

## Conoramides A–C, New Zwitterionic Alkaloids from the Fungus *Irpex consors*

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### ABSTRACT

In our ongoing search for new secondary metabolites from fungi, a basidiomycete fungus *Irpex consors* was selected for mycochemical investigation, and three new zwitterionic alkaloids (**1–3**) and five known compounds (**4–8**) were isolated from the culture broth (16 l) of *I. consors*. The culture filtrate was fractionated by a series of column chromatography including Diaion HP-20, silica gel, and Sephadex LH-20, Sep-Pak C<sub>18</sub> cartridge, medium pressure liquid chromatography (MPLC), and high pressure liquid chromatography (HPLC) to yield eight compounds (**1–8**). The structures of the isolated compounds were elucidated by the interpretation of nuclear magnetic resonance (NMR) spectra and high-resolution mass spectrometry (HR-MS). Their antioxidant and antibacterial activities were examined. The zwitterionic structures of three new sesquiterpene alkaloids (**1–3**) were determined together with five known compounds identified as stereumamide E (**4**), stereumamide G (**5**), stereumamide H (**6**), stereumamide D (**7**), and sterostrein H (**8**). This is the first report of the zwitterionic alkaloids in the culture broth of *I. consors*. Three new zwitterionic alkaloids were named as conoramides A–C (**1–3**).

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Mushrooms are a good source of functional foods and traditional therapeutic agents [1]. They produce a wide range of biologically active compounds with unique chemical structures [2–4]. The mushroom *Irpex consors*, belonging to the family Meruliaceae, is distributed in India and East Asian countries such as Korea and Japan [5]. Previous investigations of *I. consors* have reported that it possesses tricyclic sesquiterpene derivatives with anti-bacterial and anti-tumor activities [6–8]. In our ongoing search for new secondary metabolites from fungal strains, three new zwitterionic alkaloids (**1–3**) together with five known compounds (**4–8**) were isolated from the culture broth of the fungus *I. consors*. Herein, we describe the isolation and structure determination of these compounds (Figure 1).

Fungal strain *Irpex consors* was obtained from Rural Development Administration, Korea. The fungal strain *I. consors* was cultured on potato dextrose agar at 27 °C for two weeks. Small pieces of fresh mycelium were inoculated into 40 1-l flasks containing 400 ml of potato dextrose broth and cultured on a rotary shaker of 120 rpm at 27 °C for four weeks.

