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STRUCTURES AND SYNTHESIS OF SOME NEW ANTIBACTERIAL SESQUITERPENOIDS FROM THE GORGONIAN CORAL *PSEUDOPTEROGORGIA RIGIDA*[1]

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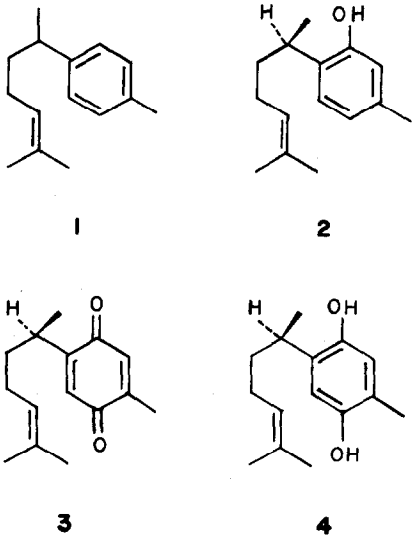
Abstract—The antibacterial properties of extracts of the Caribbean gorgonian *Pseudopterogorgia rigida* have been shown to originate in three previously undescribed derivatives of the aromatic sesquiterpene α-curcumene. (-)-Curcuphenol, (-)-curcuquinone, and (-)-curcuhydroquinone were isolated in high yield (30% extract) and structurally defined by inter-conversion and by reduction of the mesylate derivatives to yield the parent hydrocarbon (-)-α-curcumene. A high yielding synthesis (86% overall) of curcuphenol, the most active antibacterial metabolite, is described.

As part of our general interest in the isolation and characterization of new, biologically active compounds from marine organisms, we have investigated the Caribbean gorgonian sea plume *Pseudopterogorgia rigida* (Bielschowsky) (Octocorallia, Coelenterata). In preliminary biotesting, $CHCl_3$–CH_3OH extracts of *P. rigida* showed modest antibacterial activity against *Staphylococcus aureus* and the marine pathogen *Vibrio anguillarum*.

While *P. rigida* is virtually inseparable morphologically from most other *Pseudopterogorgia* species, particularly the more abundant *P. americana*, it can readily be recognized based upon its rather distinct lemon-like odor. In general, the odors of gorgonians have been attributed to their unusually high concentrations of sesquiterpene hydrocarbons. *P. americana*, for example, is reported to contain two aristolene sesquiterpenes, (+)-α-maaliene and (+)-β-gorgonene.[2] We report here that the characteristic sesquiterpene components of *P. rigida* are the α, β, and γ-bisabolenes and the corresponding aromatic analog α-curcumene **1**. The bisabolenes and α-curcumene have been recognized as odor components of the distantly related gorgonians *Plexaurella dichotoma*, *P. grisea* and *P. fusifera*.[3] At the same time we wish to report the structures of three new compounds from this gorgonian, for which we suggest the trivial names (-)-curcuphenol **2**, (-)-curcuquinone **3**, and (-)-curcuhydroquinone **4**. These compounds are, collectively, responsible for the antibiotic properties of *P. rigida*.

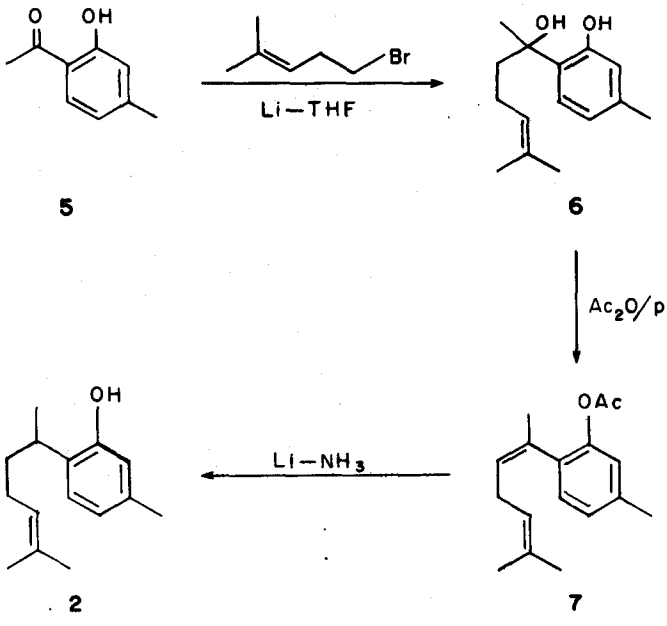
Conventional silica gel column chromatography of the $CHCl_3$–CH_3OH extract of freshly alcohol-preserved *P. rigida*[4] gave first a small hydrocarbon fraction (2% extract), which by GC–MS was recognized to be composed of numerous isomeric $C_{15}H_{24}$ (M^+ $m/e = 204$) hydrocarbons and a single $C_{15}H_{22}$ (M^+ $m/e = 202$) hydrocarbon. Without further separation, these sesquiterpenes could be assigned as mixtures of the α and β-bisabolenes (35% mixture), γ-bisabolenes (40%) and α-curcumene (1,25%). Assignments of these hydrocarbons were greatly facilitated by comparisons of their spectral data (MS, PMR) and GC retention times with those of authentic samples (Givaudan Corp.). Further elution of the column gave, initially, a 1:1 mixture of (-)-curcuphenol **2** and (-)-curcuquinone **3** (12% extract), and finally pure (-)-curcuhydroquinone (**4**, 18% extract).



