# Aromatic Disabolenes from an Australian Marine Sponge, Arenochalina sp.

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# AROMATIC BISABOLENES FROM AN AUSTRALIAN MARINE SPONGE, *ARENOCHALINA* SP.

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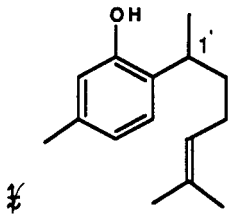
ABSTRACT.—Three new isomeric sesquiterpenes **6**, **7**, and **8** have been isolated from an Australian marine sponge, *Arenochalina* sp., and their structures determined by spectroscopic analysis and chemical correlation.

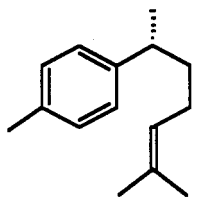
The aromatic bisabolene sesquiterpene (−)-curcuphenol [**1**] was first reported (1) as the most active antibacterial constituent of the gorgonian *Pseudopterogorgia rigida*, and the structure was confirmed by synthesis. The absolute stereochemistry of **1** was secured by chemical interconversion to (−)-α-curcumene [**2**], a known aromatic bisabolene (2) also found as a co-metabolite with **1**. Almost a decade later simultaneous reports appeared describing the isolation of (+)-curcuphenol [**3**] from two marine sponges, *Didiscus flavus* from the Bahamas (3) and *Epipolasis* sp. from Japan (4). The antipode **3** was said to have a variety of biological properties, including cytotoxic activity against in vitro tumor cell lines (P-388 murine leukemia, A-549 lung, HCT-8 colon, MDAMB mammary) and antifungal activity (*Candida albicans*), and was acclaimed as the first marine natural product to inhibit H,K-ATPase activity. Co-occuring in *D. flavus* was (+)-curcudiol [**4**], while in *Epipolasis* sp. **3** was accompanied by **4** and dehydrocurcuphenol [**5**]. In this report we describe the re-isolation of **3** and **4** together with three new isomeric minor metabolites **6–8** from an Australian marine sponge, *Arenochalina* sp. (Poecilosclerida: Mycalidae).

The crude EtOH extract of a specimen of *Arenochalina* sp. proved toxic to brine shrimp and also showed nonselective inhibition of isolated guinea pig ileum contractions evoked by various agonists including acetylcholine, histamine, and serotonin. Partitioning of this extract into $CH_2Cl_2$ solubles and $CH_2Cl_2$ insolubles, followed by rapid (vacuum) filtration through silica and hplc (silica) of the former, yielded two major components **3** and **4** and three minor components **6–8**. The two major components were fully characterized and proved to be identical in all respects to (+)-curcuphenol [**3**] and (+)-curcudiol [**4**].

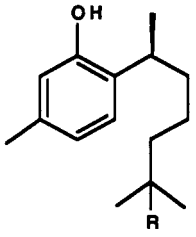
The more abundant, compound **6**, of the three minor components displayed many spectroscopic similarities with **4** and was identified as a dehydro analogue. Supportive of this was an $[M - H_2O]^+$ (*m/z* 216) 2 amu less than that of the corresponding ion in the mass spectrum of **4**, consistent with a molecular formula of $C_{15}H_{22}O_2$. A two-proton multiplet (δ 5.57) in the $^1$H-nmr spectrum ($CDCl_3$) together with an extra two $sp^2$ hybridized tertiary carbons (Table 2) indicated the presence of a disubstituted double bond. Because of almost co-incident chemical shifts for the two olefinic protons ($CDCl_3$), it was impossible to measure *J* values and determine both the position and geometry of the double bond. The $^1$H nmr spectrum in $C_6D_6$, however, was well resolved (δ 5.57, ddd, 15.5, 6.6, 7.1; δ 5.48, ddd, 15.5, 1.0, 0.7) and together with $^1$H nmr decoupling experiments clearly confirmed a $\Delta^{3',4'}$ system with an *E* geometry. Hydrogenation of **6** yielded a product identical in all respects, includ-



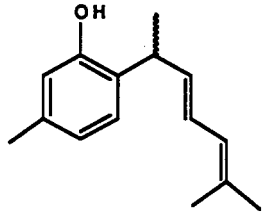
**1** (1′*R*)
**3** (1′*S*)



