Variabilin, a Chemotaxonomic Marker for the Family Irciniidae

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Z. Naturforsch. **62c**, 473–476 (2007); received December 13, 2006/January 28, 2007

The furanosesterterpene variabilin was isolated from the sponge *Sarcotragus.* From a chemical point of view, the family Irciniidae has been the source of furanosesterterpenes, and especially variabilin is an important chemotaxonomic marker for the family Irciniidae.

Key words: Irciniidae, *Sarcotragus*, Variabilin

Introduction

Marine sponges of the order Dictyoceratida have frequently provided a large number of linear sesterterpenoids (Blunt *et al*., 2006; Faulkner, 2002; Liu *et al.*, 2006a). Sponges of the genus *Sarcotragus* were reported to contain compounds such as variabilin (Perry *et al*., 1987), (7*E*,12*E*,20*E*)-variabilin, (7*E*,12*Z*,20*Z*)-variabilin, 8-hydroxy-(12*E*,20*Z*)-variabilin, 14-furan-3-yl-3,7,11-trimethyl-tetradeca-7,11-dienoic acid (Barrow *et al*., 1988), sarcochromenol sulfates A–C and sarcohydroquinone sulfates A–C (Stonik *et al.*, 1992), octa- and nonaprenylhydroquinone sulfates (Wakimoto *et al*., 1999), geranylfarnesylacetone (Ponomarenko *et al*., 1998), and sarcotragins A and B (Shin *et al*., 2001). In our previous studies on the cytotoxic compounds of two sponges of the genus *Sarcotragus*, thirty-three cytotoxic terpenoids, three cyclitols, a trisoxazole macrolide, three indole alkaloids, three glycerolipids, and a fatty acid ester were reported (Liu *et al*., 2001, 2002a, b, 2003, 2005, 2006b, c, d, e).

In our continuing investigation the furanosesterterpene variabilin (**1**) was isolated from the sponge *Sarcotragus.* Compound **1** was identified by comparison of its spectral data (^{1}H, ^{13}C NMR and MS) with previously reported data of variabilin, which was isolated from other species of the genus *Sarcotragus* (Liu *et al*., 2003).

Sponges of the order Dictyoceratida have yielded a wide range of new sesterterpenes, many of which contain both furan and tetronic acid functional groups (Liu *et al*., 2006a). Typical for these furanosesterterpene tetronic acids is variabilin, which was first isolated from the sponge *Ircinia variabilis* (Faulkner, 1973). This compound is antimicrobial and cytotoxic. Subsequently, 7*E* and 12*E* configurations were assigned (Gonzalez *et al*., 1983), and the stereochemistry at the exocyclic double bond was solved (Barrow *et al*., 1988). Variabilin is a major component in all New Zealand collections of sponges of the genera *Ircinia, Psammnocinia,* and *Sarcotragus* (Perry *et al*., 1987; Barrow *et al*., 1988).

Variabilin is a novel RGD-containing antagonist of glycoprotein IIb–IIIa and a platelet aggregation inhibitor (Wang *et al*., 1996). It is a dual inhibitor of human secretory and cytosolic phospholipase A2 with anti-inflammatory activity (Escrig *et al*., 1997).

Results

Compound **1** (Fig. 1) was isolated as a light yellow oil. The molecular formula of **1** was established as $C_{25}H_{34}O_4$ on the basis of FABMS data. A

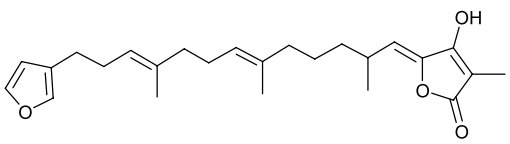

Fig. 1. (7*E*,12*E*,20*Z*)-Variabilin (**1**).

β-substituted furan unit was recognized from the broad singlets at δ_H 7.35, 7.27, and 6.28 in the ^{1}H NMR spectrum. The presence of a conjugated tetronic acid moiety was established with the aid of COSY, HMQC, and HMBC experiments.

The ^{1}H NMR spectrum of compound **1** displayed resonances consistent with the presence of three vinylic methyl groups (δ_H 1.54, 3H, s; 1.56, 3H, s; 1.83, 3H, s) and three trisubstituted double bonds (δ_H 5.23, 1H, t; 5.14, 1H, t; 5.08, 1H, t). The positions of the double bonds were confirmed by the COSY experiment. Examination of the ^{13}C NMR chemical shifts for the vinylic methyl resonance confirmed the geometry of the trisubstituted double bonds as 7*E*,12*E* and 20*Z*. The assignments of the carbon atoms and protons were supported by COSY and HMBC experiments and were similar to the literature values of the geometric isomer (7*E*,12*E*,20*Z*)-variabilin (Liu *et al*., 2001, 2002a, 2003; Choi *et al*., 2004).

The family Irciniidae comprises three genera: *Ircinia* Nardo, 1833; *Psammocinia* Lendenfeld, 1889; and *Sarcotragus* Schmidt, 1862, which together have a wide-ranging, global distribution (Cook and Bergquist, 1999). In 1978, Bergquist erected the family Thorectidae, to separate those taxa with laminated fibres and diplodal choanocyte chambers from the dictyoceratid taxa now recognized as spongiids, which are characterized by homogenous (unlaminated) fibres. Bergquist and Wells (1983) suggested that on the basis of skeletal composition and terpene chemistry, a discrete family may need to be established for *Ircinia, Psammocinia,* and *Sarcotragus.* Hooper and Wiedenmayer (1994) mistakenly assigned all thorectid taxa, including these three genera, to Irciniidae. This was rectified by Bergquist (1995) who separated this distinct group of filament-bearing genera from the Thorectidae and referred it to the family Irciniidae (Cook and Bergquist, 1999).

