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Structure of a novel multidrug resistance modulator, irciniasulfonic acid, isolated from a marine sponge, *Ircinia* sp.

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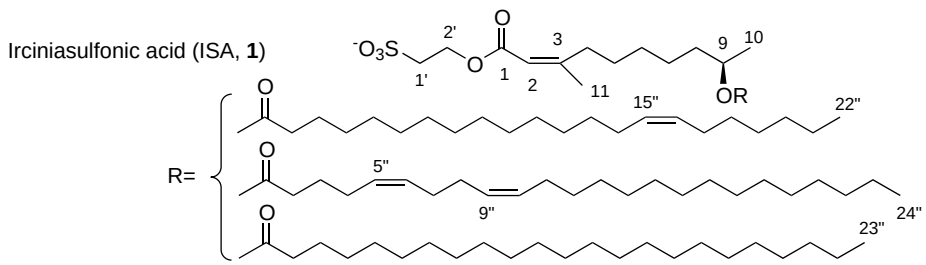
Abstract—Irciniasulfonic acid was obtained from a marine sponge of *Ircinia* sp. Spectroscopic and chemical analyses revealed its structure consists of three different kinds of acids, i.e. common fatty acids, a novel unsaturated branched C-10 fatty acid and an isethionic acid. Irciniasulfonic acid and deacyl irciniasulfonic acid reverse multidrug resistance in human carcinoma cells caused by overexpression of membrane glycoprotein.

Multidrug resistance (MDR) is one of the main causes for failure in chemotherapy treatment for cancer patients. Overexpression of a membrane glycoprotein (termed P-glycoprotein or P-gp) confers resistance by acting as an energy-dependent drug transporter or an efflux pump, which results in the decreased cellular accumulation of drugs. Since the discovery of a calcium channel blocker verapamil was recognized as an agent for reversing MDR, a variety of compounds, including cyclosporin A, have been reported to reverse MDR. For example, agosterol A,[1] patellamide D,[2] and lamellarin I[3] have recently been isolated from marine invertebrates as resistance modulators.

While searching for other MDR modulators in marine invertebrates, we isolated a novel fatty acid analogue named irciniasulfonic acid (ISA) as an active constituent from the Japanese marine sponge, *Ircinia* sp. In this paper, we will report on the isolation and structural determination of ISA.

Ircinia sp. (wet weight 2.8 kg) was collected by hand at depths of 10–15 m off Tsutsumi Island, Fukuoka prefecture, Japan, in 1999.[4] The Et_2O soluble fraction (3.0 g) of the EtOH extract obtained from *Ircinia* sp. reversed MDR at 33 μg/mL against P-gp overexpressing MDR tumor cells (KB/VJ300)[5] in the presence of



Deacyl irciniasulfonic acid (**2**): R=H

Figure 1.

Keywords: multidrug resistance; irciniasulfonic acid; *Ircinia* sp.; sulfonic acids and derivatives.
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10 ng/mL of vincristine, but no cytotoxicity was seen against parental KB cells at 100 μg/mL. Bioassay guided the separation of the active fraction by Sephadex LH-20 ($CHCl_3$:MeOH 1:1) and silica gel chromatography (EtOAc:MeOH 10:1) to give an active compound, ISA (**1**, Fig. 1) (49.5 mg, 1.7×10^{-3} % yield).

ISA (**1**)[6] was obtained as a colorless oil and showed a positive reaction to the qualitative analysis of sulfonic acid.[7] The IR spectrum revealed a strong absorption band due to ester carbonyl (1719 cm^{-1}). The ^1H and ^{13}C NMR data suggested the presence of a primary methyl, a secondary methyl, a tri-substituted olefin, an oxygenated methine, esters and a long alkyl chain. The negative-FABMS showed a series of quasi-molecular ion peaks at m/z 653, 643, 627 $[M-H]^-$, caused by the diversity of a fatty acyl moiety, together with a base peak at m/z 289 $[M-\text{fatty acids}]^-$, and a fragmentation peak at m/z 80 $[SO_3]^-$. Methanolysis with 5% HCl/MeOH gave deacyl ISA (**2**),[8] a mixture of fatty acid methyl ester, and a small amount of isethionic acid ($HO_3SCH_2CH_2OH$).[9]

