Journal of Natural Products
Vol. 50, No. 5, pp. 976-978, Sep-Oct 1987

(+)-CURCUPHENOL AND (+)-CURCUDIOL, SESQUITERPENE PHENOLS FROM SHALLOW AND DEEP WATER COLLECTIONS OF THE MARINE SPONGE *DIDISCUS FLAVUS*

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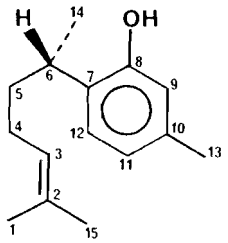
From our efforts to identify antifungal agents from marine organisms, we have isolated and identified (+)-curcuphenol [**1**] and (+)-curcudiol [**2**] two sesquiterpene phenols, from both deep and shallow water collections of the sponge *Didiscus flavus* van Soest (fam. Latrunculiidae). (−)-Curcuphenol has been reported previously as a metabolite of the gorgonian soft coral *Pseudopterogorgia rigida* (1) and the terrestrial plant *Lasianthaea podocephala* (2,3). The spectral and physical data observed for **1** differed from the reported values only in the sign of the optical rotation, indicating that the sponge metabolite is enantiomeric with the previously reported metabolite.[1] The structure of **2** was determined by spectral methods and confirmed by dehydration of **2** to form (+)-curcuphenol.

The first of the two shallow water collections of *D. flavus* was made adjacent to Cape Santa Maria, Long Island, Bahamas, at a depth of 3 m and is a new record of occurrence for this organism. The second collection was made by scuba at a depth of 15 m adjacent to Turneffe Island, Belize. The deep water specimen of *D. flavus* was collected at a depth of 139 m (a new depth record for this species) on Hogsty Reef, Bahamas, using Harbor Branch Oceanographic Institution's submersible, the Johnson Sea-Link II.

High resolution eims of **2** gave a molecular formula of $C_{15}H_{24}O_2$ (236.1777 observed, 236.1778 calculated). The ^{1}H- and ^{13}C-nmr spectra observed for the aromatic portions of **1** and **2** are very similar. The primary difference between the nmr spectra of **1** and **2** is the replacement of the resonances observed for the C2-C3 olefin of **1** by those of a quaternary carbon bearing an oxygen (71.53 ppm, C-2) and a methylene group (43.28 ppm, C-3; 1.44 and 1.31, H-3a and b) suggesting that **2** is the naturally occurring hydration product of **1**. This was confirmed by the facile dehydration of **2** on treatment with *p*-toluenesulfonic acid in refluxing toluene to form material spectroscopically and chromatographically identical to (+)-curcuphenol.



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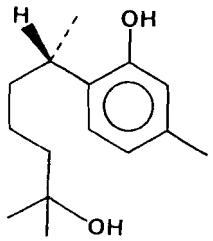

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[1]McEnroe and Fenical (1) report a specific rotation of −7° for (−)-curcuphenol whereas Ghisalberti *et al.* (3) report a specific rotation of −23.6° for optically pure, synthetic material. A specific rotation of +24.6±2° was observed for (+)-curcuphenol.

(+)-Curcuphenol is cytotoxic against in vitro tumor cell lines. An IC_{50} of 7 μg/ml is observed against P-388 murine leukemia and minimum inhibitory concentrations (MIC) of 10, 0.1, and 0.1 μg/ml are observed against the human tumor cell lines A-549 (lung), HCT-8 (colon), and MDAMB (mammary), respectively. (+)-Curcuphenol also inhibits growth of the fungus *Candida albicans* with an MIC of 8 μg/ml. (+)-Curcudiol has an MIC of 250 μg/ml against *C. albicans*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Spectral data were measured on the following instruments: ir, Perkin Elmer 1310; uv/visible Perkin-Elmer Lamda 3B; nmr, Bruker AM360 with the Aspect 3000 computer; ms curcuphenol [**1**] was measured by ei at 8.8 eV on a MS-30 mass spectrometer (Shrader Labs), curcudiol [**2**] was measured by ei at 70 eV on a VG-7070E mass spectrometer (University of Illinois, Urbana-Champaign). Nmr chemical shifts are reported as δ values in ppm relative to TMS (0 ppm). Optical rotations were measured on an Optical Activity Ltd. Polarimeter type AA-5.

