

ORIGINAL ARTICLE

# Isolation and structure elucidation of izuminosides A–C: a rare phenazine glycosides from *Streptomyces* sp. IFM 11260

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Three new glycosylated phenazine derivatives, named izuminosides A–C (1–3) have been isolated from the ethyl acetate extract of *Streptomyces* sp. IFM 11260. The structures of the new compounds were determined on the basis of their spectral data. Compounds 1–3 were evaluated for their activity in overcoming tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in human gastric adenocarcinoma cells. Compounds 2 (10  $\mu\text{M}$ ) and 3 (60  $\mu\text{M}$ ) in combination with TRAIL showed synergistic activity in sensitizing TRAIL-resistance AGS cells.

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**Keywords:** glycosides; phenazine; *Streptomyces*; TRAIL

## INTRODUCTION

Phenazines comprise a large group of nitrogen-containing heterocyclic compounds that differ in their chemical and physical properties based on the type and position of functional groups present. Bacteria are the only known source of natural phenazines. The Gram-negative pathogen *Pseudomonas* was known as the first natural source that produces phenazine pigments.<sup>1</sup> Other phenazine producers include *Nocardia*, *Sorangium*, *Brevibacterium*, *Burkholderia*, *Erwinia*, *Pantoea agglomerans*, *Vibrio*, *Pelagibacter*<sup>2,3</sup> and members of the actinomycetes, especially *Streptomyces*.<sup>4–6</sup> More than 100 different phenazine structural derivatives have been identified in nature, and over 6000 compounds that contain phenazine as a central moiety have been synthesized. However, the carbohydrate-containing phenazine natural products are rare in nature. Only a few examples exist, all derived from 6-deoxy-L-glycopyranosides and exhibiting antibacterial activity.<sup>7</sup>

In the course of our screening program for bioactive natural products from actinomycetes,<sup>8</sup> we collected soils and seawaters from different areas of Japan. From a soil sample collected from Izumi forest at Chiba city (Japan), we isolated the *Streptomyces* sp. IFM 11260. Its crude extract showed on the TLC several relatively polar yellow bands, which seems dark under UV light (254 nm). These bands gave a dark red color with concentrated sulfuric acid and turned to reddish brown after spraying with the Dragendorff's reagent. In this paper, we report isolation, structure determination and biological activity of three new glycoconjugated phenazines designated as izuminosides A–C (1–3) from the culture extract of *Streptomyces* sp. IFM 11260 (Figure 1).

