The Journal of Antibiotics (2010) 63, 371–373

www.nature.com/ja

NOTE

New tensidols, JBIR-86 and JBIR-87, isolated from *Aspergillus* sp. fJ80

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The Journal of Antibiotics (2010) **63,** 371–373; doi:10.1038/ja.2010.45; published online 12 May 2010

Keywords: *Aspergillus*; *Candida albicans*; furopyrrole; miconazole; tensidol

Aspergillus is a member of the phylum Ascomycota. There are over 185 known species of ascomycetes, about 20 of which are known to be harmful to humans and other animals. The *Aspergillus* species is known to produce more than 1000 secondary metabolites containing bioactive compounds, such as mevinolin, aflatoxin and citrinin.[1] In a previous study, we reported that a fungus belonging to the *Aspergillus* species produces a novel secondary metabolite, JBIR-15.[2] Therefore, in this study, we screened for novel secondary metabolites from cultures of *Aspergillus* species and succeeded in discovering novel furopyrrole compounds, JBIR-86 (**1**) and JBIR-87 (**2**), in addition to the known compounds, tensidols A and B (Figure 1a). In this study, we report the fermentation, isolation and structural determination of **1** and **2**, and briefly describe their biological activities.

Aspergillus sp. fJ80 was isolated from a soil sample collected in Akita Prefecture, Japan. The strain was cultivated in 50 ml test tubes containing 15 ml of the seed medium (24 g l^{-1} potato dextrose broth; BD Biosciences, San Jose, CA, USA). The test tubes were shaken on a reciprocal shaker (355 r.p.m.) at 27 °C for 3 days. Aliquots (5 ml) of the seed culture were inoculated into 500 ml Erlenmeyer flasks containing the production medium (15 g brown rice and 45 ml solution containing 0.67 g l^{-1} yeast extract, 0.33 g l^{-1} sodium tartrate dihydrate and 0.33 g l^{-1} K_2HPO_4) and incubated in static culture at 27 °C for 14 days.

The production culture (10 flasks) was extracted with 80% aqueous Me_2CO (1 l) and concentrated *in vacuo*. The aqueous concentrate (200 ml) was extracted with EtOAc (200 ml×5). After drying over Na_2SO_4, the organic layer was evaporated to dryness. The residue (0.52 g) was subjected to normal-phase, medium-pressure liquid chromatography (Purif-Pack SI-60; Moritex, Tokyo, Japan) and successively eluted with gradient systems of *n*-hexane–EtOAc (0–30% EtOAc) and $CHCl_3$–MeOH (0–50% MeOH). The 1% MeOH-eluted fraction (70.0 mg) was purified by preparative reversed-phase HPLC using an L-column2 ODS column (20 i.d. ×150 mm; Chemical Evaluation and Research Institute, Tokyo, Japan) with 55% MeOH–H_2O containing 0.1% formic acid (flow rate 10 ml min^{-1}) to yield **2** (0.99 mg, retention time (Rt) 12.6 min), tensidol B (3.22 mg, Rt 9.1 min) and a crude fraction of **1** (7.25 mg, Rt 4.3 min). The eluate containing **1** was further purified by preparative reversed-phase HPLC using an L-column2 ODS column (20 i.d. ×150 mm) with 50% MeOH–H_2O containing 0.1% formic acid (flow rate 10 ml min^{-1}) to yield **1** (1.02 mg, Rt 13.0 min) and tensidol A (3.81 mg, Rt 11.0 min).

