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Torrubiellutins A–C, from insect pathogenic fungus *Torrubiella luteorostrata* BCC 12904

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ABSTRACT

Investigation of the insect pathogenic fungus *Torrubiella luteorostrata* led to the isolation of three new macrocyclic torrubiellutins A–C (**1**–**3**) and the known pyrone diterpene **4**. Structures were elucidated by spectroscopic data including 1D, 2D NMR, and MS spectral data. The absolute stereochemistry was determined by chemical means using Mosher reactions and Marfey's reagent, together with NOESY spectral data. Torrubiellutin C showed biological activities against KB, MCF-7, NCI-H187, and Vero cell lines with IC_{50} varying from 0.78 to 4.36 μg/mL, while compound **4** exhibited antimalaria and anti-inflammatory activity with IC_{50} values of 3.49 and 1.21 μg/mL, respectively.

1. Introduction

Insect fungi have consistently been shown to be a good source of bioactive metabolites.[1] Several compounds isolated from insect fungi in our BIOTEC Culture Collection were described together with their biological activities. As a part of our on-going search for bioactive substances from microorganisms, the crude extracts of the insect fungus *Torrubiella luteorostrata* BCC 12904 exhibited biological activity against human breast cancer (MCF-7) and human epidermoid carcinoma (KB) with IC_{50} values of 1.03–10.37 and 1.27–15.52 μg/mL, respectively. Further investigation led to the isolation of new macrocyclic torrubiellutins A–C (**1**–**3**), and the pyrone diterpene **4**. Biological activity of isolated compounds was also evaluated.

2. Results and discussion

The extract obtained from culture broth was subjected to Sephadex LH20 chromatography followed by either silica column or reversed-phase HPLC to afford pure torrubiellutins A (**1**) and B (**2**). In addition, purification of the mycelium extract yielded zeorin [$C_{30}H_{52}O_2$, 215–217 °C],[2] torrubiellutins B (**2**) and C (**3**), and pyrone diterpene (**4**).

Compound **1**, obtained as a colorless solid, the molecular formula of which was determined as $C_{26}H_{37}NO_5$ based on HRESIMS showing *m*/*z* peak at 466.2577 $[M+Na]^+$, Δ +1.3 mmu. ^{13}C NMR spectrum together with DEPT-135 spectral data gave 24 signals of 6 methyl, 1 methylene, 13 methine, and 4 quaternary carbons. Two aromatic methine signals at δ_C 128.2 and 129.3 contained two carbons each. COSY and HMBC spectra gave, respectively, ^1H–^1H and ^1H–^{13}C correlations as shown in Table 1. The spectral information gave the partial structures from C-1 to C-11 and from C-14 to aromatic ring. The correlation of methyl at δ_H 2.79 to the C-1 in HMBC spectrum indicated an amide linkage. In addition, the methine at H-11 (δ_H 5.05), suggesting a connection to an oxygen, together with the remaining carbon at δ_C 170.4 (C-13) led to the chemical structure of compound **1**, named as torrubiellutin A. Torrubiellutin A (**1**) is a macrocyclic compound containing an amino acid unit. The configuration of amino acid, *N*-methyl-phenylalanine (*N*MePhe), was determined by acid hydrolysis of **1** with 6 N HCl and then derivatization with Marfey's reagent (FDAA).[3,4] The sample was co-injected with L-FDAA derivatives of standard *N*MePhe (D- and L-forms) and analyzed by reversed-phase HPLC. The result indicated the L-form of *N*MePhe. The molecule has stereogenic centers at C-4, C-5, C-8, C-9, C-10, and C-11. The absolute configurations at C-5 and C-9 were assigned by employing the Mosher method.[5] Treatment of **1** with *R*- and *S*-MTPACl afforded compounds **5** and **6**, respectively. The differences in chemical shift values ($\Delta\delta_{R-S}$) of diester derivatives **5b** and **6b** (given in Fig. 1) were

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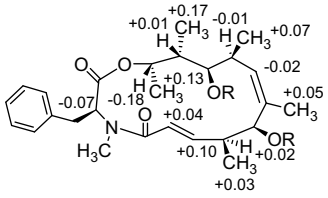
Table 1
1D and 2D NMR assignment of torrubiellutin A in acetone-d_6