1 **2** **3** **4**

Further chromatography of the phenol–quinone mixture on neutral alumina (II) afforded purified samples of each compound.

(-)-Curcuphenol **2** was obtained as an optically active oil ($[\alpha]_D = 7.0°$) which showed classic UV absorptions for the phenol functionality at 217 and 276 nm ($\epsilon = 4690$, 2400). These absorptions were shifted in base to 237 and 290 nm ($\epsilon = 5200$, 3520). The IR spectrum of **2** further illustrated the existence of the hydroxyl functionality ($\gamma_{O-H} = 3250\ cm^{-1}$). The high resolution mass spectrum of **2** established the molecular formula of $C_{15}H_{22}O$ and contained an intense peak (95% base) corresponding to benzylic cleavage of an isoprenoid C_8 side-chain. The PMR spectrum of **2** could be readily interpreted to suggest the presence of the very familiar terminal isopropylidene group (vinyl proton δ 5.03, dd, J = 7, 7 Hz and two vinyl methyl singlets at δ 1.64 and 1.48), an aromatic methyl group (δ 2.12, s) and a benzyl substituted secondary methyl group (a 3H doublet at δ 1.16, J = 7, which was coupled to a methine multiplet at δ 2.91). Also, distinct aromatic bands were observed characteristic of 1,2,4-trisubstitution (δ 6.35, 1H, s; δ 6.87, 1H, d, J = 7.8 Hz; δ 6.53, 1H, d, J = 7.8 Hz). These overall spectral features indicated that **2** was the phenolic derivative of the already isolated α-curcumene,



however, two hydroxyl substitution isomers exist for this structure. To discern unambiguously between these possibilities, the most likely isomer was synthesized by a regiospecific route in 86% overall yield (Scheme 1). The product, racemic curcuphenol, was identical in all respects with the naturally-occurring compound.

The starting materials for the synthesis were the readily available 2-hydroxy-4-methyl acetophenone and 5-bromo-2-methyl-2-pentene,[6] which, when condensed in $Li/NH_3/THF$ solution, gave the benzyl alcohol **6** in 90% yield. Contrary to our predictions, the benzyl-substituted hydroxyl group in **6** could not be dehydrated with strong acids. However, on base-catalyzed acetylation (Ac_2O/pyridine, RT) the tertiary alcohol dehydrated quantitatively, yielding the styrene acetate **7**. The conjugated olefin and the ester functionality of **7** could then be readily reduced with lithium in ammonia to give 97% yields of racemic curcuphenol **2**. The absolute stereochemistry at the lone asymmetric carbon of natural **2** was established as *R* by an overall reduction to obtain the optically active parent hydrocarbon. Lithium in ammonia reduction of the corresponding mesylate derivative[7] afforded hydrogenolysis of the substituent to yield α-curcumene which showed $[\alpha]_D - 32.4°$, in close agreement with the (−) and *R* enantiomer as isolated from *Curcuma aromatica* ($[\alpha]_D - 34°$).[8]

(−)-Curcuquinone **3** was isolated as a viscous yellow oil which showed quinoid absorptions in its IR ($\gamma_{c=o}$ 1650 cm^{-1}) and UV (λ_{max} 253 nm, $\epsilon = 10{,}200$) spectra. High resolution mass spectrometry indicated the parent ion composition of $C_{15}H_{20}O_2$ for this compound. The PMR spectrum of **3** was clearly analogous to that from curcuphenol showing an isopropylidene group (δ 5.01, 1H, dd, J = 7, 7 Hz; δ 1.63, 3H, s; δ 1,53, 3H, s), a secondary benzylic methyl (δ 1.10, 3H, d, J-7 Hz; δ 2.84, 1H, m), but only two aromatic protons (δ 6.42, 1H, s; δ 6.52, 1H, q, J = 1.6 Hz). An aromatic methyl was also observed at δ 1.98, but in this case it was coupled (J = 1.6 Hz) to the adjacent aromatic proton.

