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Bioactive Polyacetylenes from Stony Corals

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Four new polyacetylenes have been isolated from three species of hermatypic corals, Montipora sp, M. mollis, and Pectinia lactuca. These compounds, representing the first such metabolites of coelenterates, exhibited ichthyotoxicity and inhibited the growth of some bacteria and fungi.

In contrast to soft corals which have been extensively studied since 1960s, hard corals have rarely been objects of investigation by organic chemists.[1] This is partly based on the supposition that a hard coral would hardly produce organic extract for it is consisted mostly of calcarious skeleton. However, in the course of our study for bioactive marine metabolites we obtained an antiviral compound, tubastrine (**1**) in a high yield from the hard coral Tubastraea aurea.[2] Encouraged by this finding we started to look into other hard corals. In this communication we report the isolation of polyacetylene alcohols (**2**, **3**) and related esters (**4**, **5**) having ichthyotoxicity and antimicrobial activity from three species of hermatypic (reef-building) corals.

A sample (13.2 kg) of Montipora sp. was extracted by steeping in methanol. The extract was concentrated, and the resulting residue was taken up in ethyl acetate to give an oil (55 g) which was fractionated by flash chromatography on silica gel with hexane-acetone. Since the UV absorbing constituents appeared to be responsible for the antimicrobial activity of the crude oil, fractions exhibiting UV absorbing spots on TLC were isolated by successive chromatography on a Lowbar column (Si-60, hexane-ethyl acetate) and HPLC (RP-8, Methanol-water 10:1) to furnish compounds **2**, **3**, **4**, and **5** in the yields of 29.7, 30.4, 10.3, and 28.7 mg, respectively. Compounds **2** and **5** were also isolated from M. mollis, while compounds **2** and **3** were found in Pectinia lactuca. All the compounds showed ichthyotoxicity against guppies at concentration levels of 1-5 ppm. Compounds **2** and **3** were active at 10 μg/disc against Bacillus subtilis, Staphylococcus aureus, Aspergillus sp., and Cladosporium sp., while compounds **4** and **5** were inactive against these microbes at 10-100 μg/disc.

The molecular formula of compound **2** was deduced as $C_{14}H_{20}O$ from HR EIMS (M^+, m/z 204.1523, Δ 2.0 mmu). The IR spectrum (CCl_4)[3] reveals the presence of a disubstituted acetylene (2260 cm^{-1}), a hydroxyl (3620, 3420, 1020 cm^{-1}), and a monosubstituted olefin (3070, 1640, 990, 910 cm^{-1}). The ^{13}C NMR spectrum[3] shows

the presence of only one olefinic bond (δ 139.0 d, 114.0 t) and two acetylenic bonds (δ 81.5 s, 73.5 s, 70.6 s, 64.4 s) that fulfil the unsaturation requirement of the molecule. Seven other signals, one (δ 28.8) being overlapped, were due to eight methylene carbons. Thus, compound **2** must be a linear alcohol. A two-proton singlet at δ 4.29 in the ^{1}H NMR spectrum suggests that a methylene with a hydroxyl group is bound to an acetylene. The spectrum exhibits only two other allylic methylene signals, one [δ 2.02 (2 H, q, J = 7 Hz)] due to the methylene next to the olefin and the other [δ 2.26 (2 H, t, J = 7 Hz)] next to an acetylene, indicating that the two acetylenes are conjugated each other. The UV spectrum[3] confirms the conjugation as it shows virtually identical absorption maxima with the same 2,4-diyn-1-ol system found in renierin-2.[4] Thus, the structure of compound **2** is 13-tetradecene-2,4-diyn-1-ol.