Compound **1** was isolated as a colorless oil that yielded an $[M + H]^+$ ion at *m/z* 244.0986 in the HR-ESI-MS corresponding to a molecular formula of $C_{14}H_{12}NO_3$ (calcd for $C_{14}H_{13}NO_3$, 244.0974). Furthermore, **1** displayed the following UV and IR spectra: UV ($CHCl_3$) $\lambda_{max}(\varepsilon)$ 241 (11 750), IR (KBr) ν_{max} 1670 cm^{-1}. The ^1H and ^{13}C NMR spectral data for **1** are listed in Table 1. The structural information on **1** was obtained by a series of 2D NMR analyses such as heteronuclear single-quantum coherence, HMBC and double-quantum filtered (DQF)-COSY spectra (Figure 1b). The spin couplings observed in the DQF-COSY spectrum among aromatic protons 9/13-H (δ_H 7.24), 10/12-H (δ_H 7.32) and 11-H (δ_H 7.32) revealed the existence of a phenyl residue. In the HMBC spectrum, a singlet methylene proton 7-H (δ_H 3.83) was ^1H–^{13}C long-range coupled to aromatic methine carbons C-9/13 (δ_C 129.4). The ^1H–^{13}C long-range couplings from 9/13-H to aromatic carbon C-11 (δ_C 128.0) and to methylene carbon C-7 (δ_C 40.0) established the presence of a benzyl moiety, as shown in Figure 1b. In addition to the resonances ascribed to the benzyl moiety, **1** showed seven additional ^{13}C resonances. Long-range couplings from aromatic protons 2-H (δ_H 8.43) and 5-H (δ_H 6.21) to each of the aromatic carbons C-3 (δ_C 163.9), C-3a (δ_C 119.8), C-4 (δ_C 175.0) and C-6a (δ_C 167.6) were observed. Moreover, the long-range coupling between the methoxyl proton 3-OMe (δ_H 3.87) and C-3 revealed that the methoxyl group is substituted at the C-3 position. A comparison of the ^{13}C chemical shifts with those of

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Received 6 March 2010; revised 15 April 2010; accepted 16 April 2010; published online 12 May 2010

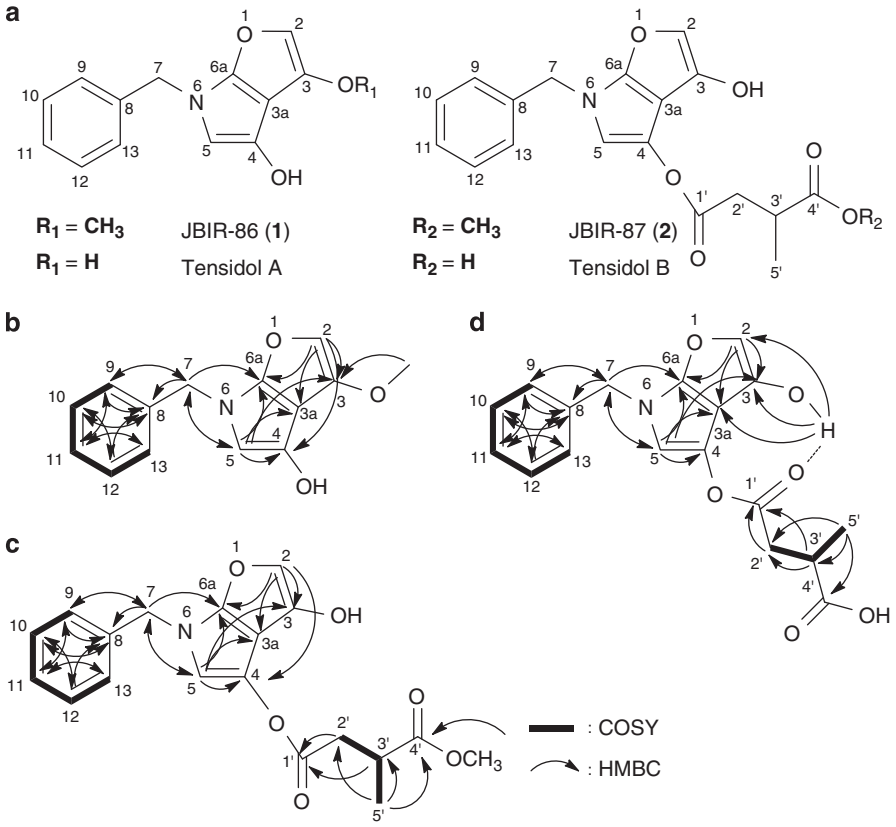

Figure 1 (**a**) Structures of tensidols, JBIR-86 (**1**) and JBIR-87 (**2**). (**b**) Key correlations in DQF-COSY (bold lines) and HMBC (arrows) spectra of **1**. (**c**) Key correlations in DQF-COSY (bold lines) and HMBC (arrows) spectra of **2**. (**d**) Key correlations in DQF-COSY (bold lines) and HMBC (arrows) spectra of tensidol B.

