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Phenatic Acids A and B, New Potentiators of Antifungal Miconazole Activity Produced by *Streptomyces* sp. K03-0132

Takashi Fukuda, Atsuko Matsumoto, Yoko Takahashi, Hiroshi Tomoda, Satoshi Ōmura

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Abstract Two new phenols, designated phenatic acids A and B, were isolated along with known actiphenol, from the culture of *Streptomyces* sp. K03-0132 by solvent extraction, silica gel column chromatography and HPLC. Their structures were elucidated by spectroscopic analyses including mainly various NMR experiments. They have a common 1-hydroxy 2, 4-dimethyl benzene ring. These compounds potentiate miconazole activity against *Candida albicans*. Phenatic acid B also showed moderate antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Bacteroides fragilis* and *Acholeplasma laidlawii*.

Keywords phenatic acids, phenol, azole potentiator, actinomycetes, actiphenol

Introduction

Opportunistic infections caused by certain fungi, in particular the problematic *Candida albicans*, have increased recently and become a public concern [1]. Patients with compromised immune systems, *e.g.* patients receiving organ transplants and cancer chemotherapy, or those infected by human immunodeficiency virus (HIV), are particularly prone to such infections [1]. Azole derivatives, which inhibit fungal ergosterol biosynthesis by blockade of the cytochrome P-450 reaction involved in 14-α demethylation, are the most commonly used agents. In the last few years, several antifungal combination therapies have been evaluated against infections. However, new antifungal agents of a different mechanism of action have been sought extensively.

On the basis of the new concept "antiinfective drugs" [2], we discovered actofunicone [3] and beauvericins D, E and F [4, 5] from a fungal origin as potentiators of antifungal miconazole activity. The compounds showed no antimicrobial activity themselves, but reinforced miconazole activity against the growth of *C. albicans*. During the course of the continuous screening program, three 2,4-dimethyl phenols (Fig. 1) were isolated from the culture broth of strain *Streptomyces* sp. K03-0132. Two were found to be new compounds, designated phenatic acids A and B, although one additional compound was identified as actiphenol which was previously isolated as an antifungal antibiotic and antitumor props produced by *Streptomyces* sp. [6, 7]. They all potentiated miconazole activity against *C. albicans*. In this paper, the taxonomy of the producing strain, fermentation, isolation, structure elucidation and biological properties of phenatic acids A and B are described.

Materials and Methods

General Experimental Procedures

The strain K03-0132 was isolated from soil collected at Mt. Daisetsu, Hokkaido, Japan and was used for production of phenatic acids A and B. *C. albicans* ATCC 64548 (KF-1, wild type) was purchased from ATCC (Virginia, USA). For determination of the amount of phenatic acids A and B in the culture broths, the samples were dissolved in methanol

H. Tomoda (Corresponding author)**, T. Fukuda, A. Matsumoto, Y. Takahashi, S. Ōmura:** Kitasato Institute for Life Sciences & Graduate School of Infection Control Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan, and The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan, E-mail: tomoda@lisci.kitasato-u.ac.jp

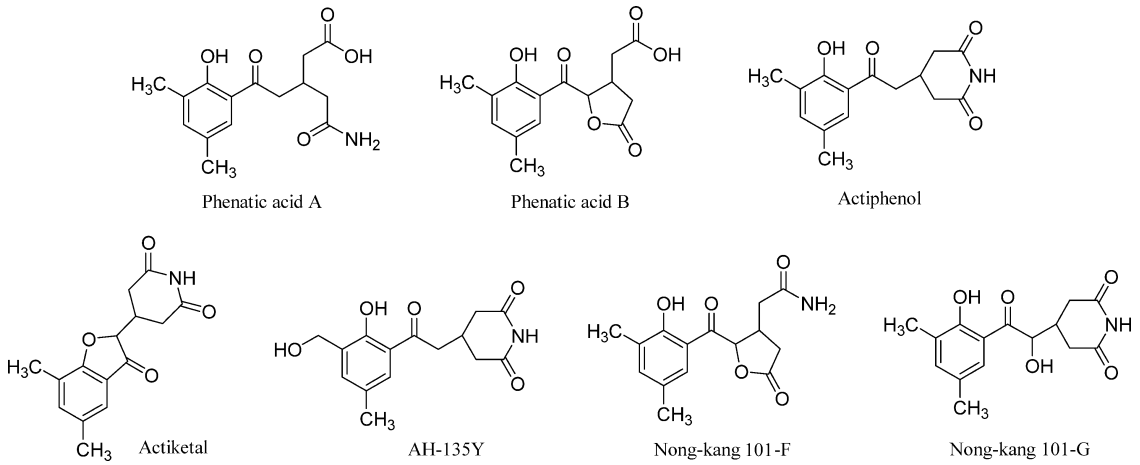


Fig. 1 Structures of phenatic acids A and B and structurally related known compounds.

