Pelorol from the Tropical Marine Sponge *Dactylospongia elegans*

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From the dichloromethane solubles of the tropical marine sponge *Dactylospongia elegans*, a new aromatic substituted sesquiterpene, pelorol (**1**), and the known sesquiterpene, ilimaquinone (**2**), were isolated. The structures of **1** and **2** were deduced from their spectroscopic data. The biological activities of compounds **1** and **2** were assessed in a variety of bioassays, and both compounds were found to have weak antitrypanosomal and antiplasmodial effects.

Sponges of the families Dysideidae, Thorectidae, Spongiidae, and Haploscleridae are known to produce a variety of sesquiterpene hydroquinone and sesquiterpene quinone metabolites, as summarized by Rodriguez et al.[1] All of these compounds possess a 4,9-friedodrimane or a drimane skeleton, which is substituted with a hydroquinone or quinone moiety. From *Dactylospongia elegans* Thiele, 1899 (Spongiidae) about 25 substances with such a diterpene skeleton have been isolated. Of these, 17 were isolated from samples of *D. elegans* collected from Fiji, Papua New Guinea, and Thailand.[1,2] In 1994, Lopez et al.[3] isolated tetronic acid derivatives with a drimane skeleton from *D. elegans* collected in Malaysia, together with several known sesquiterpene quinones, for example, ilimaquinone, a metabolite frequently encountered in these sponges. Recently, two new sesquiterpene lactones were isolated by Lal et al.,[4] from *Dactylospongia* sp. collected from New Caledonia. In the current work, the new compound pelorol (**1**), which is related to the sesquiterpene hydroquinones mentioned above, and ilimaquinone (**2**) were isolated from a sample of *D. elegans* collected from the Great Barrier Reef.

Mass spectral analysis of compound **1** indicated it to have the molecular formula $C_{23}H_{32}O_4$. From its ^{13}C NMR data four double bonds (3 × CC and 1 × CO) were deduced as the only multiple bonds within the molecule and accounted for four of the eight degrees of unsaturation implied by the molecular formula; **1** is thus tetracyclic. A singlet ^1H NMR resonance at δ 7.07 for an aromatic proton indicated the presence of a penta-substituted aromatic ring and accounted for the three carbon−carbon double bonds and one of the rings within **1**. The remaining functionality was deduced as two aromatic OH groups [δ 140.8 (s), 149.4 (s), 3440 cm^{-1}] and a methyl ester [δ 3.80 (3H, s), 51.7 (q), 168.4 (s), 1730 cm^{-1}], accounting for all of the oxygen within the molecule and the final double bond. From the HMBC spectrum of **1** (see Table 1) it was possible to construct fragment 1 of **1**. Thus, long-range CH correlations were observed from H_3-12 and H_3-13 to C-3, C-4, and C-5. As both H_3-12 and H_3-13 are singlet resonances and because H_2-3 and H-5 do not proton−proton couple, C-12 and C-13 must bond to C-4, as must C-3 and C-5. Further, H_3-14 long-range CH couples with C-1, C-5, C-9, and C-10. As H_3-14 is a singlet resonance and because H_2-1, H-5, and H-9 do not proton−proton couple, C-14 must bond to C-10, as must C-1, C-5, and C-9. Finally, long-range CH correlations between H_3-15 and C-7, C-8, C-9, and C-21, together with the information that H_3-15 is a singlet resonance and the fact that H_2-7 and H-9 do not proton−proton couple, indicated C-15 to bond to C-8, as must C-9 and C-21. This molecular fragment was further extended to fragment 2 of **1** on the basis of $^1H-^1H$ COSY cross-peaks between the resonances for H_2-1 and H_2-2, H_2-2 and H_2-3, H-5 and H_2-6, and H_2-6 and H_2-7. This left only C-11 without a bonding partner; clearly C-11 had to be bonded to C-16. With the planar structure of the carbocyclic skeleton of **1** complete, the regiochemistry of the aromatic ring and the relative stereochemistry at the four chiral centers needed solving. On the basis of NOE difference measurements, it was evident that the single aromatic proton was located at C-19 and the methyl ester at C-20, leaving the two OH groups to be positioned at C-17 and C-18. Thus, irradiation at the resonance frequency of H_3-23 caused enhancement of the resonances associated with H_2-7 and H-19. The β orientation of H_3-12, H_3-14, and H_3-15, and thus the stereochemistry of the bridge-head in the decalin system, was evident from irradiation of the resonance for H_3-14, which caused enhancement of the resonances for H-6β, H_3-12, and H_3-15, giving the decalin system the fairly typical transfused double-chair conformation. For **1**, the trivial name of pelorol is proposed.

It is clear that compound **1** has many structural elements in common with the dorisenones,[5] and even more so with smenodiol (**3**), an isolate from a Seychellean sponge belonging to the genus *Smenospongia*.[6] In a formal sense, **3** could be considered a possible direct precursor of **1**, as it is a simple cyclization product thereof.