Deacyl ISA (**2**) showed the presence of sulfonic acid, and the UV spectrum indicated the presence of α,β-unsaturated carbonyl group [λ_{max} 220 nm (ε 6300)]. The IR spectrum showed the absorption bands due to hydroxyl (3412 cm^{-1}) and ester carbonyl (1705 cm^{-1}). The HR-API-TOFMS showed the molecular ion peak at m/z 307.1218 (Δ+0.3 mmu) corresponding to the molecular formula of $C_{13}H_{23}O_6S$. The ^1H, ^{13}C NMR and HSQC spectral data suggested the presence of one secondary methyl, one olefinic methyl, seven methylenes, one oxygenated methine, one tri-substituted olefin and one conjugated ester. The ^1H–^1H COSY and TOCSY spectra of **2** afforded partial structures A and B and these two partial structures and the conjugated ester were merged by the aid of the HMBC experiment as shown in Fig. 2. The geometry of the tri-substituted olefin was assigned as *Z* from the ^{13}C-chemical shifts value ($\delta_C = 23.8$).[10]

The absolute configuration at C-9 was investigated by application of modified Mosher's method.[11] Deacyl ISA was treated with *S*-(−)- or *R*-(+)-2-methoxy-2-phenyl-2-trifluoromethyl-acetyl chloride to furnish the 9-*O*-*R*-(+) (**2a**) and 9-*O*-*S*-(−)-MTPA ester (**2b**). The absolute configuration at C-9 was determined to be *R* on the basis of $\Delta\delta$ values, which were obtained from the proton signals of **2a** and **2b**[12] as shown in Fig. 3.

Fatty acid methyl esters (FAMs) were purified by reversed phase HPLC, and each FAM was analyzed by GC–MS. The positions of double bonds were determined by the mass fragmentation of their DMDS derivatives,[13] and their geometry was elucidated by the allylic ^{13}C carbon signals.[14] Thus, the three kinds of constituent fatty acid components were identified as tricosanoic acid,[15a] (*Z*)-15-docosenoic acid,[15b] and (5*Z*,9*Z*)-5,9-tetracosenoic acid.[15c] Accordingly, the final structure of **1** was determined as shown in Fig. 1.

ISA reversed the multidrug resistance to vincristine in KB/VJ300 cells at 25 μg/mL (ca. 38 μM), which corresponds to 1 μM of verapamil. However, **2** was 13-fold stronger than **1** (ca. 3 μM), but isethionic acid had no activity. The details of the biological activities will be reported elsewhere.

To our knowledge, irciniasulfonic acid is the first naturally occurring product composed of normal fatty acids, isethionic acid and a unique fatty acid, 9-hydroxy-3-methyl-2-decenoic acid. Irciniasulfonic acid and its analogues will be useful for human cancer chemotherapy as MDR modulators in a clinical cure.

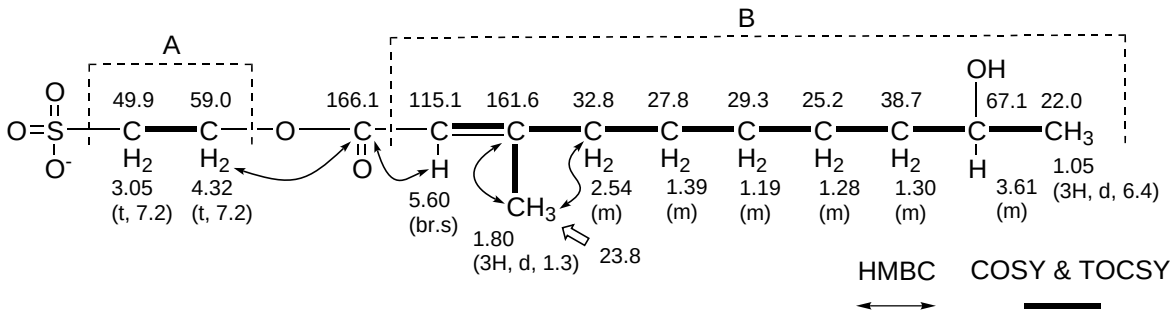

Figure 2. ^1H–^1H COSY, TOCSY and HMBC correlations of **2**.

