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CYTOTOXIC AND ANTIMICROBIAL ALKALOIDS FROM THE FIJIAN SPONGE *XESTOSPONGIA CAYCEDOI*

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Sponges of the genus *Xestospongia* have yielded a variety of bioactive metabolites. These metabolites can be placed in three categories, exemplified by xestospongin C [**1**] (1), halnaquinone [**2**] (2-4), and the dibromoacetylenic acid **3** (5). We wish to report the isolation of a new *Xestospongia* metabolites exemplified by the previously unknown metabolite renierol [**4**] and the known metabolite mimosamycin [**5**], isolated from a hard blue sponge *Xestospongia caycedoi* Zea and Van Soest, collected at Sand Island, Suva Harbor, Fiji.

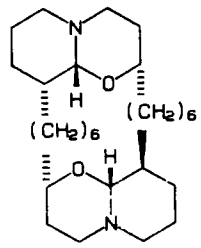
Crude methanolic extracts of *X. caycedoi* showed significant in vitro activity against the L1210 murine leukemia line (LC_{50} 0.05 μg/ml), insecticidal activity against the tobacco budworm (*Heliothis virescens*) and antimicrobial activity against *Staphylococcus aureus* (SA-27661), *Bacillus subtilis* (BS-6051), *Escherichia coli* (EC-14948), and *Saccharomyces cerevisiae* (SC-9763).

The methanolic extract from the lyophilized sponge (161 g, dry wt.) was partitioned according to a Kupchan scheme (6) to give hexane, CCl_4, $CHCl_3$, and MeOH soluble materials (1.7, 2.4, 2.1, and 10.7 g, respectively). Antimicrobial activity was concentrated in the $CHCl_3$ fraction. Column chromatography of the $CHCl_3$ layer on Sephadex LH-20 (MeOH-$CHCl_3$, 1:1; 2×106 cm) followed by hplc (Whatman Partisil 10, EtOAc-TMP, 1:1) yielded pure renierol [**4**] (11.3 mg, 7×10^{-3}%) as a reddish-brown powder, and mimosamycin [**5**] (15 mg, 9.3×10^{-3}%) as a yellow powder.

Renierol showed antibiotic activity against *S. aureus* (100 μg on 6-mm disk gave a 10-mm ring of inhibition) and mild cytotoxicity against the L1210 cell line (IC_{50} 3.0 μg/ml). Renierol was assigned the molecular formula $C_{12}H_{11}NO_4$ by high resolution mass measurement (obsd 233.0687, calcd 233.0688). The ir spectrum of **4** showed a strong broad band at 3600-2850 cm^{-1} indicating a hydroxyl group and a band at 1665 cm^{-1} indicating a quinone carbonyl. Uv absorptions at λ (max) (MeOH) 314.8 (ε=4100), 241.4 (ε=13000), and 205.6 nm (ε=15000) indicated a *para*-benzoquinone moiety that was further conjugated (7). Low resolution eims gave a M^+ ion at *m/z* 233 as the base peak and major fragment ions at *m/z* 208 (M^+-CH_3), 190 (M^+-C_2H_3O), and 162 (190-CO). The ^{1}H-nmr spectrum showed two adjacent aromatic protons at δ 8.93 (d, 1H, J=4.61 Hz) and 7.93 (d, 1H, J=4.61 Hz); a broad exchangeable singlet at 4.85 ppm assigned to the hydroxyl proton and three additional singlets at 5.19, 4.15, and 2.10 ppm assigned to -OCH_2, -OCH_3, and Ar-CH_3 groups, respectively.

The ^{13}C-nmr spectrum (Table 1) contained two quinone carbonyls at 184.05 and 181.18 ppm, two protonated sp^2 carbons at 117.77 and 152.32 ppm assigned to C-4 and C-3, respectively, and five additional sp^2 carbons at 159.87, 157.74, 138.62, 130.27, and 121.28 ppm. In addition, the spectrum contained two carbons bearing oxygen at 63.64 and 60.88 ppm assigned to -OCH_2 and -OCH_3 groups, respectively, and an aromatic methyl at 8.64 ppm. These data are consistent with isoquinoline quinones and are virtually identical with data reported for re-

[1]Alfred P. Sloan Foundation Fellow, 1985-1987.



