3-Acetoxyspathulenol, a New Aromadendrane-Type Natural Product from the Soft Coral *Parerythropodium fulvum*

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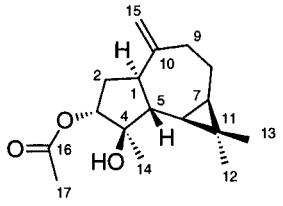
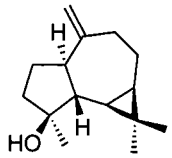
From the lipophilic extract of the soft coral *Parerythropodium fulvum* a new sesquiterpene with an aromadendrane-type carbon skeleton, 3-acetoxyspathulenol (**1**), was isolated, and the known compounds spathulenol (**2**) and tridensenone (**3**) were identified. The structure of the new compound was determined by interpretation of its spectroscopic data, including 1D and 2D ^{1}H and ^{13}C NMR (COSY, HMQC, HMBC) and MS.

Chemical investigations have revealed soft corals as a rich source of secondary metabolites, mainly sesqui- and diterpenes.[1,2] Some of these investigations focused on the ecological function of soft coral terpenoids and showed them to have important roles in interactions between marine organisms[3] or indicated the terpenoid content to be useful in the taxonomic identification of soft corals.[4] Other studies reported significant biological activities for several compounds.[2,5] Terpenes of octocorallian origin have been shown to have antiinflammatory, antibacterial, and neuromuscular activities.[2] Many of the compounds are isolated from soft corals of the family Alcyoniidae, mainly from the genera *Sinularia*, *Sarcophyton*, and *Lobophytum*, with very few investigations being carried out on *Parerythropodium* sp.[1,2]

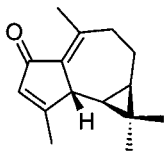
In the present study the secondary metabolite content of the soft coral *Parerythropodium fulvum* (Forskal, 1775) (family Alcyoniidae), collected from the reef fringing Phantom Island, Great Barrier Reef, Australia, was investigated. The CH_2Cl_2 extract of this animal was found to contain the new compound, **1**, and the known compounds spathulenol (**2**) and tridensenone (**3**). Fractionation and purification of the CH_2Cl_2 extract were guided by TLC and ^{1}H NMR and were carried out by both vacuum liquid chromatography (VLC) and HPLC.



1 3-Acetoxyspathulenol



2 Spathulenol



3 Tridensenone

The stereochemistries shown for compounds **1**-**3** are all relative.

The ^{13}C NMR and mass spectral data of **1** indicated it to have the molecular formula $C_{17}H_{26}O_3$ and thus have five elements of unsaturation. Analysis of its ^{13}C NMR spectrum showed the presence of one carbonyl group, as an acetate (171.0 ppm), and one 1,1-substituted C−C double bond (152.8, s; 106.2, t, ppm), as the only multiple bonds within the molecule; thus, the molecule is tricyclic. Further analysis of the ^{13}C NMR data revealed the presence of two further quaternary carbons (81.4 [bearing OAc or OH], 20.4 ppm), five methines (82.4 [bearing OAc or OH], 52.5, 48.3, 28.8, 27.5 ppm), three further methylenes (38.9, 31.4, 24.9 ppm), and four methyl groups (28.7, 21.2, 19.9, 16.2 ppm). Analysis of the ^{1}H NMR spectrum showed all the resonances for the methyl groups to be present as singlets (2.08, 1.22, 1.07, 1.03 ppm), indicating them to be bonded to quaternary carbons. Also evident from the ^{1}H NMR data was the presence of a cyclopropyl moiety (0.74 [ddd, 1H, J = 5.9, 9.4, 11.2 Hz], 0.58 [dd, 1H, J = 11.1, 9.4 Hz] ppm).

After all protons had been assigned to their directly bonded carbon atoms, aided by an HMQC spectrum, it was possible to deduce one molecular fragment. Thus, from the ^{1}H−^{1}H COSY spectrum, couplings in the form of cross-peaks were observed between H-3 and H_2-2, H_2-2, and H-1, and H-1 and H-5. Furthermore, H-5 coupled with H-6, and H-6 with H-7; H-7 also coupled with H_2-8, which further coupled with H_2-9, thus completing the molecular fragment H-3 to H_2-9, via H-5. This information in conjunction with the HMBC spectral data finally led to the planar structure of **1** being deduced. Thus, H_3-14 showed heteronuclear multiple bond couplings (HMBCs) with C-3, C-4, and C-5, indicating it to bond directly to C-4, which bonds to both C-3 and C-5, and in so doing completed the five-membered ring. Further, the ^{1}H NMR resonances of methyl groups CH_3-13 and CH_3-12 showed HMBC cross-peaks to the resonances of each other's C-atoms as well as to those of C-6, C-7, and C-11, thus completing the cyclopropyl ring. HMBC correlations observed from the resonances associated with H_2-15 to those of C-1 and C-9 positioned the exomethylene group between these two carbon atoms and in so doing gave rise to the third and final ring. Finally, the cross-peak between the resonance of H-3 and the resonance of C-16 clearly showed the acetate function to reside at C-3 and the OH group at C-4 (81.4 ppm).