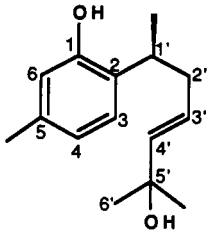
**2**



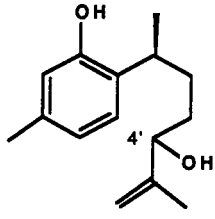
**4** R=OH
**9** R=H



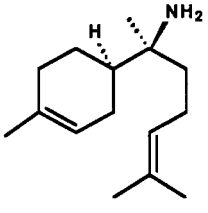
**5**



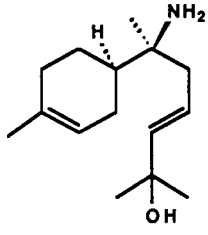
**6**



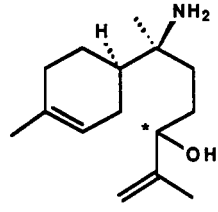
**7** and **8** (epimers about C-4′)



**10**



**11**



**12** and **13** (epimers about carbon with asterisk)

ing [α]D, to **4**, thereby establishing a common 1′*S* absolute stereochemistry.

The two remaining minor components **7** and **8** were isomeric with **6**; however, unlike **6** both incorporated a geminally disubstituted olefin ($\Delta^{5',6'}$) substituted by a methyl and a hydroxymethine (Tables 1 and 2). This array of functional groups, together with other structural features in **7** and **8**, was confirmed by interpretation of $^{13}$C-nmr spectra, in particular comparison with the related compounds described above. Given that **7** and **8** were stereoisomers but not enantiomers, it follows that with only two chiral centers they must be

TABLE 1. $^1$H-nmr ($CDCl_3$) Data for Three Isomeric Dehydrocurcudiols **6**, **7**, and **8**.

| Proton | Compound | | | |
|---|---|---|---|---|
| | **6** | **6** ($C_6D_6$) | **7** | **8** |
| H-3 | 7.03 (d, 7.8) | 6.99 (d, 7.8) | 7.02 (d, 7.8) | 7.03 (d, 7.8) |
| H-4 | 6.73 (bd, 7.8) | 6.68 (bd, 7.8) | 6.72 (bd, 7.8) | 6.72 (bd, 7.8) |
| H-6 | 6.57 (bs) | 6.05 (bs) | 6.64 (bs) | 6.61 (bs) |
| 5-Me | 2.26 (s) | 2.10 (s) | 2.27 (s) | 2.27 (s) |
| H-1′ | 3.07 (tq, 6.8, 6.8) | 3.18 (tq, 6.8, 6.8) | 3.16 (tq, 6.8, 6.8) | 3.07 (tq, 6.8, 6.8) |
| H-2′ | 2.25 (m) | 2.23 (dddd, 0.7, 6.8, 7.1, 13.4) | 1.49 (m) | 1.45–1.63 (m) |
| | 2.32 (m) | 2.36 (dddd, 1.0, 6.6, 6.8, 13.4) | | |
| H-3′ | 5.58 (m) | 5.57 (ddd, 6.6, 7.1, 15.5) | 1.65 (m) | 1.45–1.63 (m) |
| H-4′ | 5.58 (m) | 5.48 (ddd, 0.7, 1.0, 15.5) | 4.16 (dd, 4.6, 8.1) | 4.13 (dd, 4.2, 6.6) |
| H-6′ | 1.26[a] (s) | 1.12[a] (s) | 4.81 (m, W½ = 4.6) | 4.86 (m, W½ = 4.8) |
| | | | 4.94 (m, W½ = 3.8) | 4.97 (m, W½ = 3.5) |
| 1′-Me | 1.23 (d, 6.8) | 1.23 (d, 6.8) | 1.25 (d, 6.8) | 1.23 (d, 6.8) |
| 5′-Me | 1.25[a] (s) | 1.13[a] (s) | 1.68 (bs) | 1.68 (bs) |
| 1-OH | 4.73[b] (s, $D_2O$ ex) | 6.95[b] (s, $D_2O$ ex) | [c] | [c] |
| 5′-OH | 3.50[b] (s, $D_2O$ ex) | 4.10[b] (s, $D_2O$ ex) | | |

[a,b]Assignments with identical superscripts may be interchanged.
[c]Not observed.

epimers. Under hydrogenation conditions pure samples of both **7** and **8** yielded the dihydro hydrogenolysis product **9**. The spectroscopic and chiroptical properties of **9** produced from either **7**, **8**, or **3** were identical, thereby confirming a common 1′*S* stereochemistry. Because of a lack of ma-

