

# WISTARIN, A TETRACYCLIC FURANOSESTERTERPENE FROM THE MARINE SPONGE *IRCINIA WISTARII*

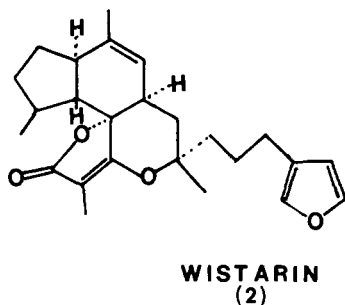
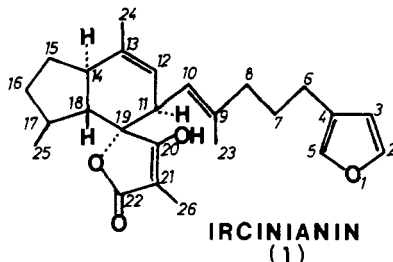
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ABSTRACT.—The structure of a new C<sub>25</sub> furanoterpene, wistarin (2), isolated from the marine sponge *Ircinia wistarii*, has been determined by spectral analysis.

Sesterterpenes are a rare group of natural products, but they are often encountered as secondary metabolites in sponges of the order Dictyoceratida (1, 2). Linear sesterterpenes are common in the genus *Ircinia* and a tetronic acid moiety if often present (3). Hofheinz (4) reported the isolation and X-ray structural determination of ircinianin (1) from *Ircinia* sp. Subsequently, this sponge, which was collected from Heron Island on the Great Barrier Reef, was found to be a new species and named *Ircinia wistarii* Bergquist (5). Other samples of *I. wistarii* from The Great Barrier Reef and The Great Australian Bight have been examined in our laboratories and found to contain relatively large amounts of 1. A recent collection of *I. wistarii* from the Swain Reef area of The Great Barrier Reef also contained 1 and, in addition, wistarin (2) a cyclic ether isomer of 1.

## IRCINIA WISTARII



## RESULTS

Column chromatography followed by hplc on silica gel of the dichloromethane extract of lyophilized *I. wistarii* afforded ircinianin (1) and wistarin (2). Spectral data on 1 was difficult to obtain as it decomposed readily, whereas 2 was relatively stable. Wistarin was not detectable in the decomposition products of 1, and 1 did not cyclize to 2 on treatment with dilute acid, base or Lewis acids such as boron trifluoride etherate. Rapid extraction and chromatography of *I. wistarii* revealed

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the presence of **2**, and this observation provides further evidence in support of **2** as a natural product and not an artifact of the isolation procedure.

The  $^1\text{H}$  nmr spectra of **1** and **2** were similar but differed in that the doublet at  $\delta$  5.12 (H10) and multiplet at  $\delta$  3.14 (H11) in **1** were absent in **2**, and one olefinic methyl group at  $\delta$  1.6 (C23) in **1** was replaced by a methyl group at  $\delta$  1.08 (broad singlet) in **2**. A comparison of the mass spectra of **1** and **2** revealed that they possessed the same molecular ion (396,  $\text{C}_{25}\text{H}_{32}\text{O}_4$ ), base peak (135,  $\text{C}_9\text{H}_{11}\text{O}$ ), and a similar fragmentation pattern. The infrared spectrum of **2** displayed absorptions at 3,000–2,800, 1750, 1680  $\text{cm}^{-1}$  which were consistent with an  $\alpha,\beta$ -unsaturated- $\gamma$ -lactone containing an isolated double bond (6) and which contrasted with 3,400–2,600, 1709, 1659  $\text{cm}^{-1}$  for the tetric acid moiety and homoconjugated double bonds of **1**, (4). Absence of the tetric acid group in **2** was indicated by its relatively lower polarity compared to **1**.

The  $^{13}\text{C}$  nmr spectra of **1** and **2**, previously unreported, were compared and the structural elucidation of **2** was confirmed. Resonances were assigned to all carbons, but the assignments to C15, C16 and C11, C18 could be interchanged in both **1** and **2**. Resonances attributable to the furan carbons and C6 in **1** and **2** are essentially identical to each other in each case and to those reported (7) for furo-spongolide, which contains this functional group. In **1** C9, C10 and C23 resonate at 136.7 (s), 124.2 (d) and 16.3 (q) but in **2** they occur at 81.4 (s), 42.6 (t) and 23.5 (q) confirming that the ether link is at C9 not C10. Carbons 21 and 11 have significantly different chemical shifts in **1** and **2** in accordance with the ring closure, but, as expected, the other carbons in the tetrahydroindan group are essentially unaffected by the cyclization and are very similar in **1** and **2**.

A study of molecular models of **1** revealed that C9 can readily approach the proximity of the C20 oxygen atom, therefore, it is reasonable to assume that **2** is derived from **1** by cyclization *in vivo*. The stereochemistry represented in the illustration of **2** is based on this assumption and the close similarity of the spectral parameters of **1** and **2**.

## EXPERIMENTAL<sup>2</sup>

**COLLECTION AND EXTRACTION.**—*I. wistarii* was collected at 21–27 meters depth from Horse-shoe Reef, Swain Reefs, off Gladstone, Queensland. The organism was stored frozen then cryogenically ground and lyophilized. The dry organism (573 g) was percolated in a column with dichloromethane (10 liters), and the solvent was evaporated; a dark green extract (24.85 g, 4.3%) was obtained.