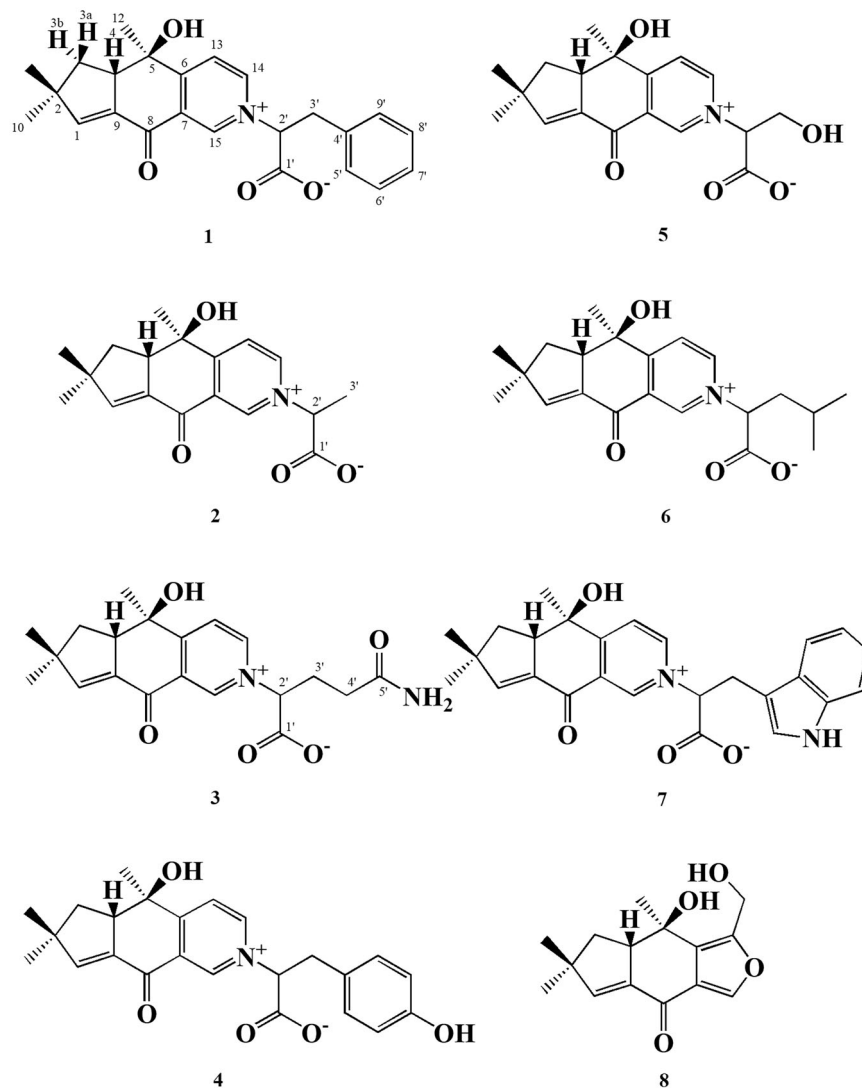
The culture broth (about 16 l) was filtered to remove mycelia. The culture filtrate was fractionated by Diaion HP-20 column chromatography eluted with a mixture of methanol-water (30:70–100:0, v/v, stepwise), followed by silica gel column chromatography with stepwise chloroform-methanol (30:1–0:100, v/v) to afford four fractions (Fractions A–D). Fraction A was subjected to Sephadex LH-20 column chromatography, followed by medium pressure liquid chromatography (MPLC) to give two fractions A1 and A2. Fraction A1 was purified by Sep-Pak C<sub>18</sub> cartridge eluted with 20% aqueous methanol to obtain two compounds **1** (4.3 mg) and **2** (12.9 mg). Fraction A2 was further separated by a Sep-Pak C<sub>18</sub> cartridge eluted with 15% aqueous methanol to obtain compound **7** (4.5 mg). Fraction B was fractionated by Sephadex LH-20 column chromatography, followed by preparative reversed-phase high pressure liquid chromatography (HPLC) eluted with 18% aqueous methanol to yield two compounds **4** (9.3 mg) and **6** (9.5 mg). Fraction C was subjected to MPLC, followed by preparative reversed-phase HPLC eluted with 18% aqueous methanol to

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 Supplemental data for this article can be accessed [here](#).

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**Figure 1.** Structures of compounds 1-8.

provide two compounds **5** (12.0 mg) and **8** (9.0 mg). Fraction D was separated by MPLC eluted with a gradient of increasing methanol (20–100%) in water, followed by preparative reversed-phase HPLC eluted with 27% aqueous methanol to obtain a compound **3** (4.8 mg).

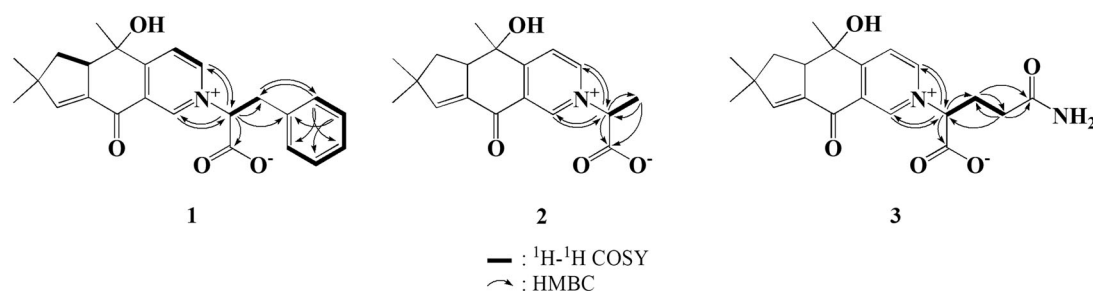
Compound **1** was obtained as a brown powder with a specific rotation value of  $-92.8^\circ$  ( $c=0.1$ ,  $24.5^\circ\text{C}$ , methanol). Its molecular formula was established as  $\text{C}_{24}\text{H}_{25}\text{NO}_4$  by a high-resolution fast atom bombardment (FAB)-mass measurement ( $m/z$  392.1849  $[\text{M} + \text{H}]^+$ ,  $\Delta -1.2$  mmu). The  $^1\text{H}$  NMR spectrum of **1** revealed the presence of a substituted benzene moiety at  $\delta$  7.18 ( $\times 2$ ), 7.14, and 7.09 ( $\times 2$ ), a 3,4-disubstituted pyridine moiety at  $\delta$  9.05, 8.99, and 8.27, a olefinic methine at  $\delta$  6.93, two methines at  $\delta$  5.59 and 3.69, two methylenes at  $\delta$  3.88/3.47 and 2.10/1.97, and three methyls at  $\delta$  1.28, 1.24, and 1.19 (Table 1). In the  $^{13}\text{C}$  NMR spectrum, twenty-four carbons including one ketone carbon at  $\delta$  181.1, one carbonyl carbon at  $\delta$  171.0, nine  $sp^2$  methine carbons at  $\delta$  155.7, 148.6, 145.7, 130.1 ( $\times 2$ ), 129.9 ( $\times 2$ ), 128.6, and 125.0, four  $sp^2$

