7-Methyl-9-oxo-dec-7-eneoic acid from the Red Sea sponge *Ircinia* sp

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An NMR and ultra-high resolution mass investigation of secondary metabolites from a Red Sea sponge *Ircinia* sp. led to the isolation and characterisation of a new ketone. The structure has been assigned on the basis of detailed spectroscopic analysis (^{1}H, ^{13}C, DEPT, COSY, HMQC and HMBC NMR spectra) as 7-methyl-9-oxo-dec-7-eneoic acid (**1**). Ultra-high resolution FTMS was utilised to facilitate the formulation and structure assignment because there was a broad and undetectable carbon signal in the NMR spectra.

Keywords: Irciniidae, *Ircinia* sp., 7-methyl-9-oxo-dec-7-eneoic acid

The marine environment has proven to be a rich source of unique metabolites that have demonstrated significant activities in cytotoxicity, immunomodulation and cognitive disease targets.[1] In the sponge phylum Porifera, the order Dictyoceratida have attracted interest for their bioactive sesterterpene secondary metabolites, mainly from the genera *Ircinia*.[2] The metabolites from *Ircinia* sp. display a wide range of bioactivity including the inhibition of fertilised starfish egg cell division, protein phosphatase inhibition and anti-inflammatory activities.[3] In the course of our research regarding bioactive compounds from coral reef organisms, we found that the EtOAc extract from a sponge of the genus *Ircinia* showed moderate activity on Alzheimer's diseases. NMR guided chromatographic separation of this extract led to the isolation of a new ketone (**1**). In this paper, we report the isolation, structure elucidation of **1** using NMR and ultra-high resolution mass spectrometry. The biological activity of this metabolite is also reported.

The sample of sponge was collected from the Red Sea and exhaustively extracted with acetone. The EtOAc soluble part of the acetone extract was subjected to silica gel vacuum-liquid chromatography followed by column chromatography and reverse-phase HPLC to yield compound **1**.

Compound **1** was obtained as colourless oil. The ^{13}C NMR data of **1** showed the presence of 11 carbon signals, which were identified by the assistance of a DEPT spectrum as carbonyl groups at δ 198.5, a long chain of methylene carbons at δ 23.5–43.1, olefenic carbons at δ 159.5 and 117.2, and methyl groups at δ 30.9 (terminal) and 24.9. The sample (~15 μg) was dissolved into 1:1 water/methanol solution and injected into an actively shielded 9.4 Tesla superconducting mass spectrometer using nanoelectrospray in the positive detection mode. Four major peaks were detected with *m/z* 221.11454 (calcd. 221.11481), 243.09662 (calcd. 243.09676), 441.22236 (calcd. 441.22235) and 463.20434 (calcd. 463.20430). These four ions could be assigned as the $[M + Na]^+$, $[M + 2Na\text{-}H]^+$, $[2M + 2Na\text{-}H]^+$ and $[2M + 3Na\text{-}2H]^+$ ions based on the relation and differences between these *m/z* values. An ion at *m/z* 197.11818 (calcd. 197.11832) was detected in the negative mode experiment and was assigned as the $[M–H]^-$ molecular ion. Therefore the molecular formula of **1** was clearly $C_{11}H_{18}O_3$ for the neutral molecule. This is one additional carbon unit more than was observed using ^{13}C NMR due to line broadening of one carbon resonance. Although this cluster of positively charged species is rare for most compounds, mass measurement accuracy strongly supports this interpretation. Loss of H_2O and CO_2 from the $[M + 2Na\text{-}H]^+$ ion at *m/z* 243 was observed in the SORI-CID experiment suggesting a COOH moiety and this is in agreement with the observation of the strong preference of sodium by this compound. Finally, the detected and simulated isotopic distributions agree very well with each other based on the proposed molecular formula $C_{11}H_{18}O_3$. The IR spectrum (KBr) of compound **1** showed absorption bands at 1662 cm^{-1} further supporting the presence of a carbonyl functionalities. The ^{1}H NMR spectrum exhibited two-proton signals at δ 2.13 (30.9, C-10) and δ 1.90 (24.9, C-11), each integrating for three protons, and these assignments were confirmed using the HMQC and HMBC spectrums. The presence of a methyl-substituted olefin was suggested by the olefinic proton resonance observed at δ 5.67 (br s), which showed one-bond coupling to the carbon observed at δ 117.2 (d) and long-range coupling to the carbons observed at δ 159.5 (s) and 24.9 (q) and to the methyl protons observed at δ 1.90 (s) in the appropriate proton homonuclear or long- and short-range C–H two dimensional NMR correlation experiments. Five additional methylene doublets were observed at δ 2.62 (2H, dd, J = 15.2/7.6 Hz), 2.43 (2H, dd, J = 14.8/7.6 Hz), 1.59 (2H, ddd, J = 14.8/7.6/7.2 Hz), 1.48 (2H, ddd, J = 14.8/7.6/7.2 Hz) and 1.33 (2H, ddd, J = 14.8/7.6/7.2 Hz) in the proton NMR spectrum of **1**. The sequence of C_2–C_6 was unambiguously assigned by analysis of the HMQC, HMBC (Fig.1) and ^{1}H–^{1}H COSY spectra. A connection was assigned from an HMBC correlation between H-2 [δ 2.43, dd (t)] and C-3 (δ 23.5, t); H-2 and C-4 (δ 28.9, t); H-3 (δ 1.59, ddd) and C-4; H-6 [δ 2.62, dd (t)] and C-4; H-6 and C-5 (δ 27.7, t), establishing the gross structure. The carbon resonance for the carbonyl group (C-1) was not be observed, however, mass measurement strongly supports this interpretation and it is in accord with the presence of methylene group linked to carbonyl. The complete assignments of all proton and carbon resonances were based on the ^{1}H, ^{1}H COSY, HMQC and HMBC experiments. The structure of **1** was determined to be 7-methyl-9-oxo-dec-7-eneoic acid.