Compound **3** has the molecular formula $C_{16}H_{22}O$ as determined by HR EIMS (M^+ at m/z 230.1649, Δ -2.0). Spectral data[5] reveal the presence of the same 2,4-diyn-1-ol unit [3630, 2260 cm^{-1}; δ 4.32 (2 H, s); δ 81.9 s, 73.7 s, 71.1 s, 64.6 s, 51.6 t]. The other end of the molecule is consisted of a diene as demonstrated by five interacting olefinic signals [δ 6.64 (1 H, ddd, J = 17.1, 10.1, 11.0 Hz, H-15), 6.00 (1 H, dd, J = 11.0, 10.7 Hz, H-14), 5.46 (1 H, dt, J = 10.7, 7.6 Hz, H-13), 5.19 (1 H, d, J = 17.1 Hz, H-16), 5.09 (1 H, d, J = 10.1 Hz, H-16)]. The coupling constant (10.7 Hz) between H-13 and H-14 indicates that the geometry of the double bond is cis. Therefore, the structutre of compound **3** is (Z)-13,15-hexadecadiene-2,4-diyn-1-ol.

The molecular formula of compound **4** was deduced as $C_{15}H_{20}O_3$ from HR EIMS (M^+ at m/z 248.1405, Δ -0.5). The IR spectrum[6] shows no hydroxyl but an ester (1760, 1210 cm^{-1}), acetylenic (2260 cm^{-1}), and monosubstituted olefinic group (1640, 990, 910 cm^{-1}). The alcoholic portion of the ester contains the same 2,4-diyn-1-ol functionality. The end of the alcohol chain is a vinyl group (δ 138.8 d, 114.5 t). The acid portion of the ester is shown to be methoxyacetate by NMR data[6] [δ 4.22 or 4.37 (2 H, s), 3.78 (3 H, s); δ 170.3 s, 66.3 t or 59.0 t, 52.0 q]. Thus, the structure of **4** is deduced as 11-dodecene-2,4-diynyl methoxyacetate.

Compound **5**,[7] $C_{16}H_{24}O_3$ (M^+, m/z 264.1723, Δ 0 mmu), is also a methoxyacetate of a 2,4-diyn-1-ol. The ending group of the alcohol chain is a methyl group [δ 0.80 (3 H, t, J = 7 Hz); δ 13.9 q]. The structure of **5** can therefore be described as 2,4-tridecadiynyl methoxyacetate.

A number of polyacetylenes have so far been reported from marine sponges and nudibranchs that prey on sponges.[8] However, this is the first report on the isolation of polyacetylenes from coelenterates and presumably from any other marine organisms than the former two. Isolation of the same compounds from different coral genera suggests that they are metabolites of a common symbiotic organism such as a zooxanthella. Ichthyotoxicity and antimicrobial activity imply that these compounds play defensive role in the corals.

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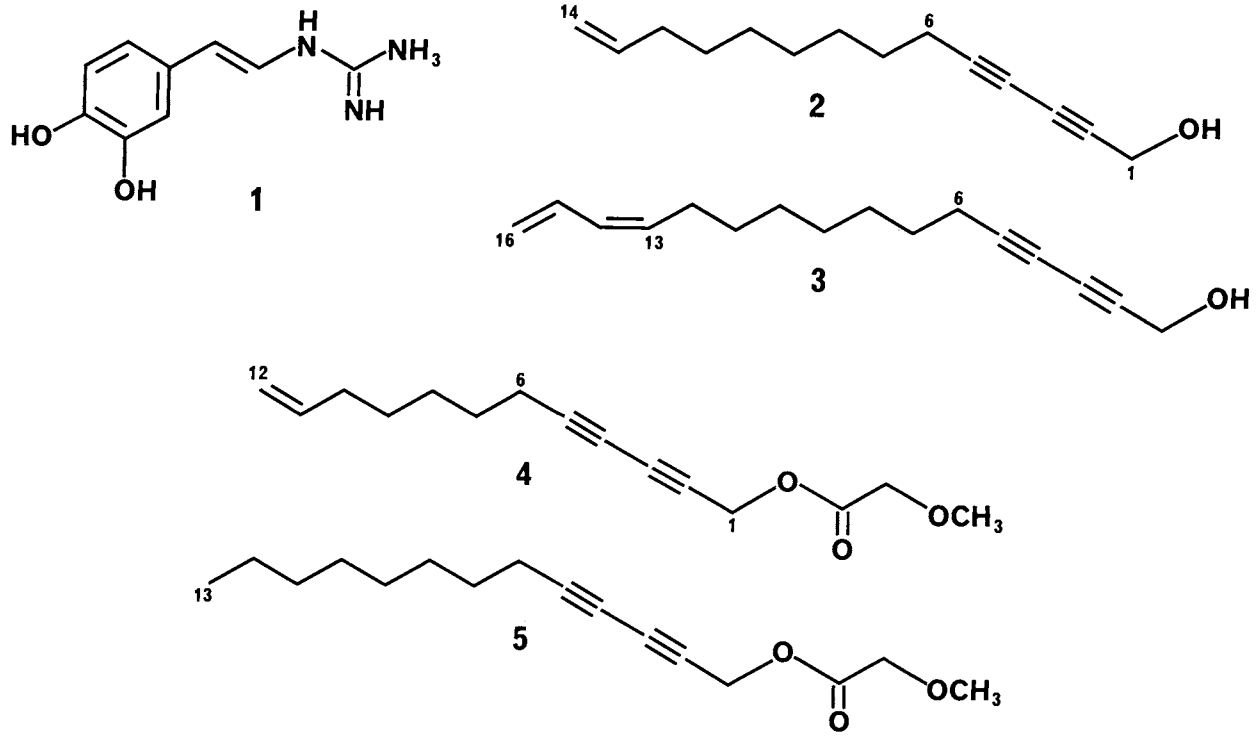

