Structure of a Novel Epidithiodioxopiperazine, Emethallicin A, a Potent Inhibitor of Histamine Release, from *Emericella heterothallica*[1)]

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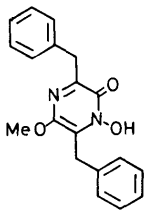
A new epidithiodioxopiperazine derivative, emethallicin A (1), was isolated along with ergosterol from the mycelial extract of the heterothallic fungus, *Emericella heterothallica* (mating type A). The structure of emethallicin A (1) was established on the basis of chemical and spectroscopic investigations and finally by chemical transformation to apoaranotin (11). Emethallicin A (1) has the same basic skeleton as apoaranotin (11), with C_6–C_2 carboxylic acid (mandelic and phenylacetic acids) diester moieties, and showed a potent inhibitory activity on histamine release from mast cells.

Keywords *Emericella heterothallica*; heterothallic fungus; epidithiodioxopiperazine; emethallicin A; aranotin; mandelic acid; phenylacetic acid; histamine release inhibition

Recently we reported the isolation of the pyrazinone derivative, emeheterone (**2**),[2)] and two sulfur-containing dioxopiperazines, emethacins A (**3**) and B (**4**),[1)] along with two dioxopiperazines, (*S*)-3-benzyl-6-[(*Z*)-benzylidene]-2,5-dioxopiperazine (**5**) and (3*S*,6*S*)-3,6-dibenzyl-2,5-dioxopiperazine (**6**), from the culture filtrate of the heterothallic fungus, *Emericella heterothallica* (KWON, FENNELL *et* RAPER) MALLOCH *et* CAIN (anamorph: *Aspergillus heterothallicus* KWON, FENNELL *et* RAPER) (mating type a), strain ATCC 16824. Moreover, 3,6-di[(*Z*)-benzylidene]-2,5-dioxopiperazine (**7**)[1)] was isolated from the mycelial chloroform extract of the above strain. All of the above compounds (**2**—**7**) are expected basically to be biosynthesized from two molecules of phenylalanine. During our search for sulfur-containing compounds related to **2**—**7**, a novel compound designated as emethallicin A (**1**) was isolated from the mycelial chloroform extract of *E. heterothallica* (mating type A), strain ATCC 16847: the strain of another mating type of the above fungus. The structural elucidation of the compound **1** is reported in this paper.



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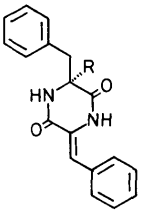
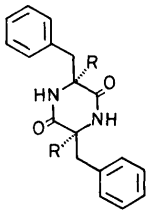


3 : R=SMe
5 : R=H



4 : R=SMe
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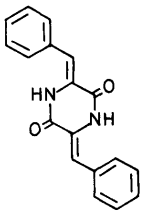

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Emethallicin A (**1**), $[\alpha]_D$ −220° ($CHCl_3$), gave a molecular ion peak at *m/z* 657 $(M+1)^+$ by fast-atom bombardment (FAB) mass spectrometry, and elemental analysis confirmed the molecular formula as $C_{34}H_{28}N_2O_8S_2$. A positive coloration with silver nitrate (dark brown-black)[3)] suggested the presence of a dithio bond in **1**. The absorptions at 1740 and 1700 cm^{-1} in the infrared (IR) spectrum of **1** suggested the presence of ester and amide moieties, respectively. Carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals at δ 162.06 and 163.45, and 170.90 and 171.70 were assigned to two ester and two amide carbons, respectively, in view of the presence of two nitrogen atoms in the molecule of **1**.

On acetylation, emethallicin A (**1**) afforded a monoacetate (**8**), mp 147—149 °C, $[\alpha]_D$ −214° ($CHCl_3$), $C_{36}H_{30}N_2O_9S_2$, which showed a proton nuclear magnetic resonance (^1H-NMR) signal at δ 2.200, assigned to the methyl protons of an acetoxyl group. The ^1H-NMR signal of the proton attached to the carbon bearing the hydroxyl group at δ 5.187 (d, J=6.1 Hz), which was coupled with the hydroxyl proton, in **1** shifted downfield to δ 6.084 (s) after acetylation. The IR spectrum of **8** showed no absorption at 4000—3000 cm^{-1}. These results confirmed that emethallicin A (**1**) possesses only one secondary alcohol.

