

Ircinianin Sulfate from the Marine Sponge *Ircinia (Psammocinia) wistarii*

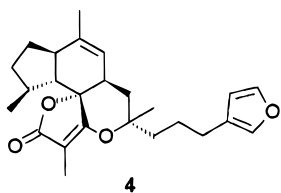
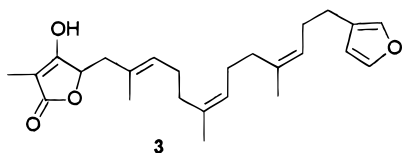
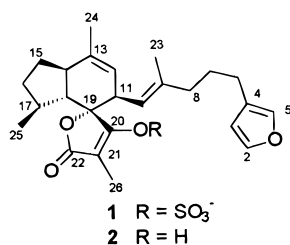
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Received June 12, 1997[®]

Extraction of *Ircinia (Psammocinia) wistarii* Wilkinson from Lamont Reef, Great Barrier Reef, with MeOH has yielded the very unstable sulfate ester (**1**) of the known sesterterpene ircinianin (**2**).

Marine sponges of the order Dictyoceratida have attracted interest for their unusual sesterterpene secondary metabolites. The genus *Ircinia* in particular has yielded many linear sesterterpenes, which contain both furan and tetronic acid moieties, for example, palominin (**3**).¹ The most unusual of the *Ircinia* sesterterpene metabolites, however, has been the tetracyclic furanoterpene ircinianin (**2**), first reported from *Ircinia wistarii* by Hofheinz and Schonholzer,² and its pentacyclic isomer wistarin (**4**), more recently isolated³ by Gregson and Ouvrier from one of a number of specimens of *I. wistarii* collected at various locations around Australia.



Extraction in our laboratory of a specimen of *I. wistarii* Wilkinson (Irciniidae) collected from the Great Barrier Reef resulted in isolation of a polar, very unstable, major secondary metabolite. This compound had better stability when the sponge was extracted with MeOH, and because it decomposed immediately when dissolved in CHCl₃, NMR spectra were obtained in CD₃-OD solutions. Rapid step-gradient column chromatography on Si gel of the Et₂O-soluble portion of the MeOH extract gave ircinianin sulfate (**1**) as a light yellow gum.

Smaller amounts of the known compounds ircinianin and wistarin were also recovered from earlier column fractions and identified by comparison of their ¹H- and ¹³C-NMR spectra with those reported in the literature.^{2,3}

The ¹H- and ¹³C-NMR spectra of **1** showed a sesterterpene containing furan (β -substituted) and tetronic acid functionalities, with the DEPT135 spectrum indicating a probable ten degrees of unsaturation, six of which had already been accounted for. An additional two degrees of unsaturation were satisfied by carbon-carbon double bonds, implying a tetracyclic structure for **1**. The presence of an oxygenated quaternary carbon at 87.7 ppm suggested a possible spiro ring junction at C-19. Comparison of the spectra of **1** with those reported for ircinianin (**2**) revealed close similarities. Assignments for **1** (e.g., C-8 and C-17), which differ significantly from those reported³ for **2**, are supported by DEPT135 and COSY, HMBC, and HMQC 2D-NMR correlations, and by comparison with the ¹³C- and DEPT135-NMR spectra of **2** in MeOH and CD₃CN in our laboratory. We believe the anomalies between the ¹³C spectra of **1** and **2** are due to assignment errors rather than to any differences in structure. Assignments of the ¹³C-NMR spectrum of **1**, and our partial reassignment of signals for **2**, are shown in Table 1.

The greater separation of the C-20 and C-22 carbon signals in **1**, and the downfield shift of C-26, are typical of C-20 esters of the tetronic acid moiety. However, as there are no more carbons in **1** than in **2**, an inorganic ester, most likely a sulfate, is required. This was confirmed by the negative ion HRMS of **1**, which gave a molecular ion at m/z 475 for C₂₅H₃₁O₇S.

The rarity of sesterterpene sulfate esters was noted by Faulkner⁴ in his paper describing the isolation of the halisulfates. Our finding that ircinianin occurs as a tetronic acid sulfate may indicate that the linear sesterterpene compounds such as **3** are also present in the living sponge as very unstable sulfate esters.

Our study of ircinianin sulfate also casts some doubt on the true status of both ircinianin and wistarin as natural products. Although Gregson and Ouvrier noted³ that wistarin might arise from ircinianin during isolation, they reported that ircinianin did not cyclize to wistarin with either acid, base, or Lewis acid catalysis. We have observed, however, that, although only trace amounts of wistarin are isolated directly from the sponge, it can be isolated in 18% yield from the decomposition of ircinianin sulfate in CHCl₃, and hence both wistarin and ircinianin could be formed from ircinianin sulfate during extraction of *I. wistarii*. The

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[®] Abstract published in *Advance ACS Abstracts*, October 15, 1997.

