Bioactive Isoquinoline Quinone from an Undescribed Philippine Marine Sponge of the Genus *Xestospongia*

Ru Angelie Edrada,[†,‡] Peter Proksch,*[,‡] Victor Wray,[§] Ruprecht Christ,[§] Ludger Witte,[⊥] and Rob W. M. Van Soest[∥]

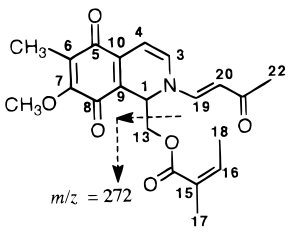
Julius-von-Sachs-Institut für Biowissenschaften, Lehrstuhl für Pharmazeutische Biologie, Mittlerer Dallenbergweg 64, D-97082 Würzburg, Germany, Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124, Braunschweig, Germany, Institut für Pharmazeutische Biologie Mendelssohnstr. 1, D-38106, Braunschweig, Germany, and Instituut voor Systematiek en Populatie Biologie, Zoölogisch Museum, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands

Received February 21, 1996[⊗]

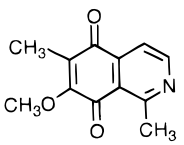
An undescribed Philippine marine sponge of the genus *Xestospongia* afforded four isoquinoline quinones: the new *N*-ethylene methyl ketone derivative of renierone (**1**), as well as the known compounds 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**2**), renierone (**3**), and mimosamycin (**4**). Compound **2** exhibited insecticidal activity toward neonate larvae of the polyphagous pest insect *Spodoptera littoralis* (EC_{50} of 35 ppm and LC_{50} of 521 ppm) when incorporated into artificial diet. Compounds **1** and **3** were only weakly active toward *S. littoralis*, while compound **4** was found to be inactive. All four isolated compounds were active against the Gram-positive bacteria *Bacillus subtilis* and *Staphyloccoccus aureus*. Compound **4** was also active against the fungus *Cladosporium cucumerinum*.

A variety of bioactive metabolites has been isolated from sponges of the genus *Xestospongia* (Family Petrosiidae, Order Haplosclerida), exemplified by xestospongin C,[1] halenaquinone,[2−4] dibromoacetylenic acid,[5] β-carboline alkaloids,[6,7] and isoquinoline quinones.[8,9,15] Naturally occurring isoquinoline quinones have also been isolated from other marine sponges as well as from Actinomycetes.[20] The isoquinoline quinones possess significant biological activity,[8−10] which suggests potential value as lead structures for the development of new pharmaceuticals or agrochemicals. In this paper, we describe the isolation and structure elucidation of a new isoquinoline quinone derivative, the *N*-(3-oxo-1-butenyl) derivative of renierone (**1**), and three other known congeners: 1,6-dimethyl-7-methoxy-5,8-dihydroisoquinoline-5,8-dione (**2**), renierone (**3**), and mimosamycin (**4**). These were obtained from a hitherto undescribed blue Philippine marine sponge of the genus *Xestospongia*, and we report on their insecticidal, antibacterial, and antifungal properties. Compounds **2** and **3** have been previously isolated from sponges of the genus *Reniera*[10,11] but not from the genus *Xestospongia*, while compound **4** was reported to occur in blue sponges of both genera, *Reniera* and *Xestospongia*. Compound **4** is a known metabolite of the terrestial bacterium *Streptomyces lavendulae*,[12] and it has previously been speculated[13,14] that a similar bacterium is epiphytic or symbiotic with the marine sponges.

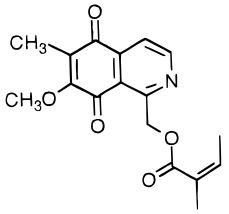
A blue *Xestospongia* sp., which was collected in the Philippines, was found to have no other available matching descriptions in the systematic literature on Indo-Pacific sponges. From this, we conclude that this material belongs to a new species, which will be described elsewhere.