and analyzed with an HP 1100 system (Hewlett Packard Inc., Germany) under the following conditions: column, Symmetry (2.1×150 mm, Waters Inc., Missouri, USA); flow rate, 0.2 ml/minute; mobile phase, a 20-minute linear gradient from 60% CH_3CN/0.05% H_3PO_4 to 100% CH_3CN/0.05% H_3PO_4; detection, UV at 210 nm. Phenatic acids A and B were eluted with retention times of 6.0 and 11.7 minutes, respectively.

Optical rotations were recorded with a DIP-370 digital polarimeter (JASCO, Tokyo, Japan). Melting points were measured with a micro melting apparatus (Yanaco, Kyoto, Japan). FAB-MS spectrometry was conducted on a JMS-AX505H spectrometer (JEOL, Tokyo, Japan). UV and IR spectra were measured with a DU640 spectrophotometer (Beckman, California, USA) and an FT-210 Fourier transform infrared spectrometer (Horiba, Kyoto, Japan), respectively. The various NMR spectra were measured with an XL-400 spectrometer (Varian, California, USA).

Taxonomic Studies

The International *Streptomyces* Project (ISP) media recommended by Shirling and Gottlieb [7] and media recommended by Waksman [8] were used to investigate the cultural and physiological characteristics. Cultures were routinely observed after the incubation for two weeks at 27°C. Color names and hue numbers were determined according to the Color Harmony Manual [9]. The utilization of carbon sources was tested by growth on Pridham and Gottlieb's medium containing 1% carbon at 27°C [10]. The morphological properties were observed with a scanning electron microscope (model JSM-5600, JEOL). The type of diaminopimelic acid (DAP) isomers was determined by the method of Becker *et al.* [11]. Menaquinones were extracted and purified by the methods of Collins *et al.* [12], then analyzed by HPLC equipped with a CAPCELL PAK C18 column (Shiseido) [13].

Assay for Miconazole-potentiating Activity

C. albicans KF-1 (wild strain) was inoculated into a 50-ml test tube containing 10 ml of seed medium (potato extract containing peptone 0.5% and glucose 1%), and were grown for 24 hours on a rotary shaker at 210 rpm. The seed culture of *C. albicans* (0.1%, v/v) was transferred to the two different agar plates, GY agar (glucose 1%, yeast extract 0.5% and agar 0.8%) (Plate A) and GY agar plus miconazole (0.06 μM) (Plate B). The concentration (0.06 μM) of miconazole is one fourth of the MIC value against *C. albicans*, showed no effect on the growth of *C. albicans*. Paper disks (8 mm, ADVANTEC, Tokyo, Japan) containing 10~50 μg of a sample were put on Plates A and B, which were incubated at 27°C for 24 hours. Samples showing inhibition zones selectively on Plate B were selected as potentiators of miconazole activity against *C. albicans*.

Antimicrobial Activity

Antimicrobial activity against 14 species of microorganisms was measured. The microorganisms were as follows; *Bacillus subtilis* PCI 219, *Staphylococcus aureus* FDA 209P, *Micrococcus luteus* PCI 1001, *Mycobacterium smegmatis* ATCC 607, *Escherichia coli* NIHJ, *Pseudomonas aeruginosa* P-3, *Xanthomonas campestris* pv. *Oryzae* KB 88, *Bacteroides fragilis* ATCC 23745, *Acholeplasma laidlawii* PG 8, *Pyricularia oryzae* KF 180, *Aspergillus niger* ATCC 6275, *Mucor racemosus* IFO 4581, *C. albicans* ATCC 64548 and *Saccharomyces cerevisiae*. Media for microorganisms were as follows: GAM agar (Nissui Seiyaku Co.) for *B. fragilis*; Bacto PPLO agar (Difco) supplemented with 15% horse serum, 0.1% glucose, 0.25% phenol red (5 mg/ml) and 1.5% agar for *A.*

laidlawii; Taiyo agar (Shimizu Syokuhin Kaisya Ltd.) for the other bacteria; a medium composed of 1.0% yeast extract, and 0.8% agar for fungi and yeasts. A paper disk (i.d. 6 mm, ADVANTEC) containing 10 μg of a sample was placed on an agar plate. Bacteria except *X. oryzae* were incubated at 37°C for 24 hours. Yeasts and *X. oryzae* were incubated at 27°C for 24 hours. Fungi were incubated at 27°C for 48 hours. Antimicrobial activity was expressed as diameter (mm) of the inhibitory zone.

