504d has an additional hydroxy group compared with KS-504a and KS-504b. The ^{13}C NMR spectrum of KS-504d does not exhibit a large coupling ($J_{C-H}=220$ Hz) of epoxide methine carbon observed in KS-504a and KS-504b. Alkaline treatment of KS-504d gave KS-504a which was identified with HPLC, ^1H NMR, and $[\alpha]_D$. These results suggested the structure of KS-504d to be an addition product of hydrogen chloride to the epoxide of KS-504a.

The location of newly produced hydroxy group was determined to 1-position, because 6-H exhibited a sharp singlet and no coupling was observed between 6-H and hydroxy proton in ^1H NMR. Thus the structure of KS-504d was determined to be a halohydrine derivative having tertiary hydroxy group at 1-position (Fig. 1).

Biological Activities

KS-504 compounds inhibited CaM-dependent activity of bovine brain CaM-PDE dose-dependently. The potency of KS-504a, KS-504b and KS-504e were similar to each other: The IC_{50} values were 122, 109 and 139 μM, respectively (Table 6). However, KS-504d at 500 μM inhibited merely 33% of the activity (Table 6). The CaM-independent activity (basal activity) was not inhibited by KS-504a, KS-504b and KS-504e at 250 μM (Table 6). The compounds showed similar effects on bovine heart CaM-PDE (Table 6). Bovine heart CaM-independent PDE was not inhibited by KS-504a and KS-504b at 250 μM, but inhibited by KS-504e with the IC_{50} value of 57 μM (Table 6).

The antimicrobial activities of KS-504 compounds were shown in Table 7. KS-504a, KS-504b and KS-504d had weak activities against, *Staphylococcus aureus*, *Streptococcus faecium*, *Bacillus subtilis*, *Proteus vulgaris* and *Candida albicans*. KS-504e showed relatively wide and strong activities. The LD_{50} values of these compounds were higher than 300 mg/kg in rats by po administration.

Discussion

We found novel CaM-PDE inhibitors, KS-504 compounds, from microbial origin. These compounds have unique characteristics in their structures: They contained 7 or 8 atoms of chlorine in a molecule (66% or 69% by weight percent). Such high content of chlorine in the compounds was surprising in natural organic products. *M. ventosa* KAC-1148 produced substantial amounts of the compounds; the chlorine atoms that could be derived from chloride ions in the fermentation medium. This suggests that the chlorination activity of the fungus is markedly high.

KS-504a and KS-504b inhibited CaM-dependent activities of CaM-PDE's but did not affect their calmodulin-independent (basal) activities and CaM-independent PDE activity. These results supported the idea that the compounds interacted with CaM to inhibit Ca^{2+}/CaM-dependent enzymes. KS-504e, however, showed inhibition against CaM-independent PDE, indicating low selectivity of the compound. A variety

of compounds have been found to inhibit Ca^{2+}/CaM-dependent enzymes, including phenothiazines[11], naphthalenesulfonamides[12], alkaloids[13,14], peptides[15], antimycotic agents[16,17], and others[18~20]. The structures of KS-504 compounds are quite different from these calmodulin inhibitors.

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