# Monoamine Oxidase Inhibitory Constituents from *Anixiella micropertusa*

Haruhiro FUJIMOTO,*,[a] Yoko SATOH,[a] Kentaro YAMAGUCHI,[b] and Mikio YAMAZAKI[a]

*Faculty of Pharmaceutical Sciences,[a] and Analysis Center,[b] Chiba University, 1–33, Yayoi-cho, Inage-ku, Chiba 263–8522, Japan.* Received May 6, 1998; accepted June 23, 1998

**Two known naturally-occurring constituents, 1,7-dihydroxy-3-methylxanthone and emodin, were isolated from an Ascomycete *Anixiella micropertusa* as inhibitors of mouse monoamine oxidase (MAO), together with four non-MAO inhibitory components, including two novel compounds. 1,7-Dihydroxy-3-methylxanthone and emodin inhibited MAO moderately, and $IC_{50}$ values were calculated to be $2.06\times10^{-5}$ and $3.70\times10^{-5}$ M, respectively.**

**Key words** fungal metabolite; Ascomycete; *Anixiella micropertusa*; monoamine oxidase inhibitor; xanthone; anthraquinone

In our screening program for monoamine oxidase (MAO) inhibitory components from fungi, several metabolites have already been isolated from *Emericella navahoensis*,[1a] *Talaromyces luteus*,[1b] *Talaromyces helicus*,[1c] Mycelia Sterilia from *Gelasinospora pseudoreticulata*,[1d] *Monascus anka*,[1e] and *Coniochaeta tetraspora*.[1f] In a continuation of these studies, we have now found that the AcOEt soluble portion of the MeOH extract of an Ascomycete, *Anixiella micropertusa* HORIE et UDAGAWA IFM4495[2] appreciably inhibited mouse liver MAO in a modified Kraml's assay.[3] Fractionation guided by the MAO inhibitory activity, afforded six constituents, compounds **1**—**6**, among which, **1** and **3** showed significant MAO inhibitory activity. This report describes the isolation, structure elucidation, and MAO inhibitory activity of these six components isolated from *A. micropertusa*.

## Results and Discussion

The MeOH extract of *A. micropertusa* IFM4495[2] cultivated on sterilized rice was partitioned between AcOEt and $H_2O$ into AcOEt and aqueous layers. After evaporation, the AcOEt layer inhibited MAO by 33% at a concentration of $5.0\times10^{-5}$ g/ml, whilst the aqueous layer inhibited by −7% at the same concentration. Repeated chromatography of the AcOEt layer afforded six constituents, compounds **1**—**6** [yields (%) from the AcOEt layer, **1**: 0.021, **2**: 0.034, **3**: 0.0085, **4**: 0.084, **5**: 0.0053, and **6**: 0.080].

Compound **2** was obtained as optically inactive yellow needles, positive to the $FeCl_3$ reagent. IR and UV spectra suggested the presence of a hydrogen-bonded phenolic hydroxyl and a ketone carbonyl conjugated with a benzene ring. The $^1$H- and $^{13}$C-NMR spectra of **2** suggested the presence of one aromatic methyl, six aromatic hydrogens, one hydrogen-bonded phenolic hydroxyl, seven $sp^2$ quaternary carbons, among which three bore oxygen and one was ketone carbonyl. The $^1$H- and $^{13}$C-NMR spectral data, including spin-decoupling $^1$H-NMR and two-dimensional (2D) $^1$H-$^1$H shift correlation spectroscopy ($^1$H-$^1$H COSY), $^{13}$C-$^1$H COSY and $^1$H-detected heteronuclear multiple-bond correlation (HMBC) NMR data suggested that **2** was composed of two moieties, *a* and *b*, as shown in Chart 1. The partial structures *a* and *b* were connected to construct the whole molecular structure of **2**, taking into consideration that an absorption due to a ketone carbonyl conjugated with a benzene ring was present in the IR spectrum. The constructed structure was 1-hydroxy-3-methylxanthone (**2**), which was also supported by the presence of the molecular ion corresponding to $[C_{14}H_{10}O_3]^{\cdot+}$ in the electron-impact MS (EI-MS) spectrum. 1-Hydroxy-3-methylxanthone (**2**) is already known as a synthetic compound whose melting point reported in the literature[4] was very close to that of **2** (see Experimental). To our knowledge, this is the first time that 1-hydroxy-3-methylxanthone (**2**) has been isolated as a natural product.

Compound **1** was obtained as optically inactive yellow needles, positive to the $FeCl_3$ reagent. The IR and UV spectra