Results

Taxonomy of the Producing Strain K03-0132

The vegetative mycelia grew abundantly on yeast extract-malt extract agar and others, and did not show fragmentation into coccoid forms or bacillary elements. The aerial mycelia grew abundantly on yeast extract - malt extract and others. The spore chains were spirals and each had more than 20 spores per chain. The spores were cylindrical in shape, 0.7×1.2 μm in size and had a smooth surface (Fig. 2). Whirls, sclerotic granules, sporangia and flagellate spores were not observed.

The isomer of DAP in whole-cell hydrolysates of strain K03-0132 was determined to be LL-form. Major menaquinones were MK-9 (H_6) and MK-9 (H_8).

The cultural characteristics, the physiological properties and the utilization of carbon sources are shown in Tables 1, 2 and 3. The color of vegetative mycelia showed yellow to brown and the aerial mass color showed brown to gray. Melanoid pigment was produced on tyrosine agar.

Based on the taxonomic properties described above, strain K03-0132 was considered to belong to the genus *Streptomyces* [14].

Fermentation

A slant culture of the strain K03-0132 grown on Seino agar (starch 1.0%, N–Z amine 0.3%, yeast extract 0.1%, meat extract 0.1%, $CaCO_3$ 0.3%, agar 1.0%, pH 7.0) was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of the seed medium (glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, $CaCO_3$ 0.4%, pH 7.0). The flask was shaken on a reciprocal shaker for 4 days at 27°C. One ml of the seed culture was incubated into a 500-ml Erlenmeyer flask containing 100 ml of the production medium (starch 2.4%, glucose 0.1%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, $CaCO_3$ 0.4%, trace metals 5 ml/liter, pH7.0). The fermentation was carried out at 27°C for 6 days.

A typical time course of the fermentation is shown in Fig. 3. Phenatic acids A and B were detected in the culture broth from day 2 after inoculation. The concentration of phenatic acids A and B on day 6 reached levels of 6.7 and 19.7 μg/ml, respectively.

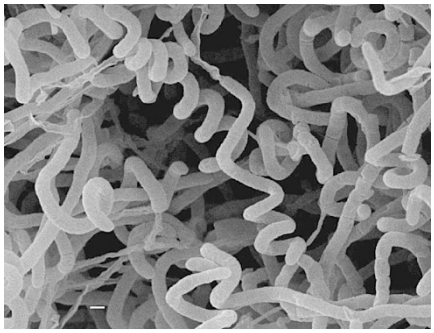


Fig. 2 Scanning electron micrograph of *Streptomyces* sp. K03-0132 grown on yeast extract - malt extract agar at 27°C for 14 days. Bar represents 1 μm.

Isolation

To the 6-day old culture broth (1 liter) was added 1 liter of ethanol. After the ethanol extracts were filtered and concentrated, the resulting aqueous solution was extracted with 1 liter of ethyl acetate. The ethyl acetate layer was dried over Na_2SO_4 and concentrated *in vacuo* to dryness to yield an oily material (362.1 mg). The material was dissolved in a small volume of $CHCl_3$, applied on a silica gel column (20 g, 2.5×12 cm, 70~230 mesh, Merck), and eluted stepwise with 100% $CHCl_3$, 100 : 1, 50 : 1, 10 : 1, 5 : 1 and 1 : 1 (v/v) of $CHCl_3$–CH_3OH solvents and 100% MeOH (200 ml each). The antimicrobial activity against *S. aureus* was observed in the three fractions 100 : 1 (I), 5 : 1 (II) and 1 : 1 (III) which were concentrated to give a white powder I (37.4 mg), brown material II (41.0 mg) and brown material III (40.7 mg), respectively. The white powder I was recrystallized from MeOH to give actiphenol (5.5 mg) as white needles. The brown material III was purified by HPLC; ODS column (4.6×250 mm, Pegasil, Senshu Sci. Co. Tokyo, Japan), a 30-minute linear gradient from 10 to 50% CH_3CN in 0.05% TFA, 1.0 ml/minute, and UV at 210 nm. Under the conditions, phenatic acid A was eluted as a peak with a retention time of 20 minutes. The fraction was concentrated to yield pure phenatic acid A (4.0 mg) as colorless needles. The brown material II was purified by HPLC under the same conditions. Phenatic acid B was eluted as a peak with a retention time of 28 minutes to give pure phenatic acid B (4.0 mg) as colorless needles.