Reductive methylation[3)] of **1** and its acetate (**8**) gave deepidithiobis(methylthio)emethallicin A (**9**), mp 185—187 °C, $C_{36}H_{34}N_2O_8S_2$, and acetyldeepidithiobis(methylthio)emethallicin A (**10**), $C_{38}H_{36}N_2O_9S_2$, respectively. Both of these compounds have two methylthio groups in the molecule, because ^1H-NMR signals at δ 2.121 and 2.350 in **9** and at δ 2.149 and 2.286 in **10**, and ^{13}C-NMR signals at δ 14.25 and 14.74 in **9** and at δ 14.20 and 14.92 in **10** were observed. The circular dichroism (CD) curve of **1** was closely similar to those of epidithiodioxopiperazines, as described later. Thus, the structure of emethallicin A (**1**) was suggested to contain an epidithiodioxopiperazine moiety with two trisubstituted nitrogen atoms.

TABLE I. ^1H-NMR Chemical Shifts of the Basic Skeleton of Emethallicin A (**1**) and Related Compounds in $CDCl_3$

Proton	1	11	12
1-H	6.627	6.643	6.61
3-H	6.262	6.318	6.30
4-H	4.417	4.616	4.67
5-H	5.680	5.725	5.68
5a-H	5.122	5.120	5.10
8α-H	2.843	2.861	2.81
8β-H	3.772	3.737	3.88
9-H	5.942[a)]	5.965	6.00
10-H	5.479	5.779	5.57[b)]
11-H	5.982[a)]	5.965	6.00[b)]
12-H	6.014	4.799	6.03
12a-H	5.020	4.799	5.01
15α-H	2.970	3.083	3.00
15β-H	4.028	4.033	4.02

a) Assignments may be reversed. *b*) Assignments were revised.

The ^{1}H-NMR signals of the basic skeleton of emethallicin A (**1**) (Table I) corresponded well, including their coupling patterns, to those of apoaranotin (**11**), originally isolated from *Arachniotus aureus* (EIDAM) SCHROETER[4] as an antiviral antibiotic,[5] and its acetate (**12**). The ^{13}C-NMR signals of **1** (Table II) and the homonuclear ^{1}H–^{1}H shift correlation spectrum of **8** also suggested the presence of the above skeleton in **1**. The alkaline hydrolysis of **1** with sodium hydroxide followed by acetylation gave compound **11**, which was identical with naturally occurring apoaranotin[4] including the optical rotation. The comparison of the CD curves of **1** [219 (negative), 268 (positive), and 338 nm (negative)] and **8** [218 (negative), 267 (positive), and 338 nm (negative)] with those of acetylapoaranotin (**12**) [230 (negative), 269 (positive), and 343 nm (negative)], derived from **11**, confirmed that these compounds had the same configuration about the epidithiodioxopiperazine ring.[6] Therefore it is clear that emethallicin A (**1**) is the diester of the deacetyl derivative of **11**, including the absolute stereochemistry.

TABLE II. ^{13}C-NMR Chemical Shifts of Emethallicin A (**1**) and Its Derivative in $CDCl_3$