Variabilin was found only in the morphologically similar genera *Ircinia, Psammocinia* and *Sarcotragus.* Variabilin occurs in two New Zealand *Sarcotragus* sp. (Perry *et al*., 1987; Barrow *et al*., 1988) and two Korean *Sarcotragus* sp. (Liu *et al*., 2003). Variabilin occurs as an antibiotic from the sponge *Ircinia variabilis* (Faulkner, 1973), and was also found in the morphologically similar genus *Ircinia* (Perry *et al*., 1987), *Ircinia campana* (Martínez *et al*., 1997a; Pawlik *et al*., 2002), *Ircinia felix* (Martínez *et al*., 1995, 1997a, b; Pawlik *et al*., 2002), *Ircinia strobilina* (Martínez *et al*., 1997a; Rothberg and Shubiak, 1975; Davis and Capon, 1994; Pawlik *et al*., 2002; Epifanio *et al*., 1999), *Ircinia oros* (Höller *et al*., 1997), and *Ircinia* sp. (Barrow *et al*., 1989). Variabilin also occurs as cytotoxic component in the sponge *Psammocinia* (Choi *et al*., 2004).

To the best of our knowledge, from a chemical point of view, the family Irciniidae (order Dictyoceratida) is the source of furanosesterterpenes, especially of variabilin and its analogues. The family Irciniidae is frequently difficult to differentiate due to its morphological characteristics. Thus, the use of chemical criteria may provide a valuable clue for taxonomic classification.

Experimental

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200, Varian Unity Plus 300, and Unity INVOA 500 instruments. Chemical shifts are reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). Optical rotations were obtained using a JASCO DIP-370 digital polarimeter. HRFABMS data were obtained on a JEOL JMS-SX-101A instrument. HPLC was performed with an YMC ODS-H80 (semipreparative, 250 × 10 mm i. d., 4 μm, 8 μm; preparative, 250 × 20 mm i. d., 4 μm, 8 μm) and a YMC-Pack CN (250 × 10 mm i. d., 5 μm, 12 μm) column using a Shodex RI-71 detector.

Animal material

The sponge was collected in July 1998 (15–25 m depth), off the coast of Jeju Island, Korea. The specimen was identified as *Sarcotragus* sp. by Prof. Chung Ja Sim, Hannam University, Daejon, Korea. A voucher specimen of the sponge (registry No. Por. 33) was deposited in the Natural History Museum, Hannam University, and has been described elsewhere (Liu *et al*., 2001).

*Extraction, isolation and characterization of compound **1***

The frozen sponge (7 kg) was extracted with MeOH at room temperature. The MeOH extract of the sponge displayed moderate cytotoxicity against five human tumour cell lines (ED_{50} values for A549, SK-OV-3, SK-MEL-2, XF498, and HCT15 were 19.0, 20.3, 11.8, 15.5, and 12.6 μg/mL, respectively) and toxicity to brine shrimp larvae

(LD_{50} 93 μg/mL). The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between 90% methanol and *n*-hexane to yield 90% methanol- (54 g) and *n*-hexane-soluble (13 g) fractions. As described in our previous report (Liu *et al.*, 2001), the 90% methanol fraction was subjected to reversed-phase flash column chromatography using a YMC Gel ODS-A column (60 Å, 500/400 mesh), eluted with the solvent system 25 to 0% H_2O/MeOH, to afford 20 fractions (Fg1–Fg20). These fractions were evaluated for activity in the brine shrimp assay, and fractions Fg6–Fg9 were found active. Compound **1** (5.0 mg) was obtained by purification of fraction Fg9 by ODS HPLC.

(7E,12E,20Z)-Variabilin (**1**): Light yellow oil; $[\alpha]_D^{25}$ +40.8° (*c* 0.01, MeOH). – ^{1}H NMR (500 MHz, CD_3OD): δ = 7.35 (1H, brs, H-1), 6.28 (1H, brs, H-2), 7.27 (1H, brs, H-4), 2.42 (2H, t, *J* = 7.5 Hz, H-5), 2.22 (2H, q, *J* = 7.5 Hz, H-6), 5.14 (1H, t, *J* = 7.0 Hz, H-7), 1.56 (3H, s, H-9), 1.95 (2H, m, H-10), 2.06 (2H, m, H-11), 5.08 (1H, t, *J* = 6.0 Hz, H-12), 1.54 (3H, s, H-14), 1.95 (2H, m, H-15), 1.35 (2H, m, H-16), 1.32 (2H, m, H-17), 2.72 (1H, m, H-18), 1.05 (3H, d, *J* = 7.0 Hz, H-19), 5.23 (1H, d, *J* = 10.0 Hz, H-20), 1.83 (3H, s, H-25). – ^{13}C NMR (50 MHz, CD_3OD): δ = 143.7 (C-1), 112.0 (C-2), 126.2 (C-3), 140.1 (C-4), 26.0 (C-5), 29.6 (C-6), 125.2 (C-7), 136.5 (C-8), 16.1 (C-9), 40.4 (C-10), 27.4 (C-11), 125.6 (C-12), 135.8 (C-13), 16.0 (C-14), 40.7 (C-15), 26.8 (C-16), 37.6 (C-17), 31.9 (C-18), 21.0 (C-19), 115.6 (C-20), 145.1 (C-21), 165.1 (C-22), 98.7 (C-23), 173.7(C-24), 6.0 (C-25). – FABMS: *m*/*z* = 421 $[M+Na]^+$.

Acknowledgements

This study was supported by a grant from Marine Bio 21, the Ministry of Maritime Affairs and Fisheries, Korea, a grant from Knowledge Innovation Program (KZCX2-YW-216) and a grant from Hundred Talents Project of Chinese Academy of Sciences.

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