Table 1 ^{1}H and ^{13}C NMR spectral data for JBIR-86 (1), JBIR-87 (2) and tensidol B

	1		***2***		*Tensidol B*	
Position	^{13}C	^{1}H *(J in Hz)*	^{13}C	^{1}H *(J in Hz)*	^{13}C	^{1}H *(J in Hz)*
2	161.7	8.43, s	165.3	8.80, s	163.4	8.72, s
3	163.9[a]		162.7[a]		161.5	
3a	119.8		119.5		118.8	
4	175.0[a]		179.7[a]		178.0	
5	117.3	6.21, s	116.5	6.38, s	116.3	6.30, s
6a	167.6		171.9		169.6	
7	40.0	3.83, s	40.0	4.06, s	39.8	3.88, s
8	134.1		135.9		133.7	
9	129.4	7.24, m	130.3	7.34, m	130.3	7.22, m
10	129.3	7.32, m	130.0	7.36, m	129.4	7.36, m
11	128.0	7.32, m	128.7	7.32, m	128.7	7.30, m
12	129.3	7.32, m	130.0	7.36, m	129.4	7.34, m
13	129.4	7.24, m	130.3	7.34, m	130.3	7.22, m
1′			174.4		172.9	
2′			42.6	2.90, dd (4.8, 18.0)	41.9	2.86, dd (4.7, 17.9)
				3.16, dd (8.4, 18.0)		3.24, dd (8.4, 17.9)
3′			36.3	2.98, m	35.1	3.02, m
4′			177.8		180.8	
5′			17.4	1.22, d (7.2)	17.2	1.26, d (7.2)
3-OCH_3	52.8	3.87, s				
4′-OCH_3			52.4	3.66, s		
3-OH				11.71		11.72

^{13}C (150 MHz) and ^{1}H (600 MHz) NMR spectra were recorded using NMR System 600 NB CL (Varian, Palo Alto, CA, USA) in $CDCl_3$ with the residual solvent peak being used as an internal standard (δ_C 77.0, δ_H 7.24 p.p.m.).
[a]These assignments are exchangeable.

tensidol A suggested the existence of a 4-methoxy-6*H*-furo[2,3-*b*]pyrrol-3-ol moiety[3] (Figure 1b). Finally, the ^{1}H–^{13}C long-range couplings from 7-H to C-5 and C-6a and from 5-H to C-7 established a relation between these partial structures. Although four-bond HMBC correlations from H-2 to C-4 and from H-5 to C-3 were observed, and the ^{13}C chemical shifts were identical to the tensidol A chemical shifts, we could not determine the exact assignment of the C-3 and C-4 signals.