Carbon No.	1	8	9	10
1	139.39 (Dm)	139.20 (Dm)	137.81 (Dm)	137.52 (Dm)
3	141.45 (Dm)	141.22 (Dm)	140.08 (Dm)	139.89 (Dm)
4	104.20 (Dm)	104.22 (Dm)	104.28 (Dm)	104.81 (Dm)
5	71.55 (Dm)	70.85 (Dm)	72.92 (Dm)	73.19 (Dm)
5a	62.74 (Dm)	62.35 (Dm)	60.80 (Dm)	59.94 (Dm)
7	163.45 (d)	162.94 (d)	165.24 (t)	165.01 (t)
7a	78.34 (dd)	78.32 (dd)	74.27 (m)	74.16 (m)
8	36.08 (Td)	35.82 (Td)	40.81 (Tdd)[a]	41.45 (Tm)
8a	132.07 (m)	132.62 (m)	133.97 (m)	133.94 (m)
9	119.99 (Dm)	119.81 (Dm)	119.85 (Dm)	119.93 (Dm)
10	124.45 (Ddd)	124.53 (Ddd)	125.07 (Ddd)	125.13 (Ddd)
11	127.64 (Dm)	127.49 (Dm)	127.88 (Dm)	127.96 (Dm)
12	74.37 (Ddd)	74.51 (Ddd)	75.65 (Ddd)	75.74 (Ddd)
12a	64.40 (Dm)	64.37 (Dm)	64.55 (Dm)	64.57 (Dm)
14	162.06 (d)	162.32 (d)	164.67 (t)	164.51 (t)
14a	75.73 (dd)	75.60 (dd)	70.43 (m)	70.81 (m)
15	34.53 (Tdd)	34.77 (Tdd)	40.19 (Tdd)[a]	40.09 (Tm)
15a	112.73 (m)	112.90 (m)	109.45 (m)	109.82 (m)
1′	171.70 (dd)	167.59 (dd)	172.39 (dd)	167.95 (dd)
2′	72.86 (Dt)	73.37 (Dt)	72.72 (Dm)	73.86 (Dt)
3′	138.10 (m)	134.16 (m)	138.07 (m)	134.00 (m)[a]
4′ (8′)	126.54 (Dm)	127.80 (Dddd)	126.65 (Dddd)	127.73 (Dddd)
5′ (7′)	128.35 (Dd)[a]	128.64 (Dd)	128.31 (Dd)[b]	128.70 (Dd)
6′	128.20 (Dt)	128.93 (Dt)	128.35 (Dt)	129.05 (Dt)
1″	170.90 (td)	170.93 (td)	171.05 (td)	171.14 (td)
2″	41.28 (Tt)	41.31 (Tt)	41.21 (Tt)	41.15 (Tt)
3″	134.09 (m)	134.16 (m)	133.63 (m)	134.04 (m)[a]
4″ (8″)	129.52 (Dm)	129.55 (Dm)	129.63 (Dm)	129.66 (Dm)
5″ (7″)	128.40 (Dd)[a]	128.40 (Dd)	128.44 (Dd)[b]	128.31 (Dd)
6″	126.92 (Dm)	126.89 (Dm)	126.92 (Dt)	126.89 (Dt)
MeCOO		20.90 (Q)		20.72 (Q)
MeCOO		169.61 (qd)		169.82 (qd)
SMe			14.25 (Q)	14.20 (Q)
			14.74 (Q)	14.92 (Q)

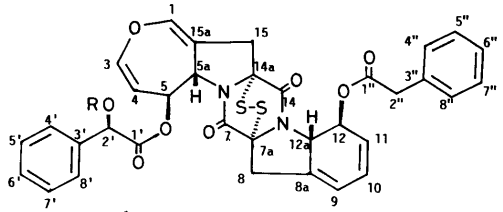
a, *b*) The assignments may be reversed.

The acids, which were expected to be connected at C-5 and C-12 of the basic skeleton of **1**, were assummed to be one phenylacetic acid and one mandelic acid, from the analysis of the ^{1}H-NMR and ^{13}C-NMR spectra (Tables I and II) of **1**. In order to determine the exact positions of mandelate and phenylacetate, the heteronuclear ^{1}H–^{13}C long-range shift correlation (COLOC) spectrum of **8** (Fig. 1) was examined. The carbonyl carbon signal of phenylacetate at δ 170.93, which was coupled with the signal of the methylene protons at C-2″ (δ 3.739), was also correlated to the ^{1}H-NMR signal at δ 6.031 assigned to the proton at C-12, whereas the carbonyl carbon signal of mandelate at δ 167.59, which was coupled with the proton at C-2′ (δ 6.084), was also correlated to the ^{1}H-NMR signal at δ 5.727 assigned to the proton at C-5. A correlation peak was also observed between the carbon signal of the acetate (δ 169.61) and the proton signal at δ 6.084 described above. The above results confirmed the positions of the esters in **1**, *i.e.*, mandelate was attached to the dihydrooxepine ring and phenylacetate to the dihydrobenzene ring. (−)-Methyl mandelate, $[\alpha]_D$ −125° (MeOH),[7] was obtained by alkaline hydrolysis of **1** followed by methylation with diazomethane. Therefore the absolute configuration of methyl mandelate derived from **1** was *R*.[8] Consequently, the structure of emethallicin A (**1**) was confirmed, including its absolute stereochemistry.