No.	δ_H (int., mult., J in Hz)[a]	δ_C[b]	COSY	HMBC (H→C#)
1	—	166.5	—	—
2	6.28 (1H, d, 15.0)	119.2	—	1, 4
3	7.07 (1H, dd, 15.0, 10.3)	149.1	—	1
4	2.68–2.71 (1H, m)	39.5	H-5	18
5	3.94 (1H, d, 5.1)	77.7	—	6, 7
6	—	136.0	—	—
7	5.66 (1H, dd, 11.0, <1)	122.8	—	5, 19
8	2.58–2.62 (1H, m)	34.2	H-7	—
9	3.15 (1H, ddd, 11.0, 9.3, 1.1)	76.4	—	7
10	1.56–1.58 (1H, m)	41.3	—	—
11	5.05 (1H, dq, 6.5, 3.7)	72.6	—	—
13	—	170.4	—	—
14	3.74 (1H, dd, 10.5, 4.4)	67.7	—	—
16	3.24 (1H, dd, 13.8, 4.4), 3.33 (1H, dd, 13.8, 10.5)	35.2	H-14	1′, 2′, 6′
17	2.79 (3H, s)	38.2	—	1
18	1.18 (3H, d, 7.0)	18.8	H-4	3, 4, 5
19	1.56 (3H, d, 0.9)	14.5	—	5, 6, 7
20	1.01 (3H, d, 6.8)	17.8	H-8	7, 8, 9
21	0.69 (3H, d, 6.8)	9.3	H-10	9, 10, 11
22	1.10 (3H, d, 6.5)	12.6	H-11	10, 11
1′	—	139.6	—	—
2′/6′	7.19–7.30 (2H, m)	129.3×2	H-3′, H-5′	4′, 16
3′/5′	7.19–7.30 (2H, m)	128.1×2	H-2′, H-4′, H-6′	1′
4′	7.19–7.30 (1H, m)	126.1	—	—

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.

calculated in order to assign the absolute configurations at C-5 and C-9 as *S*- and *R*-, respectively. Due to the limited amount of compound **1**, the absolute configurations of C-4, C-8, C-10, and C-11 were determined by NOESY spectral data and coupling constant values (J). In the presence of D_2O, H-5 and H-9 appeared as a singlet and double doublet (J=11.0, <1 Hz), respectively. The former indicated no coupling between H-4 and H-5. In addition, the conformation of H-4 and H-5 was restricted by the configuration of two olefins at C-2 and C-6. The olefinic proton at H-2 coupled to H-3 with a coupling constant of 15.0 Hz indicated a *trans*-configuration and the cross-peak correlation showing in NOESY spectrum between H-7 and H_3-19 indicated a *cis*-configuration of the olefin at C-6. Moreover, the NOESY spectral data displayed cross-peak correlations from H-2 to H-4; H-3 to H-10; H-4 to H_3-19, suggesting *R*-configuration at C-4. The double doublet of H-9 (J=11.0, <1 Hz) was changed to a doublet with a coupling constant of 11.0 Hz after irradiation at H-8. Thus, it was implied that the coupling constant between H-9 and H-10 is 11.0 Hz and that between H-8 and H-9 is small (J<1 Hz). Together with the cross-peak in NOESY spectrum, which correlated H-9 to H_3-21; H-8 to H_3-19 and H_3-21, this indicated an *anti*-relationship between H-9 and H-10 and a *syn*-relationship between H-8 and H-9. The absolute configurations at C-8 and C-10 can therefore be assigned as *S*- and *R*-, respectively. The coupling constant agreed with the dihedral angle of 95.2° between H-8 and H-9 as calculated from the most favorable conformation shown in Figure 2. The absolute configuration at C-11 was assigned as *S*- based on the evidence from NOESY correlation between H-3 and H-10 and the coupling constant of 3.7 Hz between H-10 and H-11. The dihedral angle of −85.8° was also calculated based on the conformation shown in Figure 2. Therefore, six stereogenic centers can now be assigned as 4*R*, 5*S*, 8*S*, 9*R*, 10*R*, and 11*S*, respectively. The chemical structure of torrubiellutin A (**1**) can be depicted as shown in Figure 3.



