Journal of Natural Products
Vol. 58, No. 4, pp. 609-612, April 1995

THE CYCLORENIERINS, SESQUITERPENOID QUINOLS FROM THE SPONGE *HALICLONA* SP. COLLECTED IN VANUATU

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ABSTRACT.—Two sesquiterpene quinols, cyclorenierins A [**1**] and B [**2**], which are closely related to panicein A_2 [**3**] and renierin A [**4**], were isolated as an inseparable mixture from the marine sponge *Haliclona* sp. collected in Vanuatu. Structures were proposed using nmr shift analogies to **3** and **4** and confirmed with 2D nmr data.

During field expeditions to Indo-Pacific coral reefs we have placed a priority on the collection of flattened sponges, either cup or fan shaped or encrusting (sometimes called foliose sponges). Taxa with this physical shape are considered by Wilkinson to have a high potential for phototrophic nutrition by prokaryotic symbionts (1). There is much speculation, but little proof, that such microorganisms might contribute additional diversity to the range of metabolites isolated from the macroorganism host tissue. The present study began when we located a colony of sponges in Vanuatu which were 1–2 cm thick and plate-like in shape. Taxonomic examination of the voucher sample indicated it was a *Haliclona* sp. (family Chalinidae, order Haplosclerida) and ^{1}H-nmr spectra of the semipurified polar extract fractions contained resonances indicative of a terpenoid major component. Reported herein are the isolation and structure elucidation of two inseparable sesquiterpene quinols, cyclorenierins A [**1**] and B [**2**], which are analogous to compounds reported from other Haplosclerida sponges.

The initial processing of the *Haliclona* sp. was conducted according to procedures described previously (2). Visual inspection of the crude extract solvent partition fractions did not reveal significant quantities of chlorophyll. Initially, the CH_2Cl_2 solvent partition fraction (1.38 g) was selected for further work and this proved to be beneficial. Subjecting it to Sephadex LH-20 chromatography (MeOH) followed by ODS hplc (40% aqueous MeOH) yielded a mixture of cyclorenierins A [**1**] and B [**2**]. Many further attempts were made to separate these compounds, but no successful method could be found. That these compounds were isomers was indicated by the ^{1}H- and ^{13}C-nmr resonances of this sample which contained clusters of peaks as illustrated in Table 1. This circumstance considerably hindered the progress of the structure elucidation. Further analy-

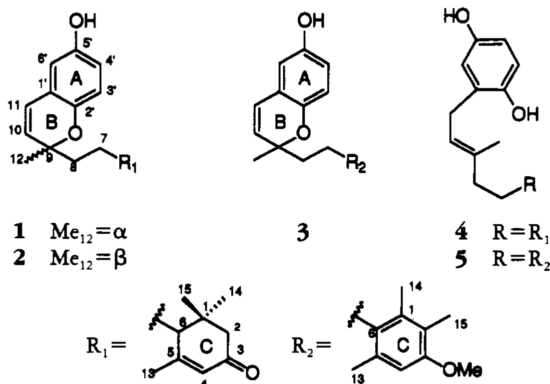

TABLE 1. Nmr Data for Compounds **1/2**, **3**, and **4**.

Position	^{1}H (500 MHz) **1/2** ($CDCl_3$)	HMBC, C→H[a] **1/2**	^{13}C (125 MHz) **1/2** ($CDCl_3$)	^{13}C (100 MHz) **3** (CD_3OD) (6)	^{13}C (100 MHz) **4** (CD_3OD) (6)
1		2,14	36.40 s		37.4
2	2.36/2.34 d/d J=17.0; 2.02/2.01 d/d J=17.0	14	47.45/47.38 t		48.1
3		2	200.30 s		202.3
4	5.82/5.80 br s/br s	13	125.37/125.27 d		125.4
5		6,13	165.67 s		170.1
6	1.83 m	2,14	51.21 d		51.5
7	1.54 m	6,8	24.31/24.26 t		29.6
8	1.70 m	12	40.40/40.36 t		40.7
9		10,11,12	78.16/78.04 s	78.7	
10	5.53/5.52 d/d J=10.0		130.37/130.32 d	130.6	
11	6.30/6.29 d/d J=10.0		123.40/123.32 d	123.3	
12	1.32 s		26.20/26.08 q	26.5	
13	1.95/1.94 d/d J=1.0	6	24.67/24.60 q		24.8
14	1.02/1.00 s/s	15	27.31/27.27 q		27.4
15	0.99/0.98 s/s	2,14	28.95/29.02 q		28.9
1′		10	121.80/121.75 s	122.3	
2′		3′,4′,6′,11	146.50/146.41 s	150.9	
3′	6.62 d J=8.5		116.77/116.72 d	116.7	
4′	6.58 dd J=8.5, 3.0	6′	115.72 d	115.6	
5′		3′	149.60 s	146.4	
6′	6.29 dd J=3.0, 1.0		113.11 d	113.0	

