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Irciniasulfonic acid B, a novel taurine conjugated fatty acid derivative from a Japanese marine sponge, *Ircinia* sp.

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Abstract—Irciniasulfonic acid B (**1**) was obtained from a marine sponge, *Ircinia* sp., together with a known multi-drug resistance modulator irciniasulfonic acid (**2**). Spectroscopic and chemical analyses revealed structure consisting of common unsaturated fatty acids, a novel unsaturated branched fatty acid, and a taurine. Final separation of irciniasulfonic acid B (**1**) was achieved with its methylated derivatives. Irciniasulfonic acid B (**1**) reversed the multi-drug resistance in the same way as irciniasulfonic acid.

1. Introduction

In our continuing research for bioactive compounds from marine invertebrates, we isolated a novel fatty acid derivative, irciniasulfonic acid (ISA, **2**), which reverses multi-drug resistance (MDR) in human carcinoma cell lines caused by overexpression of membrane glycoprotein (P-gp) from a marine sponge *Ircinia* sp.[1] Further investigation has resulted in isolation of a novel taurine conjugated fatty acid derivative, irciniasulfonic acid B (ISA-B, **1**) from the same marine sponge. In this paper, we will describe the isolation, structure elucidation, and biological activity of ISA-B.

2. Results and discussion

2.1. Isolation and structure elucidation

The gray-colored sponge *Ircinia* sp., collected off Tsuzumi Island, Fukuoka Prefecture, Japan in 2004 was extracted with EtOH. The extract was partitioned between Et_2O and H_2O, and the aqueous layer was further extracted with *n*-BuOH. By means of TLC analysis, the *n*-BuOH extract was found to contain ISA, and its related compound was in the polar fraction. The *n*-BuOH extract was subjected to flash silica gel column chromatography (EtOAc/MeOH), reversed phase column chromatography (RP-18), and preparative TLC (EtOAc/MeOH) to give ISA (8.2 mg) and ISA-B (3.5 mg). ISA (17.3 mg) and ISA-B (8.4 mg) were also purified from the Et_2O extract in a similar manner as above.

The negative FABMS of ISA-B showed quasi-molecular ion peaks at *m/z* 652 and 626 $[M-H]^-$, caused by the diversity of a fatty acid moiety, and these molecular ion peaks were one mass unit, which is less than those of ISA. The HR-ESI TOFMS gave their molecular formula for $C_{37}H_{66}NO_6S$ and $C_{35}H_{64}NO_6S$. The fragment ion peaks at *m/z* 288 $[M-\text{fatty acids}]^-$ and *m/z* 80 $[SO_3]^-$ were also observed and these fragment ion peaks were the same as those for ISA. In a comparison of ^1H NMR spectral data for ISA-B and ISA, an oxygenated methylene proton at δ_H 4.32 (2H, t) in an isethionic acid moiety of ISA was upfield shifted at δ_H 3.51 (2H, t) in ISA-B. Furthermore, the ^1H NMR spectrum of ISA-B measured in CD_3OH showed an amide proton signal at δ_H 7.55 (1H, br s). These spectral data suggested that ISA-B consists of taurine in place of isethionic acid in ISA. Further structural analysis was achieved with a deacyl derivative of ISA-B (deacyl ISA-B, **3**). Methanolysis of ISA-B with 1.0% HCl/MeOH gave **3** and a mixture of fatty acid methyl esters (FAMEs).

Deacyl ISA-B (**3**) showed a quasi-molecular ion peak at *m/z* 306 $[M-H]^-$, IR absorption bands due to hydroxyl (3359 cm^{-1}), amide (1659, 1632 cm^{-1}) functionalities, and a UV absorption band due to α,β-unsaturated carbonyl functionality (λ_{max} 220 nm). These spectral features were quite similar to those of deacyl ISA (**4**). The ^1H, ^{13}C NMR, and HSQC spectral data indicated the presence of one primary methyl, one vinyl methyl, five aliphatic methylenes, one nitrogenous methylene, one sulfur-bearing methylene, one oxygenated methine, one tri-substituted olefin, and one amide carbonyl (Table 1). The ^1H–^1H COSY and TOCSY spectra of **3** afforded two partial structures A and B. The correlation from a terminal methyl (δ_H 0.83, H-10) to an allylic methylene (δ_H 2.51, H-4) gave the partial structure of A. The correlation between a nitrogenous methylene

