Structures of a New Dihydroxanthone Derivative, Nidulalin A, and a New Benzophenone Derivative, Nidulalin B, from *Emericella nidulans*

Nobuo KAWAHARA,*[,a] Setsuko SEKITA,[a] Motoyoshi SATAKE,[a] Shun-ichi UDAGAWA,[b] and Ken-ichi KAWAI[c]

National Institute of Health Sciences (NIHS),[a] Kamiyoga 1–18–1, Setagaya-ku, Tokyo 158, Japan, Nodai Research Institute, Tokyo University of Agriculture,[b] Sakuragaoka 1–1–1, Setagaya-ku, Tokyo 156, Japan and Faculty of Pharmaceutical Sciences, Hoshi University,[c] Ebara 2–4–41, Shinagawa-ku, Tokyo 142, Japan.
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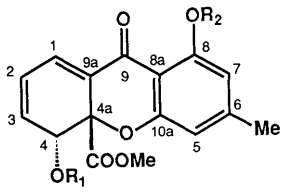
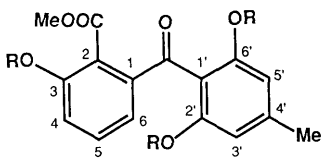
Two new compounds, designated nidulalins A (1) and B (2), were isolated along with emestrin and sterigmatocystin from the extract of rice culture of *Emericella nidulans* var. *lata*. The structures of nidulalins A (1) and B (2) were determined on the basis of chemical and spectroscopic investigation and X-ray crystallographic study, together with the application of the modified Mosher's method.

Keywords *Emericella nidulans*; dihydroxanthone; benzophenone; nidulalin A; nidulalin B; X-ray analysis; Mosher's method modified

Recently we reported[1] the isolation of two adenosine derivatives, 3′-deoxyadenosine and 5′-acetyl-3′-deoxyadenosine, from the rice culture of an ascomycetous fungus, *Emericella nidulans* (EIDAM) VUILL. var. *lata* (THOM *et* RAPER) SUBRAM. (anamorph: *Aspergillus nidulellus* SAMSON *et* W. GAMS), strain IN-68, which was isolated from an Indonesian medicinal plant, *Trigonella foenum-graecum*, used as a carminative and tonic.[2] In the course of a search for other metabolites of the above fungus, two new compounds designated as nidulalins A (**1**) and B (**2**) were isolated, along with emestrin and sterigmatocystin, from the dichloromethane extract of the rice culture. The structural elucidation of the above compounds **1** and **2** is reported in this paper.

Nidulalin A (**1**), mp 119—121 °C, $[\alpha]_D$ −426° ($CHCl_3$), gave a molecular ion peak at *m/z* 302 in electron impact ionization mass spectrometry (EIMS), and elemental analysis confirmed the molecular formula as $C_{16}H_{14}O_6$. The absorptions at 3400, 1740 and 1650 cm^{-1} in the infrared (IR) spectrum of **1** suggested the presence of hydroxyl, ester and chelated carbonyl moieties, respectively. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) signals at δ 170.4 and 184.8 were assigned to ester and chelated carbonyl carbons, respectively (Table I). The proton nuclear magnetic resonance (^{1}H-NMR) signals at δ 2.30 (3H, br s), 3.65 (3H, s), 6.33 (1H, br d, J=1.3 Hz), 6.38 (1H, br d, J=1.3 Hz) and 12.29 (1H, s) were assigned to an aromatic methyl, a methoxyl, two aromatic protons with *meta*-coupling, and a hydrogen-bonding phenolic hydroxyl group, respectively (Table II). Decoupling experiments showed that each *meta*-coupling proton was coupled to the methyl group and their chemical shifts indicated the presence of a phenolic ring. The ester carbonyl carbon signal at δ 170.4 (q) suggested the presence of methoxycarbonyl group.