**ISOLATION OF IRCINIANIN AND WISTARIN.**—A solution of the extract (19.8 g) in dichloromethane (40 ml) was applied to a column (13 x 10 cm) of Merck Kieselgel (type 60, 200 g). A vacuum applied to the column outlet gave a flow rate of 15 ml  $\text{min}^{-1}$ ; the column was eluted successively with dichloromethane, dichloromethane-ethyl acetate 9:1, 5:1, 1:1, ethyl acetate, ethyl acetate-methanol 9:1, 4:1, 1:1 and methanol. Analysis of these fractions by tlc (dichloromethane-ethyl acetate 9:1) revealed the presence of **1** in fractions 2–8 and **2** in fractions 6 and 7. Fraction 6 (1.24 g) was subjected to hplc in dichloromethane-ethyl acetate (20:1), and ircinianin (**1**) was isolated as an unstable crystalline solid (42 mg, 0.009%). Attempts to recrystallize **1** were unsuccessful as it decomposed. It had Rf 0.50 (dichloromethane-ethyl acetate, 20:1). The  $^1\text{H}$  nmr, ir, and ms data of (**1**) agree with that reported previously (4). In addition the  $^{13}\text{C}$  nmr ( $\text{CD}_3\text{CN}$ ) was as follows:  $\delta$  176.2, s, C22; 174.3, s, C20; 143.8, d, C2; 139.9, d, C5; 137.5, s, C13; 136.7, s, C9; 126.2, s, C4; 124.2, d, C10; 123.0, d, C12; 112.0, d, C3; 98.2, s, C21; 85.1, s, C19; 50.9, d, C18; 48.2, d, C11; 45.7, d, C14; 40.1, d, C17; 33.0, t, C8; 32.7, t, C15; 29.0, t, C16; 29.0, t, C16; 26.8, t, C7; 24.8, t, C6; 20.5, q, C24; 20.5, q, C25; 16.3, q, C23; 6.4, q, C26.

Fraction 7 [ethyl acetate-methanol, (4:1)] (1.48 g) was applied to a Merck LiChroprep Si 60 column (40–63  $\mu\text{m}$ , size C) in dichloromethane and eluted at 10 ml  $\text{min}^{-1}$ . Elution of the column with dichloromethane:ethyl acetate, 20:1, afforded a fraction of pure wistarin (**2**) (58.5 mg, 0.012%) as a colorless oil. Wistarin chromatographed as a single component in dichloromethane-ethyl acetate (20:1) on tlc (Rf 0.9) and hplc, and as a single component in

<sup>2</sup>Tlc and hplc were carried out on silica gel (Merck) plates and a solution of vanillin in methanol (3%) followed by sulfuric acid (50%) was used to visualize the furans as a red coloration. Hplc was performed on a Whatman Partisil M9 10/50 silica column coupled to a Waters Differential Refractometer R404 and pumped at 5 ml  $\text{min}^{-1}$ .  $^1\text{H}$ nmr and  $^{13}\text{C}$ nmr spectra were recorded on Jeol MH-100 and Jeol FX60 spectrometers, respectively. Mass spectra were measured with a VG Micromass 70-70 instrument. Infrared spectra were obtained with a Hitachi 285 spectrometer.

di-isopropyl ether-petroleum ether (3:1) on tlc (Rf 0.4) and hplc. It exhibited the following data:  $[\alpha]_{589}^{20} +130^\circ$  (C 0.25, dichloromethane); ms (e.i.)  $m/e$  (%): found 396.2295 (15) ( $C_{25}H_{32}O_4$  requires 396.2298) 381 (3), 315 (4), 287 (5), 246 (8), 229 (6), 135 (100) (found 135.0803,  $C_9H_{11}O$  requires 135.0809), 122 (30), 107 (20), 83 (42), 81 (24), 79 (10), 41 (21); ir (neat) 3,000–2,800 (br), 1750, 1680, 1500, 1390, 1355, 1270, 1010, 870;  $^1H$  nmr ( $CDCl_3$ )  $\delta$  0.84, d,  $J=5.5$  Hz, 25 Me; 1.08, brs, 23 Me; 1.64, m, 24 Me, 26 Me; 2.4, 4H, m; 5.00, m, H12; 6.20, m, H3; 7.16, 7.22, brs, H2, H5;  $^{13}C$  nmr ( $CD_3CN$ ) 175.9, s, C22; 174.3, s, C20; 143.8, d, C2; 140.0, d, C5; 138.8, s, C13; 125.9, s, C4; 122.6, d, C12; 111.9, d, C3; 107.4, s, C21; 87.6, s, C19; 81.4, s, C9; 51.8, d, C18; 45.9, d, C11; 42.6, d, C14; 40.8, d, C17; 31.5, t, C8; 32.7, t, C15; 27.3, t, C16; 25.5, t, C7; 24.6, t, C6; 23.5, q, C23; 20.9, q, C24; 20.6, q, C25; 6.4, q, C26.

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