Monoamine Oxidase-Inhibitory Components from an Ascomycete, *Coniochaeta tetraspora*

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Two cyclopentabenzopyran-4-ones tentatively named CT-2 and -3 have been isolated as new monoamine oxidase (MAO)-inhibitory components from an Ascomycete, *Coniochaeta tetraspora*, together with a new chlorinated pigment tentatively named CT-1. The structures of CT-2 and -3 have been elucidated, and these products were shown to be identical with coniochaetones A and B, respectively, which have recently been isolated as antifungal components from *Coniochaeta saccardoi*. CT-1 appears to be a *seco*-anthraquinone.

Key words fungal metabolite; Ascomycete; *Conoichaeta tetraspora*; monoamine oxidase-inhibitory activity; cyclopentabenzopyran-4-one; coniochaetone

Several components having a monoamine oxidase (MAO)-inhibitory effect have been isolated from *Emericella navahoensis*,[1] *Talaromyces luteus*,[2] *Talaromyces helicus*,[3] and Mycelia Sterilia derived from *Gelasinospora pseudoreticulata*[4] in our laboratory. We also found that the AcOEt extract from mycelia of *Coniochaeta tetraspora* CAIN has significant inhibitory effect against mouse liver MAO. Two metabolites (tentatively named CT-2 and -3) among three isolated from the extract showed MAO-inhibitory activity, and this report deals with their identification.

Results and Discussion

From the defatted portion of the AcOEt extract of *C. tetraspora*, which showed MAO-inhibitory activity (48% at 1.0×10^{-4} g/ml), three metabolites tentatively named CT-1 (**1**), -2 (**2**), and -3 (**3**) have been isolated. CT-2 and CT-3 showed MAO-inhibitory activity.

CT-3 (**3**), white solid, $C_{13}H_{12}O_4$, $[\alpha]_D^{23.5}$ +95.6°, was positive in the $FeCl_3$ reaction. From the UV, IR, and mainly ^{1}H- and ^{13}C-NMR spectral data (Table 1), including spin-decoupling ^{1}H-NMR and two-dimensional ^{1}H–^{1}H (^{1}H–^{1}H COSY), ^{13}C–^{1}H shift correlation (^{13}C–^{1}H COSY) NMR, and C–H long-range coupling with J_2 and/or J_3 (8 Hz) in a heteronuclear multiple-bond correlation (HMBC) NMR experiment, two possible cyclopentabenzopyran-4-one structures, **3** ($a+b_1$) and **3a** ($a+b_2$) were considered for CT-3 (Chart 1). Structure **3** has a phenolic OH group in *a* and a *sec*-alcoholic OH group in b_1, while **3a** has a phenolic OH group in *a* and a *sec*-alcoholic OH group in b_2.

Compound CT-2 (**2**), white needles, $C_{13}H_{10}O_4$, $[\alpha]_D^{24}$ 0°, also gave a positive $FeCl_3$ reaction. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **3** indicated that CT-2 might be a didehydro derivative of CT-3, in which the alcoholic OH moiety is replaced with a >C=O.

Very recently, we became aware that two new metabolites belonging to the cyclopentabenzopyran-4-one