Structure Elucidation

Physico-chemical Properties of Phenatic Acids

Physico-chemical properties of phenatic acids are summarized in Table 4. Similarity in their data indicated

Table 1 Cultural characteristics of strain K03-0132

Medium	Cultural characteristics	Medium	Cultural characteristics
Yeast extract - malt extract agar [a]	G: Good, yellow maple (3ng) R: Clove brown (3pl) AM: Abundant, dark covert gray (2ih) SP: Trace, golden brown (3pg)	Tyrosine agar [a]	G: Good, light mustard tan (2ie) R: Mustard tan (2lg) AM: Moderate, silver gray (3fe) SP: Trace, light mustard tan (2ie)
Oatmeal agar [a]	G: Good, mustard (2le) R: Dark covert gray (2ih) AM: Moderate, beige brown (3ig) SP: None	Sucrose - nitrate agar [b]	G: Good, pearl (2ba) R: Silver gray (3fe) AM: Poor, beige (3ge) SP: None
Inorganic salts - starch agar [a]	G: Good, mustard (2le) R: Light mustard tan (2ie) AM: Abundant, covert gray (2fe) SP: Trace, light mustard tan (2ie)	Glucose - nitrate agar [b]	G: Moderate, pearl (2ba) R: Pearl (2ba) AM: Poor, beige (3ge) SP: None
Glycerol - asparagine agar [a]	G: Good, camel (3ie) R: Adoobe brown (3lg) AM: Abundant, beige gray (3ih) SP: Trace, mustard	Glycerol - calcium malate agar [b]	G: Good, pearl pink (3ca) R: Pearl pink (3ca) AM: None SP: None
Glucose - asparagine agar	G: Moderate, pearl (2ba) R: Pearl (2ba) AM: None SP: None	Glucose - peptone agar [b]	G: Good, ivory (2db) R: Mustard (2le) AM: None SP: None
Peptone - yeast extract - iron agar [a]	G: Good, bamboo (2gc) R: Honey gold (2ic) AM: None SP: Trace, honey gold (2ic)	Nutrient agar [b]	G: Good, cream (11/2ca) R: Cream (11/2ca) AM: Poor, natural (2dc) SP: None

a; Medium recommended by ISP, b; Medium recommended by S. A. Waksman.
Abbreviations: G, growth of vegetative mycelium; R, reverse side color; AM, aerial mycelium; SP, soluble pigment.

Table 2 Physiological properties of strain K03-0132

Melanin formation	
Tyrosine agar	positive
Peptone - yeast extract - iron agar	negative
Tryptone - yeast extract broth	negative
Gelatin medium	negative
Reduction of nitrate	positive
Liquefaction of gelatin (21～23°C)	positive
Hydrolysis of starch	positive
Coagulation of milk (37°C)	negative
Peptonization of milk (37°C)	negative
Decomposition of cellulose	negative
Temperature range for growth	7～38°C

Table 3 Utilization of carbon sources by strain K03-0132

Utilized:	D-Glucose, Melibiose, L-Rhamnose
Weakly utilized:	D-Fructose, Raffinose, Sucrose, D-Xylose
Not utilized:	L-Arabinose, *myo*-Inositol, D-Mannitol

that they are structurally related. IR spectrum of phenatic acid A showed absorptions at 3440, 3212, 3137, 1712, 1660 and 1633 cm^{-1}, suggesting the presence of an amide carbonyl, a carboxylic acid and a carbonyl residue. That of phenatic acid B showed absorptions at 3100, 3037, 1781, 1708 and 1637 cm^{-1}, suggesting the presence of a carboxylic acid and two carbonyl residues.