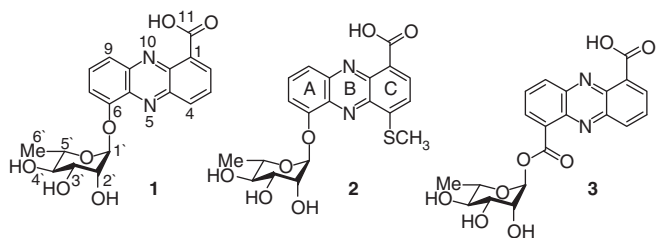
## RESULTS AND DISCUSSION

### Structure elucidation

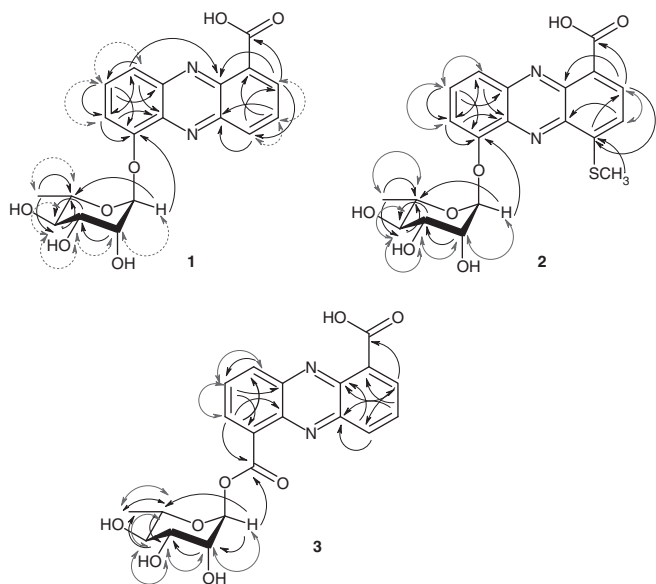
The terrestrial *Streptomyces* sp. IFM 11260 was cultured in Waksman medium<sup>9</sup> at 28 °C for 5 days on a rotary shaker. After centrifugation, extraction and evaporation, working up of the crude extract resulted in the isolation of izuminosides A–C (1–3). Compound 1 was isolated as a yellow solid. The UV spectrum showed maxima at 365 and 259 nm. The ESI-MS of 1 displayed in the positive ion mode a mass of  $m/z$  409.1 [M+Na]<sup>+</sup>, suggesting a molecular weight of 386 Da. The molecular formula was determined as C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>7</sub> by HRESI-MS  $m/z$  409.1042 [M+Na]<sup>+</sup> (calcd 409.1012,  $\Delta$ +3.1 mmu). The <sup>1</sup>H NMR of 91 (Table 1) showed six aromatic protons in two 1,2,3-trisubstituted aromatic spin systems (H-2, H-3 and H-4,  $\delta_{\text{H}}$  7.80 (d, 8.0), 7.77 (t, 8.0) and 8.13 (d, 8.0); H-7, H-8 and H-9,  $\delta_{\text{H}}$  7.46 (d, 7.7), 7.69 (t, 7.7) and 7.88 (d, 7.7)). The aliphatic pattern revealed signals between  $\delta_{\text{H}}$  5.70–3.45 and one methyl doublet typical for 6-deoxyhexapyranose at  $\delta_{\text{H}}$  1.15. The <sup>13</sup>C NMR spectrum revealed 19 signals, which were assigned to one carbonyl ( $\delta_{\text{C}}$  176.1), one oxygenated  $sp^2$  carbon ( $\delta_{\text{C}}$  152.8), six  $sp^2$  aromatic methine carbons ( $\delta_{\text{C}}$  131.6, 131.5, 129.7, 128.3, 124.6 and 114.2) and five  $sp^2$  quaternary carbons ( $\delta_{\text{C}}$  145.4, 143.2, 142.0, 137.8 and 131.0). In the sugar region, one-anomeric carbon signal ( $\delta_{\text{C}}$  101.3), four oxygenated methine carbons ( $\delta_{\text{C}}$  73.9, 72.3, 71.9 and 71.2) and one methyl group ( $\delta_{\text{C}}$  18.0) were observed. The structure of 1 was clarified by analyses of the 2D NMR spectra (<sup>1</sup>H-<sup>1</sup>H COSY, HMQC and HMBC). From the <sup>1</sup>H-<sup>1</sup>H COSY, two spin aromatic systems were established (Figure 2). The HMBC spectrum of 1 also suggested that izuminoside A (1) had 6-hydroxy-phenazine-1-carboxylic acid aglycone (H-2/C-11, C-10a, C-4; H-3/C-1; H-4/C-4a,

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**Figure 1** Structures of izuminosides A-C (1-3).



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

C-2; H-7/C-9, C-6, C-5a; H-8/C-9a, C-6 and H-9/C-10a, C-7, C-5a). The aglycone was also confirmed by comparison of the spectral data with the literature values.<sup>10</sup> From the analysis of  $^1\text{H}$ - $^1\text{H}$  COSY spectra and the coupling pattern of each H-signal, the *O*-glycosidic moiety in compound **1** could be deduced as 6-deoxyhexapyranose. The connectivity of the 6-deoxy sugar to the aglycone was confirmed from the HMBC long-range coupling of H-8 ( $\delta_{\text{H}}$  7.69) and H-1' ( $\delta_{\text{H}}$  5.70) to the C-6 ( $\delta_{\text{C}}$  152.8) of the phenazine nucleus.