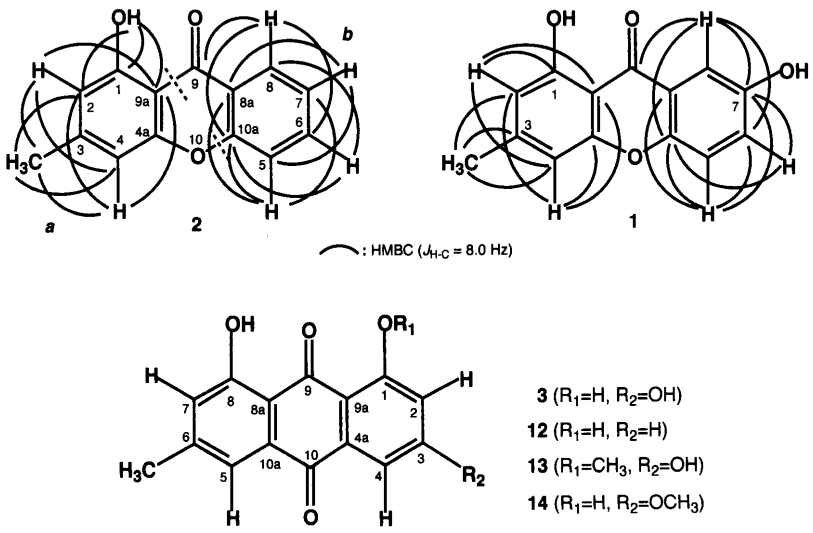



Chart 1. Structures of Compounds **1**, **2**, **3**, Chrysophanol (**12**), Questin (**13**), and Physcion (**14**)

* To whom correspondence should be addressed.

Table 1. $^1$H-NMR and $^{13}$C-NMR Data for Compounds **1** and **2**, $\delta$ (ppm) from Tetramethylsilane (TMS) as an Internal Standard [Coupling Constants (Hz) in Parentheses]

| Position | **1** (acetone-$d_6$) | | **2** ($CDCl_3$) | |
|---|---|---|---|---|
| | $^1$H-NMR | $^{13}$C-NMR | $^1$H-NMR | $^{13}$C-NMR |
| 1 | 12.60 (br s, OH) | 162.48 (s) | 12.54 (s, OH) | 161.07 (s) |
| 2 | 6.58 (br d, 0.7) | 111.36 (d) | 6.62 (br d, 1.2) | 110.70 (d) |
| 3 | | 149.84 (s) | | 148.36 (s) |
| $H_3C$-3 | 2.41 (3H, br s) | 22.41 (q) | 2.42 (3H, br s) | 22.01 (q) |
| 4 | 6.79 (br d, 0.7) | 108.07 (d) | 6.74 (br d, 1.2) | 106.90 (d) |
| 4a | | 157.24 (s) | | 155.65 (s)[a] |
| 5 | 7.46 (d, 9.1) | 120.09 (d) | 7.44 (dd, 8.2, 0.8) | 134.70 (d) |
| 6 | 7.38 (dd, 9.1, 2.9) | 125.83 (d) | 7.72 (ddd, 8.2, 7.2, 1.7) | 117.23 (d) |
| 7 | 8.91 (br s, OH) | 150.96 (s) | 7.37 (ddd, 8.0, 7.2, 0.8) | 123.35 (d) |
| 8 | 7.57 (d, 2.9) | 109.23 (d) | 8.25 (dd, 8.0, 1.7) | 125.36 (d) |
| 8a | | 121.90 (s) | | 120.12 (s) |
| 9 | | 182.39 (s) | | 181.20 (s) |
| 9a | | 107.18 (s) | | 106.44 (s) |
| 10a | | 154.94 (s) | | 155.58 (s)[a] |

*a*) The assignments may be reversed in each column.

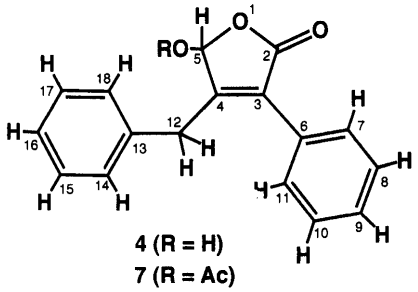
were very similar to those of **2**. Comparison of the $^1$H-NMR spectrum of **1** with that of **2** suggested that the structure of **1** was the same as **2** except that the hydrogen at position 7 in **2** was replaced with a hydroxyl in **1**. This finding was supported by comparison of the $^{13}$C-NMR data (see Table 1) and also by the presence of a molecular ion corresponding to $[C_{14}H_{10}O_4]^{\cdot+}$ in the EI-MS spectrum. Isolation of 1,7-dihydroxy-3-methylxanthone (**1**) has already been reported from a higher plant, *Cassia occidentalis*, although without mention of physicochemical data.[5] To our knowledge, this is the first time that 1,7-dihydroxy-3-methylxanthone (**1**) has been isolated as a MAO inhibitory principle from a natural source.

Compound **3** was obtained as optically inactive orange needles, positive to the $FeCl_3$ reagent. The physicochemical and spectral data of **3** were quite similar to those of an anthraquinone, emodin (**3**), which has been isolated from many fungi.[6] Direct comparison of compound **3** with an authentic sample of emodin (**3**) indicated that they were identical. To our knowledge, this is the first time that emodin (**3**) has been isolated as a MAO inhibitory principle from a natural source.