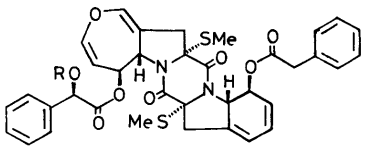
Emethallicin A (**1**) has a potent inhibitory activity upon compound 48/80-induced histamine release from mast cells and a strong inhibitory activity toward 5-lipoxygenase. The IC_{50} values for inhibition of histamine release and inhibition of 5-lipoxygenase were determined as 3.0×10^{-8} and 1.7×10^{-6} M, respectively.[9]

Experimental

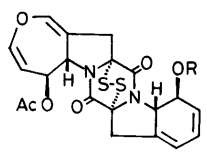
Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 spectrometer. Field desorption (FD) and FAB mass spectra were taken with a JEOL JMS-DX-303 spectrometer and a JEOL JMX-HS-110 spectrometer, respectively. IR and ultraviolet (UV) spectra were recorded on a JASCO IR-810 spectrophotometer, and a Hitachi 124 or Hitachi U-3210 spectrophotometer, respectively. ^{1}H-NMR spectra were measured with a JEOL JNM-GX 270 spectrometer at 270.17 MHz or with a JEOL JNM-GX 400 spectrometer at 399.78 MHz, whereas ^{13}C-NMR spectra were recorded on a JEOL JNM-GX 400 spectrometer at 100.43 MHz, using tetramethylsilane as an internal standard. The coupling patterns are indicated as follows: singlet = s, doublet = D or d, triplet = T or t, quartet = Q or q, multiplet = m, and broad = br. Capital letters refer to the patterns resulting from directly bonded coupling ($^1J_{C,H}$). CD curves were determined on a JASCO J-40 spectrophotometer. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low-pressure liquid chromatography (LPLC) was performed with a Chemco



1 : R=H
8 : R=Ac



9 : R=H
10 : R=Ac



11 : R=H
12 : R=Ac

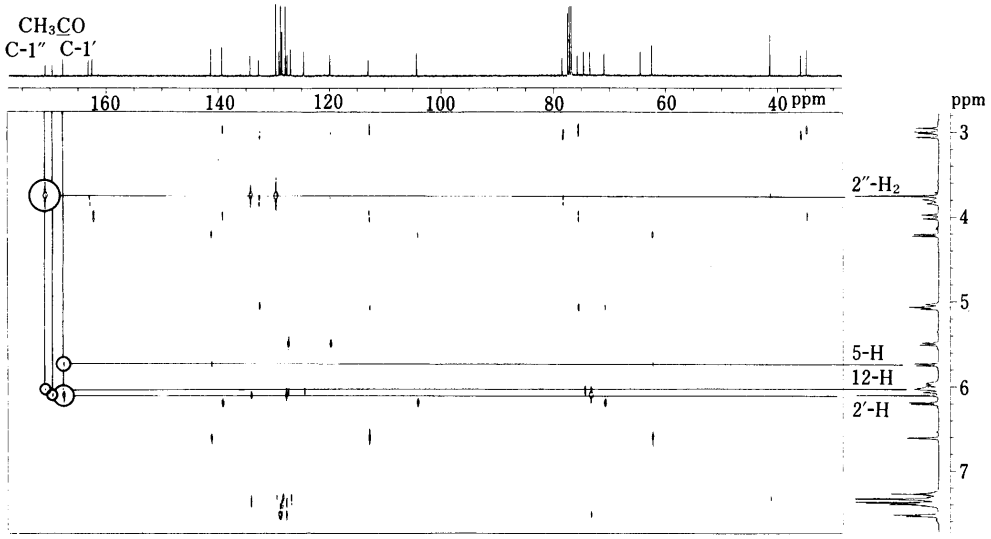


Fig. 1. COLOC Spectrum of Emethallicin A Monoacetate (**8**) in $CDCl_3$

Low-Prep pump (81-M-2) and a glass column (200 × 10 or 15 mm) packed with Silica gel CQ-3 (30—50 μm; Wako). Thin layer chromatography (TLC) was conducted on precoated Kieselgel 60 GF_{254} plates (Art. 5715; Merck). Spots on TLC were detected under UV light, and/or by spraying 5% silver nitrate.