Compound **2** was obtained as an orange solid. It gave a yellow fluorescent spot under UV light at 366 nm. The molecular weight of **2** was determined as 432 Da based on the (+)-ESI-MS. The molecular formula was estimated as  $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_7\text{S}$  by HRESI-MS  $m/z$  455.0890  $[\text{M}+\text{Na}]^+$  (calcd 455.0889,  $\Delta+0.1$  mmu). The ion peak fragment at  $m/z$  309.03264  $[\text{M}+\text{Na}]^+$  (calcd for  $\text{C}_{14}\text{H}_{10}\text{N}_2\text{O}_3\text{S}$ ,  $\Delta+1.7$  mmu) was assigned to the aglycone of **2**. The UV spectrum recorded in MeOH (maxima at 271, 366 and 454 nm) suggested a phenazine nucleus. The  $^1\text{H}$  NMR data of **2** (Table 1) recorded in  $\text{DMSO}-d_6$  indicated one  $\text{D}_2\text{O}$  exchangeable proton (11-OH,  $\delta_{\text{H}}$  14.70 (brs)), two *ortho*-coupled aromatic signals (H-2 and H-3,  $\delta_{\text{H}}$  8.52 (d, 8.1) and 7.74 (d, 8.1)), three aromatic protons in ABC system (H-7, H-8 and H-9,  $\delta_{\text{H}}$  7.62 (d, 8.6), 8.00 (t, 8.6) and 8.03 (d, 8.6)), many aliphatic signals typical for 6-deoxy sugar residue ( $\delta_{\text{H}}$  5.81–1.09) and one singlet attributable to  $-\text{SCH}_3$  group ( $\delta_{\text{H}}$  2.66 (s)). The  $^{13}\text{C}$  NMR spectrum of **2** showed only one carbonyl ( $\delta_{\text{C}}$  166.1), one  $sp^2$  carbon connected to sulfur atom ( $\delta_{\text{C}}$  148.7), five  $sp^2$  methine carbons ( $\delta_{\text{C}}$  134.6, 132.8, 122.2, 121.3 and

114.9) and also four quaternary  $sp^2$  carbons ( $\delta_{\text{C}}$  141.5, 141.3, 139.7 and 135.4). The spectrum also revealed the six carbons for the 6-deoxy sugar and one methyl group ( $\delta_{\text{C}}$  13.9). Several of the relevant carbon chemical shifts were, however, obtained only by interpretation of HMBC and HMQC NMR data. Interpretation of the HMBC spectrum of **2** confirmed the structure of ring A (1,2,3-trisubstituted benzene ring) as in **1**. The *ortho*-positioned aromatic protons H-2 ( $\delta_{\text{H}}$  8.52) and H-3 ( $\delta_{\text{H}}$  7.74) showed a long-range HMBC correlations to the C-11 ( $\delta_{\text{C}}$  166.1), C-10a ( $\delta_{\text{C}}$  139.7), C-3 ( $\delta_{\text{C}}$  122.2); and C-4a ( $\delta_{\text{C}}$  138.5), respectively. As the aromatic proton H-2 ( $\delta_{\text{H}}$  8.52) and the  $-\text{SCH}_3$  group ( $\delta_{\text{H}}$  2.66) coupled to C-4 ( $\delta_{\text{C}}$  148.7), the methylsulfanyl group must be located at C-4 in a *para*-position to the carboxylic acid group in ring C. These couplings construct the aglycone of compound **2** as shown in Figure 2. The position of the 6-deoxy sugar was determined from the interpretation of the HMBC spectrum, which exhibited correlation from the anomeric proton H-1' ( $\delta_{\text{H}}$  5.81) to the C-6 ( $\delta_{\text{C}}$  151.2) of the phenazine moiety.

Compound **3** was isolated as yellow solid. It was revealed to have the molecular formula of  $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_8$  on the basis of HRESI-MS, at  $m/z$  437.0975 ( $[\text{M}+\text{Na}]^+$ ,  $\Delta+1.4$  mmu). The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of **3** (Table 1) were similar to those of **1** except in the presence of an extra carbonyl ( $\delta_{\text{C}}$  164.8) for **3** in place of oxygenated  $sp^2$  carbon at C-6 for **1**. The HMBC correlations of **3** allowed the construction of phenazine-1,6-dicarboxylic acid (H-2/C-11, C-10a, C-4; H-3/C-4a, C-1; H-4/C-10a, C-4a, C-2; H-7/C-12, C-9, C-5a; H-8/ C-9a, C-6 and H-9/ C-7). The long-range HMBC couplings of the aromatic signal H-7 ( $\delta_{\text{H}}$  8.42) and the anomeric proton ( $\delta_{\text{H}}$  6.18) with the ester carbonyl carbon ( $\delta_{\text{C}}$  164.8) indicated that the sugar unit linked to the phenazine nucleus through an ester linkage.