Compound **2** was obtained as a colorless oil ($[\alpha]_D$ + 6.0, *c* 0.1, MeOH), and it displayed a UV spectrum ($\lambda_{max}(\varepsilon)$ 239 (14 050), in $CHCl_3$) and an IR spectrum (KBr, ν_{max} 1730 and 1670 cm^{-1}). The molecular formula of **2** was established as $C_{19}H_{18}NO_6$ by HR-ESI-MS (*m/z* 358.1286 $[M + H]^+$, calcd for $C_{19}H_{19}NO_6$, 358.1291). The NMR data were similar to those of **1** except for the appearance of two carbonyl carbons C-1′ (δ_C 174.4) and C-4′ (δ_C 177.8), a methine carbon C-3′ (δ_C 36.3), a methylene carbon C-2′ (δ_C 42.6) and a methyl carbon C-5′ (δ_C 17.4), as listed in Table 1. The sequence from doublet methyl protons H-5′ (δ_H 1.22) to methylene protons H-2′ (δ_H 2.90, 3.16) through a methine proton 3′-H (δ_H 2.98) was observed in the DQF-COSY spectrum. The long-range couplings from methoxyl protons 4′-*O*Me (δ_H 3.66) to C-4′; from 5′-H to C-2′, C-3′ and C-4′; and from 2′-H and 3′-H to C-1′ established the presence of a 4-methoxy-3-methyl-4-oxobutanoate moiety, as shown in Figure 1c. Because the long-range couplings from 3-OH (δ_H 11.71) were not the same as those observed in **1**, we could not determine the substituted position of the side-chain moiety. To determine the assignment of C-3 and C-4, which would establish the substituted positions of the methyl residue and the side-chain moiety in **1** and **2**, respectively, detailed NMR analyses of tensidol B isolated from *Aspergillus* sp. fJ80 were carried out (Figure 1d). Using the analysis results, we could confirm that the long-range couplings from a hydrogen-bonded phenolic hydroxy proton 3-OH (δ_H 11.72) to C-2 (δ_C 163.4), C-3 (δ_C 161.5) and C-3a (δ_C 118.8) established the exact assignment of all the signals of tensidol B and 3-OH was found to be substituted at the C-3 position. Taking into consideration the ^{13}C chemical shifts at C-3 and C-4 in both **1** and **2**, we conclude that the methyl residue and 4-methoxy-3-methyl-4-oxobutanoate moiety of **1** and **2** are substituted at the C-3 and C-4 positions, respectively. Note that the assignment of the ^{1}H and ^{13}C NMR signals published by Fukuda *et al.*[3] should be corrected as per the results of this study. The identical $^{1}J_{C-H}$ values at C-2 and C-5 in tensidol B, **1** and **2** ($^{1}J_{2C-2H}$: tensidol B 200.6 Hz, **1** 197.4 Hz, **2** 198.0 Hz; $^{1}J_{5C-5H}$: tensidol B 167.5 Hz, **1** 165.4 Hz, **2** 168.0 Hz) provided further evidence in support of the proposed structures of **1** and **2**. Thus, **1** and **2** were established as 3-*O*-methyl tensidol A and 4′-*O*-methyl tensidol B, respectively.

Because it has been reported that tensidols A and B potentiate miconazole activity against *Candida albicans*,[3] we tested the miconazole-potentiating activities of **1** and **2** using the previously reported paper disk method.[3,4] As in the case of the tensidols, **1** and **2** did not show an inhibition zone against *C. albicans* at 50 μg per disk (plate A) and exhibited miconazole-potentiating activity in a dose-dependent manner on plate B (plate A containing 0.06 μM miconazole), as listed in Table 2. However, the activity of **1** was weaker than that of tensidol A. In addition, the cytotoxic activity of **1** and **2** against several cancer cell lines was tested by the WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) colorimetric assay (Cell Counting Kit; Dojindo, Kumamoto, Japan). However, **1** and **2** showed no cytotoxicity even at a concentration of 50 μg ml^{-1} for 48 h.

Table 2 Miconazole-potentiating activity of tensidols, JBIR-86 (1) and JBIR-87 (2)

Compounds	Concentration μg per disk (6 mm)	Inhibition zone (mm) Plate A	Plate B
Tensidol A	50	—	21
	25	—	14
	10	—	8
1	50	—	11
	25	—	7
	10	—	—
Tensidol B	50	—	14
	25	—	9
	10	—	—
2	50	—	16
	25	—	10
	10	—	—

Plate A is a GY agar plate containing a 0.1% aliquot of *C. albicans* NBRC 1594 culture, and plate B is a GY agar plate containing *C. albicans* and 0.06 μm miconazole.

ACKNOWLEDGEMENTS

This work was supported by a grant from the New Energy and Industrial Technology Department Organization (NEDO) of Japan.

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