1: R_1, R_2 = H
3: R_1, R_2 = Ac
5: R_1 = (+) - (*R*) - MTPA, R_2 = H
6: R_1 = (-) - (*S*) - MTPA, R_2 = H

2: R = H
4: R = Ac

Chart 1

On acetylation, nidulalin A (**1**) afforded a diacetate (**3**), pale yellow amorphous powder, $[\alpha]_D$ −551° ($CHCl_3$), $C_{20}H_{18}O_8$, and a triacetate (**4**), colorless needles, mp 98—100 °C, $C_{22}H_{20}O_9$. Nidulalin A diacetate (**3**) showed ^{1}H-NMR signals at δ 2.06 (3H, s) and 2.41 (3H, s) due to the methyl protons of an aliphatic acetoxyl group and those of a phenolic acetoxyl group. The ^{1}H-NMR signal at δ 4.69 (dd, J=5.2, 6.4 Hz), which was coupled with the hydroxyl proton, in **1** shifted downfield to δ 5.93 (d, J=5.6 Hz) after acetylation. The IR spectrum of **3** showed no absorption at 4000—3000 cm^{-1}. These results confirmed that nidulalin A (**1**) possesses only one secondary hydroxyl group and one phenolic hydroxyl group. Nidulalin A triacetate (**4**) showed three acetoxyl methyl groups at δ 1.99 (6H, s) and 2.42 (3H, s), and three adjacent aromatic proton signals at δ 7.29 (1H, dd, J=1.4, 7.8 Hz), 7.40 (1H, dd, J=1.4, 7.7 Hz) and 7.48 (1H, dd, J=7.7, 7.8 Hz) in the ^{1}H-NMR spectrum, whereas the proton signal of **1** at δ 4.69, which was shifted downfield in nidulalin A diacetate (**3**), was not observed in the ^{1}H-NMR spectrum of **4**. This suggested that nidulalin A (**1**) had been isomerized to an aromatic compound on acetylation.

The partial structure =CH–CH=CH–CH(OH)– in **1** was demonstrated by decoupling experiments, using the homonuclear ^{1}H–^{1}H shift correlation (^{1}H–^{1}H COSY) spectrum and the heteronuclear ^{1}H–^{13}C shift correlation (^{1}H–^{13}C COSY) spectrum as shown in Tables I and II. All these data together with the ultraviolet (UV) absorption maxima at 341 nm indicated that the compound has a dihydroxanthone moiety.

In order to determine the exact structure of **1**, an X-ray structure analysis was undertaken. Crystals were grown as orange prisms from *n*-hexane solution. The molecular structure of **1** is illustrated in Fig. 1.[3] Therefore, the

TABLE I. ^{13}C-NMR Data for Nidulalins and Their Acetates[a]

Carbon No.	1[b]	3[c]	Carbon No.	2[b]	4[c]
1	133.6 (Ddd)	128.8 (Ddd)[d]	1	147.6 (d)	140.4 (d)
2	126.4 (Dd)	128.5 (Dd)	2	118.5 (br s)	126.3 (br s)
3	135.2 (Dm)	131.2 (Ddd)[d]	3	162.4 (br d)	148.8 (dd)
4	66.2 (Ddd)	64.9 (Ddd)	4	118.6 (Dt)	127.5 (Dd)[d]
4a	84.7 (m)	81.6 (m)	5	136.2 (D)	131.0 (D)
5	109.4 (Dqd)	117.1 (Dqd)[e]	6	118.2 (Dd)	126.9 (Dd)[d]
6	152.4 (br q)	148.7 (q)	1′	109.2 (br s)	122.7 (br s)
7	111.6 (Dqd)	119.1 (Dqd)[e]	2′	163.2 (s)	149.7 (s)
8	164.3 (dd)	151.0 (d)	3′	109.3 (Dqd)	121.8 (Dqd)
8a	106.4 (m)	112.3 (t)	4′	150.1 (q)	144.0 (q)
9	184.8 (d)	177.3 (d)	5′	109.3 (Dqd)	121.8 (Dqd)
9a	128.6 (m)	129.1 (m)	6′	163.2 (s)	149.7 (s)
10a	160.3 (d)	160.3 (d)	CO	202.0 (d)	191.5 (d)
4a-COOMe	53.8 (Q)	53.8 (Q)	2-COOMe	52.7 (Q)	52.8 (Q)
4a-COOMe	170.4 (q)	168.7 (q)	2-COOMe	171.3 (q)	169.5 (q)
6-Me	22.5 (Qt)	22.1 (Qt)	4′-Me	22.1 (Qt)	21.5 (Qt)
OCOMe		21.0 (Q)	OCOMe		20.4 (Q)
		21.3 (Q)			20.4 (Q)
					20.6 (Q)
OCOMe		170.5 (q)	OCOMe		166.3 (q)
		170.7 (qd)			169.4 (q)
					169.4 (q)

a) The multiplicity of the signals was determined from the proton-coupled ^{13}C-NMR spectra. 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 pattern resulting from directly bonded coupling ($^1J_{C,H}$). *b*) Measured in acetone-d_6. *c*) Measured in $CDCl_3$. *d, e*) The assignments may be reversed in each column.

