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TETRAHEDRON

Structures of TMC-120A, B and C, Novel Isoquinoline Alkaloids from *Aspergillus ustus* TC 1118

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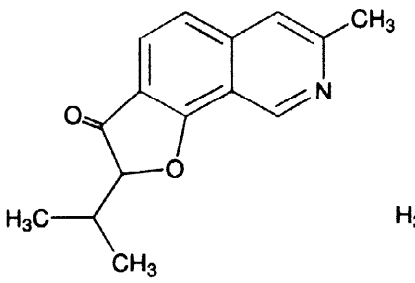
Abstract: Three novel isoquinoline alkaloids, TMC-120A, B and C (**1**-**3**) have been isolated from a fermentation broth of *Aspergillus ustus* (Bain.) Thom & Church TC 1118, a fungus isolated from rhizosphere of grass. Their structures were determined through extensive spectroscopic analyses and chemical studies. TMC-120s (**1**-**3**) are found to be the first furo[3,2-*h*]isoquinoline-type alkaloids isolated from natural sources.

Keywords: TMC-120A, B and C; furo[3,2-*h*]isoquinoline; alkaloid; *Aspergillus ustus*

TMC-120A(**1**), B(**2**) and C(**3**) are novel isoquinoline alkaloids produced by *Aspergillus ustus* (Bain.) Thom & Church TC 1118, discovered as inhibitors of interleukin-5 mediated eosinophil viability. In this paper, we report the structure determination of TMC-120A, B and C. The production, isolation and biological activities will be reported in a separate paper.[1]



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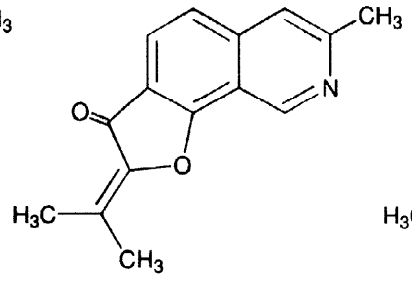

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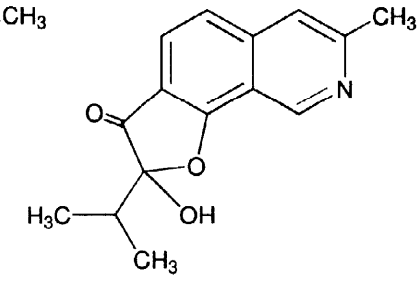

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Results

TMC-120A(**1**) had the molecular formula of $C_{15}H_{15}NO_2$, determined by its high resolution electron spray ionization (HRESI)-MS [*m/z* found 240.1017 $(M-H)^-$, calcd 240.1024], and ^{13}C and ^1H NMR spectral data. The ^{13}C and ^1H NMR data of **1** obtained from DEPT, HMQC and HMBC spectra are shown in Table 1. The ^{13}C NMR spectrum displayed three methyl, one methine, one oxygenated methine, nine aromatic sp^2 and one carbonyl carbons. The DQF-COSY and HMBC experiments clarified the connectivity of the nine sp^2 carbons and the four sp^2 protons of the aromatic moiety as shown in Figure 1. 3-Methyl isoquinoline nucleus was demonstrated by the following observations, together with the presence of a nitrogen: i) the characteristic large coupling constant $^1J_{C\text{-}9,H\text{-}9}$ = 181.8 Hz, ii) the HMBC correlation from the low field proton H-9 (δ 9.56) to C-5a, C-9a and C-9b, iii) the correlation from H-9 and the methyl proton H-10 (δ 2.76) to C-7 (δ 157.5).

Table 1. The ^{13}C and ^1H NMR Data of **1-3** in $CDCl_3$.

