Rigidone, a Sesquiterpene *o*-Quinone from the Gorgonian *Pseudopterogorgia rigida*

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Received September 25, 1996[⊗]

As part of a search for novel biologically active compounds in the Macrophage Scavenger Receptor (MSR) assay, the EtOAc extract of a gorgonian coral, *Pseudopterogorgia rigida*, was shown to be active. Bioassay-guided fractionation of the extract yielded curcuphenol, curcuhydroquinone, curcuquinone, and a novel *o*-quinone, rigidone (**1**). The structure and stereochemistry of **1** was determined by interpretation of spectral data and chemical transformation.

Macrophage-derived foam cells are hallmarks of early, as well as advanced, atherosclerotic lesions, and these cells arise by uptake and accumulation of modified low-density lipoprotein (LDL) through the action of non-downregulating macrophage scavenger receptors (MSR), which recognize all forms of modified LDL.[1,2] An MSR inhibitor, by preventing the binding of modified LDL to the receptor, should prevent the development of foam cells, inhibit the progression of atherosclerotic lesions, and induce their regression.

As part of our continuing search for biologically active natural products with potential utility in the treatment of atherosclerosis, we initiated a high throughput screen to evaluate the ability of natural product extracts to inhibit MSR. Several hundred plant and marine extracts were screened. The ethyl acetate extract of the gorgonian coral *Pseudopterogorgia rigida* (Bielschowsky) (Gorgoniidae) exhibited a moderate MSR inhibitory activity and as a result was selected for fractionation.

The freeze-dried gorgonian was extracted sequentially with EtOAc and MeOH. The active EtOAc extract was chromatographed over a RP-18 Si gel column. Final purification by RP-18 Si gel PTLC followed by Si gel HPLC afforded a novel *o*-quinone, for which we propose the name rigidone (**1**), plus the previously reported[3,4] curcuquinone, curcuphenol, and curcuhydroquinone.

The *o*-quinone, **1**, $[\alpha]_D$ +8.3° (*c* , 0.37, $CHCl_3$), was isolated as pale red powder, in which the presence of a phenolic group was indicated by a positive ferric chloride test. The presence of a quinone feature was suggested by the pale red color of the compound as well as by IR (1627 and 1608 cm^{-1}) and UV [λ_{max} (MeOH) 264, 408 nm] absorptions. The low resolution DCI mass spectrum of rigidone (**1**) exhibited a strong molecular ion at *m/z* 248 (one exchangeable hydrogen), which corresponded to a molecular formula of $C_{15}H_{20}O_3$. The ^1H NMR spectrum displayed a narrow quartet at δ 6.46 and a triplet at δ 5.04. In addition, it contained a methine multiplet, two methylene pairs, and four methyl signals in the aliphatic region, which were very similar to those of known compounds isolated earlier from *P. rigida*.[3] A ^{13}C GASPE NMR spectrum of **1** confirmed the existence of four methyls, two methylenes, three methines, and four quaternary carbons, in addition to the two quinone carbonyls at δ 184.3 and 187.4. These chemical shifts of C-5 and C-6 together with IR spectral data supported the presence of a quinone.[5] The COSY data indicated that H-7 in the side chain was coupled to the CH_3-14 doublet and to the prenyl moiety, which included the H-8 and H-9 methylenes, the H-10 olefinic triplet of multiplets, and two methyl multiplets. NOEs observed between H-10 and CH_3-12 and between H-9 and CH_3-13 were consistent with this proposal.

The H-3 aromatic quartet at δ 6.46 in rigidone (**1**) shared a 1.5 Hz coupling with the CH_3-15 doublet and three-bond HMBC correlations with C-1 (δ 124.6), carbonyl C-5 (δ 184.3), and CH_3-15 (δ 14.7). The CH_3-15 protons correlated to C-3 (δ 135.8) and carbonyl C-5 and C-4 (δ 140.5), indicating that this methyl group was situated between the carbonyl and aromatic proton, H-3. In similar fashion, the H-7 methine at δ 3.05 correlated to the ring carbons C-1 (δ 124.6), carbonyl C-6 (δ 187.4), and phenolic C-2 (δ 151.0), indicating that the C-7 aliphatic side chain must be attached to the ring at C-1. These correlations would be consistent with either the 2,5-*p*-quinone or the 5,6-*o*-quinone.