TABLE II. ^{1}H-NMR Data for Nidulalins and Their Acetates

Proton	1[a]	3[b]	Proton	2[a]	4[b]
1-H	7.37 (dd, 5.4, 1.4)	7.26 (dd, 5.5, 1.1)	4-H	6.76 (dd, 7.5, 1.0)	7.29 (dd, 7.8, 1.4)
2-H	6.43 (dd, 9.6, 5.4)	6.43 (dd, 6.0, 5.5)	5-H	7.54 (dd, 8.5, 7.5)	7.48 (dd, 7.8, 7.7)
3-H	6.49 (ddd, 9.6, 5.2, 1.4)	6.30 (ddd, 9.0, 5.6, 1.1)	6-H	7.00 (dd, 8.5, 1.0)	7.40 (dd, 7.7, 1.4)
4-H	4.69 (dd, 6.4, 5.2)	5.93 (d, 5.6)			
4-OH	4.79 (d, 6.4)		3-OH	10.77 (s)	
5-H	6.33 (br d, 1.3)	6.58 (br d, 1.1)	3′-H	6.25 (br s)	6.91 (s)
6-Me	2.30 (br s)	2.35 (br s)	4′-Me	2.23 (s)	2.31 (s)
7-H	6.38 (br d, 1.3)	6.76 (br d, 1.1)	5′-H	6.25 (br s)	6.91 (s)
8-OH	12.29 (s)		2′-OH	10.89 (s)	
			6′-OH	10.89 (s)	
4a-COOMe	3.65 (s)	3.66 (s)	2-COOMe	3.67 (s)	3.79 (s)
OCOMe		2.06 (s)	OCOMe		1.99 (s)
		2.41 (s)			1.99 (s)
					2.42 (s)

Coupling constants (J in Hz) are given in parentheses. *a*) Measured in acetone-d_6. *b*) Measured in $CDCl_3$.

relative structure of nidulalin A was confirmed as **1**.

The absolute configuration of **1** has been determined by ^{1}H-NMR analysis of the (+)-(*R*)- and (−)-(*S*)-α-methoxy-α-trifluoromethylphenyl acetates (MTPA esters) of **1** (namely by the modified Mosher's method[4]). As given in Table III, the ^{1}H-NMR signals at 4a-COOMe, 5-H, 6-Me, 7-H and 8-OH of (+)-(*R*)-MTPA ester (**5**) were observed at lower fields than those of (−)-(*S*)-MTPA ester (**6**), while the signals at 1-H, 2-H and 3-H of **5** appeared at slightly higher fields as compared to those of **6** (Fig. 2). Consequently, the configuration of C-4 was confirmed to be *R*.

The circular dichroism (CD) spectra of secalonic acids, fungal tetrahydroxanthone derivatives, showed a large Cotton effect at 330—335 nm, which is correlated with the stereochemical environment of the methoxycarbonyl group at C-10a (C-10a′).[5,6] Secalonic acids A (**7**) and G (**8**), with *S* configuration at C-10a (C-10a′), gave a negative Cotton effect [$\Delta\varepsilon$ (molar circular dichroism) −18.9 and $\Delta\varepsilon$ −15.8,[6,7] respectively], whereas secalonic acids D (**9**) and F (**10**), with *R* configuration at C-10a

TABLE III. ^{1}H-NMR Data for the (+)-(*R*)-MTPA Ester (**5**) and (−)-(*S*)-MTPA Ester (**6**) in $CDCl_3$

Proton	5	6
1-H	7.29 (dd, 5.5, 1.1)	7.31 (dd, 5.6, 1.1)
2-H	6.48 (dd, 9.6, 5.5)	6.54 (dd, 9.5, 5.6)
3-H	6.36 (ddd, 9.6, 5.6, 1.1)	6.40 (ddd, 9.5, 5.7, 1.1)
4-H	6.15 (d, 5.6)	6.17 (d, 5.7)
5-H	6.27 (br d, 1.4)	6.18 (br d, 1.4)
6-Me	2.31 (br s)	2.29 (br s)
7-H	6.42 (br d, 1.4)	6.38 (br d, 1.4)
8-OH	12.08 (s)	12.03 (s)
4a-COOMe	3.71 (s)	3.71 (s)

Coupling constants (J in Hz) are given in parentheses.