Together with **1**, ilimaquinone (**2**) was also isolated, a compound distinguished by its two distinctive pH-dependent colors, yellow (pH < 7) and violet (pH > 7). In antitrypanosomal[7] and antiplasmodial[8] bioassays, compounds **1** and **2** were shown to have weak effects (see Table 2). The antimicrobial and antialgal activities of **1** and **2** were investigated in agar diffusion tests,[9] which showed them to have very weak effects or none at all. In ELISA-based assays, inhibition of tyrosine kinase (TK) and human immunodifficiency virus type 1 reverse transcriptase were studied. Ilimaquinone (**2**), inhibited TK activity by 87% at a concentration of 1 μg/μL.[10]

Experimental Section

General Experimental Procedures. These were performed as previously reported.[11]

Animal Material. Animal material was obtained in August 1990, from Pelorus Island, the Great Barrier Reef, Queensland,

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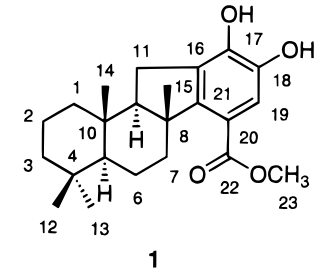
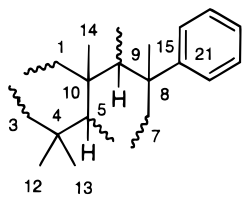
Table 1. ^{1}H and ^{13}C NMR Data for Compound **1** (300 MHz and 75.5 H_2, respectively, $CDCl_3$)

carbon	δ ^{13}C	δ ^{1}H[a]	δ ^{1}H (600 MHz, CD_3OD)	CH long-range correlations	diagnostic NOEs[b]
1	40.2 t[c]	0.96*, 1.56*	*d*		
2	18.4 t	1.43*	*d*		
3	42.5 t	1.12*, 1.38*	*d*		
4	33.1 s				
5	57.1 d	0.91*	1.02 (dd, J = 3.3, 12.4 Hz)		
6	19.6 t	1.54*, 1.65*	*d*		
7	36.5 t	1.38*, 2.49*	*d*		
			2.60 (ddd, J = 3.3, 3.3, 12.5 Hz)		
8	48.5 s				
9	65.2 d	1.64*	1.65 (dd, J = 6.0, 13.0 Hz)		
10	37.2 s				
11	24.4 t	2.49*, 2.62*	2.49 (dd, J = 13.0, 14.6 Hz)		
			2.67 (dd, J = 6.0, 14.6 Hz)		
12	21.1 q	0.84 (s)	0.93 (s)	3, 4, 5,13	
13	33.4 q	0.85 (s)	0.91 (s)	3, 4, 5, 12	
14	16.3 q	1.03 (s)	1.14 (s)	1, 5, 9, 10	1β, 6β, 11β, 12, 15
15	19.9 q	1.21 (s)	1.26 (s)	7, 8, 9, 21	6β, 7β, 11β, 14
16	130.1 s				
17	143.9 s				
18	140.8 s				
19	115.0 d	7.07 (s)	7.01 (s)		23
20	118.0 s				
21	149.4 s				
22	168.4 s				
23	51.7 q	3.80 (s)	3.82 (s)	22	7, 19

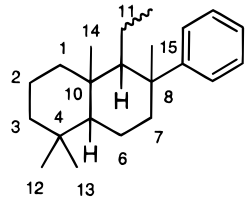
[a] Resonances marked with an asterisk were all broad, showing no fine structure, thus coupling constants could not be determined. [b] NOEs were recorded in CD_3OD and at 600 MHz. [c] Implied multiplicity by DEPT (C = s, CH = d, CH_2 = t, CH_3 = q). [d] Resonance is located between δ 1.05 and 2.50.



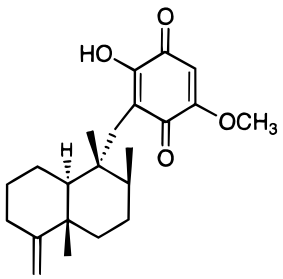
1



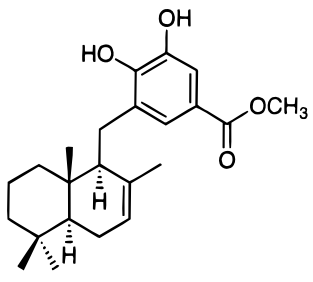
Fragment 1 of **1**



Fragment 2 of **1**



2 Ilimaquinone



3 Smenodiol

Australia. Animals growing at a 9-m depth were collected, deep frozen, and, on return to the laboratory, freeze-dried. A voucher specimen is deposited at the Institute for Pharmaceutical Biology, University of Bonn (voucher number CT902C).