(−)-Curcuhydroquinone **4** was isolated last from the column as a colorless viscous oil which showed strong hydroxyl bands in its IR spectrum at 3250 cm^{-1}. Mass spectrometry established this compound as the $C_{15}H_{22}O_2$ hydroquinone reduction product of **3** by exhibiting similar fragmentations including the expected benzylic cleavage of the C_8 substituent. The PMR features of **4**, as in **2** and **3**, were readily interpreted in support of the hydroquinone structure. In confirmation, the hydroquinone was cleanly oxidized to (−)-curcuquinone **3** with Jones' reagent in acetone. Synthetic **3** showed $[\alpha]_D - 1.5°$ in close agreement to the value of $[\alpha]_D - 1.3°$ as recorded for the natural quinone. Hence, the absolute configurations of **3** and **4** are identical. Further, lithium in ammonia reduction of the dimesylate derivative[7] of **4** also gave (−)-α-curcumene($[\alpha]_D - 27°$) thus establishing that the asymmetric centers in **3** and **4** also possess the *R* configuration.

The curcumene derivatives **2–4** all showed weak antibacterial activities *Staphylococcus aureus* and *Vibrio anguillarum*, with curcuphenol **2** being the most active. No activity was observed for any of these compounds against the bacterium *Escherichia coli*.

Antibacterial bioassay. (agar plate-assay disc method). Zones of inhibition (mm) against:

Compound	μg (conc/disc)	*S. aureus*	*V. anguillarum*
2	(7)	4	slight
2	(70)	11	3
3	(18)	3	0
3	(180)	3	0
4	(9)	slight	slight
4	(90)	3	2

EXPERIMENTAL

IR spectra were recorded on a Perkin–Elmer 137 instrument, UV spectra were run in MeOH on a Perkin–Elmer Coleman 124 spectrophotometer. PMR and decoupling experiments were performed on a Varian HR-220 spectrometer in CCl_4 with TMS as internal standard, with $\delta = 0$. CMR spectra were determined on a Varian CFT-20 spectrometer in $CDCl_3$ with TMS as internal reference at $\delta = 0$. The off-resonances multiplicities are shown in

parentheses. Low resolution mass spectra were recorded at 70 eV on a Hewlett–Packard Model 5930A mass spectrometer. High resolution mass spectra were obtained at The California Institute of Technology. Silica gel, Grade 62, 60–200 mesh (W. R. Grace) and alumina, 80–325 mesh, Activity II (Matheson, Coleman and Bell) were used for column chromatography. GC data were obtained on a Hewlett–Packard Model 5710A gas chromatograph using a 4-ft × 1/4-in 3% SP-2250 column at 60 ml/min He flow.

Isolation. P. rigida, collected near Carrie Bow Cay, Belize, May, 1976, was immediately chopped into fine pieces and stored in isopropyl alcohol for shipment. On arrival the alcohol was decanted and the remaining parts were re-extracted twice by warming and blending in 1:1 $CHCl_3$: MeOH. The combined extracts from two quarts of animals yielded 17 g of hexane soluble material. The entire extract was then chromatographed on a 5 × 60 cm silica gel column using various hexane–benzene mixtures. Pure hexane eluted a small hydrocarbon fraction (400 mg, 2% extract) which showed a minimum of eight peaks by GC analysis. Comparisons of the mass spectra of these compounds with literature data and with those from authentic samples ("bisabolene" mixture for Givaudan Corporation) allowed the assignments of the peaks as α and β-bisabolenes (35% fraction), γ-bisabolenes (40%) and lastly α-curcumene (25%). Further elution first with 10% benzene gave 1:1 mixtures of (−)-curcuphenol **2** and (−)-curcuquinone **3** (2 g, 12% extract), and next with 30% benzene in hexane, gave (−)-curcuhydroquinone **4** (3 g, 18% extract).