	^{13}C			^1H		
Position	**1**	**2**	**3**	**1**	**2**	**3**
2	91.5 d	145.6 s	110.3 d	4.68 (1H, d, 3.9)	---	---
3	199.6 s	182.1 s	198.5 s	---	---	---
3a	117.5 s	119.3 s	115.6 s	---	---	---
4	123.9 d	124.1 d	124.3 d	7.72 (1H, d, 8.5)	7.80 (1H, d, 8.5)	7.53 (1H, d, 8.3)
5	120.1 d	120.5 d	119.4 d	7.31 (1H, d, 8.5)	7.35 (1H, d, 8.5)	6.99 (1H, d, 8.3)
5a	142.3 s	141.3 s	142.0 s	---	---	---
6	119.5 d	119.5 d	119.4 d	7.54 (1H, s)	7.52 (1H, s)	7.14 (1H, s)
7	157.5 s	156.7 s	156.8 s	---	---	---
9	146.6 d	146.2 d	146.5 d	9.56 (1H, s)	9.52 (1H, s)	9.49 (1H, s)
9a	115.2 s	114.6 s	114.6 s	---	---	---
9b	173.9 s	164.0 s	171.5 s	---	---	---
10	24.7 q	24.7 q	23.8 q	2.76 (3H, s)	2.74 (3H, s)	2.51 (3H, s)
11	31.1 d	133.7 s	33.9 d	2.48 (1H, m)	---	2.42 (1H, m)
12	15.7 q	17.5 q	[a]16.0 q	0.94 (3H, d, 6.9)	2.43 (3H, d, 0.7)	0.99 (3H, d, 6.3)
13	18.8 q	20.4 q	[a]15.6 q	1.25 (3H, d, 6.9)	2.25 (3H, d, 0.7)	1.24 (3H, d, 6.6)
OH	---	---	---	---	---	7.75 (1H, brs)

[a] may be exchangeable.

The correlation from the aromatic proton H-4 (δ 7.72) and the oxygenated methine proton H-2 (δ 4.68) to the carbonyl carbon C-3 (δ 199.6) indicated that the 1-methylethyl unit

was connected to the isoquinoline unit at C-3 through the carbonyl group. In addition , the correlation from H-2 to the aromatic carbon C-9b (δ 173.9) suggested that the 1-methylethyl unit was also linked by ether bond with the isoquinoline unit at C-9b. The structure of **1** was thus established to be 7-methyl-2-(1-methylethyl)-furo[3,2-*h*]isoquinoline-3-one. In spite of our utmost efforts, the absolute configuration at C-2 of **1** has not yet been ascertained, up to the present.

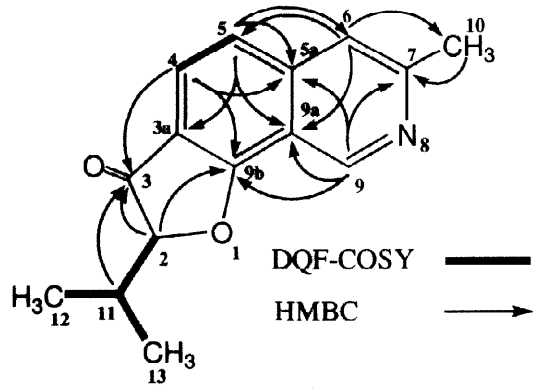

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Figure 1. 2D NMR Experiments for **1**.

The molecular formula of **2** ($C_{15}H_{13}NO_2$, HRESI-MS: *m/z* found 238.0904 $(M-H)^-$, calcd 238.0868) suggested that **2** was the didehydro analog of **1**. Two olefinic carbons C-2 (δ 145.6) and C-11 (δ 133.7) in **2** were observed in place of the corresponding methine carbons (δ 91.5 and 31.1) in **1**. Moreover, the carbonyl carbon C-3 (δ 182.1) in **2** was shifted to higher field relative to that of **1** (δ 199.6), suggesting the presence of the conjugated carbonyl group. Compound **2** is thus presumed to be a C-2, 11-didehydro analog of **1**. Finally, the single crystal X-ray analysis has confirmed unambiguously the structure of **2** (Figure 2).

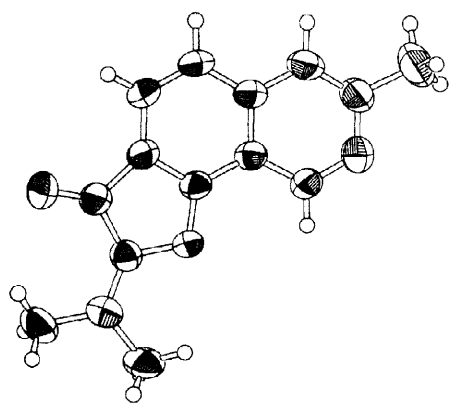

Figure 2. *ORTEP* II diagram of **2**.

The hydrogenation of **2** over palladium on charcoal gave racemic **1**, which further confirmed the planner structure of **1**.