Table 1. Comparison of ^{13}C Spectra of **1** and **2**

carbon	1 (CD ₃ OD)	2 (CD ₃ CN)	carbon	1 (CD ₃ OD)	2 (CD ₃ CN)
2	143.9	143.8	15	27.4	26.8 (C-7) ^c
3	112.4	112.0	16	34.0	33.0 (C-8) ^c
4	126.9	126.2	17	33.5	32.7 (C-15) ^c
5	140.4	139.9	18	52.6	50.9
6	25.8	24.8	19	87.7	85.1
7	29.4	29.0 (C-16) ^c	20	177.8	174.3 ^a
8	40.8	40.1 (C-17) ^c	21	102.9	98.2
9	137.4 ^a	136.7	22	171.2	176.2 ^a
10	124.2 ^b	124.2	23	16.6	16.3
11	50.1	48.2	24	20.6	20.5
12	124.4 ^b	123.0	25	21.1	20.5
13	137.3 ^a	137.5	26	9.6	6.4
14	46.6	45.7			

^{a,b} Signals may be interchanged. ^c Previous assignments.³

true status of wistarin and ircinianin may have to await examination of the living animal.

Experimental Section

General Experimental Procedures. ^1H - and ^{13}C -NMR spectra were recorded in CD₃OD with a Bruker AMX-300 NMR at 300 and 75 MHz, respectively, calibrated to 3.5 and 49.3 ppm, respectively. Negative ion LREIMS were recorded on a VG Quattro mass spectrometer and negative ion HRMS on a Bruker BioApex 47e FTMS. FTIR spectra were recorded on a Perkin-Elmer 1600 spectrometer and optical rotations on a Perkin-Elmer 341 polarimeter. Column chromatography was performed on Merck Kieselgel 60 (230–400 mesh).

Animal Material. A specimen of *Ircinia (Psammocinia) wistarii* Wilkinson was collected by scuba diving in about 5 m of water from Lamont Reef in the Capricorn Bunker Group, Great Barrier Reef, in March 1994. The sponge was kept frozen prior to extraction. Voucher specimen QMG306975 has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. A sample (12.5 g) of the frozen sponge was extracted with MeOH (3 × 100 mL) and the extract volume reduced under vacuum to a thick oil. This was suspended in a NaHCO₃ solution (40 mL, 30% w/v) and partitioned against Et₂O (3 × 30 mL). The organic phase was dried over Na₂SO₄ and evaporated to dryness. The extract was chromatographed on a Si gel column using step-gradient elution from light petroleum through CH₂Cl₂ to EtOAc. All collection tubes contained a small volume of MeOH to stabilize ircinianin sulfate after elution.

Ircinianin sulfate (1): isolated as a light yellow gum (2.4% by dry wt); mp 138–140 °C (dec); $[\alpha]_D^{20} -160$ (*c* 0.02, MeOH); IR ν_{max} (thin film) 3150, 3100, 2973, 2870, 1732, 1654, 1501, 1446, 1385, 1332, 1264 cm⁻¹; ^1H NMR (CD₃OD) 1.18 (3H, d, *J* = 6 Hz, H-25), 1.5 (2H, m), 1.78 (3H, s, H-23), 1.85 (2H, m), 1.89 (3H, s, H-24),

2.05 (2H, m), 2.20 (3H, s, H-26), 2.27 (2H, t, *J* = 8 Hz, H-8), 2.57 (2H, t, *J* = 8 Hz, H-6), 2.59 (1H, m, H-14), 3.28 (1H, br d, *J* = 11 Hz, H-11), 5.21 (1H, br s, H-12), 5.29 (1H, d, *J* = 10 Hz, H-10), 6.52 (1H, br s, H-3), 7.57 (1H, m, H-5), 7.65 ppm (1H, m, H-2); ^{13}C -NMR data, see Table 1; negative ion HRESIMS *m/z* 475.1810 (calcd for C₂₅H₃₁O₇S, 475.1785), 395.2231 (calcd for C₂₅H₃₁O₄, 395.2216); negative ion ESIMS *m/z* 475 [M⁻] (100), 395 [M⁻ - SO₃] (8).

Ircinianin (2): isolated from the CH₂Cl₂ column fraction as a white solid (0.26%); $[\alpha]_D^{20} -167$ (*c* 0.004, MeOH); ^{13}C NMR (CD₃OD) 6.2, 16.5, 20.8, 20.9, 25.5, 27.4, 29.7, 33.4, 33.8, 40.7, 46.3, 49.5, 52.2, 87.1, 97.5, 112.2, 123.7, 125.2, 126.7, 136.8, 137.3, 140.4, 144.2, 178.0, 179.5 ppm.

Wistarin (4): isolated in trace amounts from the light petroleum–MeOH column fraction, and from decomposition of **2**, as white solid; $[\alpha]_D^{20} +134$ (*c* 0.007, MeOH); ^{13}C NMR (CD₃OD) 6.4, 20.9 (C-24), 21.3 (C-25), 24.0 (C-23), 25.4, 26.1 (C-6), 28.0 (C-15), 32.2 (C-17), 33.4 (C-16), 41.5 (C-11), 42.0 (C-10), 43.2 (C-8), 46.6 (C-14), 52.9, 82.9 (C-19), 88.7 (C-9), 108.1, 112.1, 123.1, 126.3, 139.4, 140.5, 144.4, 176.9, 177.9 ppm, other assignments as per Gregson and Ouvrier.³

Acknowledgment. We thank Dr. David Bourne of the Australian Institute of Marine Science for the HRMS data, Dr. Margaret Sheil of the Chemistry Department, Wollongong University, for the LRMS, and Drs. Ernestina Tentori and Sabine Duquesne for assistance with collection. We also thank the Australian Research Council for financial support.

References and Notes

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NP970287M