To determine the relative stereostructure of **1**, NOE difference experiments were performed. Thus, irradiation at the resonance frequency of H-3 resulted in an enhancement of the resonances associated with H-5 and H_2-2, indicating H-3 and H-5 to be on the same side of the molecule. The magnitude of the coupling constants of H-5

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(δ 1.40 [dd, 1H, J = 9.8, 11.1 Hz]) indicated it to have two diaxial interactions and showed H-1 and H-6 to be on the same side of the molecule and on the opposite side from H-5. Irradiation at the resonance frequency of H-1 (δ 2.50 [ddd, 1H, J = 8.0, 9.6, 9.8 Hz]) resulted in NOEs being detected between it and H-6 and H_3-12 and showed them to be on the same side of the molecule and on the opposite side of H-3 and H-5. Further NOEs observed between H-6 and H-7, H-7 and H_3-12, H-6 and H_3-12, and H-5 and H_3-13 enabled all of the remaining stereochemistries to be assigned and the relative configuration of **1** to be deduced as shown in **1**.

Together with **1** (3-acetoxyspathulenol) the known compounds spathulenol (**2**) and tridensenone (**3**) were tentatively identified by GC−MS analysis of a fraction resulting from the isolation of **1**. The physical (GC−MS) and spectral (^{1}H NMR) data of **2** and **3** were in good agreement with those reported in the literature.[6−8]

Compound **1** is a new sesquiterpene with an aromadendrane-type carbon skeleton similar to the one reported from another investigation of *Parerythropodium* sp.[9] In that study it was found that most of the sesquiterpenoid compounds were lost during freeze-drying or through simple evaporation, a phenomenon that has been reported earlier[4,10] and which is directly related to the fact that soft corals have a high content of volatile compounds, mainly sesquiterpenes. Even though the currently investigated sample was freeze-dried, the three sesquiterpene compounds **1**−**3** were successfully isolated with apparent minimal, if any, losses as a result of evaporation probably due to the polar nature of their functionalities. Clearly, however, it is also possible that a great many other less polar metabolites were not found as a result of the sample preparation methodologies used. Compound **2**, first isolated from *Eucalyptus spathulata*,[11] has been described from a variety of other terrestrial plants too.[12] Compound **3** was also first isolated from a terrestrial source, the Japanese liverwort *Bazzania tridens*.[8] This report is the first of spathulenol (**2**) and tridensenone (**3**) or stereoisomers thereof as being constituents of marine organisms.

Compounds **1**−**3** (**2** and **3** as part of a mixture) were tested for their antimicrobial activity against two bacteria (*Escherichia coli, Bacillus megaterium*), four fungi (*Eurotium repens, Fusarium oxysporum, Microbotryum violacea, Mycotypha microspora*), and the green alga *Chlorella fusca*. Their abilities to inhibit the enzymes reverse transcriptase of the human immunodeficiency virus type 1 (HIV-1-RT) and p56^{lck} tyrosine kinase (TK) were also assessed. Finally, their effects toward a nematode (*Caenorhabditis elegans*) and brine shrimp (*Artemia salina*) were also investigated. In all of the applied test systems no positive activities were observed.

Since the current work was completed a paper by Liu et al.,[13] which contained the structure of the new natural product *ent*-3β-hydroxyspathulenol, was published. The NMR data of this compound further support the relative steroechemistry proposed for **1**.

Experimental Section

General Experimental Procedures. The general experimental procedures were carried out as previously described.[14]

Animal Material. *Parerythropodium fulvum* (Alcyoniidae) was collected by hand using scuba at depths between 2 and 8 m from the reef fringing Phantom Island, North Queensland, Australia, in September of 1990. Collected material was stored at −20 °C until used. A voucher specimen, number (NTM C13070), is stored at the Museum and Art Gallery of the Northern Territory, Darwin, Australia.

Table 1. ^{1}H NMR[a] and ^{13}C NMR[b] Data of Compound **1**[c]

position	δ ^{1}H	δ ^{13}C
1	2.50 (ddd, 1H, *J* 8.0, 9.6, 9.8)	48.3 d[d]
2	2.27 (ddd, 1H, *J* 5.9, 9.6, 13.5)	31.4 t
	1.72 (ddd, 1H, *J* 4.7, 8.0, 13.5)	
3	4.95 (dd, 1H, *J* 4.7, 5.9)	82.4 d
4		81.4 s
5	1.40 (dd, 1H, *J* 9.8, 11.1)	52.5 d
6	0.58 (dd, 1H, *J* 9.4, 11.1)	28.8 d
7	0.74 (ddd, 1H, *J* 5.9, 9.4, 11.2)	27.5 d
8	2.00 (m, 1H)	24.9 t
	1.01 (m, 1H)	
9	2.44 (ddd, 1H, *J* 1.4, 6.4, 13.4)	38.9 t
	2.05 (m, 1H)	
10		152.8 s
11		20.3 s
12	1.07 (s, 3H)	28.7 q
13	1.03 (s, 3H)	16.2 q
14	1.22 (s, 3H)	19.9 q
15	4.68 (br s, 2H)	106.2 t
16		171.0 s
17	2.08 (s, 3H)	21.2 q

[a] 300 MHz, δ in ppm relative to $CHCl_3$ in $CDCl_3$ = 7.26; J in Hz. [b] 75.5 MHz, δ in ppm relative to $CDCl_3$ = 77.0. [c] All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY). [d] Implied multiplicities determined by DEPT (s = C, d = CH, t = CH_2, q = CH_3).