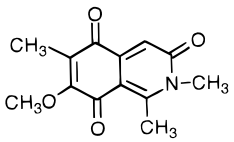
1



2



3



4

From the petroleum ether-soluble material of a crude extract from the sponge, two yellow bands were obtained by column chromatography on Si gel, yielding the known compounds **2** and **3**. From the ether-soluble extract, a blue band and an orange band were obtained, affording the new compound **1** and the known compound **4**, respectively. The known compounds were readily identified from their spectroscopic data and by comparison with published data.[8−12]

Compound **1** was obtained as a blue gummy film. Low-resolution EIMS gave the molecular ion peak M^+ at *m/z* 385 and the base peak at *m/z* 272 [M − CH_2-OCOC[CH_3]=$CHCH_3$]$^+$. The IR spectrum of **1** showed a band at 1717 cm^{-1} indicating an unsaturated ester and a band at 1648 cm^{-1} indicating both the quinone functionalities and the alkene group that is conjugated with the nitrogen and the carbonyl group. Compound **1** is a derivative of renierone, and this was confirmed from a comparison of the NMR data of **1** with those of compound **3** (Table 1). The ^{1}H NMR spectrum of **1** showed an additional pair of signals in the olefinic

* To whom correspondence should be addressed. Phone: 0049/931-8886174. FAX: 0049/931-8886182.
† Permanent Address: College of Pharmacy, University of the Philippines, Manila, Padre Faura, Manila, Philippines.
‡ Julius-von-Sachs-Institut für Biowissenschaft.
§ Gesellschaft für Biotechnologische Forschung mbH.
⊥ Institut für Pharmazeutische Biologie.
∥ Instituut voor Systematiek en Populatie Biologie.
⊗ Abstract published in *Advance ACS Abstracts,* September 1, 1996.

S0163-3864(96)00258-3 CCC: $12.00

Table 1. ^{13}C- and ^{1}H- NMR Data of Compounds **1** and **3** in CD_2Cl_2

	1			3	
	δC	δH	carbon correlations[a]	δC	δH
1	51.8 d	5.53 br dd ($\Sigma J \approx 10.0$ Hz)	C3, C9, C10, C13	159.0 s	
3[b]	138.3 d	6.85 d ($J = 7.9$ Hz)	C1, C4, C10, C19	154.3 d	8.93 d ($J = 4.8$ Hz)
4	99.7 d	5.98 d ($J = 7.8$ Hz)	C3, C5, C9	118.7 d	7.89 d ($J = 4.8$ Hz)
5	185.6 s			184.9 s	
6	127.8 s			123.1 s	
7	156.8 s			157.2 s	
8	180.6 s			182.2 s	
9	120.4 s			128.3 s	
10	136.3 s			139.4 s	
11	8.7 q	1.96 s	C5, C6, C7	8.0 q	2.12 s
12	61.4 q	4.04 s	C7	60.9 q	4.15 s
13A	61.2 t	4.30 dd ($J = 6.2$, 12.0 Hz)	C1, C9, C14	65.7 t	5.80 s
13B		4.12 dd ($J = 4.5$, 12.0 Hz)	C9, C14		
14	167.5 s			168.0 s	
15	127.4 s			131.1 s	
16	140.2 d	6.11 qq ($J = 7.2$, 1.5 Hz)	C14, C17, C18	138.0 d	6.15 qq($J = 7.2$, 1.4 Hz)
17-Me	20.7 q	1.82 dq ($J = 1.5$,1.5 Hz)	C14, C15, C16	20.7 q	1.97 dq($J = 1.5$, 1.5 Hz)
18-Me	15.9 q	1.96 dq ($J = 7.2$, 1.5 Hz)	C15, C13	15.8 q	2.01 dq($J = 7.3$, 1.5 Hz)
19	145.9 d	7.42 d ($J = 13.8$ Hz)	C1, C3, C21		
20	105.7 d	5.93 d ($J = 13.8$ Hz)	C19, C21, C22		
21	195.9 s				
22-Me	29.1 q	2.21 s	C20, C21		