ANIMAL COLLECTION AND EXTRACTION.—*D. flavus* was collected by scuba in June and November 1985, adjacent to Cape Santa Maria, Long Island, Bahamas and Turneffe Island, Belize, respectively. The deep water sample was collected in August 1985, at a depth of 139 m on Hogsty Reef, Bahamas, using the Harbor Branch Oceanographic Institution's submersible, the Johnson Sea-Link II. The sponge identifications were made by S.A. Pomponi as per the descriptions for *D. flavus* (4). EtOH-fixed vouchers of the sponges are stored in Ft. Pierce at the Harbor Branch Oceanographic Institution. Samples for extraction were frozen immediately after collection. Extracts were prepared by homogenizing the frozen sponge with EtOAc. The sponge residue was filtered off and the extract concentrated by distillation under reduced pressure to yield a crude brown oil (1% of frozen wt).

ISOLATION OF (+)-CURCUPHENOL [**1**].—The residue from the EtOAC extraction was chromatographed on Si gel using a step gradient of EtOAc/heptane. Semipure (+)-curcuphenol was eluted with 20% EtOAc/80% heptane. Hplc of this fraction on a Partisil-10 column (Whatman) with 2.5% iPrOH/ 97.5% heptane as the eluent, yielded pure (+)-curcuphenol (0.4% of frozen wt). (+)-Curcuphenol was identified by comparison of the uv, ir, ms, ^{1}H-nmr, and ^{13}C-nmr values with those observed for authentic (−)-curcuphenol isolated from *Pseudopterogorgia rigida*. ^{13}C nmr: ($CDCl_3$) δ 17.65 (q C1 or C15), 20.85 (q C13), 21.06 (q C14), 25.69 (q C1 or C15), 26.03 (t C5), 31.30 (d C6), 37.24 (t C4), 116.13 (d C9), 121.66 (d C11), 124.56 (d C3), 126.79 (d C12), 129.94 (s C7), 131.98 (s C2), 136.47 (s C10), 152.80 (s C8).

ISOLATION OF (+)-CURCUDIOL [**2**].—(+)-Curcudiol was eluted with 40% EtOAc/60% heptane in the vacuum flash separation of the residue from the EtOAc extract described above. Hplc of this fraction on a Partisil 10 column (Whatman) with 5% iPrOH/95% heptane as the eluent yielded pure curcudiol as a colorless oil: $[\alpha]^{22}D = +9.2°$ (c=10.8, $CHCl_3$); uv nm λ max (MeOH)=208, 276 (ξ=3433, 1438), base shift (incomplete conversion) λ max=240, 290 (ξ=10,476, 6349); ir ν max cm^{-1} (CCl_4) 3600, 2950, 1430, 1410, 1360, 1280, 930, 905; ^{1}H nmr ($CDCl_3$) δ 7.02 (d J=7.5 Hz, H12), 6.70 (d J=7.5 Hz, H11), 6.56 (s H-9), 5.28 (s exchangeable), 3.45 (bs exchangeable), 2.99 (m H6), 2.09 (3H s H13abc), 1.52 (m H5a), 1.44 (m H3a), 1.38 (m H5b), 1.31 (m H3b), 1.21 (2H m H4ab), 1.09 (3H d J=7.2 Hz, H14abc), 1.07 (3H s H1abc or H15abc), 1.04 (3H s H1abc or H15abc); ^{13}C nmr δ ($CDCl_3$) 153.10 (s C8), 136.25 (s C10), 130.61 (s C7), 126.72 (d C12), 121.39 (d C11), 116.28 (d C9), 71.53 (s C2), 43.28 (t C3), 37.69 (t C5), 31.10 (d C6), 29.48 (q C1 or C15), 28.68 (q C1 or C15), 22.03 (t C4), 20.99 (q C14), 20.84 (q C13); lrcims (CH_4) *m/z* 237, 219, 215, 163, 149, 135, 121, 109, 95.

CONVERSION OF **2** INTO **1**.—(+)-Curcudiol [**2**] (17 mg) was dissolved in 5 ml of toluene and placed into a 10-ml pear-shaped flask fitted with a reflux condenser. Dry *p*-toluenesulfonic acid (5 mg) was added to the flask and the solution refluxed for 30 min. The solution was added to 10 ml of Et_2O and extracted successively with saturated $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated by distillation under reduced pressure to yield after hplc, 9 mg of (+)-curcuphenol, spectroscopically identical with the natural compound.

ACKNOWLEDGMENTS

We thank Drs. J.E. Armstrong and K.L. Rinehart, Jr. for sponge collection. We also thank D. Forleo for assay against the tumor cell lines.

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Received 15 April 1987