(C-10a'), showed a positive Cotton effect [$\Delta\varepsilon$+13.5 and $\Delta\varepsilon$+17.0,[8] respectively]. A large negative Cotton effect at 336 nm ($\Delta\varepsilon$ −18.6), which was observed in the CD spectrum of **1**, suggested the configuration of C-4a to be *S*. This strongly supported the results of the modified Mosher's method. From the above results, the structure of nidulalin A (**1**) was established, including its absolute stereochemistry.

Nidulalin B (**2**), mp 141—143 °C, gave a molecular ion peak at *m/z* 302 in EIMS, and elemental analysis confirmed the molecular formula as $C_{16}H_{14}O_6$. The absorptions at 3300, 1680 and 1640 cm^{-1} in the IR spectrum of **2** also suggested the presence of hydroxyl, chelated ester and carbonyl moieties, respectively. The ^{1}H-NMR signals (Table II) at δ 3.67 (3H, s), 6.76 (1H, dd, J=1.0, 7.5 Hz), 7.00 (1H, dd, J=1.0, 8.5 Hz), 7.54 (1H, dd, J=7.5, 8.5 Hz) and 10.77 (1H, s) were assigned to a methoxyl, three adjacent aromatic protons and a hydrogen-bonding phenolic hydroxyl group, respectively. On the other hand, the characteristic fragment ions at *m/z* 151 $[CH_3C_6H_2(OH)_2C{\equiv}O]^+$ and 179 $[HOC_6H_3(COOCH_3)C{\equiv}O]^+$ in the EIMS spectrum of **2** suggested the presence of a substituted benzophenone skeleton in the molecule. Further, the ^{1}H-NMR signals at δ 10.89 (2H, s), 6.25 (2H, br s) and 2.23 (3H, s) indicated the presence of a 2,6-dihydroxy-4-methylbenzoyl moiety in **2**.

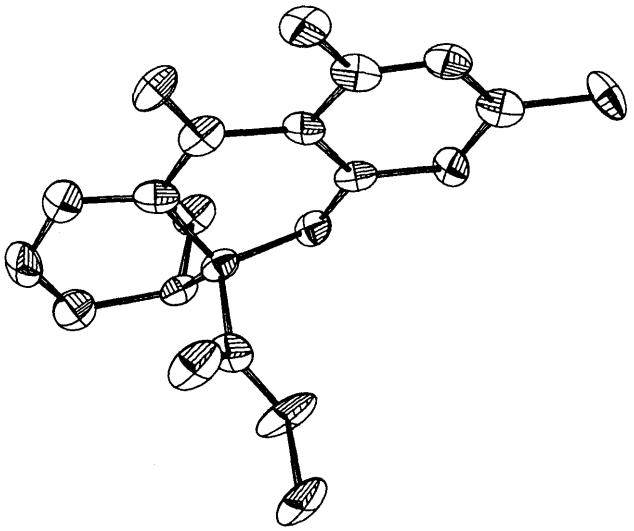


Fig. 1. Perspective View of the Crystal Structure of Nidulalin A (**1**)

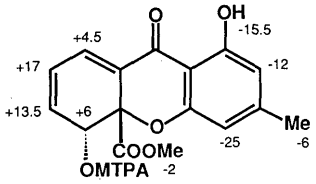


Fig. 2. ^{1}H Chemical Shift Changes Observed in the MTPA Ester (**5** and **6**)

Values [$\Delta\delta=\delta(S)-\delta(R)$] are given in Hz.

On acetylation, nidulalin B (**2**) afforded a triacetate (**4**), colorless needles, mp 98—100 °C, $C_{22}H_{20}O_9$, which was identical with nidulalin A triacetate (**4**). Thus, the structure of nidulalin B (**2**) was confirmed as 2',3,6'-trihydroxy-2-methoxycarbonyl-4'-methylbenzophenone.