[a] Long -range $^{13}C-^{1}H$ correlations, over two and three bonds, observed for **1** in the 2D ^{1}H-detected multiple ^{13}C multiple-quantum coherence spectrum. In all cases the direct correlation over one bond was also observed. [b] H3 also showed a four-bond correlation to C9.

region, δ 7.42 (d, $J = 13.8$ Hz) and δ 5.93 (d, $J = 13.8$ Hz), which are indicative of a substituted *N*-vinyl substituent with an *E*-configuration.[16] The signals of the aromatic protons H-3 and H-4, at δ 6.85 (d, $J = 7.9$ Hz) and δ 5.98 (d, $J = 7.8$ Hz), respectively, were at a higher field than those in **3**. The signal for H-1 appeared as a broad double doublet at δ 5.53 as it was coupled with both protons of the CH_2O group. Hence, in contrast to the singlet found in **3**, the protons of the latter group of the angelate ester are nonequivalent and appeared as a pair of double doublets due to vicinal (to H-1) and geminal couplings. A unique methyl ketone signal was found at δ 2.21, while the signals of the arylmethyl, methoxyl, and methyl groups of the angelate ester resembled those of **3.** The ^{13}C NMR spectrum of **1** was comparable with that of **3**, except for additional signals at δ 195.9 of an α,β-unsaturated ketone and two olefinic doublet signals at δ 145.9 and 105.7 corresponding to the substituted *N*-vinyl carbons. The signal of the methine carbon C-1 occurred at δ 61.8, and a methyl ketone signal was observed at δ 29.1.

Finally, a 2D ^{1}H-detected multiple-bond ^{13}C multiple-quantum coherence spectrum[17] of **1** afforded an independent, unambiguous confirmation of the signal assignments, substituent positions, and total structure (Table 1). In particular, long-range correlations of H19, H20, and H22 to C-21 established the nature of the *N*-substituent, while the correlations of H1 to C19 and H19 to C1 and C-3 confirmed its point of attachment to N2. Correlations of H13A and H13B to C14, and internal fragment correlations, confirmed the attachment of the angelate ester at C13.

All compounds isolated were analyzed for insecticidal activity and antibacterial activity, as well as fungicidal activity. Insecticidal activity was studied by incorporating each compound into an artificial diet at an arbitrarily chosen concentration (270 ppm) and offering the spiked diet to neonate larvae of the vigorous pest insect *S. littoralis* in a chronic feeding experiment. After 6 days of exposure, larval survival and larval weight were monitored and compared to controls. All compounds except **4** were active and inhibited the growth (40−100% larval weight compared to controls) of the larvae of *S. littoralis*. Compound **2** caused 93% growth inhibition at a concentration of 270 ppm, while **3** caused 60% growth inhibition at the same concentration. The new compound **1** was weakly active, causing 50% inhibition at the same concentration. In a subsequent experiment the most active compound **2** was analyzed for insecticidal activity at a range of concentrations (26−132 ppm). From the dose-response curves obtained, the EC_{50} for growth inhibition and LC_{50} were calculated by probit analysis as 35 ppm [±0.43 (SE)] and 521 ppm [±0.65 (SE)] ppm, respectively.

All isolated compounds were also tested for antibacterial activity, and they were all found to be active against the Gram-positive bacteria. No inhibition, however, was observed for *E. coli*. Compound **4** was found to be most active, causing an inhibition zone of 22 mm in diameter with *B. subtilis* and 17 mm with *S. aureus*, followed by compounds **3** and **1**, and compound **2** with the weakest activity of 12 mm and 9 mm diameter for *B. subtilis* and *S. aureus*, respectively (Table 2). The results were comparable to that of gentamycin, which was used as the positive control, causing an inhibition zone of 16 and 17 mm diameter for *B. subtilis* and *S. aureus*, respectively.