Phenatic Acid A

The molecular formula of phenatic acid A was determined to be $C_{15}H_{19}NO_5$ on the basis of HRFAB-MS measurement (Table 4). The ^{13}C NMR spectrum (in $CDCl_3$) showed 15 resolved signals, which were classified into two methyl carbons, three methylene carbons, one methine carbon, three sp^2 methine carbons, four quaternary carbons and three carbonyl carbons by analysis of DEPT spectra. The

^{1}H NMR spectrum (in $CDCl_3$) showed two methyl signals, two methylene signals, one methine signal, two aromatic signals and two N–H signals. The connectivity of proton and carbon atoms was established by the ^{13}C-^{1}H HMQC spectrum as shown in Table 5. Analysis of the ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC spectra revealed the partial structure (Fig. 4).

The ^{13}C-^{1}H long range couplings of ^{2}J and ^{3}J observed in the ^{13}C-^{1}H HMBC experiments (Fig. 5) gave the following results. 1) The cross peaks from 3-H (δ 7.09) to C-1 (δ 159.0) and C-5 (δ 127.3), from 5-H (δ 7.43) to C-1 and C-3 (δ 138.6), from 14-H_3 (δ 2.14) to C-1, C-2 (δ 127.2) and C-3 and from 15-H_3 (δ 2.20) to C-3, C-4 (δ 127.5) and C-5 supported the partial structure I. 2) The cross peaks from 8-H_2 (δ 3.12) to C-6 (δ 118.5), C7 (δ 206.0), C-9 (δ 29.2), C-10 (δ 38.1) and C-12 (δ 39.3), from 9-H (δ 2.86) to C-8 (δ 42.0), C-10, C-11 (δ 174.2), C-12 and C-13 (δ 174.4), from 10-H_2 (δ 2.45) to C-8, C-9, C-11 and C-12, from 12-H2 (δ 2.32) to C-8, C-9, C-10 and C-13 supported the partial structure II. 3) The cross peaks from 5-H to C-7 and 8-H_2 to C7 showed that the partial structure I is linked to the partial structure II. 4) From the data of IR, the chemical sifts and molecular formula, C-11 should be the carboxylic acid carbon and C-13 is the amino carbonyl carbon.

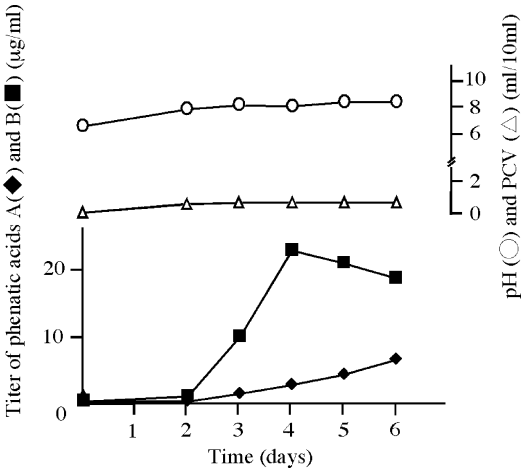


Fig. 3 A typical time course of production phenatic acids A and B by *Streptomyces* sp. K03-0132. The amounts of phenatic acids A (◆) and B (■) in culture broths were determined by HPLC as described in Materials and Methods. PCV, packed cell volume (ml) from 10 ml of the whole culture broth after centrifugation at 3000 rpm.

Taken together, the structure of phenatic acid A was elucidated as shown in Fig 1. All the data assigned here is very reasonable in comparison with those of actiphenol reported previously [6].

Phenatic Acid B

The molecular formula ($C_{15}H_{16}O_6$) of phenatic acid B is NH_3 smaller and O bigger than that of phenatic acid A. The difference between phenatic acids A and B is due to the partial structure III for phenatic acid B, which was elucidated by the ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC experiments (Fig. 6), in place of the partial structure II for phenatic acid A. The cross peaks from 8-H (δ 5.70) to C-7 (δ 198.5), C-9 (δ 34.7), C-10 (δ 37.0) and C-13 (δ 175.0), from 10-H_2 (δ 2.52, 2.65) to C-8 (δ 80.7), C-9, C-11