quaternary carbons at  $\delta$  171.4, 137.4, 137.3, and 131.6, one oxygenated quaternary carbon at  $\delta$  74.1, two methine carbons at  $\delta$  78.8 and 53.8, one quaternary carbon at  $\delta$  47.0, two methylene carbons at  $\delta$  40.7 and 40.6, and three methyl carbons at  $\delta$  28.6, 27.6, and 25.2 were evident (Table 1). In the  $^1\text{H}$ - $^1\text{H}$  correlated spectroscopy (COSY) spectrum, correlations between H-3 and H-4 and between H-13 and H-14 were observed, and the long-range correlations from H-1 to C-8 and C-9, from H-3 to C-5 and C-9, from H-10 and H-11 to C-1, C-2, and C-3, from H-12 to C-4, C-5, and C-6, from H-13 to C-5 and C-7, from H-14 to C-6 and C-15, and from H-15 to C-6 and C-8 established the presence of sterostrein Q moiety. The long-range correlations from H-2' to C-1', C-3' and C-4', from H-8' to C-4', and from H-9' to C-3' and C-7' as well as  $^1\text{H}$ - $^1\text{H}$  COSY correlations of H-5'/H-6'/H-7'/H-8'/H-9' and H-2'/H-3' revealed the presence of a phenylalanine moiety. Finally, the long-range correlations from H-14 and H-15 to C-2' and from H-2' to C-14 and C-15 indicated that the phenylalanine moiety was connected to sterostrein Q *via* carbon-nitrogen bond

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of compounds 1-3 in methanol- $d_4$ .

No.	<b>1<sup>a</sup></b>		<b>2<sup>a</sup></b>		<b>3<sup>a</sup></b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult, <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult, <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult, <i>J</i> in Hz)
1	155.7	6.93 (d, 2.7)	155.6	6.98 (d, 2.5)	155.8	6.99 (d, 2.3)
2	47.0		47.0		47.1	
3a	40.6	2.10 (dd, 13.0, 8.2)	40.6	2.14 (dd, 13.0, 8.3)	40.6	2.15 (dd, 13.5, 7.9)
3b		1.97 (dd, 13.0, 8.2)		2.05 (dd, 13.0, 8.3)		2.05 (dd, 13.5, 7.9)
4	53.8	3.69 (td, 8.2, 2.7)	53.9	3.77 (td, 8.3, 2.5)	53.9	3.78 (td, 8.2, 2.3)
5	74.1		74.2		74.3	
6	171.4		171.1		172.3	
7	131.6		132.0		132.2	
8	181.2		181.6		181.3	
9	137.4		137.6		137.5	
10	28.6	1.28 (s)	28.6	1.31 (s)	28.6	1.31 (s)
11	27.6	1.19 (s)	27.7	1.22 (s)	27.6	1.22 (s)
12	25.2	1.24 (s)	25.2	1.34 (s)	25.2	1.36 (s)
13	125.0	8.27 (d, 6.4)	125.2	8.38 (d, 6.1)	125.6	8.43 (d, 6.1)
14	148.6	8.99 (dd, 6.4, 1.3)	147.9	9.08 (d, 6.1)	148.6	9.13 (d, 6.1)
15	145.7	9.05 (d, 1.3)	145.3	9.25 (s)	146.2	9.32 (s)
1'	171.0		172.6		170.5	
2'	78.8	5.59 (dd, 11.0, 4.8)	73.0	5.41 (q, 7.5)	75.0	5.63 (m)
3a'	40.7	3.88 (m)	19.3	1.94 (d, 7.5)	29.6	2.80 (m)
3b'		3.47 (m)				2.60 (m)
4'	137.3				32.5	2.38 (t, 6.8)
5'	129.9	7.09 (d, 6.8)			176.3	
6'	130.1	7.18 (d, 7.5)				
7'	128.6	7.14 (t, 7.5)				
8'	130.1	7.18 (d, 7.5)				
9'	129.9	7.09 (d, 6.8)				