References

1) There are a few reports on the secondary metabolites of hard corals. See M. Alam, R. Sanduja, and G. M. Wellington, Heterocycles, 27, 719 (1988); R.Sanduja, M. Alam, G. M. Wellington, J. Chem. Res.(S), 1986, 450; R. Sanduja, G. E. Martin, A. J. Weinheimer, M. Alam, M. B. Hossain, and D. van der Helm, J. Heterocycl. Chem., 21, 845 (1984); N. Fusetani, M. Asano, S. Matsunaga, and K. Hashimoto, Comp. Biochem. Physiol., 85B, 845 (1986).

2) R. Sakai and T. Higa, Chem. Lett., 1987, 127.

3) **2**: UV (MeOH) λ_{max} 220 (ε 340), 230 (445), 242 (430), 256 nm (250); IR (CCl_4) 3620, 3420, 3070, 2930, 2850, 2260, 1640, 1380, 1020, 990 (sh), and 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.79 (1 H, ddt, J = 17, 10, 7 Hz), 4.98 (1 H, d, J = 17 Hz), 4.92 (1 H, d, J = 10 Hz), 4.29 (2 H, s), 2.26 (2 H, t, J = 7 Hz), 2.03 (2 H, q, J = 7 Hz), 1.51 (2 H, quintet), 1.37 (4 H, m), and 1.28 (4 H, m); ^{13}C NMR ($CDCl_3$) δ 139.0 d, 114.0 t, 81.5 s, 73.5 s, 70.6 s, 64.4 s, 51.2 t, 33.8 t, 28.9 t, 28.8 (2 C, t), 28.7 t, 28.1 t, and 19.2 t; EIMS m/z 204 (M^+, 0.4), 203 (1.2), 189 (14), 161 (7.7), 147 (11.7), 145 (10.7), 143 (12), 133 (19), 131 (24.4), 129 (18), 121 (15.3), 119 (22.5), 117 (32.5), 107 (32), 105 (45.3), 95 (24.6), 94 (22), 93 (45.8), 92 (22.6), 91 (99.7), 81 (47.2), 80 (22), 79 (100), 78 (26.8), 77 (68.9), 67 (91.8), 66 (34.1), 65 (53.3), 55 (90.3), 53 (38.4), and 51 (28.2 rel %).

4) G. Cimino and S. De Stefano, Tetrahedron Lett., 1977, 1325.