Extraction and Isolation. The sponge tissue (96.0 g) was exhaustively extracted with CH_2Cl_2 (2 L) and MeOH (2 L), to yield 3.1 g (3.2%) of a CH_2Cl_2-soluble material. Vacuum liquid chromatography (VLC) of the crude extract over Si gel, using hexane with increasing proportions of $(CH_3)_2O$ as eluent, followed by MeOH, and finally MeOH containing 1% HOAc, afforded 25 fractions, each of 80 mL. TLC and ^{1}H NMR examination of these fractions indicated VLC fractions 9−14 to be of further interest.

Table 2. Antitrypanosomal and Antiplasmodial Activities of Compounds **1** and **2**

compound	antitrypanosomal activity[a] IC_{50} μg/mL	antiplasmodial activity[b] IC_{50} ng/mL Clone K1	Clone NF54
melarsoprol	0.0026		-
chloroquine		91	4.6
qinghaosu		1.4	2.5
1	17.4	786	1911
2	7.7	1743	949

[a] Test organism was *Trypanosoma rhodesiense*. [b] Test organisms were two strains of *Plasmodium falciparum*.

VLC fractions 9−13 were predominantly compound **2**. HPLC separation (RP-C_{18}, MeOH−H_2O, 5:1) of VLC fraction 14 afforded compounds **1** and **2**.

Pelorol (1): a white amorphous solid (20 mg, 0.02%); $[\alpha]^{22}_D$ −69.4° (c 0.1, $CHCl_3$); UV/vis λ_{max} (MeOH) 286 (sh, ϵ 2380), 256 (ϵ 4840) nm; IR ν_{max} (film) 3440, 2920, 1730, 1640, 1465, 1380, 1290, 1190 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS m/z $[M]^+$ 372 (60), 357 (100), 325 (40), 221 (40), 219 (60); HREIMS m/z 372.230 (calcd for $C_{23}H_{32}O_4$, 372.230).

Ilimaquinone (2): an amorphous powder, yellow when isolated from acidic medium and violet when isolated from alkaline medium (+500 mg, +0.52%); $[\alpha]^{22}_D$ −30° (c 0.1, $CHCl_3$), lit.[12,13] −23.2°; remaining physical and spectroscopic properties identical with those previously reported.[12]

Bioassays. Antitrypanosomal and antimalarial testing followed the procedures of Desjardins et al.[7] and Ridley et al.,[8] respectively. The agar diffusion assays and ELISA based assays were performed as previously described.[9,10]

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References and Notes

(1) Rodriguez, J.; Quinoa, E.; Riguera, R.; Peters, B. M., Abrell, L. M.; Crews, P. *Tetrahedron* **1992**, *48*, 6667−6680.
(2) Kushlan, D. M.; Faulkner, D. J. *Tetrahedron* **1989**, *45*, 3307−3312.
(3) Lopez, M. D.; Quinoa, E.; Riguera, R.; Omar, S. *J. Nat. Prod.* **1994**, *57*, 992−996.
(4) Lal, A. R.; Cambie, R. C.; Rickard, C. E. F.; Bergquist, P. R. *Tetrahedron Lett.* **1994**, *35*, 2603−2606.
(5) Miyamoto, T.; Sakamoto, K.; Arao, K.; Komori, T.; Higuchi, R.; Sasaki, T. *Tetrahedron* **1996**, *52*, 8187−8198.
(6) Venkateswarlu, Y.; Faulkner, D. J.; Steiner, J. L. R.; Corcoran, E.; Clardy, J. *J. Org. Chem.* **1991**, *56*, 6271−6274.
(7) Desjardins, R. E.; Canfield, C. J.; Haynes, D.; Chulay, J. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.
(8) Ridley, R. G.; Hofheinz, W.; Matile, H.; Jaquet, C.; Dom, A.; Masciadri, R.; Jolidon, S.; Richter, W. F.; Guenzi, A.; Girometta, M. A.; Urwyler, H.; Huber, W.; Thaithong, S.; Peters, W. *Antimicrob. Agents Chemother.* **1996**, *40*, 1846−1854.
(9) Schulz, B.; Sucker, J.; Aust, H. J.; Krohn, K.; Ludewig, K.; Jones, P. G.; Döring, D. *Mycol. Res.* **1995**, *99*, 1007−1015.
(10) Wessels, M.; König, G. M.; Wright, A. D. *J. Nat. Prod.* **1999**, *62*, 927−930.
(11) Wright, A. D.; König, G. M.; Angerhofer, C. K.; Greenidge, P.; Linden A.; Desqueyroux-Faundez, R. *J. Nat. Prod.* **1996**, *59*, 710−716.
(12) Luibrand, R. T.; Erdman, T. R.; Vollmer, J. J.; Scheuer, P. J.; Finer, J.; Clardy, J. *Tetrahedron* **1979**, *35*, 609−612.
(13) Capon, R. J.; MacLeod, J. K. *J. Org. Chem.* **1987**, *52*, 5059−5060.

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