The isolated compounds were also tested for fungicidal activity against the fungus *Cladosporium cucumerinum*. Only compound **4** was found to be active at concentrations of 150 and 75 μg, causing inhibition zones of 18 and 15 mm in diameter, respectively.

From the results in the bioassays described (Table 2), it is obvious that the antibacterial and fungicidal activity of the compounds under study is not paralleled by the insecticidal activity toward neonate larvae of *S. littoralis*. Indeed, an inverse correlation appears to be the case. The attachment of an angelate ester to the nitrogen ring decreases the antibacterial and fungicidal activity, while the absence of the carbonyl substituent

Table 2. Bioactivities of the Compounds Isolated from *Xestospongia* sp.

comp no.	growth inhibition of larvae *S. littoralis* (dose = 270 ppm) (%)	zone of inhibition fungicidal activity against *C. cucumerinum* (dose = 150 μg) (mm diam)	zone of inhibition fungicidal activity against *C. cucumerinum* (dose = 75 μg) (mm diam)	zone of inhibition antibacterial activity *B. subtilis* (dose = 100 μg) (m diam)	zone of inhibition antibacterial activity *S. aurues* (dose = 100 μg) (mm diam)
1	50.0			15.0	15.0
2	93.0			12.0	9.0
3	60.0			15.0	14.0
4	15.0	18.0	15.0	22.0	17.0

results in a weakening of the antibacterial activity and a loss of fungicidal activity. However, the absence of a carbonyl substituent in the nitrogen ring system strengthens the insecticidal activity of the respective isoquinoline quinones. In this study, it is remarkable that the observed antibacterial activity and fungicidal activity of **4** or the insecticidal activity of **2** is likewise not caused by general toxicity but is rather due to a different mode of action and specific target requirement, which is apparently strongly influenced by the chemical structure of the studied compounds.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra (chemical shifts in ppm) were recorded on Bruker ARX 400 or DMX 600 NMR spectrometers, respectively. High-resolution mass spectra were recorded on a Finnigan MAT 95 mass spectrometer, with isobutane as reactant gas and low-resolution mass spectra (EI, CI-MS and FAB-MS (NBA as matrix)) on a Finnigan MAT 8430 mass spectrometer. The infrared spectrum was recorded on a Nicolet DX20 FTIR spectrometer using KBR disks as beamsplitter. The CD spectrum was recorded on a CD6 ISA Jobin Yvon Division d'Instrument S.A. using ethanol as solvent. For HPLC analysis, samples were injected into a HPLC system (Pharmacia, LKB, Sweden) coupled to a photoiode-array detector (Waters Millipore GmbH, Eschborn, Germany) which recorded the UV spectra on-line. Routine detection was at 254 nm. The separation column (125 × 4 mm, i.d.) was prefilled with Nova-Pak C-18 (μm) (Waters Milipore GmbH, Eschborn, Germany). Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on precoated TLC plates with Si gel 60 F_{254} (Merck, Darmstadt, Germany). The compounds were detected by their absorbance under UV_{254} or UV_{366}.

Animal Material. Specimens of blue *Xestospongia* sp. were collected by snorkelling off the shores of Mindoro Island, Philippines, in April 1994. The specimen is a massive sponge with tube-shaped oscules. Consistency is firm to hard, and the surface is optically smooth but velvety. The surface skeleton is barely developed and mostly absent. The interior is cavernous, and the choanosomal skeleton is irregularly alveolar, obscuring ill-defined longitudinal paucispicular tracts. Spicules are exclusively oxcas, typically 200 × 8−10 μm in size. The samples were frozen immediately upon collection and then freeze-dried prior to transport to University of Würzburg, Germany. A voucher specimen preserved in alcohol is kept under registration no. ZMA POR.10922 in the Zoölogisch Museum, Amsterdam.

