ANTINEOPLASTIC AGENTS 385. THE ISOLATION AND STRUCTURE OF A SCALARANE-TYPE SESTERTERPENE FROM THE INDIAN OCEAN PORIFERA *Hyrtios erecta**

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Dedicated to Dr Jan Fajkos on the occasion of his 75th birthday.

Bioassay-guided separation techniques were employed to isolate ($3.3 \cdot 10^{-7}$% yield) a new murine P388 lymphocytic leukemia cell line inhibitor (ED_{50} 2.9 μg/ml) 12-*O*-deacetyl-19-deoxyscalarin from extracts of the Republic of Maldives marine sponge *Hyrtios erecta*. The structure was deduced using high field 2D NMR and high resolution mass spectral methods and confirmed by X-ray crystal structure determination.

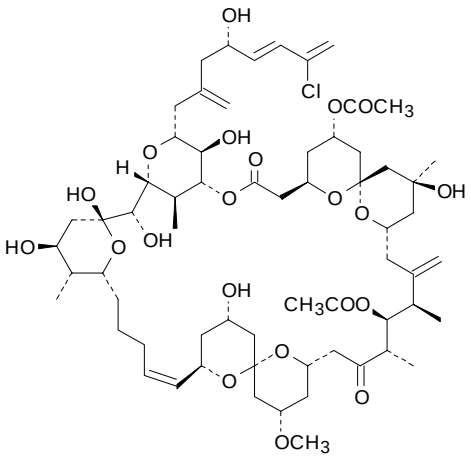
Key words: Natural products; Terpenes; Sesterterpenes; Scalarolides; Marine sponge; NMR spectroscopy; X-Ray diffraction; Crystal structure.

Marine sponges in the class Demospongiae are now very well established as productive sources of biologically active substances bearing novel structures[2–5]. Several of the families in the order Dictyoceratida such as Dysideidae (genera such as *Dysidea*), Spongiidae (*e.g. Spongia*), and Thorectidae (*e.g. Hyrtios* or *Heteronema*) enjoy a broad geographic distribution in tropical to temperate ocean areas[5] and a number of such species are now known to provide cancer cell growth inhibitory substances. Illustrative is a *Hyrtios* species[2] we located (in 1986) in the Republic of Maldives and in which we discovered the powerful anticancer agents[2,6,7] spongistatin 1–3. As part of research directed at increasing the availability of spongistatin 1 (**1**), we have been investigating a 1994 recollection of the Maldive *Hyrtios erecta* (refs[2,6,7]) and have located a new sca-

* For part 384 in the series Antineoplastic Agents see ref.[1].

larane-type[8–14] pentacyclic sesterterpene **2** with activity (ED_{50} 2.9 μg/ml) against the murine P388 lymphocytic leukemia.

A small portion (600 kg wet weight) of our 1994 recollection of *Hyrtios erecta* was extracted with methanol, concentrated and partitioned to yield a 203 g (cancer cell line P388, ED_{50} 0.31 μg/ml) dichloromethane fraction. Sesterterpene **2** (2.0 mg) was isolated in $3.3 \cdot 10^{-7}$% yield from this fraction as colorless needles melting at 274–275 °C. The HRFAB mass spectrum of terpene **2** gave $[M + Na]^+$ at *m/e* 409.2723, corresponding to molecular formula $C_{25}H_{38}NaO_3$. The ^1H and ^{13}C NMR, APT, and HMQC spectra



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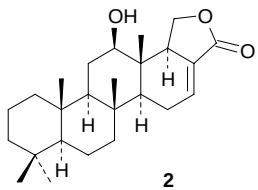

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exhibited five methyl signals as singlets, eight methylenes, six methines and six quaternary carbons (Table I). In turn that composition agreed with the seven unsaturation units indicated by the molecular formula and suggested a cyclic sesterterpene structure. The 2D TOCSY and COSY NMR spectra were used to establish four carbon chain units (C-1–C-3, C-5–C-7, C-9–C-11–C-12, and C-14–C-16). With the assistance of HMBC data (Table I), the carbon units were found to be connected by quaternary carbons joined to five methyl groups to form four fused six-membered rings characteristic of a scalarane-type[8–14] sesterterpene with 12-hydroxyl (δ 81.42/3.42, 1 H, d, J = 8) and $\Delta^{16,17}$ olefin (δ 135.53/6.79, 1 H, dd, J = 7.5/4.0; δ 127.70) groups.