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Table 1. ^{1}H and ^{13}C NMR data of deacyl ISA-B (**3**) and deacyl ISA (**4**) in CD_3OD

No.	3		4	
	^{1}H	^{13}C	^{1}H	^{13}C
1		169.1 (s)		166.1 (s)
2	5.55 (1H, br s)	119.7 (d)	5.60 (1H, br s)	115.1 (d)
3		155.7 (s)		161.6 (s)
4	2.51 (2H, t, 7.2)	33.9 (t)	2.54 (2H, m)	32.8 (t)
5	1.39 (2H, m)	29.4 (t)	1.39 (2H, m)	27.8 (t)
6	1.27, 1.39 (each 1H, m)	26.8 (t)	1.19 (2H, m)	29.3 (t)
7	1.29, 1.37 (each 1H, m)	37.8 (t)	1.28 (2H, m)	25.2 (t)
8	3.34 (1H, m)	73.8 (d)	1.30 (2H, m)	38.7 (t)
9	1.30, 1.39 (each 1H, m)	31.0 (t)	3.61 (1H, m)	67.1 (d)
10	0.83 (3H, t, 7.2)	10.4 (q)	1.05 (3H, d, 6.4)	22.0 (q)
11	1.74 (3H, d, 1.2)	24.8 (q)	1.80 (3H, d, 1.3)	23.8 (q)
1′	2.87 (2H, t, 6.6)	51.6 (t)	3.05 (2H, t, 7.2)	49.9 (t)
2′	3.51 (2H, t, 6.6)	36.3 (t)	4.32 (2H, t, 7.2)	59.0 (t)

(δ_H 3.51, H′-2) and a sulfur-bearing methylene (δ_H 2.87, H′-1) gave the partial structure of B. These two partial structures, a tri-substituted olefin and an amide carbonyl were merged by the aid of the HMBC experiment. The HMBC correlation between an olefinic methyl (δ_H 1.74, H-11) and an allylic methylene (δ_C 33.9, C-4) gave the connectivity of partial structure of A and the substituted olefin. The correlations from a nitrogenous methylene (δ_H 3.51, H′-1) and an olefinic proton (δ_H 5.55, H-2) to an amide carbonyl (δ_C 169.1, C-1) gave the connectivity of partial structures B and A. The geometry of the tri-substituted olefin was assigned as *Z* based on the NOESY spectral data and the ^{13}C chemical shifts with the value of olefinic methyl (δ_C 24.8, C-11)[2] as shown in Figure 1.

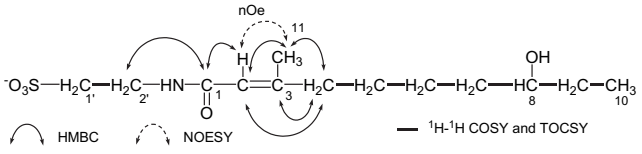

Figure 1. Plane structure of deacyl ISA-B (**3**), based on 2D NMR correlations.

The absolute configuration at C-8 was investigated by application of a modified Mosher's method.[3] Deacyl ISA-B (**3**) was esterified with (*R*)- or (*S*)-MTPA to furnish the 8-*O*-*R* (**5a**) and 8-*O*-*S*-MTPA ester (**5b**). The absolute configuration at C-8 was determined to be *R* on the basis of $\Delta\delta$ values, which were obtained from the proton signals of **5a** and **5b** as shown in Figure 2.

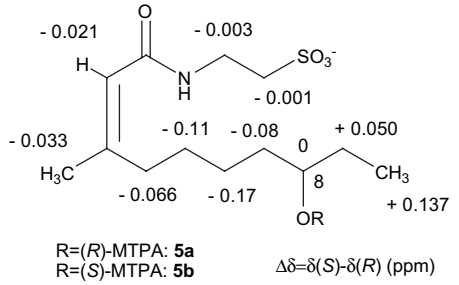

Figure 2. Application of a modified Mosher's method to **5a** and **5b**.