Isolation of Emethallicin A (1) from *E. heterothallica* *E. heterothallica* (mating type A), strain ATCC 16847, was cultivated at 27 °C for 14 d in 30 Roux flasks containing 250 ml of Czapek-Dox medium supplemented with 0.1% yeast extract in each flask. The dried mycelia (53 g) were pulverized and extracted with chloroform at room temperature. The residue (4.2 g) obtained by evaporation of the extract was chromatographed on silica gel with benzene–acetone (100 : 1) to give ergosterol (280 mg), and with benzene–acetone (50 : 1) to obtain emethallicin A (**1**) (950 mg).

Emethallicin A (**1**): White amorphous powder, $[\alpha]_D^{20}$ −220° (c=1.46, $CHCl_3$). FAB-MS (*m*-nitrobenzyl alcohol as the matrix) m/z: 657 $[(M+1)^+]$. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1740 (COO), 1700 (CON). UV λ_{max}^{MeOH} nm (log ε): 225 sh (4.17), 262 (3.89). *Anal.* Calcd for $C_{34}H_{28}N_2O_8S_2$: C, 62.18; H, 4.30; N, 4.27; S, 9.77. Found: C, 62.16; H, 4.35; N, 4.09; S, 9.67. ^{1}H-NMR ($CDCl_3$) δ: 2.843 (1H, br d, J=17.7 Hz, 8α-H), 2.970 (1H, br d, J=18.3 Hz, 15α-H), 3.659 (1H, d, J=6.1 Hz, 2′-OH), 3.732 (2H, br s, 2″-H_2), 3.772 (1H, br d, J=17.7 Hz, 8β-H), 4.028 (1H, ddd, J=18.3, 2.4, 1.2 Hz, 15β-H), 4.417 (1H, dd, J=8.0, 1.8 Hz, 4-H), 5.020 (1H, br d, J=13.5 Hz, 12a-H), 5.122 (1H, ddd, J=8.5, 2.4, 2.4 Hz, 5a-H), 5.187 (1H, d, J=6.1 Hz, 2′-H), 5.488 (1H, br d, J=9.8 Hz, 10-H), 5.680 (1H, ddd, J=8.5, 1.8, 1.8 Hz, 5-H), 5.942 (1H, m, 9- or 11-H), 5.982 (1H, m, 11- or 9-H), 6.014 (1H, br d, J=13.5 Hz, 12-H), 6.262 (1H, dd, J=8.0, 1.8 Hz, 3-H), 6.627 (1H, dd, J=2.4, 1.2 Hz, 1-H), 7.26—7.40 (8H, m, Ar-H), 7.505 (2H, br d, J=7.3 Hz, 4′-H, 8′-H). CD (c=4.04 × 10^{-4}, dioxane) [θ] (nm): −14.37 × 10^4 (219), +3.00 × 10^4 (268), −1.9 × 10^3 (338).