Further proof that compound **1** was either a 2,5-*p*-quinone or a 5,6-*o*-quinone was provided by the condensation of **1** with *o*-phenylenediamine to give a single predominant phenazine product, **2**, which was purified by PTLC. Compound **2** had a molecular ion at *m/z* 336 (2 exchangeables, $C_{21}H_{24}N_2O_2$), indicating that during the condensation reaction an unexpected oxidation of the rigidone phenyl ring also occurred at C-3. The reason for the formation of the C-3 hydroxyl group is unclear. The resulting ^1H NMR had four new overlapping multiplets between δ 8.01 and 7.49, while the high-field aliphatic region remained almost unaffected.

FT-IR microscopy data was useful in determining that **1** was the 5,6-*o*-quinone rather than the 2,5-*p*-quinone. Since *p*-quinones typically exhibit strong IR absorptions near 1650 cm^{-1}, it was unclear whether this band in compound **1** was attributable to a *p*-quinone carbonyl absorption or to the isolated double bond in the side chain, which would also give rise to an absorption at that position. However, the observation of two additional strong IR absorptions at 1627 and 1608 cm^{-1}

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⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1997.

S0163-3864(96)00653-2 CCC: $14.00 © 1997 American Chemical Society and American Society of Pharmacognosy

could only be attributed to the *o*-quinone. Furthermore, if **1** were the 2,5-*p*-quinone, one would expect a very strong 1560 cm^{-1} IR band arising from the hydrogen-bonded 5-carbonyl. No such an absorption was observed.

The absolute stereochemistry of **1** was determined by comparison of its optical rotation with that of the known (−)-curcuphenol (−7°), (−)-curcuquinone (−1.3°), (−)-curcuhydroquinone (−21°), and (−)-curcuhydroquinone 1-monoacetate (−3.8°).[3,4] The $[\alpha]_D$ of **1** was +8.3°, indicating an inversion of the stereochemistry at C-7.

Rigidone (**1**) and the phenazine analog (**2**) have IC_{50}'s of 5.6 and 71 μM, respectively. Although rigidone (**1**) showed reasonable potency, it was found to be inactive in the functional assay and, therefore, was not considered as an MSR lead.

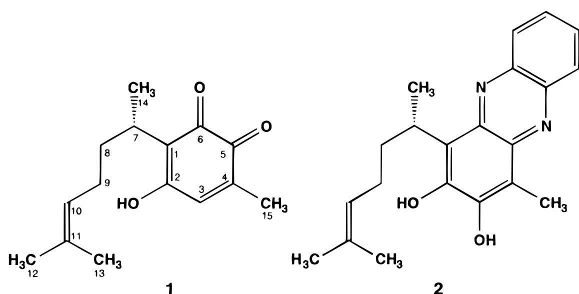

Table 1. ^1H and ^{13}C NMR Assignments for **1** in $CDCl_3$

no.	^{13}C	^1H
1	124.6	
2	151.0	
3	135.8	6.46 (1H, q, J = 1.5 Hz)
4	140.5	
5	184.3	
6	187.4	
7	29.3	3.05 (1H, m)
8	34.1	1.80 (1H,m)
		1.57 (1H, m)
9	26.7	1.87 (2H, m)
10	124.5	5.04 (1H, tm, J = 7.0 Hz)
11	131.3	
12	25.7	1.62 (3H, m)
13	17.6	1.51 (3H, m)
14	18.2	1.18 (3H, d, J = 7.0 Hz)
15	14.7	2.04 (3H, d, J = 1.5 Hz)
OH-2		7.10 (1H, b)

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. All homonuclear and heteronuclear one- and two-dimensional NMR data were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$. All of the mass spectra were obtained using a Finnigan Model 4610 quadrupole mass spectrometer. Analytical and preparative TLC were carried out on precoated reversed-phase (Whatman KC18F) plates. A Rainin HPXL solvent delivery system equipped with a refractive index detector, Model 156, was used for HPLC separations employing a Lichrosorb SI 60 column. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter. Reagent-grade chemicals (Fisher and Baker) were used throughout.