Fatty acid methyl esters (FAMEs) were purified by reversed phase HPLC, and each FAME was compared with those of ISA by the retention time of GC–MS and ^{1}H NMR spectra. As a result, two kinds of constituent fatty acid components were identified as (*Z*)-15-docosenoic acid for FAME-1 (**6**) and (5*Z*,9*Z*)-5,9-tetracosadienoic acid for FAME-2 (**7**). Accordingly, the structure of ISA-B (**1**) was determined to be a mixture of constituted fatty acids as shown in Figure 3.

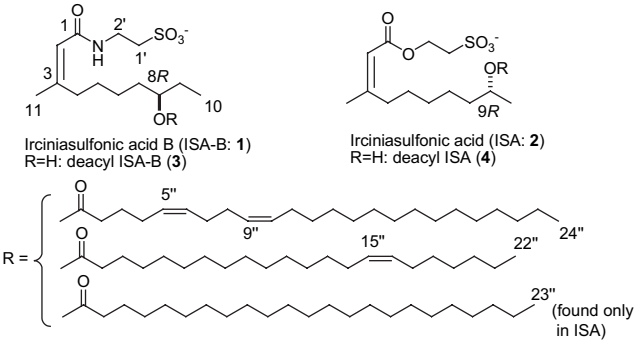

Figure 3. Structures of irciniasulfonic acids.

Because the ISA-B could not be separated into each component in an intact form, we prepared the methylated derivative of ISA-B, and applied it to HPLC separation. ISA-B was methylated with TMS–CHN_2, and successively subjected to reversed phase HPLC to give Me–ISA-B1 (**8**) and Me–ISA-B2 (**9**), respectively. The negative FABMS of **8** showed quasi-molecular ion peaks at *m/z* 666 $[M-H]^-$. The ^{1}H NMR spectrum of **8** quite resembled that of **1** except for the signals due to methoxy methyl [δ_H 3.90 (3H, s)] and the upfield shifted methylene signal [δ_H 3.31 (2H, t, H′-1)]. Me–ISA-B2 (**9**) also showed quasi-molecular ion peaks at *m/z* 640 $[M-H]^-$ in the negative FABMS and the ^{1}H NMR spectrum showed the signals due to methoxy methyl [δ_H 3.90 (3H, s)] and olefinic protons [δ_H 5.3–5.4 (2H, m)]. Thus, ISA-B (**1**) consisted of ISA-B1 and ISA-B2 (Fig. 4).

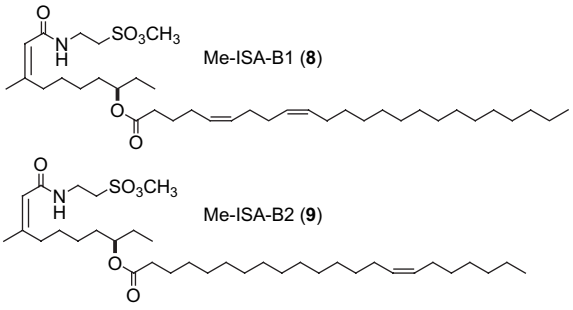

Figure 4. Structures of methylated ISA-B.

2.2. Bioactivity

ISA-B (**1**) reversed the multi-drug resistance to vincristine in KB/VJ300 cells at the concentration of 100 μM, and the reverse activity was equivalent to ISA (**2**) as shown in Figure 5.

Recently, a few taurine conjugated derivatives have been found from marine organisms.[4] Taurospongin A is the