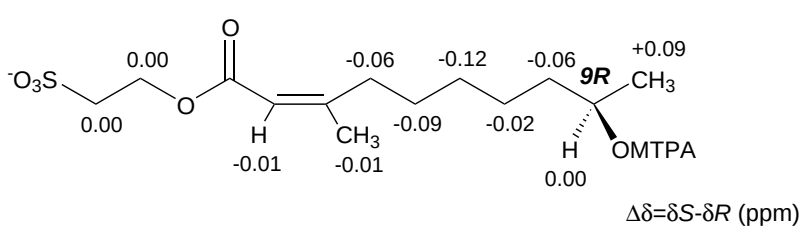

Figure 3. Application of modified Mosher's method to **2**.

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6. Irciniasulfonic acids (**1**): IR ($CHCl_3$, cm^{-1}) 2927, 2855, 1645, 1719, UV (MeOH, λ_{max}) 220 nm (ε 6100), negative-FABMS (m/z) 653, 643, 627 $[M-H]^-$, 363, 353, 337 $[R-COO]^-$, 289 [M−fatty acid]$^-$, 80 $[SO_3]^-$, ^1H NMR (CD_3OD, 600 MHz, δ): 0.77, 0.80 (t, methyls), 1.11 (3H, d, 6.6 Hz, H_3-10), 1.15–2.55 (methylenes), 1.39 (m, H_2-5), 1.80 (s, H_3-11), 3.08 (t, 7.2 Hz, H_2-1′), 4.33 (t, 7.2 Hz, H_2-2′), 4.79 (m, H-9), 5.25, 5.27 (m, -CH=CH-), 5.60 (s, H-2), ^{13}C NMR (CD_3OD, 600 MHz, δ): 13.0 (terminal methyl), 18.9 (C-10), 23.9 (C-11), 25.0–35.0 (methylenes), 27.7 (C-5), 32.7 (C-4), 34.1 (C-2″), 49.9 (C-1′), 58.8 (C-2′), 70.7 (C-9), 115.1 (C-2), 128.5, 128.7, 129.4, 129.9 (-CH=CH-), 161.6 (C-3), 165.9 (C-1), 173.0, 173.6, 173.8 (C-1″).
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8. Deacyl irciniasulfonic acid (**2**): IR ($CHCl_3$, cm^{-1}) 3412 (OH), 2931, 2857, 1705, 1645, 1170, UV (MeOH, λ_{max}) 220 nm (ε 6300), ^1H, and ^{13}C NMR (CD_3OD): summarized in Fig. 1.
9. Isethionic acid was identical with the authentic sample (Wako Pure Chemical Industries Ltd.) in spectral data; however, the *O*-methyl-9-hydroxy-3-methyl-2-decenoate could not be isolated.
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12. **2a**: ^1H NMR (CD_3OD, 600 MHz, δ): 1.15 (H_3-10), 1.27 (H_2-6), 1.37 (H_2-5), 1.51 (H_2-7), 1.57 (H_2-8), 1.79 (H_3-11), 2.52 (H_2-4), 3.03 (H_2-1′), 4.32 (H_2-2′), 5.03 (H-9), 5.60 (H-2), **2b**: ^1H NMR (CD_3OD, 600 MHz, δ): 1.24 (H_3-10), 1.14 (H_2-6), 1.28 (H_2-5), 1.49 (H_2-7), 1.51 (H_2-8), 1.78 (H_3-11), 2.46 (H_2-4), 3.03 (H_2-1′), 4.32 (H_2-2′), 5.03 (H-9), 5.60 (H-2).
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15. (a) *O*-methyl tricosanoate (41.0% by GC); EIMS (m/z) 368 (M^+), 337, 325, 87, 74; (b) *O*-methyl (*Z*)-15-docosenoate (17.1%): EIMS (m/z) 352 (M^+); ^{13}C NMR ($CDCl_3$, δ): 26.9 (-CH_2-CH=CH-CH_2-); DMDS derivative: EIMS (m/z) 446 (M^+), 301 ($C_{17}H_{33}O_2S^+$), 145 ($C_8H_{17}S^+$); (c) *O*-methyl (5*Z*,9*Z*)-5,9-tetracosenoate (41.9%); EIMS (m/z) 378 (M^+); ^{13}C NMR ($CDCl_3$, δ): 24.8, 27.2 (-CH_2-CH=CH-CH_2-); DMDS derivative: EIMS (m/z); 504 (M^+), 343 ($C_{20}H_{39}S_2^+$), 257 ($C_{16}H_{33}S^+$), 247 ($C_{11}H_{19}O_2S_2^+$), 161 ($C_7H_{13}O_2S^+$).