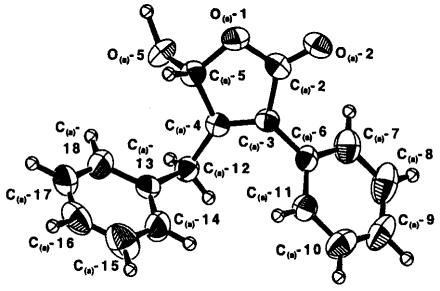
Compound **4** was obtained as optically active colorless plates, with the molecular formula $C_{17}H_{14}O_3$. The IR spectrum of **4** suggested the presence of hydroxyl and ester carbonyl. The $^1$H- and $^{13}$C-NMR spectra suggested the presence of one allylic methylene ($-C\underline{H}_2$-C=C-), one hemiacetal methine [$-$C(-O)C$\underline{H}$OH], ten aromatic protons, and five $sp^2$ quaternary carbons among which one was an ester carbonyl (see Experimental). The presence of one hemiacetal methine group in **4** was supported by the fact that acetylation with acetic anhydride and pyridine afforded a monoacetate (**7**), whose $^1$H- and $^{13}$C-NMR spectra indicated that the $-$C($-$O)CHOH group in **4** was acetylated to give a $-$C($-$O)CHOAc group. From these data, **4** was concluded to be a new aromatic compound. The structure of **4** was solved directly by X-ray crystallographic analysis, and found to be 4-benzyl-5-hydroxy-3-phenyl-2(5*H*)-furanone (**4**) (see Chart 2). It is assumed from the structure of **4** that it may be biosynthesized *via* an acid anhydride (**8**) formed from two precursors, phenylpyruvic acid (**9**) and phenylacetic acid (**10**), as shown in Chart 2. We propose to name this compound microperfuranone.

Compound **6** was obtained as an optically inactive yellow amorphous powder, positive to the $FeCl_3$ reagent. The $^1$H- and $^{13}$C-NMR spectra of **6** suggested the presence of one aromatic methyl, one methoxy methyl, five aromatic protons, and nine $sp^2$ quaternary carbons, among which three bore oxygen, one was ester carbonyl, and one was ketone carbonyl, and the IR spectrum suggested the presence of hydroxyl, ester carbonyl, and ketone carbonyl groups. The $^1$H- and $^{13}$C-NMR spectral data, including 2D NMR experiments, as in the case of **2**, indicated that **6** was composed of three moieties, *a'*, *b'*, and *c*, as shown in Chart 3. The structure of **6** was constructed from the three moieties with the aid of the $^1$H- and $^{13}$C-NMR spectral data mentioned above and also biogenetic considerations, into a *seco*-anthraquinone-type benzophenone (**6**), which was also supported by the presence of the molecular ion corresponding to $[C_{16}H_{14}O_6]$ in the EI-MS spectrum. This structure was found to be the same as that of nidulalin B (**6**),[7] which has been isolated from the Ascomycete *Emericella nidulans*, together with the *seco*-anthraquinone-type dihydroxanthone, nidulalin A (**11**)[7] (see Chart 3). Comparison of the physicochemical and spectral data of **6** with the corresponding data of nidulalin B reported in the literature[7] indicated that they were identical.

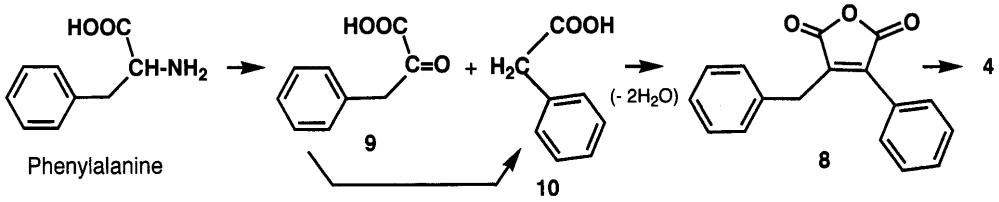
Compound **5** was obtained in low yield as an optically active white amorphous solid, positive to the $FeCl_3$ reagent. The $^1$H- and $^{13}$C-NMR spectra of **5** suggested the presence of one aromatic methyl, one methoxy methyl, one methylene, two $sp^3$ methines among which one bore oxygen, four $sp^2$ protons, one hydrogen-bonded hydroxyl, one $sp^3$ quaternary carbon bearing oxygen, and six $sp^2$ quaternary carbons among which two bore oxygen, one was ester carbonyl, and one was ketone carbonyl. The IR spectrum of **5** also suggested the presence of hydroxyl, ester carbonyl, and ketone carbonyl groups. The $^1$H- and $^{13}$C-NMR spectral data, including 2D NMR experiments, as in the case of **2**, suggested that **5** was composed of four moieties *a*, $b''_1$, $b''_2$, and *c*, among which *a* and *c* were common with the partial structures *a* in **2** and *c* in **6**, respectively, as shown in Chart 3. The partial structures *a*, $b''_1$, $b''_2$, and *c*, were connected to construct the whole molecular structure of **5**, after consideration of the fact that **5** was obtained together with nidulalin B (**6**)[7] from this fungus. The constructed structure was a novel *seco*-anthraquinone-type compound, 4a-carbomethoxy-4,8-dihy-




Structures of Compound **4** and Its acetate (**7**)



Relative Stereostructure of Compound **4** Solved by X-Ray Crystallographic Analysis [One of the Three Molecules (**a**) in an Asymmetric Unit]