TABLE 2. $^{13}$C-nmr ($CDCl_3$) Data for (+)-Curcudiol [**4**] and Three Isomeric Dehydrocurcudiols **6**, **7**, and **8**.

| Carbon | Compound | | | |
|---|---|---|---|---|
| | **4** | **6** | **7** | **8** |
| C-1 | 152.9 | 152.7 | 153.3 | 153.2 |
| C-2 | 130.4 | 129.6 | 129.7 | 129.8 |
| C-3 | 126.8 | 127.0 | 126.4 | 126.6 |
| C-4 | 121.7 | 121.6 | 121.6 | 121.6 |
| C-5 | 136.4 | 136.7 | 136.7 | 136.7 |
| C-6 | 116.3 | 116.1 | 116.7 | 116.4 |
| C-1′ | 31.4 | 32.4 | 30.8 | 31.7 |
| C-2′ | 37.6 | 39.8 | 35.1[a] | 33.4[a] |
| C-3′ | 22.1 | 125.6 | 31.8[a] | 31.8[a] |
| C-4′ | 43.3 | 139.6 | 77.0 | 76.4 |
| C-5′ | 71.3 | 70.6 | 147.7 | 147.2 |
| C-6′ | 29.6 | 29.7 | 111.0 | 110.8 |
| 5-Me | 20.9 | 20.9[a] | 21.1[b] | 20.9 |
| 1′-Me | 20.9 | 20.0[a] | 20.9[b] | 20.9 |
| 5′-Me | 28.9 | 29.7 | 17.6 | 18.0 |

[a,b]Assignments with identical superscripts may be interchanged.

terial it was not possible to determine the absolute stereochemistry about C-4′ in either epimer **7** or **8**.

It is worth noting that the new minor isomers **6–8** could be biosynthetically arrived at via oxidative elaboration of (+)-curcuphenol [**3**], and furthermore that a similarly modified series of amino bisabolenes **10–13** have been reported (5) from a *Theonella* sp. of marine sponge (Japan). The appearance of both (+) and (−) aromatic bisabolenes in marine organisms demonstrates their biosynthetic "stereo" versatility.

## EXPERIMENTAL

General experimental details have been reported elsewhere (6).

EXTRACTION AND ISOLATION.—A specimen of *Arenochalina* sp. (78.4 gm dry wt) was collected from John Brewer Reef, North Queensland, at a depth of 5–10 m in March 1986 and frozen for storage. The specimen was a massive lobate sponge with a distinct skinlike surface, shaggy reticulate choanosome, abundant fibers, large oxeas core fibers, and smaller oxeas dispersed between fibers, with abundant loose spongin. A type sample (registry number Z3720) has been lodged with the Northern Territory Museum of Arts and Sciences. The freeze-dried specimen was extracted with EtOH, and the crude extract was concentrated under reduced pressure to yield a brown gum (2.15 g). This material was partitioned into $CH_2Cl_2$ solubles and $CH_2Cl_2$ insolubles. Preliminary testing of the crude extract using guniea pig ileum showed nonselective inhibition of contractions evoked by various agonists, including acetylcholine, histamine, and serotonin. The crude extract also decreased basal tensions and contractions evoked by nerve stimulation and electrical depolarization. It also proved to be toxic to brine shrimp. Further biological screening confirmed that activity first recognized in the crude extract was only detectable in the $CH_2Cl_2$-soluble fraction. Rapid filtration (hexane to EtOAc) through a plug of silica followed by normal phase hplc (Phenomonex Spherex 5μ 250 × 10 mm, elution with 10% EtOAc/hexane) returned, in order of elution, five compounds of interest: **3** (211 mg, 0.27%), **7** (3.1 mg, 0.004%), **8** (2.9 mg, 0.004%), **6** (10.5 mg, 0.013%), and **4** (94 mg, 0.12%).

(+)-*Curcuphenol* [**3**].—The $[\alpha]_D$, ν max, λ max, $^1H$ and $^{13}C$ nmr, and eims were identical in all respects to those previously reported (3,4).

(+)-*Curcudiol* [**4**].—The $[\alpha]_D$, ν max, λ max, $^1H$ and $^{13}C$ nmr, and eims were identical in all respects to those previously reported (3,4).