Extraction and Isolation. The freeze-dried samples of *Xestospongia* sp. (54 g) were extracted successively with acetone and MeOH (300 mL × 2 for each). The total extract was evaporated under reduced pressure to a residue of 13.5 g. The residue was partitioned between petroleum ether (50 mL × 5) and 90% aqueous MeOH (50 mL). The methanolic fraction was taken to dryness and partitioned between ether (50 mL × 5) and H2O (50 mL). Both of the organic fractions were taken to dryness (3.5 g of petroleum ether-soluble and 0.5 g of ether-soluble material) and chromatographed over a Si gel column (mobile phase CH_2Cl_2−MeOH 98:2). The petroleum ether-soluble material gave two major yellow bands that yielded **2** (8.5 mg, 0.016%) and **3** (25.3 mg, 0.047%). These fractions were purified from sterols on a Si gel column with 10% ether in CH_2Cl_2. The ether-soluble material gave two major bands, a blue band yielding **1** (3.2 mg, 0.006%) and an orange band yielding **4** (4.4 mg, 0.008%). Compound **1** was separated from minor contaminants by column chromatography on Si 60 Lobar with hexane−EtOAc (2:8) as mobile phase. All fractions were evaporated under reduced pressure, and the pure compounds were obtained by further separation over a Sephadex column in CH_2Cl_2−MeOH (1:1). The identity of the fractions was confirmed by HPLC and UV spectra recorded on-line.

***N*-Ethylenemethyl ketone derivative of renierone (1)** was obtained as a blue solid film residue (R_f 0.56, CH_2Cl_2−MeOH; 98:2) having a molecular formula of $C_{21}H_{23}N0_6$ determined from the high-resolution CI mass spectral data of the molecular ion $[M]^+$ (m/z 385.1526; calcd for $C_{21}H_{23}NO_6$ 385.1525) and protonated molecular ion $[M + H]^+$ (m/z 386.1595; calcd for $C_{21}H_{23}NO_6$ 386.1604). EIMS (70eV) m/z: 385 (7), 314 (9), 272 (100). UV λ (max) (MeOH): 223 nm (ϵ 27 000), 312 nm (ϵ 23 000), 330 nm (ϵ 21 000). CD (EtOH): $\Delta\epsilon$ −17.5 (225 nm), $\Delta\epsilon$ −13.0 (268 nm), $\Delta\epsilon$ +12.5 (342 nm). FT-IR (KBr film): 3432 (br), 2927, 1717, 1648, 1527, 1452, 1376, 1263, 1230, 1189, 1135, 949, 744 cm^{-1}.

Experiments with Insects. Larvae of *S. littoralis* were from a laboratory colony reared on an artificial diet under controlled conditions as described previously.[19] Feeding studies were conducted with neonate larvae (n = 20) that were kept on an artificial diet that had been treated with various concentrations of the compounds under study. After 6 days, survival of the larvae and weight of the surviving larvae were protocolled and compared to controls.

.**Agar Plate Diffusion Assays.** Susceptibility disks (5 mm diameter) were impregnated with 100 μg of the isolated compound and placed on agar plates inoculated with the test bacteria: *B. subtilis* 168, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922. The plates were observed for zones of inhibition, after 24 h of incubation at 37 °C. Gentamycin was used as positive control.

Bioautographic Detection of Fungicidal Activity. Spores of *C. cucumerinum* were cultivated from carrot-nutrient agar and were inoculated into a liquid yeast culture medium as previously described.[17] Si gel

TLC plates were spotted with the isolated compounds at concentrations of 150 and 75 μg, and then the plates were sprayed with a suspension of spores of *C. cucumerinum* in liquid yeast culture medium. The fungitoxic compound was observed as a clear white spot of inhibition in a dark layer of the mycelia covering the TLC plate after the inoculated plates were incubated for 2 days at 25 °C.