Supposed Biosynthetic Pathway for Compound **4**

Chart 2

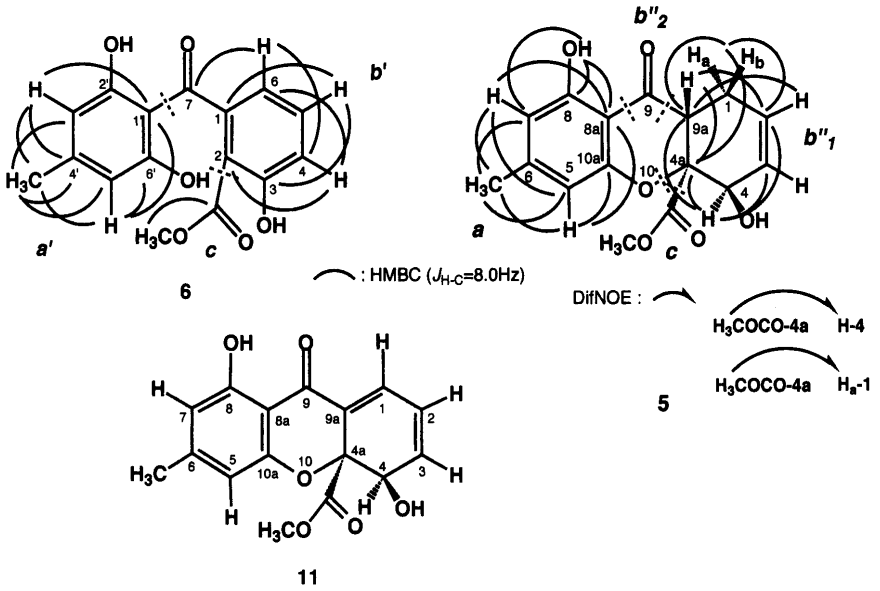



Chart 3. Structures of Compounds **5** and **6** (Nidulalin B), and Nidulalin A (**11**)

droxy-6-methyl-1,4,4a,9a-tetrahydroxanthone (**5**), which was also supported by the molecular ion corresponding to $[C_{16}H_{16}O_6]^{\cdot+}$ in the EI-MS spectrum. Comparison of the $^1$H- and $^{13}$C-NMR spectral data of **5** with those of nidulalin A (**11**) reported in the literature[7] suggested that compound **5** was a new 1,9a-dihydro derivative of **11** (see Table 2 and Chart 3). In a differential nuclear Overhauser effect (DifNOE) NMR experiment, significant NOEs observed between C$\underline{H}_3$OCO-4a ($\delta$ 3.64) and H-4 ($\delta$ 4.67) (*ca.* 1.3%), and between C$\underline{H}_3$OCO-4a and $H_a$-1 ($\delta$ 2.76) (*ca.* 2.3%), but no NOE was observed between C$\underline{H}_3$OCO-4a and H-9a ($\delta$ 2.63), suggesting that the relative configurations at positions 4, 4a, and 9a in **5** were as shown in Chart 3.

The mouse liver MAO inhibitory activities of compounds **1**—**6** were calculated to be 58, 6, 47, 1, 6, and 4% at a concentration of $1.0\times10^{-5}$ g/ml, respectively. Therefore, among these six constituents, only **1** and **3** were confirmed as MAO inhibitory principles of this fungus. The $IC_{50}$ values of **1** and **3** against MAO were calculated to be $2.06\times10^{-5}$ and $3.70\times10^{-5}$ M, respectively. Comparison of the $IC_{50}$ values of **1** and **3** with those of other MAO inhibitory components previously isolated from fungi by us indicated that the activities of **1** and **3** were lower than those of an 2-alkylanthraquinone, norsolorinic acid,[1a] 4,9-dioxonaphtho[2,3-*c*]furans, GP-A and -B,[1d] and 3-alkylazaphilones, luteusins A and B,[1b] and about equal to that of a cyclopentabenzopyran-4-one, coniochaetone A.[1f]

Comparison of the MAO inhibitory activity of **1** with that of **2** suggested that the presence of the hydroxyl at position 7 was very important for the appearance of MAO inhibitory activity in 1-hydroxy-3-methylxanthones. Meanwhile, it was found that the three fungal anthraquinones structurally related to **3**, chrysophanol (**12**),[6] questin (**13**),[6] and physcion (**14**)[6] (see Chart 1), inhibited MAO only by 2, −8, and 7% at

Table 2. $^1$H-NMR and $^{13}$C-NMR Data for Compound **5** and Nidulalin A (**11**), $\delta$ (ppm) from TMS as an Internal Standard [Coupling Constants (Hz) in Parentheses]

| Position | Compound **5** ($CDCl_3$) | | Nidulalin A (**11**)[7] (acetone-$d_6$) | |
|---|---|---|---|---|
| | $^1$H-NMR | $^{13}$C-NMR | $^1$H-NMR | $^{13}$C-NMR |
| 1 | 2.65 (m), 2.76 (m) | 24.60 (t) | 7.37 (dd, 5.4, 1.4) | 133.6 (d) |
| 2 | 6.06 (ddd, 10.0, 4.3, 2.9) | 132.56 (d) | 6.43 (dd, 9.6, 5.4) | 126.4 (d) |
| 3 | 5.89 (m) | 123.72 (d) | 6.49 (ddd, 9.6, 5.2, 1.4) | 135.2 (d) |
| 4 | 4.67 (br d, 6.2) | 66.14 (d) | 4.69 (dd, 6.4, 5.2) | 66.2 (d) |
| OH-4 | | | 4.79 (d, 6.4) | |
| 4a | | 85.24 (s) | | 84.7 (s) |
| $CH_3O\underline{C}O$-4a | | 168.37 (s) | | 170.4 (s) |
| $\underline{CH_3}OCO$-4a | 3.64 (3H, s) | 52.91 (q) | 3.65 (3H, s) | 53.8 (q) |
| 5 | 6.29 (br s) | 111.22 (d) | 6.33 (br d, 1.3) | 109.4 (d) |
| 6 | | 149.86 (s) | | 152.4 (s) |
| $CH_3$-6 | 2.27 (3H, br s) | 22.44 (q) | 2.30 (3H, br s) | 22.5 (q) |
| 7 | 6.38 (br s) | 108.09 (d) | 6.38 (br d, 1.3) | 111.6 (d) |
| 8 | | 161.39 (s) | | 164.3 (s) |
| OH-8 | 11.51 (s) | | 12.29 (s) | |
| 8a | | 105.13 (s) | | 106.4 (s) |
| 9 | | 197.66 (s) | | 184.8 (s) |
| 9a | 2.63 (m) | 40.35 (d) | | 128.6 (s) |
| 10a | | 157.65 (s) | | 160.3 (s) |