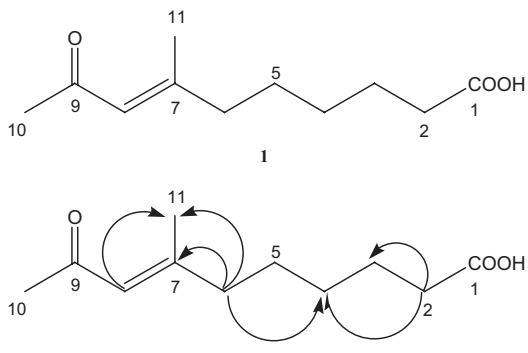

Fig. 1 Important HMBC correlations of **1**.

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The chemistry of this *Ircinia* sponge is extraordinarily rich in the range of secondary metabolite. Typical *Ircinia* constituents consist of linear furanosesterterpenes, which are typically present in very high yields[3,5]. The 7-methyl-9-oxo-dec-7-eneoic acid (**1**) represents an additional metabolite which is reported for the first time in nature. The 7-methyl-9-oxo-dec-7-eneoic acid (**1**) which was isolated did not show any significant biological activity against tumor cells, bacteria or fungi.

Experimental

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV and IR spectra were obtained using an AATI Mattson Genesis Series FTIR and a Hewlett-Packard 8452A Diode Array spectrometer. All NMR spectra were acquired on a Bruker AM-500 spectrometer using $CDCl_3$. High resolution mass spectra (HRMS) of sample were obtained using a Bruker (Billerica, MA) APEX II FTICR mass spectrometer equipped with an actively shielded 9.4 Tesla superconducting magnet (Magnex Scientific Ltd., UK), an external Bruker APOLLO ESI source, and a Synrad 50 W CO_2 CW laser. HPLC separations were carried out on a Waters 510 model system with reversed phase silica (Luna C8, 21.5 x 250 mm, 5 μm). Kieselgel 60 (230–400 mesh) silica gel was used for column chromatography. TLC was carried out on precoated silica 60 F_{254} plates and visualised with vanillin–EtOH–1% H_2SO_4.

Collection, identification and taxonomy of sponge Ircinia sp.

The marine sponge *Ircinia* sp. was collected from the Red Sea in March 1997.[3] The sponge forms a massive sphere 40 cm long, 20 cm wide and 10 cm high and was found on vertical coral faces. The surface is covered with regularly spaced tough conules 3–5 mm high. The sponge is tough, compressible, and elastic. The colour in life is light yellowish brown and darker brown in shaded areas. The skeleton consists of very robust golden ladder-like fibre fascicles with embedded sand grains, and fine collagen fibrils permeate the choanosome. The sponge is closely comparable to *Ircinia* sp. (order Dictyoceratida, family Irciniidae). A voucher specimen has been deposited at the National Institute of Water and Atmospheric Research Ltd., Auckland, New Zealand and the Department of Pharmacognosy, The University of Mississippi (97RS).

Extraction and isolation.

A sample of frozen sponge (1.4 kg wet weight) was cut into small pieces and extracted three times with acetone at room temperature. The combined extracts were concentrated and partitioned between EtOAc and H_2O. The organic layer was concentrated to give an oil (28 g) which was chromatographed on silica gel by eluting with a step gradient of hexane–CH_2Cl_2–EtOAc–MeOH. Four fractions were obtained. On the basis of the characteristic signals observed in ^1H NMR spectra, fraction III was selected for further purification using HPLC (RP-C8, AcCN–H_2O over 40 min) to afford compound **1** (1.5 mg).

7-Methyl-9-oxo-dec-7-eneoic acid (**1**): Colourless oil ($CHCl_3$), UV (EtOH) λ_{max} (log ε) 254 (3.12), 268 (2.86) nm; IR (KBr) ν_{max} cm^{-1} 2932, 1662, 1427, 1379, 1081; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.67 (1H, br s, H-8), 2.62 (2H, dd, J = 15.2, 7.6, H_2-6), 2.43 (2H, dd, J = 14.8, 7.6 Hz, H_2-2), 2.13 (3H, s, CH_3-10), 1.90 (3H, s, CH_3-11), 1.59 (2H, ddd, J = 14.8, 7.6, 7.2 Hz, H_2-3), 1.48 (2H, ddd, J = 14.8, 7.6, 7.2 Hz, H_2-5), and 1.33 (2H, ddd, J = 14.8, 7.6, 7.2 Hz, H_2-4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 198.5 (*C*O, C-9), COOH (C-1) not observed, 159.5 (C, C-7), 117.2 (CH, C-8), 43.1 (CH_2, C-2), 32.6 (CH_2, C-6), 30.9 (CO*C*H_3, C-10), 28.9 (CH_2, C-4), 27.7 (CH_2, C-5), 24.9 (CH_3, C-11), and 23.5 (CH_2, C-3); HRESIMS *m/z* 221.11454 ($C_{11}H_{18}O_3$, $[M + Na]^+$, Δ = –0.27 mmu or –1.22 ppm, calcd for 221.11481), 243.09662 ($[M + 2Na\text{-}H]^+$, Δ = –0.14 mmu or –0.58 ppm calcd. for 243.09676), 441.22236 ($[2M + 2Na\text{-}H]^+$, Δ = 0.01 mmu or 0.02 ppm, calcd. for 441.22235) and 463.20434 ($[2M + 3Na\text{-}2H]^+$, Δ = 0.04 mmu or 0.09 ppm, calcd. for 463.20430).

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