*Compound* **6**.—A pale yellow oil: $[\alpha]_D$ +19.6 ($c$ = 1.00, $CHCl_3$); λ max (MeOH) 280 nm (ε 3740); ν max (film) 3403, 1617 $cm^{-1}$; $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2; eims *m/z* (%) $[M-H_2O]^+$ 216 (26), 201 (22), 173 (13), 159 (11), 136 (10), 135 (100), 115 (13), 91 (29), 83 (13), 77 (13); hreims $[M-H_2O]^+$ 216.1514 (calcd for $C_{15}H_{20}O$, 216.1514).

*Compound* **7**.—A pale yellow oil: $[\alpha]_D$ −2.6 ($c$ = 0.31, $CHCl_3$); λ max (MeOH) 280 nm (ε 2890); ν max (film) 3359, 1650 $cm^{-1}$; $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2; eims *m/z* (%) $[M]^+$ 234 (3), 216 (6), 201 (2), 161 (4), 159 (4), 148 (9), 136 (11), 135 (100), 121 (4), 117 (4), 115 (4), 107 (2), 105 (3), 91 (8); hreims $[M]^+$ 234.1613 (calcd for $C_{15}H_{22}O_2$, 234.1620).

*Compound* **8**.—A pale yellow oil: $[\alpha]_D$ +17.7 ($c$ = 0.23, $CHCl_3$); λ max (MeOH) 280 nm (ε 2840); ν max (film) 3433, 1650 $cm^{-1}$; $^1H$ nmr see Table 1; $^{13}C$ nmr see Table 2; eims *m/z* (%) $[M]^+$ 234 (4), 216 (3), 201 (1), 161 (4), 159 (2), 148 (8), 136 (11), 135 (100), 121 (5), 117 (4), 115 (6), 107 (3), 105 (5), 91 (13); hreims $[M]^+$ 234.1626 (calcd for $C_{15}H_{22}O_2$, 234.1620).

*Chemical correlation of* **6**.—To a sample of **6** (1.8 mg) in $Et_2O$ (3 ml) was added 3% Pd/C (10 mg), and the resulting mixture was stirred under an atmosphere of $H_2$ for 16 h. Filtration of the reaction product through a plug of celite returned a quantitative yield of a dihydro product identical in all respects to **4**.

*Chemical correlation of* **8**.—A sample of **8** (2.2 mg) was hydrogenated as described above for **6** to yield after hplc (20% EtOAc/hexane on Phenomonex Spherex 5μ 250 × 10 mm, normal phase) purification compound **9** (0.9 mg): $[\alpha]_D$ +15.9 ($c$ = 0.09, $CHCl_3$); λ max (MeOH) 280 nm (ε 2950); ν max (film) 3420 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 0.83, 0.84 (2d, $J$ = 6.6, $H_3$-6′ and 5′-Me), 1.06–1.33 (m, $H_2$-3′, $H_2$-4′), 1.21 (d, $J$ = 6.8, 1′-Me), 1.45–1.65 (m, $H_2$-2′, H-5′), 2.27 (s, 5-Me), 2.98 (dt, $J$ = 6.8, 6.8, H-1′), 6.57 (bs, H-6), 6.72 (bd, $J$ = 7.6, H-4), 7.03 (d, $J$ = 7.6, H-3); $^{13}C$ nmr ($CDCl_3$) 20.9, 21.0 (2q, 5-Me, 1′-Me), 22.6, 22.7 (2q, 5′-Me, C-6′), 25.4 (t, C-3′), 27.8 (d, C-5′), 32.0 (d, C-1′), 37.4 (t, C-2′), 39.1 (t, C-4′), 116.0 (d, C-6), 121.7 (d, C-4), 126.9 (d, C-3), 130.4 (s, C-2), 136.4 (s, C-5), 152.7 (s, C-1); eims *m/z* (%) $[M]^+$ 220 (9), 136 (10), 135 (100), 121 (9), 91 (6); hreims $[M]^+$ 220.1830 (calcd for $C_{15}H_{24}O$, 220.1827).

*Chemical correlation of* **7**.—A sample of **7** (3.2 mg), treated as described above for **8**, returned **9** (2.9 mg), identical with that described above.

*Hydrogenation of* **3**.—A sample of **3** (10 mg)

was hydrogenated under the conditions described above for **6** to return a quantitative yield of **9**.

## ACKNOWLEDGMENTS

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