(−)-*Curcuphenol* **2**. Oil, $[\alpha]_D - 7.0°$ (c 3.65, $CHCl_3$), λ_{max}^{MeOH} = 217, 276 nm, ϵ = 4690, 2400, base shift λ_{max}^{MeOH} = 237, 290 nm, ϵ = 5200, 3520; IR (CCl_4): 3250 cm^{-1}; PMR: δ 1.17 (3H, d, J = 7.0 Hz), collapses to a singlet when irradiated at 2.91, 1.48 (3H, s), 1.64 (3H, s), 1.40–1.70 (2H, m), 1.82 (2H, m), 2.12 (2H, s), 2.91 (1H, m), 5.03 (1H, dd, J = 7, 7 Hz), 5.29 (1H, b), 6.35 (1H, s), 6.87 (1H, d, J = 7.8 Hz), and 6.53 (1H, d, J = 7.8 Hz); CMR: 17.66, 20.88, 21.09, 25.70, 26.16, 31.53 (d), 37.34 (t), 116.25 (d), 121.71 (d), 124.71 (d), 126.89 (d), 130.15 (s), 131.83 (s), 136.45 (s), and 152.95 (s); MS: M^+ *m/e* observed 218.165, calc. for $C_{15}H_{22}O$ 218.162; *m/e* (rel. int.) 149 (16), 135 (96), 121 (30), 115 (28), 109 (14), 107 (13), 105 (14), 95 (29), 91 (41), 79 (22), 77 (28), 69 (28), 67 (21), 65 (15), 59 (29), 57 (21), 55 (15), 43 (17), and 41 (100).

(−)-*Curcuquinone* **3**. Yellow oil, $[\alpha]_C - 1.3°$ (c 9.1, $CHCl_3$), λ_{max}^{MeOH} = 253 nm (ϵ = 10,200); IR (CCl_4) 1650 cm^{-1}, sharp and intense; PMR: δ 1.11 (3H, d, J = 7 Hz), 1.43 (2H, m), 1.52 (3H, s), 1.64 (3H, s), 1.92 (2H, m), 1.98 (3H, d, J = 1.58 Hz), collapes to a singlet when irradiated at 6.52 ppm, 2.84 (1H, m), 5.01 (1H, dd, J = 7.7 Hz), 6.42 (1H, s) 6.52 (1H, q, J = 1.58 Hz), collapses to a singlet when irradiated at 1.98 ppm: MS: M^+ *m/e* observed 232.145, calc. for $C_{15}H_{20}O_2$ 232.146; *m/e* (rel. int.) 151 (64), 150 (22), 122 (45), 91 (14), 79 (23), 77 (18), 69 (23), 67 (23), 55 (36), 53 (32), 43 (23) and 41 (100).

(−)-*Curcuhydroquinone* **4**. Viscous oil, $[\alpha]_D - 21°$ (c 0.9, $CHCl_3$); IR (CCl_4) 3250 cm^{-1}; PMR: δ 1.15 (3H, d, J = 7 Hz), 1.50 (3H, s, superimposed on 2H, m), 1.66 (3H, s), 1.87 (2H, m), 2.09 (3H, s), 2.91 (1H, m), 5.07 (1H, dd, J = 7,7 Hz), 6.41 (1H, s), and 6.44 (1H, s); MS: M^+ *m/e* observed 234.162, calc. for $C_{15}H_{22}O_2$ 234.162; *m/e* (rel. int.): 151 (65), 137 (25), 123 (10), 95 (21), 91 (11), 79 (20), 77 (21), 69 (26), 67 (23), 65 (10), 55 (38), 53 (21), 51 (11), 43 (30), and 41 (100).