5) **3**: IR (CCl_4) 3630, 3080, 3020, 2940, 2860, 2260, 1640, 1380, 1020, 990 (sh), and 900 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.64 (1 H, ddd, J = 17.1, 10.1, 11.0 Hz), 6.00

(1 H, dd, J = 11.0, 10.7 Hz), 5.46 (1 H, dt, J = 10.7, 7.6 Hz), 5.19 (1 H, d, J = 17.1 Hz), 5.09 (1 H, d, J = 10.1 Hz), 4.32 (2 H, s), 2.28 (2 H, t, J = 7Hz), 2.19 (2 H, dt, J = 7.6, 6.7 Hz), 1.54 (2 H, m), 1.39 (4 H, m), and 1.31 (4 H, m); ^{13}C NMR ($CDCl_3$) δ 133.0 d, 132.5 d, 129.4 d, 116.8 t, 81.9 s, 73.7 s, 71.1 s, 64.6 s, 51.6 t, 29.6 t, 29.1 t, 29.0 t, 28.8 t, 28.2 t, 27.8 t, and 19.4 t; EIMS m/z 230 (M^+, 0.4), 229 (1.1), 201 (2.1), 199 (9.2), 187 (2.7), 183 (2.3), 173 (5.2), 171 (4.4), 169 (7), 159 (11), 145 (20.2), 143 (17.6), 131 (28.4), 129 (36.2), 117 (53.7), 105 (37), 91 (70,9), 81 (34.7), 79 (67.7), 77 (49.1), 67 (100), 65 (43.5), and 55 (54.2 rel %).

6) **4**: IR (CCl_4) 2960, 2870, 2260, 1760, 1640, 1435, 1420, 1340, 1210, 1110, 990, and 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.80 (1 H, ddt, J = 17.1, 10.1, 6.7 Hz), 5.00 (1 H, dd, J = 17.0, 0.9 Hz), 4.95 (1 H, dd, J = 10.1, 0.9 Hz), 4.37 (2 H, s), 4.22 (2 H, s), 3.78 (3 H, s), 2.29 (2 H, t, J = 7.0 Hz), 2.06 (2 H, q, J = 6.7 Hz), 1.55 (2 H, m), and 1.40 (4 H, m); ^{13}C NMR ($CDCl_3$) δ 170.3 s, 138.8 d, 114.5 t, 81.7 s, 72.7 s, 70.5 s, 66.3 t, 64.5 s, 59.0 t, 52.0 q, 33.7 t, 28.5 (2 C, t), 28.1 t, and 19.3 t; EIMS m/z 248 (M^+, 0.8), 247 (1.1), 220 (5.5), 206 (4.5), 189 (6.2), 175 (23.7), 143 (22.8), 131 (36.4), 129 (54.4), 117 (62.4), 115 (48), 105 (50.6), 91 (94.7), 79 (59.7), 77 (63.7), 74 (100), 67 (50), and 55 (51.6 rel %).

7) **5**: UV (MeOH) λ_{max} 220 (ε 545), 231 (647), 243 (625), 256 (418), and 278 nm (417); IR (CCl_4) 2930, 2850, 2260, 1755, 1435, 1425, 1380, 1345, 1205, and 1120 cm^{-1}; ^1H NMR (CCl_4) δ 4.33 (2 H, s), 4.12 (2 H, s), 3.78 (2 H, s), 2.23 (2 H, m), 1.33 (12 H, m), and 0.80 (3 H, t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 170.0 s, 81.7 s, 72.4 s, 70.3 s, 66.0 t, 64.3 s, 58.8 t, 51.6 q, 31.6 t, 28.7 t, 28.6 t, 28.1 t, 22.5 t, 19.1 t, and 13.9 q; EIMS m/z 264 (M^+, 0.3), 249 (1.4), 221 (8.8), 177 (14), 166 (19.3), 135 (16.5), 133 (19.8), 131 (38.2), 119 (33.4), 118 (28), 117 (43.5), 105 (44.9), 91 (80), 79 (49.5), 77 (56.3), 76 (50.5), 74 (100), 67 (39.7), and 55 (45.3 rel %).

8) D. J. Faulkner, Nat. Prod. Rep., 1, 551 (1984); ibid., 3, 1 (1986); ibid., 4, 539 (1987); ibid., 5, 613 (1988); E. Quinoa and P. Crews, Tetrahedron Lett., 29, 2037 (1988); G. Cimino, A. De Giulio, S. De Rosa, and V. Di Marzo, ibid., 30, 3563 (1989).

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