The molecular formula of **3** ($C_{15}H_{15}NO_3$, HRESI-MS: *m/z* found 256.0977 $(M-H)^-$, calcd 256.0974) differed from that of **1** by an oxygen. The ^{13}C NMR data of **3** corresponded well to those of **1**, except for the carbon at C-2 and C-13. The quaternary carbon C-2 (δ 110.3) was observed in place of the corresponding oxygenated methine carbon (δ 91.5) in **1**. The signal of the methyl carbon at C-13 was shifted higher field relative to that of **1** by an additional γ-*gauche* effect of hydroxyl oxygen. These results gave us the structure of **3** to be a C-2-hydroxy analog of **1**, and is may be racemic compound ($[\alpha]_D^{26}$ 0° (*c* 0.214, methanol)).
TMC-120s are simple but unusual isoquinoline alkaloids. To our knowledge, 5-methyl-2,3-dimethyl-9-phenylfuro[3,2-*h*]-isoquinoline, chemically synthesized, was the only compound to have furo[3,2-*h*]isoquinoline moiety.[2] TMC-120s are thus the first furo[3,2-*h*]isoquinoline-type alkaloids isolated from natural sources.

Experimental Section

General Methods.

Rf values were determined with Kieselgel 60 F_{254} TLC glass plates (E. Merck, Darmstadt, Germany) in (A) CH_2Cl_2/MeOH (98:2) and (B) *n*-hexane/EtOAc (2:1). TLC spots were visualized by exposure to UV light at 254 nm or to an ammonium molibdate/H_2SO_4 spray reagent. Melting points were obtained using a Yanagimoto MP-2S micro melting apparatus and were uncorrected. Optical rotations were determined using the sodium D line on a Horiba model SEPA-200 high sensitive polarimeter in methanol at 23°C. UV spectra were measured on a Shimadzu model UV-2200A spectrophotometer in methanol. IR spectra were recorded on a JASCO model 100 infrared spectrophotometer. The samples were prepared and mounted as KBr micropellets. All mass spectra were obtained using Mstation 700 tandem type mass spectrometer (JEOL, Japan) equipped with an electrospray ionization source. Analytical HPLC were obtained using a HP1100 system (Hewlett Packard, USA). ^{13}C and ^{1}H NMR spectra were recorded on a JEOL GSX-400 NMR spectrometer at 30°C. Samples were dissolved in $CDCl_3$. The chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard. DQF-COSY, HMQC and HMBC spectra were obtained using standard pulse sequences. DQF-COSY spectrum was recorded in phase-sensitive mode. The HMQC and HMBC experiments were optimized for $^{1}J_{CH}$ = 145 Hz and $^{n}J_{CH}$ = 8.2 Hz, respectively. Typically 2048 x 128 data points were acquired and zerofilling was used in the t1 domain to 2048 points.

TMC-120A (1): (S)-7-methyl-2-(1-methylethyl)-furo[3,2-h]isoquinoline-3-one

Pale yellow solid; *Rf* 0.30 (A) and 0.37 (B); mp. 115 ~ 116°C; $[\alpha]_D^{23}$ -18° (*c* = 0.505, MeOH); UV λ_{max} 354 (4.11), 340 (4.05), 323 (sh, 3.85), 250 (4.53), 210 (4.50) nm (log ε); IR ν_{max} 3050, 3025, 2990, 2960, 2925, 2870, 1700, 1630, 1590, 1565, 1500, 1455, 1420, 1400, 1380, 1365, 1355, 1320, 1285, 1230, 1220, 1160, 1100, 1090, 1060, 965, 960, 925, 900, 875, 820, 810, 745 cm^{-1}; ESI-MS *m/z* 242 $(M+H)^+$, 240 $(M-H)^-$, 186, 158, 130; HRESI-MS *m/z* found 240.1017 $(M-H)^-$, calcd. for $C_{15}H_{14}NO_2$: 240.1024; ^{13}C and ^1H NMR data, see Table 1.

TMC-120B (2): 7-methyl-2-(1-methylethylidene)-furo[3,2-h]isoquinoline-3-one

Slightly pale yellow needles; *Rf* 0.28 (A) and 0.28 (B); mp. 176 ~ 177°C; UV λ_{max} 365 (3.89), 302 (4.33), 295 (sh, 4.33), 271 (4.37), 239 (4.11), 233 (4.11), 212 (4.54) nm (log ε); IR ν_{max} 1690, 1650, 1615, 1570, 1500, 1450, 1425, 1410, 1400, 1365, 1340, 1305, 1280, 1240, 1175, 1160, 1095, 920, 895, 865, 835, 795, 745 cm^{-1}; ESI-MS *m/z* 240 $(M+H)^+$, 238 $(M-H)^-$, 212, 197, 186, 168, 158, 130; HRESI-MS *m/z* found 238.0904 $(M-H)^-$, calcd. for $C_{15}H_{12}NO_2$: 238.0868; ^{13}C and ^1H NMR data, see Table 1.