<sup>a</sup>Measured at 600 MHz for  $^1\text{H}$  and 150 MHz for  $^{13}\text{C}$ .

**Figure 2.**  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations of compounds 1-3.

(Figure 2). The partial relative stereochemistry of **1** was established by the NOESY correlations. The cross peaks of H-4/H-3a and H-4/H-11 indicated the same face, while those of H-10/3b and H-3b/H-12 confirmed the other face. Therefore, the structure of **1** was determined as a new zwitterionic alkaloid and named consoramide A.

Compound **2** was purified as a yellow oil with specific rotation of  $-107.2^\circ$  ( $c=1.0$ ,  $25.0^\circ\text{C}$ , methanol) and exhibited UV maxima ( $\log \epsilon$ ) at 203 (3.34) and 238 (3.61) nm. Its molecular formula was determined to be  $\text{C}_{18}\text{H}_{21}\text{NO}_4$  by the high-resolution FAB-mass measurement ( $m/z$  316.1531  $[\text{M} + \text{H}]^+$ ,  $\Delta -1.8$  mmu). The 1D NMR spectra of **2** revealed that the hydroxymethyl group in **5** was replaced by a methyl group (Table 1). The long-range correlations from H-3' to C-1' and C-2' as well as  $^1\text{H}$ - $^1\text{H}$  COSY correlations between H-2' and H-3' supported the presence of an alanine moiety in **2** (Figure 2). Therefore, compound **2** was

determined to be a new zwitterionic alkaloid and named consoramide B.

Compound **3** was obtained as a yellow powder with the specific rotation of  $-89.2^\circ$  ( $c=1.0$ ,  $25.0^\circ\text{C}$ , methanol) and showed UV maxima ( $\log \epsilon$ ) at 202.0 (3.37) nm. Its molecular formula was established as  $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_5$  by the high-resolution FAB-mass measurement ( $m/z$  373.1745  $[\text{M} + \text{H}]^+$ ,  $\Delta -1.9$  mmu). The NMR spectra revealed that **3** was consisted of sterostrein Q and glutamine (Table 1). The glutamine moiety was determined by the  $^1\text{H}$ - $^1\text{H}$  COSY correlations and the long-range correlations from H-2' to C-1' and C-3' and from H-3' and H-4' to C-5' (Figure 2). Thus, compound **3** was determined to be a new zwitterionic alkaloid and named consoramide C. The configuration of all amino acid moieties in **1-3** was tentatively deduced as L-form, because the L-amino acids are abundant in nature literature [9,11].

Compounds **4-8** were identified as stereumamide E (**4**), stereumamide G (**5**), stereumamide H (**6**),

stereumamide D (7), and sterostrein H (8), respectively, by the comparison of their spectroscopic data with the literatures previously reported [9–11].

The antioxidant activities of these compounds (1–8) were evaluated by the ABTS (2,2'-azinobis[3-ethylbenzothiazoline-6-sulfonate]) and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging assays [12]. All compounds (1–8) displayed no radical scavenging activity up to 200  $\mu$ M. In the present study, all compounds exhibited no antibacterial activity up to 50  $\mu$ g/disk against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Escherichia coli*.

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### Disclosure statement

No potential conflict of interest was reported by the author(s).

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### Supplementary information

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