Table 1. ^{1}H-NMR and ^{13}C-NMR Data for CT-2 (**2**), CT-3 (**3**), CT-3 Acetate (**4**), and Coniochaetones A and B, δ (ppm) from Tetramethylsilane (TMS) as an Internal Standard in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	2		Coniochaetone A[5]		3		Coniochaetone B[5]		4	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	—	197.4 (s)	—	197.3	5.44 (br d, 6.3)	71.0 (d)	5.43 (ddd, 7.6, 3.3, 1.4)	71.2	6.25 (ddd, 7.3, 1.9, 1.7)	73.2 (d)
CH_3CO-1	—	—	—	—	—	—	—	—	2.09 (3H, s)	21.2 (q)
CH_3CO-1	—	—	—	—	—	—	—	—	—	170.6 (s)
2	2.72 (2H, m)	33.8 (t)	2.70 (2H, m)	33.8	2.04, 2.50 (each m)	29.5 (t)	2.03, 2.49 (each m)	29.4	2.08, 2.58 (each m)	28.2 (t)
3	3.09 (2H, m)	26.1 (t)	3.07 (2H, m)	26.1	2.82 (ddd, 18.3, 9.6, 5.0) 3.13 (dddd, 18.3, 9.6, 5.4, 1.3)	30.0 (t)	2.81 (ddd, 18.0, 9.3, 5.1) 3.10 (dddd, 18.0, 9.4, 5.1, 1.4)	29.9	2.85 (ddd, 18.3, 9.6, 3.3) 3.15 (dddd, 18.3, 9.2, 6.7, 1.7)	30.1 (t)
3a	—	189.7 (s)	—	189.6	—	172.1 (s)	—	171.9	—	173.7 (s)
4a	—	156.4 (s)	—	156.4	—	157.6 (s)	—	157.7	—	157.4 (s)
5	6.77 (br s)	108.0 (d)	6.77 (br s)	108.0	6.70 (br s)	107.8 (d)	6.70 (br s)	107.8	6.64 (br s)	107.7 (d)
6	—	148.2 (s)	—	148.2	—	146.8 (s)	—	146.8	—	147.0 (s)
CH_3-6	2.42 (3H, s)	22.4 (q)	2.41 (3H, s)	22.4	2.39 (3H, s)	22.3 (q)	2.38 (3H, s)	22.3	2.40 (3H, s)	22.3 (q)
7	6.68 (br s)	114.2 (d)	6.69 (br s)	114.3	6.62 (br s)	112.6 (d)	6.62 (br s)	112.6	6.72 (br s)	112.9 (d)
8	—	161.9 (s)	—	162.0	—	160.8 (s)	—	160.8	—	161.0 (s)
OH-8	12.22 (s)	—	12.21 (s)	—	12.29 (s)	—	12.25 (s)	—	12.35 (s)	—
8a	—	108.6 (s)	—	108.7 (s)	—	108.9 (s)	—	109.0	—	108.9 (s)
9	—	178.0 (s)	—	178.1 (s)	—	181.3 (s)	—	181.3	—	180.3 (s)
9a	—	117.9 (s)	—	118.0 (s)	—	121.1 (s)	—	121.1	—	117.5 (s)

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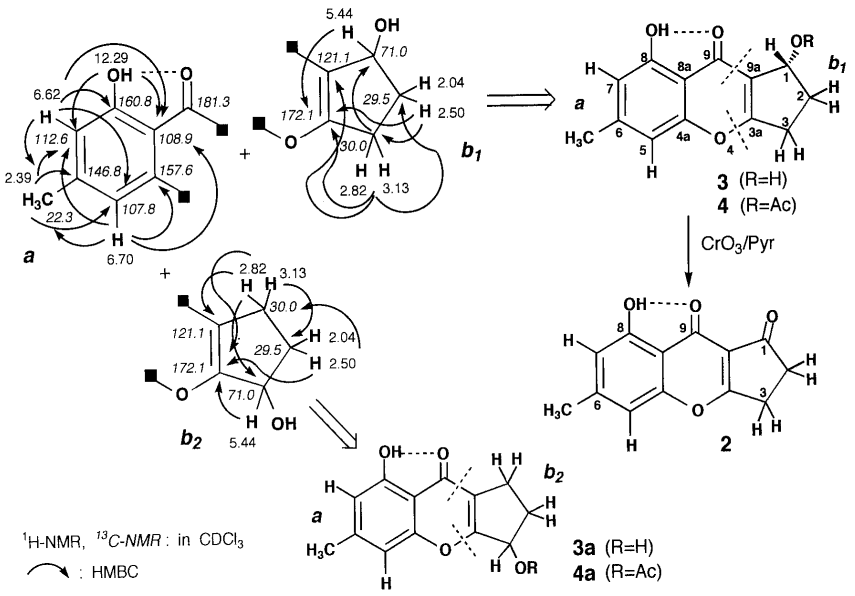