TMC-120C (3): 2-hydroxy-7-methyl-2-(1-methylethyl)-furo[3,2-h]isoquinoline-3-one

Slightly pale yellow needles; *Rf* 0.08 (A) and 0.16 (B); mp. 176 ~ 177°C (dec.); $[\alpha]_D$ 0° (*c* = 0.511, MeOH); UV λ_{max} 413 (sh, 3.05), 354 (4.01), 253 (4.50), 211 (4.51) nm (log ε); IR ν_{max} 1720, 1625, 1565, 1455, 1420, 1380, 1285, 1265, 1200, 1165, 1105, 1085, 935, 910, 880, 860, 800, 730 cm^{-1}; ESI-MS *m/z* 258 $(M+H)^+$, 256 $(M-H)^-$, 212, 197, 186, 160, 130; HREI-MS *m/z* found 256.0977 $(M-H)^-$, calcd. for $C_{15}H_{14}NO_3$: 256.0974; ^{13}C and ^1H NMR data, see Table 1.

Hydrogenation of 2

Compound **2** (39.0 mg, 0.163mmol) in EtOAc (4.0 ml) was hydrogenated with hydrogen over 10% palladium on charcoal (10 mg) for 2.5 hours. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The resulting residue (29.1 mg) was purified by preparative HPLC (55% aqueous CH_3CN). Active fractions were combined and concentrated to dryness *in vacuo* to afford 14.1 mg (35.9 %) of the dihydro derivative as pale yellow solid: $[\alpha]_D^{26}$ 0° (*c* 0.214, MeOH); *Rf* 0.30 (A) and 0.37 (B); UV λ_{max} 354 (4.11), 340 (4.06), 323 (sh, 3.85), 250 (4.52), 210 (4.49) nm (log ε); ESI-MS *m/z*, 242 $(M+H)^+$, 186, 158, 130; ^{13}C and ^1H NMR data were consistent with the data of **1**.

X-ray Crystallography of 2

A colorless needle crystal of **2** with dimensions 0.40 x 0.10 x 0.10 mm was obtained by recrystallization from methanol and used for X-ray analysis. The intensity data were collected on a Rigaku AFC5R difractometer by using graphic-monochromated Cu-Kα(λ=1.5418Å) radiation by 2θ/ω scan technique. Unit cell dimensions were determined by a least squares refinement by using the setting of 25 reflections in the range of $70°<2\theta<90°$. The crystallographic data are summarized as follows: $C_{15}H_{13}NO_2$, Mr=239.26, needle, orthorhombic, space group $Pna2_1$, a=20.037Å (1), b=18.474Å (2), c=6.661Å (3), V=2465.6(11)Å^3, Z=8, Dcalc=1.29g/cm^3, Mu=0.691mm^{-1}. The intensities of 5862 reflections with $3.3°<\theta<65.1°$, $0<h<22$, $-6<k<21$, $-7<l<7$ were measured. Three standard reflections were monitored every 200 reflection intervals and showed insignificant fluctuations. The data were corrected for Lorentz and polarization effects, but not for absorption. The structure was solved by a direct method by using SHELXS-97[3] and the subsequent difference Fourier method. The structure refinement on F^2 was carried out by a SHELXL-97[4] with anisotropic thermal parameters for all of non-hydrogen atoms. The hydrogen atoms were refined by riding with the atoms to which they were bonded. The full matrix least squares refinement varied 326 parameters and used all 4178 independent reflections weighted by $\omega=1/[\sigma^2(Fo^2)+(0.1000P)^2+0.0000P]$ where $P=(Fo^2+2Fc^2)/3$. Final $R1$=0.078, $wR2$=0.150 and *Goodness of Fit*(S)=0.89 for all data; $R1$=0.044 for 2743 reflections with $I>2(I)\sigma$. The final difference Fourier map showed maximum and minimum values of 0.13 and -0.13 e/Å^3, respectively.

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