Acetylation of Emethallicin A (1) Compound **1** (50 mg) was dissolved in pyridine (1 ml) containing acetic anhydride (0.5 ml) and the solution was kept at room temperature overnight. The reaction mixture was poured into ice-water and extracted with chloroform. The extract was evaporated and the residue was purified by LPLC using benzene as the eluting solvent, followed by recrystallization from cyclohexane–ethyl acetate (5 : 1) to give emethallicin A monoacetate (**8**) (45 mg) as colorless needles, mp 147—149 °C, $[\alpha]_D^{25}$ −214° (c=0.97, $CHCl_3$). FAB-MS (*m*-nitrobenzyl alcohol as the matrix) m/z: 699 $[(M+1)^+]$. IR ν_{max}^{KBr} cm^{-1}: 1750 sh, 1740 (COO), 1705 (CON). UV λ_{max}^{MeOH} nm (log ε): 261 (4.05). *Anal.* Calcd for $C_{36}H_{30}N_2O_9S_2$: C, 61.88; H, 4.33; N, 4.01; S, 9.18. Found: C, 61.81; H, 4.31; N, 3.88; S, 8.80. ^{1}H-NMR ($CDCl_3$) δ: 2.200 (3H, s, 2′-OAc), 2.966 (1H, br d, J=19.1 Hz, 15α-H), 3.026 (1H, br d, J=18.3 Hz, 8α-H), 3.739 (2H, s, 2″-H_2), 3.809 (1H, br d, J=18.3 Hz, 8β-H), 3.990 (1H, ddd, J=19.1, 2.4, 1.8 Hz, 15β-H), 4.203 (1H, dd, J=8.3, 1.6 Hz, 4-H), 5.027 (1H, br d, J=13.4 Hz, 12a-H), 5.061 (1H, br dd, J=8.5, 2.4 Hz, 5a-H), 5.479 (1H, br d, J=9.8 Hz, 10-H), 5.727 (1H, ddd, J=8.5, 2.1, 1.6 Hz, 5-H), 5.951 (1H, m, 9- or 11-H), 5.987 (1H, m, 11- or 9-H), 6.031 (1H, br d, J=13.4 Hz, 12-H), 6.084 (1H, s, 2′-H), 6.185 (1H, dd, J=8.3, 2.1 Hz, 3-H), 6.600 (1H, dd, J=2.0, 1.8 Hz, 1-H), 7.25—7.41 (8H, m, Ar-H), 7.515 (2H, br d, J=7.3 Hz, 4′-H, 8′-H). CD (c=2.87 × 10^{-4}, dioxane) [θ] (nm): −15.6 × 10^4 (218), +2.96 × 10^4 (267), −1.2 × 10^3 (337).

Reductive Methylation of Emethallicin A (1) Sodium borohydride (20 mg) was added to a stirred solution of emethallicin A (**1**) (60 mg) in a mixture of methanol (1 ml) and iodomethane (3 ml). After stirring for 1 h, the reaction mixture was evaporated and extracted with chloroform. The residue obtained by evaporation of the extract was purified by LPLC using chloroform to yield deepidithiobis(methylthio)emethallicin A (**9**) (40 mg) as colorless needles, mp 185—187 °C (from benzene), $[\alpha]_D^{20}$ −140° (c=0.4, $CHCl_3$). FAB-MS (*m*-nitrobenzyl alcohol as the matrix) m/z: 687 $[(M+1)^+]$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1735 (COO), 1670 (CON). UV λ_{max}^{MeOH} nm (log ε): 222 sh (4.24), 265 (3.67). *Anal.* Calcd for $C_{36}H_{34}N_2O_8S_2$: C, 62.95; H, 4.99; N, 4.08; S, 9.34. Found: C, 62.74; H, 4.96; N, 4.03; S, 9.25. ^{1}H-NMR ($CDCl_3$) δ: 2.121 (3H, s, SMe), 2.350 (3H, s, SMe), 2.861 (1H, br d, J=16.5 Hz, 8- or 15-H), 3.034 (2H, br s, 15- or 8-H_2), 3.083 (1H, br d, J=16.5 Hz, 8- or 15-H), 3.555 (1H, d, J=5.5 Hz, 2′-OH), 3.682 (1H, d, J=15.9 Hz, 2″-H), 3.759 (1H, d, J=15.9 Hz, 2″-H), 4.330 (1H, dd, J=8.6, 1.8 Hz, 4-H), 5.132 (1H, br dd, J=7.3, 2.0 Hz, 5a-H), 5.190 (1H, d, J=5.5 Hz, 2′-H), 5.249 (1H, br d, J=14.0 Hz, 12a-H), 5.552 (1H, br d, J=9.6 Hz, 10-H), 5.924 (1H, ddd, J=7.3, 2.4, 1.8 Hz, 5-H), 5.95—5.98 (2H, m, 9-H, 11-H), 6.191 (1H, br d, J=14.0 Hz, 12-H), 6.213 (1H, dd, J=8.6, 2.4 Hz, 3-H), 6.558 (1H, br s, 1-H), 7.23—7.39 (8H, m, Ar-H), 7.511 (1H, br d, J=7.9 Hz, 4′-H, 8′-H).