Benzyl alcohol **6**. 0.38 g (55 mg-atom) Li wire was hammered flat, cut into thin slivers, washed 3 times with petroleum ether, and added to 20 ml anhydrous diethyl ether in a 250 ml 3-necked round bottom flask under N_2 which was fitted with a Dewar condenser. 5-Bromo-2-methyl-2-pentene (1.96 g, 12 mmol) in 5 ml anhydrous ether was added over 3 min. After 2 h 0.90 g (6 mmol) 2-hydroxy-4-methyl acetophenone **5** in 5 ml anhydrous ether was added dropwise to the black solution, and the total volume was raised to 60 ml. After 1 h, 60 ml ammonia was distilled into the reaction vessel. After the characteristic blue color of the metal-ammonia solution was maintained for 15 min, solid ammonium chloride was cautiously added over a 10 min period to destroy the excess Li. The ammonia was allowed to evaporate and the reaction mixture was partitioned between brine and diethyl ether. The ether layer was dried over $MgSO_4$, filtered, and concentrated to yield 1.3 g of crude product. Silica gel chromatography afforded 1.2 g (90%) of **6**, an oil: IR (CCl_4) 3250–3350 cm^{-1}; PMR (CCl_4): δ 6.3–6.85 (3H, m), 5.0 (1H, m), 3.64 (1H, s), 2.20 (3H, s), 1.7–1.9 (4H, bs), 1.64 (3H, s), 1.53 (6H, bs).

Acetate **7**. A solution of 548 mg (2.35 mmol) **6**, 10 ml pyridine and 3 ml acetic anhydride were allowed to stir, under anhydrous conditions, for 5 h. The mixture was partitioned between 100 mls each of Et_2O and H_2O. The Et_2O layer was washed with 5% HCl (3 × 75 ml), 2N Na_2CO_3 (3 × 75 ml) and water (2 × 75 ml), dried over $MgSO_4$ and concentrated *in vacuo* to yield 590 mg (90%) of acetate **7**. TLC and PMR analysis indicated the product could be used without further purification.

(±) *Curcuphenol* **2**. 350 mg (50 mg-atom) Li wire was hammered flat, cut into fine slivers and washed free of oil with petroleum ether. The Li wire, 15 ml anhyd. Et_2O and 15 ml anhyd. ammonia were combined and, after the blue color had been maintained for 15 min, 100 mg (0.39 mmol) of **7** in 2 ml Et_2O was added dropwise. After 1 h excess NH_4Cl was carefully added and the ammonia was allowed to evaporate. The crude product was partitioned between Et_2O and H_2O, dried over $MgSO_4$ and concentrated *in vacuo* to yield 82 mg (97%) of racemic **2**. The synthetic product was identical (IR, PMR, MS) with natural curcuphenol.

Oxidation of (−)-*curcuhydroquinone* **4**. Dropwise addition of Jones' reagent ($CrO_3/H_2SO_4/H_2O$) to an ice-cooled acetone solution (5 ml) containing 50 mg (0.22 mmol) of the hydroquinone **4** resulted in an immediate reaction yielding green chromous salts. Water and Et_2O were immediately added, the Et_2O layer was isolated and washed with sat $NaHCO_3$, dried over $MgSO_4$ and concentrated to yield (−)-curcuquinone **3**, 46 mg (90%). Synthetic **3** showed $[\alpha]_D - 1.5°$ (c 8.5, $CHCl_3$) and was otherwise identical with the natural product.

Mesylate derivatives of **2** *and* **4**. In each case 0.35 mmol **2** or **4**, 5 ml pyridine and 1 ml methane sulfonyl chloride were combined under N_2 and stirred for 2 h. H_2O (5 ml) was next carefully added dropwise and the mixture was partitioned between Et_2O and H_2O. The Et_2O layer was removed, washed successively with H_2O (3 × 50 ml), 5% HCl (3 × 50 ml) and finally 2N Na_2CO_3 solution (2 × 50 ml). The layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield the crude mesylate derivative which was used without further purification.

(−)-α-*Curcumene* **1** *from mesylates of* **2** *and* **4**. 0.30 mmol Crude mesylate of **2** or dimesylate of **4**, in 5 ml Et_2O, was added to a solution of 175 mg (25 mg-atom) finely divided and washed Li, 10 ml Et_2O and 15 ml NH_3, under N_2. After 1 h excess NH_4Cl was cautiously added and the NH_3 was allowed to evaporate. The product was taken up in Et_2O, washed with water (3 × 50 ml), dried over $MgSO_4$ and concentrated *in vacuo*. Chromatography of the crude product on alumina (grade II) afforded a non-polar hydrocarbon identified as (−)-α-curcumene by comparison with authentic samples. The mesylate of **2** afforded the parent hydrocarbon (87% yield) which showed $[\alpha]_D - 32.4°$ (c 2.8, $CHCl_3$). The dimesylate of **4** yielded the hydrocarbon (64%) which showed $[\alpha]_D - 27°$ (c 2.1, $CHCl_3$).

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