Table 4 Physico-chemical properties of phenatic acids A and B

	Phenatic acid A	Phenatic acid B
Appearance	White needle	White needle
Melting point	147~149°C	196~197°C
$[\alpha]_D^{26}$	+2.5 (*c* 0.1, CH_3OH)	−2.2 (*c* 0.1, CH_3OH)
Molecular formula	$C_{15}H_{19}NO_5$	$C_{15}H_{16}O_6$
Molecular weight	293	292
HR-FAB-MS *m/z*		
Calcd	294.1341 $(M+H)^+$	292.0946 $(M)^+$
Found	294.1335	292.0948
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	217 (12,500), 262 (6,700), 345 (2,300)	217 (8,900), 267 (6,100), 355 (1,900)
IR ν_{max}^{KBr} cm^{-1}	3440, 3212, 3137, 1712 1660, 1633	3100, 3037, 1781, 1708 1637
Solubility		
Soluble	DMSO, CH_3OH $CHCl_3$, ethyl acetate	DMSO, CH_3OH $CHCl_3$, ethyl acetate
Insoluble	*n*-Hexane, H_2O	*n*-Hexane, H_2O

Table 5 ^{1}H and ^{13}C NMR chemical shifts of phenatic acids A and B

	Phenatic acid A		Phenatic acid B	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]
C-1	159.0		159.9	
C-2	127.2		128.0	
C-3	138.6	7.09 (1H, br.s)	139.8	7.20 (1H, br.s)
C-4	127.5		129.0	
C-5	127.3	7.43 (1H, br.s)	126.9	7.52 (1H, br.s)
C-6	118.5		114.0	
C-7	206.0		198.5	
C-8	42.0	3.12 (2H, d, *J*=6.5 Hz)	80.7	5.70 (1H, d, *J*=3.0 Hz)
C-9	29.2	2.86 (1H, ddd, *J*=6.7, 6.7, 6.5 Hz)	34.7	3.07 (1H, m)
C-10	38.1	2.45 (2H, d, *J*=6.7 Hz)	37.0	2.52 (1H, dd, *J*=7.0, 17.0 Hz)
				2.65 (1H, dd, *J*=8.0, 17.0 Hz)
C-11	174.2		172.2	
C-12	39.3	2.32 (2H, d, *J*=6.7 Hz)	39.0	2.31 (2H, dd, *J*=3.5, 17.5 Hz)
				2.82 (2H, dd, *J*=8.5, 17.5 Hz)
C-13	174.4		175.0	
C-14	15.3	2.14 (3H, s)	15.4	2.19 (3H, s)
C-15	20.4	2.20 (3H, s)	20.4	2.25 (3H, s)
NH		5.64, 6.39 (2H, br. s)		
OH		12.30 (1H, s)		11.80 (1H, s)

a) Chemical shifts are shown with reference to $CDCl_3$ as 77.0 ppm. b) Chemical shifts are shown with reference to $CDCl_3$ as 7.26 ppm.

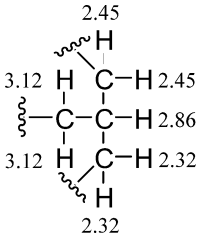
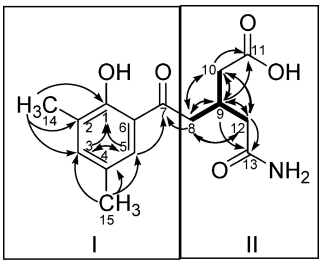


Fig. 4 Partial structure of phenatic acid A.



^{1}H-^{1}H COSY: —
^{13}C-^{1}H COSY: H → C

Fig. 5 ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC experiments of phenatic acid A.

(δ 172.2) and C-12 (δ 33.0), and from 12-H_2 (δ 2.31, 2.82) to C-9, C-10 and C-13 were observed in the ^{13}C-^{1}H HMBC experiments to give the partial structure III. The cross peaks from 5-H (δ 7.52) to C-7 and from 8-H to C-7 indicated that the partial structures I and III are connected to give the structure for phenatic acid B as shown in Fig. 1. The molecular formula supported the structure.

Biological Properties

Miconazole-potentiating Activity

The miconazole-potentiating activity of three phenatic acids and actiphenol was tested. All the compounds themselves showed no inhibition against *C. albicans* at 50 μg/disk on Plate A. Phenatic acid B showed doze-dependent inhibition against the growth of *C. albicans* on Plate B. Phenatic acid A and actiphenol inhibited the growth on Plate B (12 and 16 mm inhibition zone, respectively) at 50 μg/disk (Table 6). These results indicated that phenatic acids A and B and actiphenol potentiate the antifungal activity of miconazole against *C. albicans* strain KF-1. However, under the similar

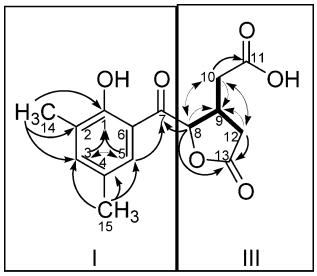


Fig. 6 ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC experiments of phenatic acid B.

conditions, no potentiation of miconazole activity by these compounds was observed against *Aspergillus niger* and *Mucor racemosus*.