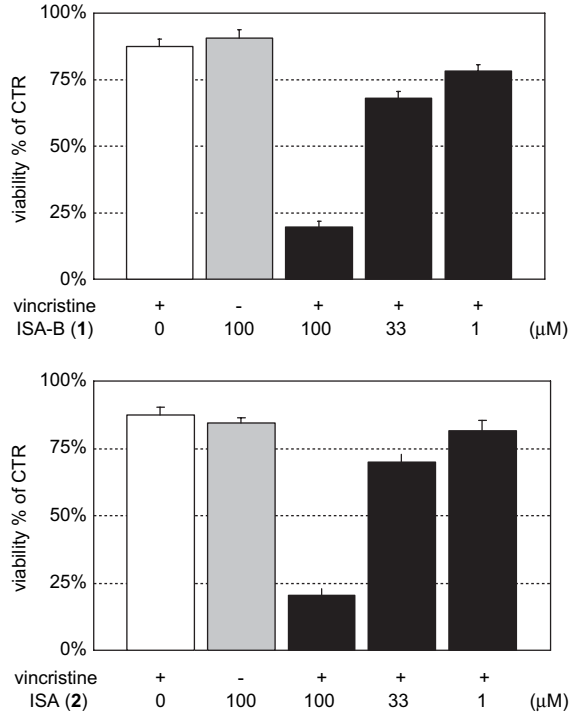

Figure 5. Reversal of MDR in KB/VJ300 cells by ISA-B (**1**) and ISA (**2**). CTR: without vincristine and testing samples, each value represents the mean±SD (n=6).

closest structural relative in the marine sponge, *Hippospongia* sp., consisting of taurine, trihydroxy fatty acid, and unsaturated fatty acid residues.[5] Taurospongin A showed a potent inhibitory activity against DNA polymerase β and HIV reverse transcriptase. Taurine is the major ingredient contained in oyster, squid, and octopus, and it is one of the free intracellular amino acids that produce oxidants and toxic substances in many tissues. It has been reported that taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed on a high-cholesterol diet.[6]

3. Experimental

3.1. General experimental procedures

NMR spectra including COSY, TOCSY, NOESY, HSQC, and HMBC experiments were recorded in CD_3OD or CD_3OH on a Varian INOVA 600 spectrometer at 600 MHz (^{1}H) or 150 MHz (^{13}C) NMR, and a Varian INOVA 400 spectrometer at 400 MHz (^{1}H), using tetramethylsilane (TMS) as an internal standard. FABMS data were obtained using a JEOL SX 102 mass spectrometer using triethyleneglycol (TEG) as a matrix. HR-ESI TOFMS was obtained on a Micromass Q-TOF Ultima LCMS spectrometer (Waters). UV spectra were recorded on a Jasco Ubest-30 Spectrometer in MeOH. IR spectra were obtained in $CHCl_3$ on a Jasco FTIR-410 spectrometer. The optical rotation was recorded on a Jasco DIP 370 digital polarimeter. The GC–MS was carried out on a Neutra BOND-5 capillary column (GL Science) with a Shimadzu QP5050A gas chromatograph mass spectrometer. Reversed phase high-performance liquid chromatography (HPLC) was carried out on a YMC-Pack Pro C_{18} column (YMC) and a Cosmosil 5C_{18} AR-II column (Nacalai tesque). Column chromatography was carried out on a silica gel 60 (70–230 mesh, Merck), and TLC was performed on precoated silica gel 60 F_{254} plates (Merck).

3.2. Collection, extraction, and isolation

Ircinia sp. (wet weight 790 g) was collected by hand at a depth of 10 m off Tsuzumi Island, Fukuoka Prefecture, Japan, in October of 2004. The sponge was homogenated and extracted with EtOH (3×1 L) and filtered. The extract was evaporated in vacuo, and the resulting aqueous suspension was diluted with H_2O (500 mL) and extracted with Et_2O (3×0.5 L) and *n*-BuOH (3×0.5 L). These organic layers were evaporated to give Et_2O extract (2.30 g) and *n*-BuOH extract (2.09 g). The *n*-BuOH extract was subjected to flash silica gel column chromatography with EtOAc/MeOH (10/0.05→10/0.5→10/1)→MeOH to give five fractions [Fr.1 (44.1 mg), Fr.2 (5.5 mg), Fr.3 (9.5 mg), Fr.4 (9.1 mg), and Fr.5 (516.8 mg)]. Fr.5 was dissolved in EtOAc/MeOH (10/1), and centrifuged. The supernatant was further chromatographed on silica gel with EtOAc/MeOH (10/1) to give three fractions [Fr.5-1 (**2**, 1.8 mg), Fr.5-2 (33.2 mg), and Fr.5-3 (124.3 mg)]. Fr.5-2 was subjected to reversed phase column chromatography (RP-18) with 90% MeOH/H_2O to give two fractions Fr.5-2-1 (15.2 mg) and Fr.5-2-2 (13.8 mg). Fr.5-2-2 was subjected to preparative TLC with EtOAc/MeOH (5/1) to give ISA (**2**, 6.4 mg) and ISA-B (**1**, 3.5 mg).