Animal tissue was freeze-dried (dry wt 91.2 g) and exhaustively extracted with CH_2Cl_2 (1.2 L) and then with MeOH (1.2 L). Solvents were removed in vacuo and the extracts filtered through a pad (5 mm thick) of Si gel. The MeOH extract was extracted with CH_2Cl_2, and the two lipophilic fractions were combined to yield 8.9 g (9.8%) of CH_2Cl_2-soluble and 7.9 g (8.7%) of MeOH-soluble material. The CH_2Cl_2-soluble fraction was applied to a vacuum liquid column (VLC, Si gel) and gradient eluted from light petroleum ether to EtOAc and thereafter with MeOH to yield 12 fractions, each of 100 mL. Repeated column chromatography, including HPLC (Si gel, petroleum ether/EtOAc) of fraction 3, and combined fractions 5 and 6 afforded compounds **1**−**3**.

3-Acetoxyspathulenol (1): oil (14.6 mg, 0.016%); $[\alpha]_D$ −0.9° (*c* 0.29, $CHCl_3$); IR ν_{max} 3470−3420, 2980, 2925, 2860, 1740, 1720, 1640, 1455 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75.5 MHz), see Table 1; EIMS *m*/*z* 278 (10) [M$^+$], 263 (5), 236 (4), 218 (24), 200 (53), 185 (26), 175 (32), 157 (27), 147 (22), 133 (20), 119 (18), 105 (24); HREIMS *m*/*z* 278.1875 (calcd for $C_{17}H_{26}O_3$, 278.1875).

Spathulenol (2): oil (≈1.3 mg, 0.0014%); with GC−MS and ^{1}H NMR in agreement with those published.[6,7]

Tridensenone (3): oil (≈2.5 mg, 0.0027%); with GC−MS and ^{1}H NMR in agreement with those published.[8]

Biological Tests. The antimicrobial,[15] RT inhibition,[16] and TK inhibition[17] assays were carried out as previously described, and the nematicidal and brine shrimp assays following the methods of Peters et al.[18]

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References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155−198, and references therein.

(2) Krebs, H. Chr. *Prog. Chem. Org. Nat. Prod.* **1986**, *49*, 151−363.
(3) Coll, J. C.; Bowden, B. F.; Heaton, A.; Scheuer, P. J.; Li, M. K. W.; Clardy, J.; Schulte, G. K.; Finer-Moore, J. *J. Chem. Ecol.* **1989**, *15*, 1177−1191.
(4) Kashman, Y.; Loya, Y.; Bodner, M.; Groweiss, A.; Benayahu, Y.; Naveh, N. *Mar. Biol.* **1980**, *55*, 255−259.
(5) Rodriguez, A. D.; Piña, I. C.; Barnes, C. L. *J. Org. Chem.* **1995**, *60*, 8096−8100.
(6) Inagaki, F.; Abe, A. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1773−1778.
(7) Maurer, B.; Hauser, A. *Helv. Chim. Acta* **1983**, *66*, 2223−2235.
(8) Toyota, M.; Asakawa, Y.; Takemoto, T. *Phytochemistry* **1981**, *20*, 2359−2366.
(9) Green, D.; Kashman, Y.; Benayahu, Y. *J. Nat. Prod.* **1992**, *55*, 1186−1196.
(10) Coll, J. C.; Bowden, B. F.; Clayton, M. N. *Chem. Br.* **1990**, 761−763.
(11) Bowyer, J. C.; Jeffries, P. R. *Chem. Ind. (London)* **1963**, 1245−1246.
(12) According to the Beilstein database (1999), there are 79 species in 57 genera described as sources of spathulenol (**2**). The genera belong mainly to the mosses, Asteraceae, and Lamiaceae.
(13) Liu, H.-J.; Wu, C.-L.; Becker, H.; Zapp, J. *Phytochemistry* **2000**, *53*, 845−849.
(14) Wright, A. D.; König, G. M.; Angerhofer, C. K.; Greenidge, P.; Linden, A.; Desqueyroux-Faundez, R. J. *J. Nat. Prod.* **1996**, *59*, 710−716.
(15) Schulz, B.; Sucker, J.; Aust, H. J.; Krohn, K.; Ludewig, K.; Jones, P. G.; Döring, D. *Mycol. Res.* **1995**, *99*, 1007−1015.
(16) Eberle, J.; Seibl, R. *J. Virol. Methods* **1992**, *40*, 347−356.
(17) Wessels, M.; König, G. M.; Wright, A. D. *J. Nat. Prod.* **1999**, *62*, 927−930.
(18) Peters, L.; König, G. M.; Wright, A. D. Unpublished data.

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