The UV absorption maximum at 222 nm (log ε 3.95) and strong IR absorption bands at 1 744 and 1 690 cm^{-1} suggested a carbonyl group conjugated with the double bond and incorporated into a lactone ring. Because of the ^1H NMR cross signals corresponding to H-18 (δ 2.84, 1 H, m) with both methylene protons (δ 4.46, 1 H, t, J = 9.5; δ 4.16, 1 H, t, J = 9.5) and the long-range olefinic coupling shown by H-16 (δ 6.79, 1 H, dd, J = 7.5/4.0) in a COSY spectrum, the bonded methylene was assigned to C-19 and the carbonyl to C-20. The stereochemistry of the C-12 hydroxyl (equatorial) and H-18 (axial) was

TABLE I

A summary of the high field (500 MHz) ^{1}H and ^{13}C NMR data (in CD_2Cl_2) corresponding to sesterterpene **2**. Chemical shifts in ppm (δ-scale), coupling constants (*J*) in Hz

Position	^{13}C	^{1}H	HMBC (C to H)
1	40.29	1.70 (1 H, m) 0.82 (1 H, m)	2-H_a, 23-H
2	18.95	1.63 (1 H, m) 1.42 (1 H, m)	
3	42.51	1.38 (1 H, m) 1.15 (1 H, dt, *J* = 3.5/13.5)	21-H, 22-H
4	33.39	–	21-H, 22-H
5	56.80	0.82 (1 H, m)	21-H, 22-H, 7-H_e
6	18.39	1.56 (1 H, m) 1.38 (1 H, m)	
7	41.82	1.72 (1 H, m) 0.92 (1 H, m)	24-H
8	37.65	–	7-H_a, 7-H_e, 24-H
9	58.98	0.92 (1 H, m)	11-H_a, 11-H_e, 23-H, 24-H
10	37.65	–	11-H_e, 23-H
11	27.71	1.67 (1 H, m) 1.42 (1 H, m)	
12	81.42	3.42 (1 H, d, *J* = 8, br)	9-H, 11-H_a, 11-H_e, 25-H
13	40.29	–	11-H_a, 11-H_e, 14-H, 25-H
14	53.58	1.26 (1 H, dd, *J* = 11/5.5)	24-H, 25-H
15	24.12	2.34 (1 H, m) 2.19 (1 H, m)	14-H
16	135.53	6.79 (1 H, dd, *J* = 7.5/4.0)	
17	127.70	–	19-H_e
18	50.86	2.84 (1 H, m)	14-H, 19-H_a, 19-H_e, 25-H
19	69.44	4.46 (1 H, t, *J* = 9.5) 4.16 (1 H, t, *J* = 9.5)	
20	170.63	–	19-H_e
21	33.39	0.85 (3 H, s)	22-H
22	21.50	0.82 (3 H, s)	21-H
23	16.78	0.87 (3 H, s)	9-H
24	16.78	0.93 (3 H, s)	9-H, 14-H
25	7.97	0.74 (3 H, s)	14-H

determined by evidence from the 2D rotational Overhauser spectrum (ROESY), where H-12 (δ 3.42) and H-18 (δ 2.84) gave NOE effects with all the nearby axial protons (9-H_a at δ 0.92; 14-H_a at δ 1.26) and with each other. The preceding observations suggested structure **2**, 12-*O*-deacetyl-19-deoxyscalarin, and that assignment was confirmed by results of the following X-ray crystal structure determination.

The X-ray crystal structure determination (Fig. 1) revealed a pentacyclic sesterterpene lactone[2], with a Δ^{16}-double bond (C_{16}–C_{17} 1.312(5) Å). Sesterterpene **2** may correspond to a previously reported terpene isolated (5 mg from 200 g of dry sponge) from the Andaman and Nicobar Islands (India) sponge *Heteronema erecta* and assigned structure **2** employing spectral data[15] (principally NMR). However, the Indian group reported this sesterterpene to be an oil that also differs from our authentic specimen by two especially marked departures in the ^{13}C NMR spectra ($CDCl_3$): namely, δ 127.23 *versus* 125.2 and 27.63 *versus* 23.7.

We have also located other scalaranes[5] from the Maldive *Hyrtios erecta*. After the present study was completed, Fusetani and coworkers[13] described three new sesterterpenes from a southern Japanese collection of *Hyrtios* cf. *erectus*. One of these with a 16β-hydroxy in place of a 16α-acetoxy group gave a P388 cell line value of ED_{50} 0.4 μg/ml. Sesterpene **2** is related to scalarolbutenolide which resulted from the pioneering research of Cimino and colleagues[8] with sponge scalarane terpenes. Presumably the moderate P388 cell line inhibitory activity shown by sesterterpene **2** and others[4,12–14] is primarily the result of Michael-type additions of biosynthetic thiol and/or related groups to the butenolide-type α,β-unsaturated carbonyl systems[14,16,17]. Further biological evaluation of sesterterpene **2** is under way.

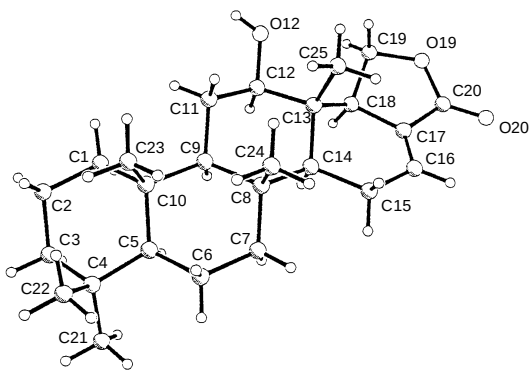

FIG. 1
Computer-generated perspective drawing of sesterterpene lactone **2**

EXPERIMENTAL

Refer to ref.[7] for leading references and a summary of the general experimental procedures and instruments employed in the present study. All ED_{50} values refer to the murine P388 lymphocytic leukemia cell line.