[a] J=9 Hz.

sis by hreims (positive-ion) was useful as a molecular formula of $C_{21}H_{26}O_3$ could be established for the isomer mixture based on the hreims $[M]^+$ of 326.1876 (Δ 1.8 ppm of calcd).

With the molecular formula in hand it was possible to begin interpreting the nmr data. In particular, this formula guided the assignment of the ^{13}C-nmr APT spectrum which was considered to be final when a count of $C_{21}H_{25}$ was derived. In an attempt to dereplicate for known compounds, this APT formula was used as input in a search of our personal database covering sponges (3). Several compounds possessing closely related structures were found including the paniceins from *Halichondria panicea* (family Halichondriidae, order Halichondrida) (4), the fulvanins from *Reniera fulva* (family Chalinidae, order Haplosclerida) (5) and the renierins from *Reniera mucosa* (family Chalinidae, order Haplosclerida) (6). Two of these sesquiterpenes, panicein A_2 [**3**] and renierin A [**4**], proved to be especially useful (6). Comparison of their nmr shift data (Table 1) facilitated the assembly of substructures for the cyclorenierins. For example, the ^1H- and ^{13}C-nmr resonances of the A and B rings of the cyclorenierins closely matched those of **3**. The chemical shifts for the C ring of **1**/**2** almost exactly corresponded to those of **4**. These similarities allowed structures **1** and **2** to be proposed for the cyclorenierins. The HMBC nmr (7) correlations listed in Table 1 provided further confirmation of these structures. Similarly consistent was the lreims ion fragment at *m/z* 161 ($C_{10}H_9O_2$), which corresponds to an oxygen directed α-cleavage at the C-8–C-9 bond.

It is possible that the cyclorenierins are artifacts produced by cyclization of **4**. The precedent for this is the cyclization of panicein A hydroquinone [**5**] to give racemic panicein A_2 [**3**] (6). The conditions for this transformation, K_2CO_3 in refluxing Me_2CO, suggest that the conversion of **4** to **1**/**2** is unlikely to occur during isolation. Finally, if the cyclorenierins were produced during isolation then this occurred during the early stages of purification as clusters of peaks were visible in the nmr spectra of the Sephadex chromatographic fractions.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded at 500 MHz for ^1H and 125 MHz for ^{13}C. Multiplicities of ^{13}C-nmr resonances were determined using APT and HMQC nmr data. Hreims and lreims data were obtained on a magnetic sector instrument. Hplc was performed using a 10 μm ODS column.

ANIMAL MATERIAL.—The sponge (coll. No. 90024) was collected in Vanuatu and identified as *Haliclona* sp. (family Chalinidae, order Haplosclerida) by Ms. M.C. Diaz (UCSC). It was plate-like in shape with an irregular weakly conulose surface. The ectosome showed tangential reticulation formed by unispicular to multispicular tracts, traveling parallel to each other. The choanosome showed mainly unispicular isodyctal reticulation, but like the ectosome there are parallel multispicular tracts. The spicules were fusiform oxea (220–300×5–10 μm), with few styloid forms, and raphids (80–200×<2 μm).

EXTRACTION AND ISOLATION.—The sponge was processed as described previously (2). The CH_2Cl_2 partitioned fraction (1.38 g) was subjected to Sephadex LH-20 chromatography (MeOH) followed by ODS hplc (40% aqueous MeOH) yielding cyclorenierins A [**1**] and B [**2**].

Cyclorenierin A [**1**] *and cyclorenierin B* [**2**].—Lreims *m/z* 326 (1.7), 161 (100); ir ν max 3600, 3306, 1602 cm^{-1}; ^1H- and ^{13}C-nmr data, see Table 1.

ACKNOWLEDGMENTS

Funding was from NIH Grant CA47135, Sea Grant R/MP-56, and a Sea Grant Traineeship awarded to PH. Additional support for LHM came from NIH MBRS grant GM08132. We thank Ms. Lisa Hunter for assisting in the collection of the sponge.

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Received 4 October 1994