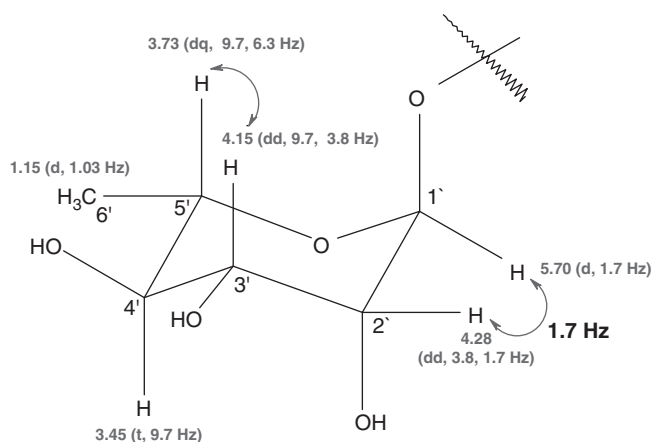
The relative stereochemistry for the 6-deoxyhexopyranose in compounds **1-3** was determined by the nuclear overhauser effect (NOE) and coupling constants. The NOE observed between 3'-H and 5'-H as well as the coupling constant between 4'-H and 5'-H ( $J_{4',5'}$  9.4–9.7 Hz) indicated that they were axially oriented. The configuration of the anomeric proton of compounds **1-3** was assigned to be  $\alpha$  based on the small coupling constant ( $J_{1',2'}$  1.1–1.7 Hz; Figure 3). The anomeric configuration for the 6-deoxy sugar was also deduced as  $\alpha$  from the one-bond coupling constant between C-1' and H-1' (Rha,  $J_{\text{C}1',\text{H}1'}$  values: 171.8–175.2 Hz observed for **1-3**; literature values<sup>11</sup>  $\alpha$ -anomer, 172.3 Hz;  $\beta$ -anomer, 160 Hz). On the basis of these results, the sugar moiety in compounds **1-3** was identified as rhamnose. It was confirmed by the HPLC analysis (SHISEIDO CO., LTD, Tokyo, Japan, 85% $\text{CH}_3\text{CN}$ ) of the crude sugar obtained by acidic hydrolysis of izuminosides A-C (**1-3**). The absolute configuration of the sugar residue was determined to be L-rhamnose by comparison with the authentic samples using a combination of the refractive index and optical rotation detectors. From all of these results, the structure of izuminosides A-C (**1-3**) was determined to be 6-(1'-*O*- $\alpha$ -L-rhamanopyranosyl)-phenazine-1-carboxylic acid, 4-methylsulfanyl-6-(1'-*O*- $\alpha$ -L-rhamanopyranosyl)-phenazine-1-carboxylic acid and 6-(1'-*O*- $\alpha$ -L-rhamano-pyranosyloxycarbonyl)-phenazine-1-dicarboxylic acid ester, respectively. From the literature, phenazoviridine was the first glycoconjugated phenazine obtained from actinomycetes.<sup>12</sup> Other examples of carbohydrate containing phenazines are aestivopheonins A-C,<sup>13</sup> 2-*O* and 3-*O*-L-quinovosyl esters of saphenic acid.<sup>14</sup>

### Biological activity

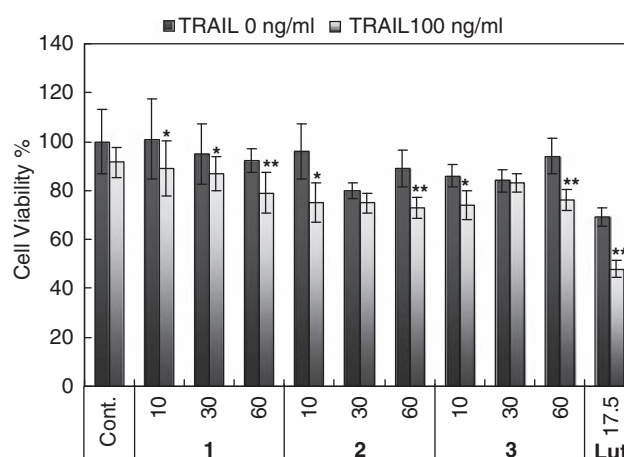
We evaluate the bioactivity of izuminosides A-C (**1-3**) for their activity in overcoming tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in AGS cells. Recently, this cell line has been widely used as a model system for evaluating