Chart 1

group, coniochaetones A and B, have been isolated as antifungal components from *Coniochaeta saccardoi* by Gloer *et al.*[5] A comparison of the physicochemical and spectral data of coniochaetones A and B described in the literature[5] with those of CT-2 and -3 indicated that our compounds are identical with coniochaetones A and B, respectively (see Table 1 and Experimental). However, some uncertainty remained concerning the position of the >CH–OH group in coniochaetone B or the >C=O group in coniochaetone A from the spectral data in the literature,[5] so we decided to confirm the position of the >CH–OH group in CT-3 by investigating the ^{13}C-NMR data of CT-3 acetate (using the acetylation shift rule[6]) and by chemical correlation between CT-3 and CT-2, as described below.

On acetylation with Ac_2O/pyridine, **3** provided a monoacetate (**4**). The ^{1}H- and ^{13}C-NMR spectra of CT-3 monoacetate showed that the >CH–OH group of CT-3 was acetylated to give **4** or **4a** (in Chart 1). Comparison of the ^{13}C-NMR spectrum of CT-3 monoacetate with that of CT-3 indicated that the signals of the α-, β_1-, and β_2-carbons to the acetoxyl (C-1, -2, and -9a) are shifted to δ 73.2 (+2.2), 28.2 (−1.3), and 117.5 (−3.6), respectively, suggesting, in accordance with the acetylation shift rule,[6] that the structure of CT-3 monoacetate should be **4**. On the other hand, if the structure of CT-3 monoacetate is postulated to be **4a**, the acetylation shift of the signals of α-, β_1-, and β_2-carbons to the acetoxyl (C-3, -2, and -3a) should be 73.2 (+2.2), 28.2 (−1.3), and 173.7 (+1.6), respectively. Accordingly, the structure of CT-3 was confirmed to be **3**. Successively, the structure of CT-2 was confirmed to be **2** from the fact that **3** gave **2** on CrO_3/pyridine oxidation (see Chart 1). These results showed that the structures of coniochaetones A (**2**) and B (**3**) presented by Gloer *et al.*[5] are indeed identical with those of CT-2 and -3. The absolute configuration at position 1 in CT-3 may be (*R*), because the configuration at position 1 in coniochaetone B has been deduced to be (*R*) by application of Horeau's method to coniochaetone B[5] and the $[\alpha]_D$ value of CT-3 is similar to that of coniochaetone B (+84.0°).[5]

Compound CT-1 (**1**), yellow solid, $C_{17}H_{13}ClO_6$, $[\alpha]_D^{24}$ 0°, was considered to be a new chlorinated phenolic compound from its physicochemical properties. The ^{1}H- and ^{13}C-NMR spectral data, including spin-decoupling ^{1}H-NMR and two-dimensional ^{1}H–^{1}H COSY, and ^{13}C–^{1}H COSY NMR indicated that **1** may be composed of three partial structures, *a*—*c*. Considering the ^{13}C–^{1}H correlation spectroscopy *via* long-range coupling (COLOC) NMR with J_2 and/or J_3, and differential nuclear Overhauser effect (diff.NOE) NMR data (see Chart 2) and likely biogenesis, a possible *seco*-anthraquinone structure **1** was constructed for CT-1, as shown in Chart 2.

Cyclopentabenzopyran-4-one compounds such as coniochaetones A and B (CT-2 and -3) are naturally rare, though benzopyran-4-ones (chromones) are widely distributed as fungal metabolites.[7] The IC_{50} value of CT-2 (**2**) against mouse liver MAO was obtained as 2.9×10^{-5} M. In contrast, CT-3 (**3**) inhibited MAO by only 19% even at 1.0×10^{-4} M, and CT-1 (**1**) showed no inhibition at 1.0×10^{-4} M. A comparison of the IC_{50} value of **2** with those of MAO-inhibitory components which we had previously isolated from fungi, such as norsolorinic acid (**5**),[1] luteusins A (TL-1) (**6**) and B (TL-2) (**7**),[2] helicusins A—D (**8**—**11**),[3] and GP-A (**12**) and -B (**13**),[4] indicated the following MAO inhibitory order: an anthraquinone **5** > two dioxonaphthofurans **13**, **12** > two azaphilones **6**, **7** > a cyclopentabenzopyran-4-one **2** > four ester-type azaphilones **8**—**11**.