Antimicrobial Activities

Phenatic acid B showed weak antibacterial activity against *B. subtilis* (inhibition zone at a concentration 10 μg/6 mm disk: 10 mm) *S. aureus* (8 mm), *B. fragilis* (13 mm) and *A. laidlawii* (12 mm), but no activity against the other microorganisms: *M. luteus*, *M. smegmatis*, *E. coli*, *P. aeruginosa*, *X. oryzae*, *P. oryzae*, *A. niger*, *M. racemosus*, *C. albicans* and *S. cerevisiae*. Phenatic acid A and actiphenol did not show antimicrobial activity against the microorganisms at 10 μg/disk (Table 7).

Discussion

As elucidated in this paper, phenatic acids A and B are new compounds belonging to 2,4-dimethyl phenol family. Structurally related known compounds (Fig. 1) show a variety of biological activities. For example, actiketal was reported as a weak inhibitor of mitogenic activity [15]. AH-135Y was reported to show the antiherpetic activity [16]. Actiphenol showed herbicidal, antifungal and antitumor activities [6, 7]. As described in this paper, phenatic acids A and B and actiphenol were found to show moderate miconazole potentiating activity (Table 6).

The three compounds described above have a common glutarimide ring at the side chain. Whereas, the glutarimide ring was opened for phenatic acid A, and further recyclized to form a γ-lactone ring at C-8 for phenatic acid B. Nong-kang 101 F and 101G, previously reported as antifungal antibiotics [17], showed very similar structures to phenatic acids A and B, respectively. Unfortunately nong-kangs have not been isolated so far from the culture broth of the phenatic acid producing strain. Taking the presence/absence of a hydroxy group at C-8 into consideration, phenatic acid A and actiphenol are structurally related, and phenatic acid B and nong-kangs are also related. Regarding the miconazole-potentiating activity (Table 6), the common 1-hydroxy 2,4-dimethyl aromatic ring is essential for exhibiting the activity. Furthermore, cyclic side chains such as glutarimide and γ-lactone rings appear to exhibit stronger activity than the linear side chains.

Table 6 Effect of phenols on miconazole activity against *Candida albicans* (KF-1)

Compound	Concentration	Inhibition zone (mm)	
	μg/disk	Plate A	Plate B
Phenatic acid A	10	—	—
	25	—	—
	50	—	12
Phenatic acid B	10	—	—
	25	—	11
	50	—	14
Actiphenol	10	—	—
	25	—	—
	50	—	16

Paper disks (8 mm i.d.) containing three concentrations of a compound were put on Plate A (*C. albicans* in GY agar) and Plate B (*C. albicans* in GY agar+0.06 μM miconazole), and incubated at 27°C. After 24 hours, the diameters of inhibition zones were measured.
The concentration (0.06 μM) of miconazole is one fourth of the MIC value against *C. albicans* showed no effect on the growth of *C. albicans*.

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Table 7 Effect of phenols against microorganisms

Microorganism	Inhibition zone (mm)		
	Phenatic acid A	Phenatic acid B	Actiphenol
Bacillus subtilis PCI 219	—	10	—
Staphylococcus aureus FDA 209 P	—	8	—
Micrococcus luteus PCI 1001	—	—	—
Mycobacterium smegmatis ATCC 607	—	—	—
Eschrichia coli NIHJ	—	—	—
Pseudomonas aeruginosa P-3	—	—	—
Xanthomonas oryzae KB 88	—	—	—
Bacteroides fragilis ATCC 23745	—	13	—
Acholeplasma laidlawii PG 8	—	12	—
Pyricularia oryzae KF 180	—	—	—
Aspergillus niger ATCC 6275	—	—	—
Mucor racemosus IFO 4581	—	—	—
Candida albicans ATCC 64548	—	—	—
Saccharomyces cerevisiae	—	—	—

Paper disks (6 mm i.d.) containing 10 μg of compound were put on plate, and incubated at 27°C or 37°C. After 24 or 48 hours, the diameters of inhibition zones were measured.

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