**Table 1**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for compounds 1–3

Position	$1^a$		$2^b$		$3^b$	
	$\delta_{\text{H}}$ (p.p.m.)	$\delta_{\text{C}}$ (p.p.m.)	$\delta_{\text{H}}$ (p.p.m.)	$\delta_{\text{C}}$ (p.p.m.)	$\delta_{\text{H}}$ (p.p.m.)	$\delta_{\text{C}}$ (p.p.m.)
1	—	131.0 <sup>c</sup>	—	135.4	—	131.0
2	7.80 d (8.0)	128.3	8.52 d (8.1)	134.6	8.50 d (7.0)	134.1
3	7.77 t (8.0)	131.5	7.74 d (8.1)	122.2	8.12 t (7.0)	133.2
4	8.13 d (8.0)	129.7	—	148.7	8.55 d (7.0)	129.5
4a	—	142.0	—	138.5 <sup>c</sup>	—	140.3
5a	—	137.8	—	141.5	—	139.7
6	—	152.8	—	151.2 <sup>c</sup>	—	130.9
7	7.46 d (7.7)	114.2	7.62 d (8.6)	114.9	8.42 d (6.9)	133.3
8	7.69 t (7.7)	131.6	8.00 t (8.6)	132.8	8.12 t (6.9)	131.3
9	7.88 d (7.7)	124.6	8.03 d (8.6)	121.3	8.56 d (6.9)	132.9
9a	—	145.4	—	141.3	—	140.5
10a	—	143.2	—	139.7	—	142.6
11	—	176.1	—	166.1	—	166.5
12	—	—	—	—	—	164.8
1'	5.70 d (1.7)	101.3	5.81 d (1.3)	97.9	6.18 d (1.1)	95.7
2'	4.28 dd (3.8, 1.7)	71.9	4.16 brd	70.0	3.90 brd	69.4
3'	4.15 dd (9.7, 3.8)	72.3	3.94 dd (9.4, 3.8)	69.4	3.76 dd (9.4, 3.8)	70.2
4'	3.45 t (9.7)	73.9	3.39 t (9.4)	71.8	3.42 t (9.4)	71.5
5'	3.73 dq (9.7, 6.3)	71.2	3.69 dq (9.4, 6.6)	69.9	4.04 dq (9.4, 5.9)	71.6
6'	1.15 d (6.3)	18.0	1.09 d (6.6)	17.9	1.24 d (5.9)	18.0
4-SCH <sub>3</sub>	—	—	2.66 s	13.9	—	—
11-OH	—	—	14.70 brs	—	14.31 brs	—
2'-OH	—	—	5.15 brs	—	5.28 brs	—
3'-OH	—	—	4.83 brs	—	4.78 brs	—
4'-OH	—	—	4.94 brs	—	5.05 brs	—

<sup>a</sup>Data was measured in CD<sub>3</sub>OD.<sup>b</sup>Data measured in DMSO-*d*<sub>6</sub>.<sup>c</sup>Values observed from HMBC.**Figure 3** Selected NOE correlations ( $\curvearrowright$ ) and coupling constant in the sugar moiety of **1**.

cancer cell apoptosis and is reported to be refractory to apoptosis induction by TRAIL.<sup>15</sup> To assess the effects of compounds **1–3** on cell viability, in the presence and absence of TRAIL, AGS cells were treated with the indicated agents and subjected to fluorometric microculture cytotoxicity assay method.<sup>16</sup> Luteolin was used as a positive control at 18.0  $\mu\text{M}$ .<sup>17</sup> The assay results (Figure 4) showed that compounds **2** (10  $\mu\text{M}$ ) and **3** (60  $\mu\text{M}$ ) exhibited 22 and 19% decreases, respectively, in cell viability in the presence

**Figure 4** Effect of the isolated compounds **1–3** on the cell viability of AGS cells in the presence and absence of TRAIL. The standard error bar represents the means ( $n=3 \pm \text{s.d.}$ ). The significance difference was determined by Student's *t*-test (\*\* $P < 0.01$ ; \* $P < 0.05$  versus control).

of TRAIL (100  $\text{ng ml}^{-1}$ ) compared with in the absence of TRAIL. Compound **1**, however, did not show TRAIL-resistance overcoming activity. These results suggest that izuminosides **B** (**2**) and **C** (**3**) had a synergistic effect in combination with TRAIL against AGS cells.