It has been suggested that fungal xanthones are biogenetically derived from anthraquinones through oxidative cleavage of the quinone ring. Kachi and Sassa reported[9] the isolation from *Monilinia fructicola* of a benzophenone derivative, moniliphenone, which was a key intermediate in the biosynthesis of xanthones. Nidulalin A (**1**) is the first example of a dihydroxanthone derivative. Its isomerization to a benzophenone derivative suggests that nidulalin A is an important intermediate in the biosynthesis of benzophenones.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-180 spectrometer. EI MS were obtained on a JEOL JMS-DX 300 spectrometer. UV and IR spectra were recorded on a Hitachi U-2000 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Gemini 300 spectrometer at 300 MHz and at 75 MHz, respectively, using tetramethylsilane as an internal standard. CD spectra were determined on a JASCO J-720 spectropolarimeter. Column chromatography was performed using Kieselgel 60 (Art. 7734, Merck). Low-pressure liquid chromatography (LPLC) was performed with a Nihon Seimitsu NP-FX-20 pump and a glass column (300 × 10 mm) packed with Silica gel CQ-3 (30—50 mm, Wako). Thin layer chromatography (TLC) was conducted on pre-coated Kieselgel 60 F_{254} plates (Art. 5715, Merck). Spots on TLC were detected under UV light.

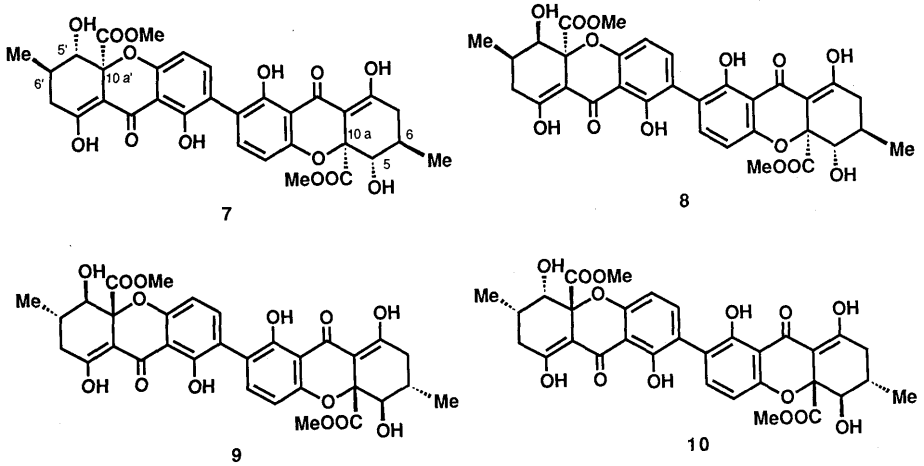


Chart 2

Isolation of Nidulalins A (1) and B (2) from *Emericella nidulans* *E. nidulans* var. *lata*, strain IN-68, was cultivated at 25 °C for 3 weeks on rice (2 kg). The rice cultures were extracted with CH_2Cl_2 at room temperature. The residue (18.1 g) obtained by evaporation of the extract was chromatographed on silica gel with $CHCl_3$ followed by LPLC with benzene–acetone (50 : 1, v/v) to give nidulalin B (**2**) (50 mg) and then benzene–acetone (30 : 1, v/v) to give nidulalin A (**1**) (240 mg).

Nidulalin A (**1**): Orange prisms, mp 119—121 °C, (from *n*-hexane), $[\alpha]_D^{25}$ −426° (c=0.47, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (COO), 1650 (CO). UV λ_{max}^{MeOH} nm (log ε): 341 (4.22). EIMS m/z (%): 302 (M^+, 24), 284 ($[M-H_2O]^+$, 62), 243 ($[M-COOMe]^+$, 100). *Anal.* Calcd for $C_{16}H_{14}O_6$: C, 63.57; H, 4.67. Found: C, 63.35; H, 4.65. CD ($c=3.31\times10^{-5}$, dioxane) [θ] (nm): -6.15×10^4 (336).

Nidulalin B (**2**): Colorless needles, mp 141—143°C, from *n*-hexane–AcOEt (5:1, v/v)). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1680 (COO), 1640 (CO). UV λ_{max}^{MeOH} nm (log ε): 284 (4.15), 316 sh (3.91). EIMS m/z (%): 302 (M^+, 41), 270 ($[M-MeOH]^+$, 71), 242 ($[M-AcOH]^+$, 100), 179 ($[HOC_6H_3(COOCH_3)C\equiv O]^+$, 22), 151 ($[CH_3C_6H_2(OH)_2C\equiv O]^+$, 17). *Anal.* Calcd for $C_{16}H_{14}O_6$: C, 63.57; H, 4.67. Found: C, 63.48; H, 4.66.