3.2.1. Irciniasulfonic acid B (1). White amorphous, IR ν_{max} ($CHCl_3$) cm^{-1}: 2928, 2855, 1721, 1658, 1461. Negative FABMS *m/z*: 652, 626, 288, 124, 80. HR-ESI TOFMS *m/z* 652.4604 [M−H]$^-$ (calcd for $C_{37}H_{66}NO_6S$, 652.4611), 626.4473 [M−H]$^-$ (calcd for $C_{35}H_{64}NO_6S$, 626.4454). ^{1}H NMR (CD_3OD) δ 0.79, 0.801 (terminal methyls), 1.11–1.60 (aliphatic methylenes), 1.73 (H-11), 1.92–2.22 (aliphatic methylenes), 2.40 (CO–CH_2), 2.52 (H-4), 2.88 (H′-1), 3.52 (H′-2), 4.88 (H-8), 5.35 (CH═CH), 5.55 (H-2), 7.58 (CO–NH, in CD_3OH). ^{13}C NMR (CD_3OD) δ 9.9 (C-10), 14.3 (terminal methyl), 23.6 (C-11), 24.0–36.0 (methylenes), 51.5 (C′-1), 76.6 (C-8), 119.6 (C-2), 129.9, 130.7, 131.2 (CH═CH), 155.6 (C-3), 169.0 (C-1), 175.1 (C═O).

3.2.2. Methanolysis of 1. ISA-B (**1**, 5.6 mg) was dissolved in 3.0 mL of 1% HCl/MeOH, and the solution was refluxed for 4 h. The reaction mixture was extracted with *n*-hexane (3×3 mL), and the remaining layer was neutralized with Ag_2CO_3, and filtrated. The *n*-hexane layer was evaporated in vacuo to give the mixture of fatty acid methyl esters (FAMEs), and subjected to reversed phase HPLC (YMC-Pack Pro C_{18}) with 100% MeOH to give FAME-1 (**6**, 0.5 mg) and FAME-2 (**7**, 1.0 mg). The filtrate was evaporated in vacuo, and subjected to silica gel column chromatography with $CHCl_3$/MeOH/H_2O (8/2/0.2) to give deacyl ISA-B (**3**, 1.6 mg).

3.2.3. Deacyl ISA-B (3). White amorphous, $[\alpha]_D$ +9.0 (*c* 0.1, MeOH). IR ν_{max} ($CHCl_3$) cm^{-1}: 3359, 2932, 2857, 1659, 1632, 1539, 1375, 1200. UV λ_{max} (MeOH) nm: 220 (ε=6490). Negative FABMS *m/z*: 306 [M−H]$^-$. ^{1}H and ^{13}C NMR, see Table 1.

3.2.4. Preparation of (*R*)- and (*S*)-MTPA esters. A solution of deacyl ISA-B (**3**, 0.7 mg, 2.3 μmol) in CH_2Cl_2 (100 μL) was added to (*R*)-α-methoxy (trifluoromethyl) phenyl acetic acid (MTPA) (5.4 mg, 23 μmol), *N*,*N*′-dicyclohexyl carbodiimide (DCC) (16.3 mg, 80 μmol), and a catalytic amount of 4-dimethylaminopyridine (DMAP). The solution was stirred for 20 h at room temperature and filtered. The filtrate was dried with N_2, and subjected to silica gel column chromatography with *n*-hexane/EtOAc (20/1) to give (*R*)-MTPA ester (**5a**, 0.8 mg, 1.5 μmol). (*S*)-MTPA ester (**5b**, 0.9 mg, 1.7 μmol) was prepared in a similar manner as (*R*)-MTPA ester. Compound **5a**, negative FABMS *m*/*z*: 522 $[M-H]^-$. ^1H NMR (CD_3OD) δ 0.703 (H-10), 1.260 (H-6), 1.380 (H-5), 1.530 (H-9), 1.580 (H-7), 1.730 (H-11), 2.514 (H-4), 2.871 (H′-1), 3.514 (H′-2), 4.931 (H-8), 5.553 (H-2). Compound **5b**, negative FABMS *m*/*z*: 522 $[M-H]^-$. ^1H NMR (CD_3OD) δ 0.840 (H-10), 1.090 (H-6), 1.270 (H-5), 1.580 (H-9), 1.500 (H-7), 1.697 (H-11), 2.448 (H-4), 2.870 (H′-1), 3.511 (H′-2), 4.931 (H-8), 5.532 (H-2). These chemical shifts were assigned by ^1H–^1H COSY and TOCSY spectral data.