Biological Assays. Human embryonic kidney (HEK) 293 cells were transfected with full-length MSRI to produce a stable cell line.[6] The cells were plated at a density of 1 × 10^5 cells/mL 4 days before use. For the assay, the cells were incubated with 6 μg/mL of the ligand diI-ac-LDL plus the test compound in serum-free growth medium; the incubation period was approximately 6 h in air at room temperature. The medium was EMEM [minimum essential media (MEM)] with Earles salts supplemented with 0.4 mg/mL of G418 (gentisin), 2 mM L-glutamine, 10 mM HEPES, and 2 mg/mL of BSA. At the end of the incubation period, the cells were washed in complete Lockes buffer [consisting of 154 nM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 8.6 mM HEPES (free acid), 5.6 mM glucose, 1.0 mM $MgCl_2$, and 2 mg/mL of BSA]. The ligand taken up by the receptors was detected fluorimetrically (excitation wavelength 544, emission wavelength 590) and the amount quantitated, compared to inhibitor-free cells and ligand-free cells.

Biological Material. The gorgonians were collected by hand using scuba at a depth of ~18 m in June 1988 at Chub Cay, Bahamas. Samples were immediately frozen and stored at −20 °C until workup. The voucher sample, No. CI88-121, has been stored at SmithKline Beecham Pharmaceuticals in King of Prussia, PA.

Extraction and Isolation. The freeze-dried samples of *Pseudopterogorgia rigida* (530 g) were extracted with EtOAC (2 × 2 L) and MeOH (2 × 2 L) to give 39.7 g and 22.2 g of extract, respectively. The EtOAc extract (2 g), which showed MSR activity, was applied to a column of RP-18 Si gel and eluted with a H_2O:CH_3CN (20:80) mixture. Several fractions (15 mL each) were collected and monitored by RP-18 TLC. Like fractions were combined to yield eight (A−H) individual fractions. The RP-18 PTLC (H_2O:CH_3CN:25:75) of the MSR active fractions (B−E) followed by Si gel HPLC (EtOAc:Hex: 15:85, flow rate of 3 mL/min, RI detection) afforded curcuquinone (0.053 g), curcuphenol (0.048 g), curcuhydroquinone (0.079 g), and the novel *o*-quinone **1** (0.086 g) in pure form.

Rigidone (1): pale red powder; $[\alpha]_D$ +8.3° (c = 0.37, $CHCl_3$); IR (microscope) 3304, 3100−2800, 1648, 1627, 1608, 1468, 1389, 1369, 1199 cm^{-1}; UV (MeOH) λ max 408, 264, 210 nm; ^1H and ^{13}C NMR data, see Table 1; LRDCIMS m/z 248; HRDCIMS 248.1457 (M^+, calcd for $C_{15}H_{20}O_3$, 248.1412).

Condensation of 1 with *o*-Phenylenediamine. To a solution of **1** (0.012 g) in MeOH (2.5 mL) was added *o*-phenylenediamine (0.005 g) with stirring at room temperature for 2 h. The solution was evaporated to dryness, and the dark red predominant component was purified by RP-18 Si gel PTLC (H_2O:CH_3CN:15:85) to give phenazine, **2** (0.016 g), as red powder: UV (MeOH) λ max 463, 397, 286, 256, 212 nm; ^1H NMR (CD_3OD) δ 8.01 (m, 1H, H-3′), 7.99 (dm, 1H, J = 8 Hz, H-6′), 7.55 (ddm, 1H, J = 6.4, 6.8 Hz, H-4′), 7.49 (ddm, 1H, J = 6.8, 8.0 Hz, H-5′), 5.09 (tm, 1H, J = 7.0, H-10), 4.19 (m, 1H, H-7), 2.54 (s, 3H, CH_3-15), 2.31, 1.78 (m, 2H, CH_2-8), 1.85, 1.80 (m, 2H, CH_2-9), 1.51 (m, 3H, CH_3-12), 1.45 (d, 3H, J = 7.1 Hz, CH_3-14), 1.23 (m, 3H, CH_3-13); LRDCIMSP m/z 336 (M^+).

Acknowledgment. The authors thank the Department of Fisheries, Ministry of Agriculture, Trade & Industry, Bahamas, for permission to collect the specimens that were identified by Professor William Fenical. We appreciate the opportunity to perform the collections

of the gorgonian aboard the R. V. Columbus Iselin, which is supported in part by NSF Grant No. CHE90-08621 to Professor William Fenical, Scripps Institution of Oceanography. We thank Priscilla Offen for IR spectra.

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NP960653M