The resistance of cancer cells toward TRAIL may occur at different points in the TRAIL-induced apoptotic pathways. Understanding the mechanisms of such resistance and developing strategies to overcome it are important for the successful use of TRAIL in cancer therapy. Combined treatment with TRAIL and chemotherapeutic agents, including natural products can overcome such resistance and sensitize TRAIL-resistant cells to enhance the therapeutic potential of TRAIL against cancer cells. Therefore, a natural product producing synergistic activity with TRAIL would be a new tool for investigating cancer cells.<sup>18</sup> In this study, we report for the first time the synergistic activity (albeit small) of glycosylated phenazine in sensitizing TRAIL-resistant AGS cells, thereby suggesting their possible use in combination with TRAIL against human gastric adenocarcinoma. The antimicrobial activity of izuminosides A–C (1–3) was also examined against *Bacillus subtilis*, but they were inactive at 50 µg per disk.

Overall, our search for new bioactive natural products from actinomycetes yielded the terrestrial *Streptomyces* sp. IFM 11260, which produced three new phenazine derivatives with 6-deoxysugar moiety for further exploration. The strain may become a valuable resource for generating novel phenazines-*O*-glycosides through combinatorial biosynthesis.<sup>19</sup>

## METHODS

### General experimental procedures

Optical rotations were measured with a JASCO P-1020 polarimeter (JASCO, Tokyo, Japan). IR spectra were measured on attenuated total reflection on a JASCO FT-IR 230 spectrophotometer (JASCO). UV spectra were measured on a Shimadzu UV mini-1240 spectrometer (Shimadzu, Kyoto, Japan). The NMR spectra were recorded on JEOL JNM-ecp600 spectrometers (JEOL, Tokyo, Japan) with a deuterated solvent, the chemical shift of which was used as an internal standard (see Supplementary Information). Mass spectra were recorded on AccuTOF-T100LP (JOEL) mass spectrometer.

### Identification of the producing strain

The strain IFM 11260 was isolated on humic acid-vitamin agar,<sup>20</sup> a medium for the selective isolation of actinomycetes, from a soil sample collected from the Izumi forest, Chiba city, Japan in 2007. It was identified as *Streptomyces* sp. and deposited at the Medical Mycology Research Center, Chiba University, Japan with the code number IFM 11260. Identification of the strain was carried out by sequence analysis of 16S ribosomal RNA gene using the DDBJ (DNA data bank of Japan)-BLAST (basic local alignment search tool) search. The strain was found to have 100% identity with the 16S ribosomal RNA of *Streptomyces spororaveus* LMG 20313.

### Fermentation and isolation

Spores of the *Streptomyces* sp. IFM 11260 growing on solid Waksman medium were inoculated into 4×500 cm<sup>3</sup> round-bottom flask each containing 100 ml of liquid medium and then incubated at 28 °C for 5 days with reciprocal shaking at 200 r.p.m. to produce seed culture. The seed culture (10 ml) was then inoculated into each of 20 of 31 flasks each containing 700 ml of liquid Waksman medium, and incubated at 28 °C for 5 days with reciprocal shaking at 200 r.p.m. to get 141 of fermentation broths. The culture broth was centrifuged at 3500 r.p.m. for 20 min then extracted three times with ethyl acetate. The solvent was concentrated in *vacuo* to yield 2.67 g extract. The mycelial cake was extracted three times with acetone. After removal of acetone, the aqueous solution was extracted three times with EtOAc to give 0.57 g residue. The extracts from water phase and mycelial cake were combined after controlling by TLC. The crude extract (3.24 g) was fractionated using silica gel flash column chromatography through a stepwise gradient solvent system of increasing polarity starting from 100% CHCl<sub>3</sub> to 80% MeOH to yield four fractions. Fraction II (540 mg) was applied to Sephadex LH-20 (GE Healthcare BioScience, Uppsala, Sweden) ( $\varphi$  15×600 mm, MeOH) to give three sub-fractions named **IIa** (129.8 mg), **IIb** (80.6 mg) and **IIc** (260.9 mg). Sub-fraction **IIb** was purified by preparative TLC (6 plates, 20×20 cm, CHCl<sub>3</sub>/16%MeOH)