Acetylation of Nidulalin A (1) Nidulalin A (**1**) (30 mg) was dissolved in pyridine (2 ml) containing acetic anhydride (1 ml) and the solution was kept at room temperature overnight. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The extract was evaporated and the residue was purified by LPLC using $CHCl_3$–*n*-hexane (1 : 1, v/v) to give nidulalin A diacetate (**3**) (20 mg), and then using $CHCl_3$–*n*-hexane (2 : 1, v/v) to give nidulalin A triacetate (**4**) (8 mg) as colorless needles.

Nidulalin A Diacetate (**3**): Pale yellow amorphous powder, $[\alpha]_D^{25}$ −551° (c=0.28, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1740 (COO), 1670 (CO). UV λ_{max}^{MeOH} nm (log ε): 313 (4.15). EIMS m/z (%): 386.1005 (M^+, 386.1002 for $C_{20}H_{18}O_8$, 3), 284 (78), 243 (100).

Nidulalin A Triacetate (**4**): Colorless needles, mp 98—100 °C from *n*-hexane–$CHCl_3$ (5 : 1, v/v). IR ν_{max}^{KBr} cm^{-1}: 1770, 1740 (COO), 1670 (CO). UV λ_{max}^{MeOH} nm (log ε): 251 (4.14), 280 sh (3.83). EIMS m/z (%): 428 (M^+, 0.3), 386 (18), 344 (47), 270 (100). *Anal.* Calcd for $C_{22}H_{20}O_9$: C, 61.68; H, 4.71. Found: C, 61.65; H, 4.73.

Acetylation of Nidulalin B (2) Nidulalin B (**2**) (15 mg) was dissolved in pyridine (2 ml) containing acetic anhydride (1 ml) and the solution was kept at room temperature overnight. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The extract was evaporated and the residue was purified by LPLC using $CHCl_3$–*n*-hexane (2 : 1, v/v) to give the triacetate (10 mg) as colorless needles. This compound was identical with nidulalin A triacetate (**4**) by comparison of the ^{1}H-NMR and IR spectra and the TLC behavior, and mixed melting point determination.

Preparation of the 2-(+)-(*R*)-MTPA Ester (5) and 2-(−)-(*S*)-MTPA Ester (6) A solution of nidulalin A (**1**) (4.6 mg) in CH_2Cl_2 (0.4 ml) was treated at room temperature (20 °C) with (+)-(*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (23.2 mg), dicyclohexylcarbodiimide (DCC) (21.6 mg), and dimethylaminopyridine (8.0 mg) for 20 min. The reaction mixture was poured into water and the whole was extracted with CH_2Cl_2. The extract was evaporated and the residue was purified by LPLC using *n*-hexane–AcOEt (8 : 1, v/v) to give the 2-(+)-(*R*)-MTPA ester (**5**) (3.5 mg). In a similar manner, the 2-(−)-(*S*)-MTPA ester (**6**) (2.3 mg) was prepared from **1** (5.0 mg).

X-Ray Structure Analysis of Nidulalin A (1) Crystals of **1** were grown from *n*-hexane as orange prisms.

Crystal Data: $C_{16}H_{14}O_6$; M_r=302.28; orthorhombic; $P2_12_12_1$; a=7.548(2), b=27.426(22), c=7.106(2) Å; V=1471.0(13) Å^3; Z=4; D_c=1.365 g·cm^{-3}; $F(000)$=632.

The diffraction intensities were collected from a nidulalin A (**1**) crystal with dimensions of 0.5×0.4×0.1 mm on a Rigaku AFC-5 FOS four-circle diffractometer using Cu K_α radiation monochromated by means of a graphite plate. Of a total of 1109 reflections, 967 were measured within a 2θ range of 110° as above the $3\sigma(F)$ level. These were used in the solution and refinement of the structure.

Determination of the Structure: The structure was solved by the direct method using MULTAN 84[10] and refined by the block-matrix least-squares method. In the final refinement, anisotropic thermal parameters were used for non-hydrogen atoms. The contribution of the hydrogen atoms was ignored. The final R factor without hydrogen atoms was 0.097.

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