a concentration of $1.0\times10^{-5}$ g/ml, respectively. Accordingly, the presence of a set of two free hydroxyls at positions 1 and 3 appears to be very important for MAO inhibitory activity of 8-hydroxy-6-methylanthraquinones.

## Experimental

The general procedures for the chemical experiments were the same as described in our previous report.[1f] The procedure for evaluation of inhibitory activity of samples against mouse liver MAO was also the same as described in our previous reports.[1a,b] Chemical shifts are expressed in $\delta$ (ppm) values from tetramethylsilane (TMS) as an internal standard.

**Isolation of Compounds 1—6** *Anixiella micropertusa* IFM4495[2] was cultivated on sterilized rice (200 g/flask×125) at 25 °C for 22 d. The moldy rice was extracted with MeOH (36.0 l) with shaking at room temperature for 6 h two times, to give a MeOH solution (72.0 l) which was evaporated *in vacuo* to give a MeOH extract (580 g). This MeOH extract was partitioned with AcOEt-$H_2O$ (1 : 1, v/v) (6.6 l) into an AcOEt layer (after evaporation *in vacuo*, 94 g) and an aqueous layer (after evaporation *in vacuo*, 295 g). The AcOEt layer, which inhibited MAO by 33% at $5.0\times10^{-5}$ g/ml, was subjected to chromatography on a silica gel column with *n*-hexane-AcOEt (20 : 1—5 : 1), (5 : 1—3 : 1), (2 : 1—1 : 1), (0.5 : 1—0 : 1), and acetone-MeOH to give five fractions I—V, respectively. Fraction II (9.3 g), which inhibited MAO by 33% at $2.5\times10^{-5}$ g/ml, was further chromatographed on a silica gel column two times and on an octadecyl silica gel (ODS) column with $H_2O$-MeOH (2.3 : 1), (1 : 1), and (0 : 1) to give six fractions IIa-f. Fraction IIb (63 mg), which inhibited MAO by 66% at $2.5\times10^{-5}$ g/ml, was further fractionated on a silica gel column with $CHCl_3$-acetone, and on a high performance liquid chromatographic (HPLC) ODS column with $H_2O$-$CH_3CN$ (1 : 1) at a flow rate of 4.0 ml/min to afford **1** (20 mg). Fraction I (9.4 g), which inhibited MAO by 11% at $2.5\times10^{-5}$ g/ml, was recrystallized repeatedly from *n*-hexane-AcOEt to afford **2** (32 mg). Fraction IIc (78 mg), which inhibited MAO by 74% at $2.5\times10^{-5}$ g/ml, was then chromatographed on a silica gel column, and on an HPLC ODS column with $H_2O$-$CH_3CN$ (1 : 1) at a flow rate of 4.0 ml/min to afford **3** (8 mg). Fraction IIa (185 mg), which inhibited MAO by 12% at $2.5\times10^{-5}$ g/ml, was further chromatographed on an HPLC ODS column with $H_2O$-$CH_3CN$ (1 : 1) at a flow rate of 4.0 ml/min to afford **5** (5 mg), **6** (75 mg) and **4** (79 mg).

Compound **1** (1,7-Dihydroxy-3-methylxanthone): Yellow needles from EtOH, mp 214—215 °C. EI-MS *m/z* (%): 242 (100, $M^+$). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3288 (O–H), 1650 (C=O), 1606 (C=C). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 232 (4.51), 257 (4.65), 288 (4.04), 379 (3.89).

Compound **2** (1-Hydroxy-3-methylxanthone): Yellow needles from *n*-hexane-AcOEt, mp 146—147 °C (lit.[4] 142—143 °C). EI-MS *m/z* (%): 226 (100, $M^+$). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3435 (O–H), 1660 (C=O), 1612 (C=C). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 233 (4.45), 251 (sh, 4.43), 253 (4.45), 289 (sh, 3.98), 298 (4.06), 359 (3.64).

Compound **3** (Emodin): Orange needles from EtOH, mp 260—263 °C. EI-MS *m/z* (%): 270 (100, $M^+$). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3425 (O–H), 1677, 1627 (C=O). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 248 (4.11), 262 (4.12), 285 (4.18), 432 (3.92). This compound was identical with an authentic sample of emodin (**3**) in terms of the $^1$H- and $^{13}$C-NMR spectra (dimethylsulfoxide (DMSO)-$d_6$) and chromatographic behavior on an HPLC ODS column, eluting with $H_2O$-$CH_3CN$ (1 : 1).