Acknowledgment. Financial support by grants from the DFG (SFB 251) and by the "Fonds der Chemischen Industrie" is gratefully acknowledged. Furthermore, we would like to thank Dr. Dieter Gross and Ms. Monica Kummer (IPB, Halle, Germany) for giving us the opportunity to perform bioassays with *C. cucumerinum* and Dr. Rolf Jansen (GBF, Braunschweig,-Germany). We thank Mr. Pedro C. Gonzales of the Zoological Division, Philippine National Museum, and Mr. Frederick Agcaoili, Ms. Gerlie C. de los Reyes, and Ms. Imelda G. Peña of the College of Pharmacy, University of the Philippines Manila, and the rest of the expedition crew, Allan M. Edrada, Gladys M. Edrada, and Arlene S. Si for their help with the collection of the sponge material. R.A.E. wishes to thank the DAAD (Deutscher Akademischer Austauschdienst) for a scholarship.

References and Notes

(1) Nakagawa, M.; Endo, M.; Tanaka, N.; Gen-Pei, L. *Tetrahedron Lett*. **1984**, *25*, 3227−3230.
(2) Roll, D. M.; Scheuer, P. J.; Matsumoto, G. K.; Clardy, J. *J. Am. Chem. Soc.* **1983**, *105*, 6177−6178.
(3) Kobayashi, M.; Shimizu, N.; Kyogoku, Y.; Kitagawa, I. *Chem. Pharm. Bull*. **1985**, *33*, 1305−1308.
(4) Nakamura, H.; Kobayashi, J.; Kobayashi, M.; Ohizuma, Y.; Hirata, Y. *Chem. Lett. (Japan)* **1985**, 713−716.
(5) Schmitz, F. J.; Gopichand, Y. *Tetrahedron Lett*. **1978**, 3637−3640.
(6) Ichiba, T.; Sakai, R.; Kohmoto, T.; Saucy, G.; Higa, T. *Tetrahedron Lett*. **1988**, *29*, 3083−3086.
(7) Kobayashi, M.; Chen, Y.; Aoki, S.; In, Y.; Ishida, T.; Kitagawa, I. *Tetrahedron* **1995**, *51*, 3727−3736.
(8) McKee, T. C.; Ireland, C. M. *J. Nat. Prod*. **1987**, *50*, 754−756.
(9) Kubo, A.; Kitahara, Y.; Nakahara, S. *Chem. Pharm. Bull*. **1989**, *37*, 1384−1386.
(10) Frinckle, J. M.; Faulkner, J. D. *J. Am. Chem. Soc.* **1982**, *104*, 265−269.
(11) He, H.; Faulkner, J. D. *J. Org. Chem*. **1989**, *54*, 5822−5824.
(12) Fukumi, H.; Kurihara, H.; Mishima, H. *Chem. Pharm. Bull*. **1978**, *26*, 2175−2176.
(13) Berquist, P. R. *Sponges*; University of California: Berkeley and Los Angeles, 1978; pp 13−15.
(14) Berquist, P. R. ; Wells, R. J. In *Marine Natural Products;* Scheuer, P. J., Ed.; Academic Press: New York, 1983; Vol. 5, pp 6−8 and 197−198.
(15) Davidson, B. S. *Tetrahedron Lett*. **1992**, *33*, 3721−3724.
(16) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: Oxford, 1969; pp 278 and 302.
(17) Summers, M. F.; Marzilli, L. G.; Bax, A. J. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.
(18) Gottstein, D.; Gross, D.; Lehmann, Hans. *Arch. Phytopathol. u. Pflanzenschutz, Berlin 20* **1984**, *2*, 111−116.
(19) Srivastava, R. P.; Proksch, *P. Entomol. Gener*. **1991**, *15*, 265−274.
(20) Arai,T.; Kubo A. In *The Alkaloids*; Brossi, A., Ed; Academic Press: New York, 1983; Vol. 21, pp 55−100.

NP960258W