5b) $R^1 = R^2$ = *S*-MTPA
6b) $R^1 = R^2$ = *R*-MTPA

Figure 1. $\Delta\delta$ values ($\Delta\delta=\delta_R-\delta_S$) obtained from **5b** and **6b**.

Compound **2**, a colorless solid, gave similar ^{1}H NMR and ^{13}C NMR spectral data to compound **1**. The ^{1}H NMR spectrum displayed two additional methyl protons at δ_H 2.11 and 2.16, which correlated in the HMBC spectrum to the carbonyl esters at δ_C 170.26 and 169.20, respectively. The data indicated the presence of two additional acetyl groups in the molecule. Also the lower field shift of two methine protons at δ_H 4.72 (H-9) and 5.07 (H-5) ($\Delta\delta$=1.57 and 1.13 ppm, respectively) suggested the two acetyl groups being substituted at H-9 and H-5, respectively. HRESIMS confirmed the molecular formula $C_{30}H_{41}O_7N$ showing m/z peak at 550.2784 $[M+Na]^+$, Δ +0.9 mmu. The methine protons at H-5 and H-9 also appeared as a singlet and a double doublet (J=11.5 and 2.4 Hz), respectively. Irradiation at H-10 resulted in a coupling constant of 2.4 Hz, suggesting a *gauche* conformation between H-8 and H-9. The result indicated an angle distortion of H-8 and H-9 in order to avoid steric effect from the acetyl group. The cross-peak correlation from H-9 to H_3-20 was also observed in NOESY spectrum. Therefore, the chemical structure of compound **2**, named as torrubiellutin B, was established as shown in Figure 3. Although, torrubiellutin B was isolated as a major product, it could not be obtained as a single crystal for X-ray crystallographic study.

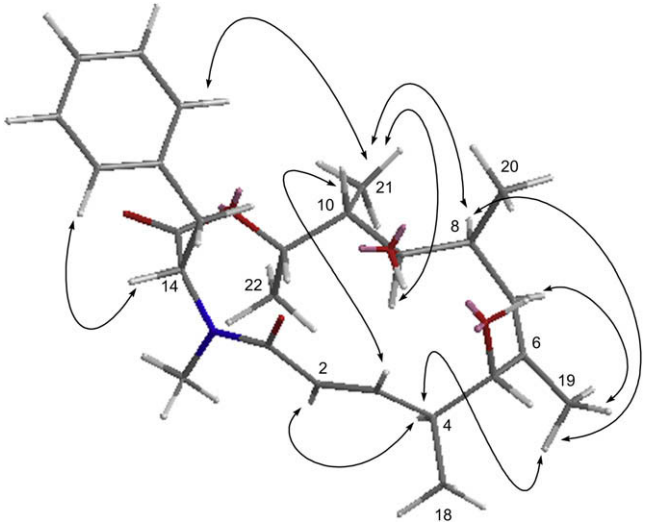


Figure 2. 3D structural conformation of torrubiellutin A generated by MM2 force field calculations for energy minimization from modeling program Chem3D Ultra 9.0 with the observed NOE correlations (arrows).

Compound **3**, named as torrubiellutin C, was obtained as a colorless solid. ^{1}H NMR and ^{13}C NMR spectral data are similar to those of **1** except for an extra methyl group at δ_H 2.17, which correlated in HMBC to the carbonyl ester at δ_C 171.27. This indicated the presence of an acetyl group in the molecule. HRESIMS determined the molecular formula $C_{28}H_{39}O_6N$, showing m/z peak at 508.2669 $[M+Na]^+$, Δ −0.6 mmu. HMBC spectral data showed correlations from H-9 to C-6; H_3-18 to C-3, C-4 and C-5; H_3-19 to H-5 and H-6; H_3-20 to H-7 and H-8. The lower field shift of H-9 at δ_H 4.77 ($\Delta\delta$=1.62 ppm) indicated the presence of an acetyl group at C-9. Coupling patterns of H-5 and H-9 were also similar to those of compound **2**. H-5 appeared as a singlet and H-9 appeared as