Hyrtios erecta Collection and Extraction

A 600 kg (wet weight) portion of a 1994 Republic of Maldives collection of *Hyrtios erecta* (KELLER, 1989, *c.f.* ref.[2]) corresponding to Queensland Museum voucher number QM G304492 was used for these experiments. The methanol–water preserving solution was decanted and the sponge compressed to extend the solvent removal. Methanol (550 l) was added and the sponge was extracted at room temperature for fifty days. The methanol extract was concentrated to an 80 l aqueous mixture that was further separated by solvent partitioning.

Solvent Partitioning Sequence

The preceding aqueous mixture was partitioned with dichloromethane (4 × 50 l) to yield a 667 g (P388, ED_{50} 0.45 μg/ml) fraction which was further partitioned between hexane (5 × 6 l) and 9 : 1 methanol–water (6 l). The methanol–water phase concentration was adjusted to 3 : 2 and partitioned with dichloromethane (5 × 6 l) to yield a brown, gummy dichloromethane fraction (203 g, P388, ED_{50} 0.31 μg/ml).

Isolation of 12-*O*-Deacetyl-19-deoxyscalarin (**2**)

The P388 active dichloromethane fraction (203 g) was further separated by gel permeation chromatography using a Sephadex LH-20 column (16 × 120 cm) in methanol. The active fractions were combined and again separated by partition chromatography on a column of Sephadex LH-20 with hexane–toluene–methanol (3 : 1 : 1). The resulting active fractions were chromatographed on a silica gel column with hexane–dichloromethane–methanol (6 : 9 : 1) as eluent. From the second fraction, sesterterpene **2** (2 mg) was obtained and recrystallized from methanol: colorless needles, m.p. 274–275 °C, $[\alpha]_D^{25}$ –2.0 (*c* 0.15, MeOH). UV spectrum (MeOH): λ_{max} 222 nm (log ε 3.95). IR spectrum ($CHCl_3$ film): ν_{max} 3 507, 2 924, 1 744, 1 690 and 1 231 cm^{-1}. HRFAB MS for $C_{25}H_{38}NaO_3$ calculated: 409.2719; found: 409.2723 $[M + Na]^+$. EI MS, *m/z* (%): 386 (M^+, 15) 368 (7), 276 (11), 261 (15), 191 (100), 175 (26), 123 (26). For the ^1H and ^{13}C NMR and HMBC data see Table I.

X-Ray Crystal Structure Determination

A large, colorless crystal of sesterterpene **2** grown from a solution of methanol was cleaved to approximate dimensions 0.60 × 0.20 × 0.18 mm and mounted on the tip of a glass fiber with Super Glue. All reflections corresponding to slightly more than a complete octant ($2\theta \leq 130°$) were measured using an $\omega/2\theta$ scan technique. Immediately following collection of each reflection, the Friedel pair reflection was also collected. Subsequent statistical analysis of the complete reflection data set using the XPREP program[18], indicated the space group was $P2_12_12_1$, the asymmetric unit of the cell containing a single molecule of the lactone. Crystal data: $C_{25}H_{38}O_3$, a = 8.899(2), b = 12.078(2), c = 20.005(3) Å, V = 2 150.2(7)Å^3, λ(CuKα) = 1.54180 Å, ρ_o = 1.122 g cm^{-3}, ρ_c = 1.194 g cm^{-3} for Z = 4 and F. W. = 386.55, F(000) = 848. After Lorentz and polarization corrections, merging of equivalent reflections and rejection of systematic absences, 3 608 unique reflections (R_{int} = 0.0663) remained, of which 3 385 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the

intensity data as well as an empirical absorption correction (based on a series of psi-scans)[19]. Structure determination was readily accomplished with the direct-methods program SIR92 (ref.[20]). All non-hydrogen atom coordinates for **2** were located in the structure solution run using the default options for that program. The remaining hydrogen atom positions were calculated at optimum positions using the program SHELXL93 (ref.[21]). The latter atoms were assigned thermal parameters fixed and equal to 1.2 the U_{iso} value of the atom to which they were attached and their coordinates were forced to ride that atom during final cycles of refinement. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process with the SHELXL93 software package. The standard residual *R* value for the model shown[22] in Fig. 1 was 0.0931 for observed data (3 385 reflections) and 0.0960 for all data (3 608 reflections). The corresponding Sheldrick *R* values were wR_2 of 0.2324 and 0.2376, respectively. Near the conclusion of the refinement, an additional empirical absorption correction was made to the reflection data with XABS2 (ref.[23]). A final refinement yielded a standard residual *R* value of 0.0797 for observed data and 0.0833 for all data. The difference Fourier map showed insignificant residual electron density, the largest difference peak and hole being 0.395 and –0.485 e/Å^3, respectively. Final bond distances and angles were all within acceptable limits.

Supplementary material: X-ray crystallographic tables of atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for **2** (7 pages) are available from the authors upon request.

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