Reductive Methylation of Emethallicin A Acetate (10) Sodium borohydride (20 mg) was added to a stirred solution of emethallicin A acetate (**8**) (50 mg) in a mixture of methanol (1 ml) and iodomethane (3 ml). After being stirred at room temperature for 30 min, the reaction mixture was evaporated and extracted with chloroform. The residue obtained by evaporation of the extract was purified by LPLC using chloroform to give deepidithiobis(methylthio)emethallicin A acetate (**10**) (35 mg) as an amorphous powder. $[\alpha]_D^{25}$ −142° (c=0.58, $CHCl_3$). FD-MS m/z (%): 728 (M^+, 100). IR ν_{max}^{KBr} cm^{-1}: 1745 (COO), 1670 (CON). UV λ_{max}^{MeOH} nm (log ε): 262 (4.03). ^{1}H-NMR ($CDCl_3$) δ: 2.149 (3H, s, SMe), 2.196 (3H, s, OAc), 2.286 (3H, s, SMe), 2.867 (1H, br d, J=15.9 Hz, 8- or 15-H), 3.023 (2H, br s, 15- or 8-H_2), 3.093 (1H, br d, J=15.9 Hz, 8- or 15-H), 3.723 (2H, br s, 2″-H_2), 4.302 (1H, dd, J=8.5, 1.8 Hz, 4-H), 5.146 (1H, br d, J=8.0 Hz, 5a-H), 5.257 (1H, br d, J=13.4 Hz, 12a-H), 5.570 (1H, m, 12-H), 5.769 (1H, ddd, J=8.0, 2.4, 1.8 Hz, 5-H), 5.93—5.98 (2H, m, 9-H, 11-H), 6.005 (1H, s, 2′-H), 6.170 (1H, dd, J=8.5, 2.4 Hz, 3-H), 6.204 (1H, br d, J=13.4 Hz, 12-H), 6.529 (1H, br s, 1-H), 7.23—7.40 (8H, m, Ar-H), 7.539 (2H, br d, J=7.9 Hz, 4′-H, 8′-H).

Hydrolysis of Emethallicin A (1) Followed by Acetylation A 1 N sodium hydroxide solution (2 ml) was added to a solution of emethallicin A (**1**) (100 mg) in acetone (2 ml) and the reaction mixture was stirred at room temperature for 30 min. The mixture was poured into water and extracted with ethyl acetate. The evaporated extract was dissolved in pyridine (1.5 ml) and acetic anhydride (2 ml) and the solution was left to stand at room temperature overnight. The reaction mixture was poured into ice-water and extracted with chloroform. The evaporated residue was purified by LPLC with benzene–acetone (50 : 1) to obtain apoaranotin (**11**) (10 mg), $[\alpha]_D^{25}$ −375° (c=0.1, $CHCl_3$). This compound was identical with authentic

apoaranotin (**11**)[4] on the basis of a comparison of the ^{1}H-NMR and IR spectra and the optical rotation.

Hydrolysis of Emethallicin A (1) Followed by Methylation A 1 N sodium hydroxide solution (2 ml) was added to a stirred solution of emethallicin A (**1**) (70 mg) in acetone (2 ml). After 30 min, the reaction mixture was poured into water and extracted with ethyl acetate. The residue obtained by evaporation of the extract was treated with diazomethane in ether at room temperature. After the removal of the ether, the residue was purified by LPLC with the solvent system of cyclohexane–chloroform (1 : 1) to give (*R*)-methyl mandelate, $[\alpha]_D^{20}$ $-125°$ ($c=0.06$, MeOH). The above compound was identical with authentic (*R*)-methyl mandelate, $[\alpha]_D^{20}$ $-143°$ (MeOH).[7,8]

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