to yield compounds **1** (8.0 mg) and **2** (1.6 mg). Purification of fraction **III** (330.2 mg) using Sephadex LH-20 ( $\varphi$  15×600 mm, MeOH) followed by preparative HPLC (Develosil ODS HG-5, Nomura Chemical Co., Ltd, Seto, Japan, 10×250 mm) afforded compound **3** (5.4 mg,  $R_t$ =21.3 min). The mobile phase was gradients of CH<sub>3</sub>CN/H<sub>2</sub>O at flow rate of 1.0 ml min<sup>-1</sup>.

### Acid hydrolysis of compounds 1–3 and determination of the absolute configuration of the sugar

Compound **1** (4.2 mg) in 1,4-dioxane (5.0 ml) and 5% aqueous H<sub>2</sub>SO<sub>4</sub> (3 ml) was heated at 100 °C under reflux for 3 h. After cooling to room temperature, water was added to the reaction mixture, and the mixture was partitioned with EtOAc. The aqueous layer containing the sugar was neutralized by passage through an Amberlite IRA-96SB column, and then analyzed by HPLC (Capcell Pak NH<sub>2</sub> UG80, 4.6×250 mm; eluent, 85% MeCN; flow rate, 0.7 ml min<sup>-1</sup>; column temperature, 40 °C; detection, refractive index; and optical rotation, JASCO OR-2090). The sugar was identified as L-rhamnose by comparing its retention time and the optical rotation detector with both of authentic sample and the literature value<sup>21</sup> ( $t_R$  7.29 min, negative peak in optical rotation detector). Compounds **2** and **3** were hydrolyzed and analyzed by the same procedures as above to give also the same sugar residue (L-rhamnose).

### Fluorometric microculture cytotoxicity assay

AGS cells were seeded in a 96-well culture plate (6×10<sup>3</sup> cells per well) in 200 µl of RPMI medium containing 10% fetal bovine serum.<sup>16</sup> Cells were incubated at 37 °C in a 5% CO<sub>2</sub> incubator for 24 h. Then the test samples with or without TRAIL (Wako, Osaka, Japan, 100 ng ml<sup>-1</sup>) at different doses were added to each well. After 24 h incubation, the cells were washed with phosphate-buffered saline, and 200 µl of phosphate-buffered saline containing fluorescein diacetate (10 µg ml<sup>-1</sup>) was added to each well. The plates were then incubated at 37 °C for 1 h, and fluorescence was measured in a 96-well scanning spectrofluorometer at 538 nm, following excitation at 485 nm.

*Izuminoside A* (**1**). Yellow solid,  $[\alpha]_D^{20} +22.3$  ( $c$  0.42, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 365 (4.2) and 259 (5.1) nm; IR  $\nu_{max}$  (attenuated total reflection) ~3360, 2917, 1561, 1407 and 737 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data in Table 1; (+)-HRESI-MS  $m/z$  409.1042 [M+Na]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>7</sub>Na, 409.1012).

*Izuminoside B* (**2**). Yellow solid,  $[\alpha]_D^{20} +23.2$  ( $c$  0.12, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 454 (4.3), 366 (4.5) and 271 (5.3) nm; IR  $\nu_{max}$  (attenuated total reflection) ~3369, 2919, 2851, 1731, 1588, 1092 and 754 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data in Table 1; (+)-HRESI-MS  $m/z$  455.0890 [M+Na]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>7</sub>SNa, 455.0889).

*Izuminoside C* (**3**). Yellow solid,  $[\alpha]_D^{20} +14.3$  ( $c$  0.14, MeOH); UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ) 206 (3.9), 250 (4.3) and 367 (4.9) nm; IR  $\nu_{max}$  (attenuated total reflection) ~3369 (br), 2917, 2677, 1706, 1530, 1263 and 752 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data in Table 1; (+)-HRESI-MS  $m/z$  437.09751 [M+Na]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>18</sub>N<sub>2</sub>O<sub>8</sub>Na, 437.0962).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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