Compound **4** (Microperfuranone): Colorless plates from EtOH, mp 106—108 °C, $[\alpha]_D^{21}$ −6.8° (c=0.60, MeOH). High resolution (HR)-FAB-MS *m/z*: 267.1011 ($C_{17}H_{15}O_3$ requires 267.1021 [$(M+H)^+$]). EI-MS *m/z* (%): 266 (18, $M^+$), 192 (20), 191 (43). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3263 (O–H), 1722 (OC=O), 1598 (C=C). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 253 (4.14). $^1$H-NMR (in $CDCl_3$): 3.88, 3.91 (each 1H, br s, $H_a$-12, $H_b$-12), 5.86 (1H, s, H-5), 7.14 (2H, br d, $J$=7.3 Hz, H-14, H-18), 7.20—7.29 (3H, m), 7.39—7.47 (5H, m). $^{13}$C-NMR (in $CDCl_3$): 32.36 (t, C-12), 96.84 (d, C-5), 129.61 (s, C-3), 158.92 (s, C-4), 171.53 (s, C-2), 127.10 (2C), 128.63 (2C), 128.78 (2C), 128.94 (2C), 128.96, 129.08 (2C), 135.95.

**Acetylation of Compound 4** A solution of **4** (7.0 mg) in acetic anhydride (40 $\mu$l) and pyridine (80 $\mu$l) was allowed to stand at room temperature for 5 h, and treated as usual to give a crude product which was then chromatographed on a silica gel column to afford **7** (5.0 mg) as a colorless oil, $^1$H-NMR ($CDCl_3$): 1.98 (3H, s, $C\underline{H_3}CO$), 3.70, 3.99 (each d, $J$=15.3 Hz, $H_a$-12, $H_b$-12), 6.71 (s, H-5), 7.04 (2H, br d, $J$=6.4 Hz, H-14, H-18), 7.18—7.38 (3H, m), 7.40—7.48 (5H, m). $^{13}$C-NMR ($CDCl_3$): 20.49 (q, $\underline{C}H_3CO$), 32.59 (t, C-12), 92.17 (d, C-5), 129.46 (s, C-3), 151.98 (s, C-4), 169.98 (2C, s, C-2 and $CH_3\underline{C}O$-), 127.43, 128.63 (2C), 128.77 (2C), 128.94 (2C), 129.11 (2C), 129.46, 132.94, 135.28.

**X-Ray Crystallographic Analysis of Compound 4** A Crystal of **4** [orthorhombic, space group *Pbca* (#61), lattice constants $a$=30.095 (2), $b$=28.390 (2), $c$=9.763 (2) Å, $V$=8341 (2) Å$^3$, $Z$=24, $D_{calcd}$ 1.272 g/cm$^3$] we used. Data from 3691 observed reflections [$I>2.50\ \sigma(I)$] within the range of $0°<2\theta<135.2°$, measured with Cu$K_\alpha$ radiation, were solved directly by the SIR92 program[8] and the solution was refined by the full-matrix least-squares method with anisotropic temperature factors for all non-hydrogen atoms, to give a final $R$ value of 0.058. All hydrogen atoms were included but not refined.[9] Three independent molecules, **a**, **b**, and **c**, were included in an asymmetric unit of the crystal. The molecular structure of one of the three molecules (**a**) in an asymmetric unit, is illustrated in Chart 2. The final fractional coordinates of all non-hydrogen atoms with estimated standard deviations are listed in Table 3.

Compound **5**: White amorphous solid from EtOH, mp 144—146 °C, $[\alpha]_D^{21}$ −431° (*c*=0.05, MeOH). HR-FAB-MS *m/z*: 305.1041 ($C_{16}H_{17}O_6$ requires 305.1025 [$(M+H)^+$]). EI-MS *m/z* (%): 304 (14, $M^+$), 227 (100), 226 (25). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3457 (O—H), 1735 (OC=O), 1639 (C=O), 1570 (C=C). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 335 (3.44), 272 (3.94).

Compound **6** (Nidulalin B): Yellow amorphous solid from EtOH, mp 144—145 °C (lit.[7] 141—143 °C). EI-MS *m/z* (%): 302 (5, $M^+$), 270 (32),

Table 3. Fractional Coordinates and Isotropic Thermal Parameters for Non-Hydrogen Atoms of Compound **4** with Estimated Standard Deviations in Parentheses