Experimental

The general procedures for the chemical experiment were the same as described in our preceding report.[2c]

Isolation of CT-1 (1), -2 (2), and -3 (3) *C. tetraspora* IFM 4660[8] was cultivated on sterilized rice (200 g/flask × 123) at 25 °C for 32 d. The moldy rice was extracted with AcOEt (30 l × 2) to give an extract (71.3 g), which was partitioned with *n*-hexane–H_2O (1 : 1) (2.4 l) into *n*-hexane-soluble (fatty) and -insoluble portions. The *n*-hexane-insoluble portion was further partitioned with AcOEt–H_2O (1 : 1) (2.4 l) into defatted AcOEt-soluble and aqueous portions. The defatted AcOEt-soluble

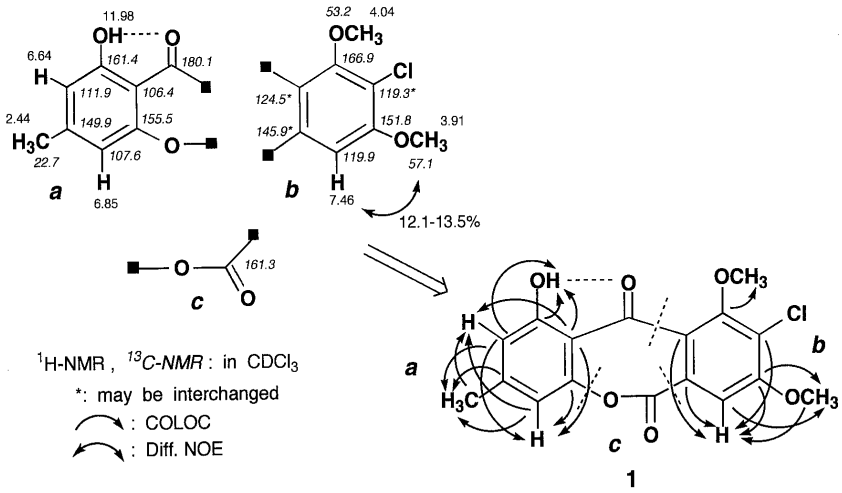

Chart 2

portion inhibited mouse liver MAO by 48% at 1.0×10^{-4} g/ml, but the other portions did not inhibit the MAO. The defatted AcOEt-soluble portion (7.4 g) was subjected to chromatography on a silica gel column to give seven fractions I—VII. Fraction V, which was eluted with *n*-hexane–acetone (1 : 1), inhibited the MAO by 32% at 1.0×10^{-5} g/ml. Fraction V (2.64 g) was further chromatographed on a silica gel column to give five fractions Va—e. Fraction Va, eluted with $CHCl_3$, was treated with $CHCl_3$ to afford a yellow powder (**1**) (163 mg). Fraction Vd, which was eluted with $CHCl_3$, inhibited the MAO by 56—60% at 1.0×10^{-5} g/ml. Fraction Vd was then chromatographed on a silica gel column to give four fractions Vd1—4. Fraction Vd3, eluted with *n*-hexane–AcOEt (1 : 2), was subjected to high-performance liquid chromatography (HPLC) on an Aquasil column (Senshu, 8 mm i.d. × 250 mm) with $CHCl_3$–MeOH–H_2O (2000 : 10 : 1) at a flow rate of 2.5 ml/min to give a solid (74 mg), which was recrystallized with $CHCl_3$ to afford white needles (**2**). Fraction Vd2, eluted with *n*-hexane–AcOEt (2 : 1), was further chromatographed repeatedly on octadecyl silica gel (ODS) columns with CH_3CN–H_2O (1 : 1) and MeOH–H_2O (1 : 1) to give a residue (27 mg), which was treated with EtOH to afford a white solid (**3**).