3.2.5. FAME-1 (6) and FAME-2 (7). FAME-1 (**6**): *O*-methyl (*Z*)-15-docosenoate, t_R=29.3 min, EIMS *m*/*z*: 352 $(M)^+$, 320 $(M-32)^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (3H, t, *J*=6.8 Hz, terminal methyl), 1.24 (aliphatic methylenes), 1.99 (4H, m, allylic methylenes), 2.28 (2H, t, *J*=7.2 Hz, CO–CH_2), 3.64(3H, s, OCH_3), 5.33 (2H, m, CH=CH). FAME-2 (**7**): *O*-methyl (5*Z*,9*Z*)-5,9-tetracosadienoate, t_R=32.7 min, EIMS *m*/*z*: 378 $(M)^+$, 346 $(M-32)^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 0.84 (3H, t, *J*=6.8 Hz, terminal methyl), 1.24 (aliphatic methylenes), 2.00 (8H, m, allylic methylenes), 2.29 (2H, t, *J*=7.2 Hz, CO–CH_2), 3.65 (3H, s, OCH_3), 5.34 (4H, m, CH=CH).

3.2.6. Methylation of 1. A solution of ISA-B (**1**, 2.5 mg) in dried MeOH (1 mL) was added to 0.1 mL of trimethylsilyldiazomethane (TMS–CHN_2), and stirred for 1 h at room temperature. The reaction mixture was dried with N_2 and subjected to silica gel column chromatography with *n*-hexane/EtOAc (20/1), then reversed phase HPLC (Cosmosil AR-II $5C_{18}$) with 100% MeOH to give Me–ISA-B1 (**8**, 0.5 mg) and Me–ISA-B2 (**9**, 0.9 mg). Compound **8**: negative FABMS *m*/*z*: 666 $[M-H]^-$, 652 $[M-15]^-$. ^1H NMR (CD_3OD) δ 0.79, 0.80 (terminal methyl, H-10), 1.25–1.60 (aliphatic methylenes), 1.75 (H-11), 1.9–2.0 (aliphatic methylenes), 2.22 (CO–CH_2), 2.53 (H-4), 3.31 (H′-1), 3.50 (H′-2), 3.90 (O–CH_3), 5.2–5.35 (4H, CH=CH), 5.56 (H-2). Compound **9**: negative FABMS *m*/*z*: 640 $[M-H]^-$, 626 $[M-15]^-$. ^1H NMR ($CDCl_3$) δ 0.86 (terminal methyl, H-10), 1.24 (methylenes), 1.82 (H-11), 2.0–2.4 (methylenes), 2.60 (H-4), 3.32 (H′-1), 3.74 (H′-2), 3.90 (O–CH_3), 4.80 (H-8), 5.3–5.4 (2H, CH=CH), 5.50 (H-2).

4. Bioassay

Multi-drug resistant KB/VJ300 cells were maintained in an EMEM medium supplemented with 10% FBS and 100 ng/mL of vincristine. The reversing activities of ISA-B and ISA were measured by means of a CellTiter 96® aqueous colorimetric assay (Promega) performed in 96-well plates. KB/VJ300 cells were seeded at 1.0×10^4 cells/mL with 180 μL of the culture medium, then grown with or without vincristine and several dilutions of testing samples with 20 μL of culture medium for 72 h (37 °C, 5% CO_2). Thereafter, 10 μL of CellTiter 96® was added to each well and incubated for a further 2 h, and the percentage of cell growth inhibition was evaluated from the absorbance at 490 nm.

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