| Atom | $x$ | $y$ | $z$ | $B_{eq}$ |
|---|---|---|---|---|
| $C_{(a)}$-2 | 0.5969 (2) | 0.1092 (2) | 1.3237 (5) | 3.6 (1) |
| $C_{(a)}$-3 | 0.6260 (1) | 0.1415 (1) | 1.3980 (4) | 3.0 (1) |
| $C_{(a)}$-4 | 0.6008 (1) | 0.1644 (1) | 1.4888 (5) | 3.0 (1) |
| $C_{(a)}$-5 | 0.5532 (1) | 0.1497 (2) | 1.4720 (5) | 3.5 (1) |
| $C_{(a)}$-6 | 0.6744 (1) | 0.1436 (2) | 1.3725 (4) | 3.3 (1) |
| $C_{(a)}$-7 | 0.6999 (2) | 0.1028 (2) | 1.3686 (5) | 4.8 (1) |
| $C_{(a)}$-8 | 0.7451 (2) | 0.1054 (2) | 1.3472 (6) | 6.0 (2) |
| $C_{(a)}$-9 | 0.7653 (2) | 0.1481 (3) | 1.3317 (5) | 5.6 (2) |
| $C_{(a)}$-10 | 0.7407 (2) | 0.1885 (2) | 1.3326 (5) | 5.1 (1) |
| $C_{(a)}$-11 | 0.6955 (2) | 0.1864 (2) | 1.3534 (5) | 4.1 (1) |
| $C_{(a)}$-12 | 0.6138 (1) | 0.1977 (2) | 1.5995 (5) | 3.6 (1) |
| $C_{(a)}$-13 | 0.5910 (1) | 0.2449 (2) | 1.5893 (5) | 3.4 (1) |
| $C_{(a)}$-14 | 0.6104 (2) | 0.2817 (2) | 1.5207 (6) | 5.2 (1) |
| $C_{(a)}$-15 | 0.5894 (2) | 0.3246 (2) | 1.5086 (6) | 6.6 (2) |
| $C_{(a)}$-16 | 0.5484 (2) | 0.3315 (2) | 1.5655 (6) | 5.6 (2) |
| $C_{(a)}$-17 | 0.5291 (2) | 0.2959 (2) | 1.6367 (6) | 5.6 (2) |
| $C_{(a)}$-18 | 0.5503 (2) | 0.2527 (2) | 1.6487 (6) | 4.9 (1) |
| $O_{(a)}$-1 | 0.55493 (9) | 0.1131 (1) | 1.3672 (3) | 3.98 (8) |
| $O_{(a)}$-2 | 0.6067 (1) | 0.0811 (1) | 1.2334 (4) | 4.61 (9) |
| $O_{(a)}$-5 | 0.53377 (9) | 0.1324 (1) | 1.5895 (3) | 4.36 (8) |
| $C_{(b)}$-2 | 0.3856 (2) | 0.2255 (2) | 1.6822 (5) | 3.7 (1) |
| $C_{(b)}$-3 | 0.3576 (1) | 0.1926 (1) | 1.6060 (4) | 3.1 (1) |
| $C_{(b)}$-4 | 0.3830 (1) | 0.1705 (1) | 1.5164 (5) | 3.1 (1) |
| $C_{(b)}$-5 | 0.4305 (1) | 0.1870 (2) | 1.5294 (5) | 3.5 (1) |
| $C_{(b)}$-6 | 0.3096 (1) | 0.1886 (1) | 1.6296 (5) | 3.3 (1) |
| $C_{(b)}$-7 | 0.2923 (2) | 0.1804 (2) | 1.7603 (5) | 4.7 (1) |
| $C_{(b)}$-8 | 0.2480 (2) | 0.1737 (2) | 1.7792 (5) | 6.1 (2) |
| $C_{(b)}$-9 | 0.2191 (2) | 0.1744 (2) | 1.6705 (6) | 6.0 (2) |
| $C_{(b)}$-10 | 0.2350 (2) | 0.1834 (2) | 1.5407 (5) | 5.5 (2) |
| $C_{(b)}$-11 | 0.2796 (1) | 0.1907 (2) | 1.5207 (5) | 4.1 (1) |
| $C_{(b)}$-12 | 0.3709 (1) | 0.1347 (2) | 1.4094 (5) | 3.8 (1) |
| $C_{(b)}$-13 | 0.3975 (1) | 0.0901 (2) | 1.4180 (5) | 3.1 (1) |
| $C_{(b)}$-14 | 0.3837 (2) | 0.0540 (2) | 1.5020 (6) | 4.9 (1) |
| $C_{(b)}$-15 | 0.4074 (2) | 0.0126 (2) | 1.5112 (6) | 6.4 (2) |
| $C_{(b)}$-16 | 0.4453 (2) | 0.0064 (2) | 1.4372 (7) | 5.6 (2) |
| $C_{(b)}$-17 | 0.4597 (2) | 0.0419 (2) | 1.3533 (7) | 5.6 (2) |
| $C_{(b)}$-18 | 0.4358 (2) | 0.0835 (2) | 1.3445 (6) | 4.8 (1) |
| $O_{(b)}$-1 | 0.42801 (9) | 0.2228 (1) | 1.6361 (3) | 4.00 (8) |
| $O_{(b)}$-2 | 0.3758 (1) | 0.2525 (1) | 1.7738 (3) | 4.91 (9) |
| $O_{(b)}$-5 | 0.44875 (10) | 0.2060 (1) | 1.4116 (3) | 4.52 (8) |
| $C_{(c)}$-2 | 0.4044 (1) | −0.0615 (2) | 1.1832 (5) | 3.3 (1) |
| $C_{(c)}$-3 | 0.3709 (1) | −0.0342 (1) | 1.1058 (5) | 3.0 (1) |
| $C_{(c)}$-4 | 0.3922 (1) | −0.0083 (2) | 1.0138 (5) | 3.2 (1) |
| $C_{(c)}$-5 | 0.4414 (1) | −0.0170 (2) | 1.0234 (5) | 3.6 (1) |
| $C_{(c)}$-6 | 0.3232 (1) | −0.0393 (2) | 1.1326 (5) | 3.3 (1) |
| $C_{(c)}$-7 | 0.3063 (2) | −0.0346 (2) | 1.2644 (5) | 5.0 (1) |
| $C_{(c)}$-8 | 0.2617 (2) | −0.0399 (2) | 1.2882 (6) | 6.7 (2) |
| $C_{(c)}$-9 | 0.2335 (2) | −0.0507 (2) | 1.1827 (7) | 6.6 (2) |
| $C_{(c)}$-10 | 0.2493 (2) | −0.0551 (2) | 1.0516 (6) | 6.1 (2) |
| $C_{(c)}$-11 | 0.2939 (2) | −0.0497 (2) | 1.0276 (5) | 4.7 (1) |
| $C_{(c)}$-12 | 0.3740 (1) | 0.0255 (2) | 0.9083 (5) | 3.9 (1) |
| $C_{(c)}$-13 | 0.3952 (1) | 0.0736 (2) | 0.9167 (5) | 3.3(1) |
| $C_{(c)}$-14 | 0.3753 (2) | 0.1082 (2) | 0.9948 (6) | 5.0 (1) |
| $C_{(c)}$-15 | 0.3944 (2) | 0.1524 (2) | 1.0057 (6) | 6.2 (2) |
| $C_{(c)}$-16 | 0.4336 (2) | 0.1623 (2) | 0.9390 (7) | 5.7 (2) |
| $C_{(c)}$-17 | 0.4525 (2) | 0.1284 (2) | 0.8585 (6) | 5.5 (2) |
| $C_{(c)}$-18 | 0.4337 (2) | 0.0843 (2) | 0.8474 (6) | 4.6 (1) |
| $O_{(c)}$-1 | 0.44522 (9) | −0.0523 (1) | 1.1316 (3) | 3.84 (8) |
| $O_{(c)}$-2 | 0.3990 (1) | −0.0888 (1) | 1.2751 (4) | 4.81 (9) |
| $O_{(c)}$-5 | 0.46075 (9) | −0.0338 (1) | 0.9035 (3) | 4.42 (9) |