CT-1 (**1**): mp 209—211 °C. HREI-MS *m/z* 348.0388 [$C_{17}H_{13}ClO_6$ requires 348.0399 (M^+)]. IR ν_{max}^{KBr} cm^{-1}: 3450, 1740, 1660, 1630, 1485, 1290, 1255, 1100. UV λ_{max}^{EtOH} nm (log ε): 240 (4.46), 265 (4.50), 298 (3.96), 388 (3.77). ^{1}H-NMR ($CDCl_3$): δ 2.44, 3.91, 4.04 (each 3H, s), 6.64, 6.85 (each br s), 7.46, 11.98 (each s). ^{13}C-NMR ($CDCl_3$): δ 22.7, 53.2, 57.1 (each q), 106.4 (s), 107.6, 111.9 (each d), 119.3 (s), 119.9 (d), 124.5, 145.9, 149.9, 151.8, 155.5, 161.4, 161.4, 166.9, 180.1 (each s). CT-2 (**2**): mp 175.5—177.5 °C (dec.) [lit.[5] 175 °C (dec.)]. HREI-MS *m/z* 230.0588 [$C_{13}H_{10}O_4$ requires 230.0579 (M^+)] (lit.[5] *m/z* 230.0579). IR ν_{max}^{KBr} cm^{-1}: 3400, 1715, 1650, 1605, 1450. UV λ_{max}^{MeOH} nm (log ε): 212 (4.26), 248 (4.30), 327 (3.58). CT-3 (**3**): mp 143.5—144.5 °C (dec.) [lit.[5] 148 °C (dec.)], $[\alpha]_D^{23.5}$ +95.6° (*c*=0.11, MeOH) [lit.[5] +84.0° (*c*=0.10, MeOH)]. HRFAB-MS *m/z* 233.0813 {$C_{13}H_{13}O_4$ requires 233.0814 [$(M+H)^+$]}. IR ν_{max}^{KBr} cm^{-1}: 3370, 1655, 1625, 1455. UV λ_{max}^{MeOH} nm (log ε): 229 (sh, 4.28), 239 (4.32), 258 (sh, 4.13), 326 (3.64).

Formation of CT-3 Monoacetate (4) from CT-3 (3) A solution of **3** (9.7 mg) in Ac_2O (15 μl) and pyridine (30 μl) was allowed to stand at room temperature for 45 min, then worked up as usual to give a product mixture, which was passed through a silica gel column with $CHCl_3$ to afford **4** (9.1 mg), white solid, mp 53.0 °C (dec.). $[\alpha]_D^{24}$ +77.3° (*c*=0.066, $CHCl_3$). EI-MS *m/z* (%): 274 (52, M^+), 231 (98), 213 (93), 203 (52). UV λ_{max}^{MeOH} nm (ε): 229 (4.19), 239 (4.24), 257 (sh, 4.09), 326 (3.53).

Oxidation of CT-3 (3) to Afford CT-2 (2) A solution of **3** (11.5 mg) in pyridine (0.2 ml) was added to a mixture of CrO_3 (50 mg) and pyridine (0.3 ml) under ice-cooling and the resultant mixture was stirred at 32 °C for 14 h. The product mixture was treated with ice-water and extracted with $CHCl_3$. Evaporation of the solvent gave a residue, which was passed through a silica gel column with *n*-hexane–AcOEt (1 : 1) to give a solid (5.0 mg), which was identical with **2** in terms of the ^{1}H-NMR spectrum and the thin layer chromatographic behavior [plate: Merck Kieselgel $60F_{254}$, solvent: *n*-hexane–AcOEt (2 : 1), identification: UV at 254 nm and 5.0% $FeCl_3$–EtOH, *Rf*: 0.27].

Bioassay The measurement of MAO-inhibitory activity and the calculation of IC_{50} value were carried out in the same manner as described in our previous reports.[1,2a]

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