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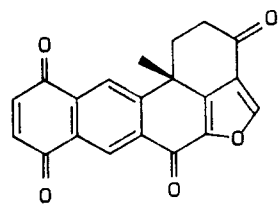

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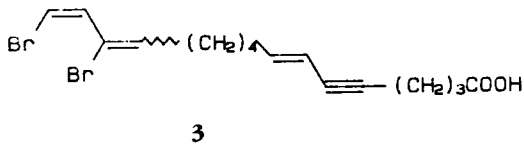

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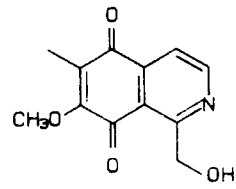

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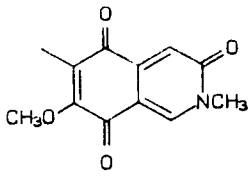

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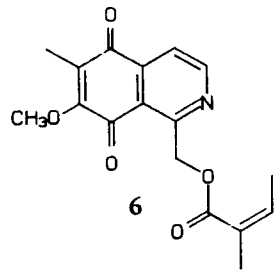

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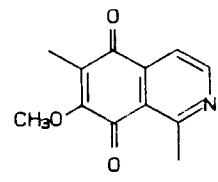

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nierone [**6**] (8) and 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione [**7**] (9) (see Table 1). All of these data are consistent for the proposed structure of renierol [**4**].

The second metabolite was assigned the molecular formula $C_{12}H_{11}NO_4$ by high resolution mass measurement (obsd 233.0679, calcd 233.0688). It showed antimicrobial activity against *S. aureus* (100 μg on 6-mm disk gave a 13-mm ring of inhibition). The ir spectrum of **5** showed two strong bands at 1688 and 1641 cm^{-1} indicating two types of carbonyls. Uv absorptions at λ (max) 315.6 (ε=12000), 230.6 (ε=14000), and 209.8 nm (ε=24000) were very similar to those found for renierol.

The ^{1}H-nmr spectrum consisted of five singlets. Two aromatic protons at 8.27 and 7.27 ppm assigned to C-1 and C-4, respectively, and aliphatic signals at 4.18, 3.67, 2.07 ppm assigned to -OCH_3, N-CH_3, and Ar-CH_3 groups, respectively. All spectral data were in good agreement with published data for

TABLE 1. Comparison of ^{13}C-nmr Data of Renierol [**4**], Renierone [**6**], and 1,6-Dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione [**7**]

Carbon Atom	Compound		
	4	**6**	**7**
1	159.87[a]	158.2	158.0
3	152.32	153.7	153.7
4	117.77	118.0	117.4
4a	138.62[b]	138.6	139.0
5	184.06	184.2	183.0
6	121.28	122.4	122.0
7	157.74[a]	156.6	158.0
8	181.18	181.4	180.0
8a	130.27[b]	130.1 or 127.6	130.2
9	63.64	65.0	29.3
Ar-Me	8.64	8.7	9.1
Ar-OMe	60.88	60.9	61.3

[a,b]May be interchanged.

mimosamycin [**5**] which was first isolated from *Streptomyces lavendulae* no. 314 (10,11), and subsequently from the *Reniera* sponge that yielded renierone [**6**] and related compounds (8,9).

Renierol bears an obvious resemblance to renierone [**6**] and 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione [**7**] isolated from a *Reniera* sp. (8,9). However, this is the first report of compounds of this type from *Xestospongia*. The fact that renierone [**6**] and renierol [**4**] were isolated from sponges from two different genera and that mimosamycin [**5**] is a known metabolite of a *Streptomyces* coupled with the extremely low yields of renierol [**4**] and mimosamycin [**5**] (7.0×10^{-3} and 9.3×10^{-3}%, respectively) suggests to us that these metabolites are of microbial origin. It is well documented that sponges can harbor large amounts of bacterial symbionts (12,13). This association has been suggested as the origin of such metabolites as tedanolide from *Tedania ignis* (14) and acanthifolicin from *Pandaros acanthifolium* (15).

EXPERMENTIAL

Specimens were collected from Sand Island, Suva Harbor, Fiji, at a depth of 2 m and immediately frozen. They were identified as *X. caycedoi* by Dr. Avril Ayling, Sea Research, Daintree, Queensland 4873, Australia. A voucher sample remains in her possession. Ir spectra were recorded on a Beckman FT-2100 on zinc selenide cells. Uv spectra were recorded on a Beckman DU-8 spectrophotometer. Low and high resolution ei mass spectra were recorded on a Varian MAT 112 and MAT 731 spectrometers, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL JNM-FX270 spectrometer; chemical shifts were reported relative to TMS.

Renierol [**4**].—^{13}C nmr ($CDCl_3$) 184.05 (s), 184.18 (s), 159.87 (s), 157.74 (s), 152.32 (d), 138.62 (s), 130.27 (s), 121.28 (s), 117.77 (d), 63.64 (t), 60.88 (q), 8.64 (q); eims *m/z*, 233 (M^+, base peak), 218, 190, 162.

Mimosamycin [**5**].—Ir (solid film) 3090, 2926, 1688, 1641, 1582, cm^{-1}; uv λ (max) (MeOH) 316 (ε=12000), 230 (ε=14000), 210 (ε=24000) nm; eims *m/z* 233 (M^+, base peak), 218, 205, 190, 162, 107.

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