242 (33) (lit.[7] 302, 270, 242). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3334 (O–H), 1677 (OC=O), 1637 (C=O) (lit.[7] 3300, 1680, 1640). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 223 (4.26), 281 (3.89), 313 (4.14) (lit.[7] 284, 316). $^1$H-NMR ($CD_3OD$): 2.20 (3H, s, $H_3$C-4′), 3.61 (3H, s, $H_3$COCO-2), 6.15 (2H, br s, H-3′ and H-5′), 6.63 (1H, dd, $J_1$=7.3, $J_2$=1.1 Hz, H-6), 6.95 (1H, dd, $J_1$=8.0, $J_2$=1.1 Hz, H-4), 7.42 (1H, dd, $J_1$=8.0, $J_2$=7.3 Hz, H-5). $^{13}$C-NMR ($CD_3OD$): 22.09 (q, $H_3$C-4′), 52.41 (q, $H_3$COCO-2), 108.90 (2C, d, C-3′ and C-5′), 109.30 (s, C-1′), 111.25 (s, C-2), 118.08 (d, C-6), 118.48 (d, C-4), 135.50 (d, C-5), 147.26 (s, C-1), 150.10 (s, C-4′), 161.82 (s, C-3), 163.08 (2C, s, C-2′, C-6′), 171.04 (s, $H_3$COCO-2), 202.16 (s, C-7).

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**References and Notes**

1) *a*) Yamazaki M., Satoh Y., Maebayashi Y., Horie Y., *Chem. Pharm. Bull.*, **36**, 670—675 (1988); Yamazaki M., Satoh Y., Horie Y., Maebayashi Y., *Proc. Jpn. Assoc. Mycotoxicol.*, **23**, 41—45 (1986); *b*) Satoh Y., Yamazaki M., *Chem. Pharm. Bull.*, **37**, 206—207 (1989); Fujimoto H., Matsudo T., Yamaguchi A, Yamazaki M., *Heterocycles*, **30**, 607—616 (1990); *c*) Yoshida E., Fujimoto H., Baba M., Yamazaki M., *Chem. Pharm. Bull.*, **43**, 1307—1310 (1995); *d*) Fujimoto H., Okuyama H., Motohashi Y., Yoshida E., Yamazaki M., *Mycotoxins.*, **41**, 61—66 (1995); *e*) Hossain C. F., Okuyama E., Yamazaki M., *Chem. Pharm. Bull.*, **44**, 1535—1539 (1996); *f*) Fujimoto H., Inagaki M., Satoh Y., Yoshida E., Yamazaki M., *Chem. Pharm. Bull.*, **44**, 1090—1092 (1996).
2) This strain had formerly been deposited at Research Institute for Chemobiodynamics, Chiba University (present name: Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University).
3) Kraml M., *Biochem. Pharmacol.*, **14**, 1684—1686 (1965).
4) Mucheli M. V. R., Kudav N. A., *Chem. Ind.* (London), **1985**, 31—32.
5) Wader G. R., Kudav N. A., *Indian J. Chem.*, **26B**, 703 (1987).
6) Turner W. B., "Fungal Metabolites," Academic Press, London, 1971, pp. 156—173; Turner W. B., Aldridge D. C., "Fungal Metabolites II," Academic Press, London, 1983, pp. 140—148.
7) Kawahara N., Sekita S., Satake M., Udagawa S., Kawai K., *Chem. Pharm. Bull.*, **42**, 1720—1723 (1994).
8) Altomare A., Burla M. C., Camalli M., Cascarano M., Giacovazzo C., Guagliardi A., Polidori G., *J. Appl. Cryst.*, **27**, 435 (1994).
9) The coordinates of all hydrogen atoms, bond distances, and bond angles for this structure have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.