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6H-anthra[1,9-bc]thiophene derivatives from a bryozoan, *Dakaira subovoidea*

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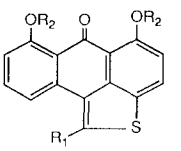
Abstract. Separation of a lipophilic extract of the bryozoan, *Dakaira subovoidea*, a common fouling organism of underwater structures, guided by inhibition of lipid peroxide formation in rat liver microsomes, gave 5,7-dihydroxy-1-hydroxymethyl-6-oxo-6H-anthra[1,9-bc]thiophene (**1a**) and its 1-methoxycarbonyl derivative (**1c**), together with the known 1,8-dihydroxyanthraquinone (**2**). The structures were determined by spectral and crystallographic analyses.

Key words. 6H-anthra[1,9-bc]thiophene derivatives; antioxidant; bryozoan; *Dakaira subovoidea*; sessile and fouling organism.

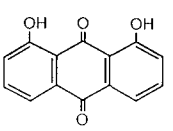
Some organisms living in shallow water, including sponges, hydrozoans, polychetes, bryozoans, bivalves, barnacles, tunicates, and algae, are called fouling or sessile organisms. They attach indiscriminantly to artificial underwater structures as well as natural rocks. Their attachment to bridges, banks, aquaculture facilities and ships reduces the economic efficiency of these structures. Many fish and shell aquaculture installations are situated in the calm bays and inland seas of central and southern Japan, and in these facilities the fouling organisms grow on aquaculture nets, ropes, or cages, and on the shells, thickly enough to make the water nearby hypoxic owing to the insufficient influx of water. Since this results in a decrease of the growth rate and increase of mortality of the animals, successful aquaculture in Japan requires the regular removal of the fouling organisms, which is a laborious process. Antifouling reagents and devices for the prevention of attachment or removal of these organisms have been investigated. However, it is also worth investigating the possibility that fouling organisms produce useful metabolites. If this is the case, their rapid and prolific growth could actually be beneficial.

In order to isolate pharmacologically active compounds from marine fouling organisms, we have carried out a systematic pharmacological screening of extracts from them. The test for inhibitory effects on lipid peroxide formation in rat liver microsomes showed that a tunicate, *Amaroucium multiplicatum* and a bryozoan, *Dakaira subovoidea*, produced active compounds. As previously reported, *A. multiplicatum* produced chromenes and hydroquinones[1]. Now we report the isolation, characterization, and activity of the active principles from the deep red colonial bryozoan, *D. subovoidea*, which is ubiquitous on rocks and shells along the open coast, and on wharf pilings and aquaculture facilities in bays in Japan.

D. subovoidea (w.wt 12 kg) was collected from the pearl aquaculture facilities in Komame Bay, Kochi, in 1986, and was extracted with methanol at room temperature. The SiO_2 chromatography of the lipophilic extract gave **1a** (64 mg)[2] and **1c** (3 mg)[4], together with a known compound, 1,8-dihydroxyanthaquinone (**2**) (128 mg) as a major metabolite.



1a:R_1=CH_2OH, R_2=H
1b:R_1=CH_2OAc, R_2=Ac
1c:R_1=CO_2CH_3, R_2=H



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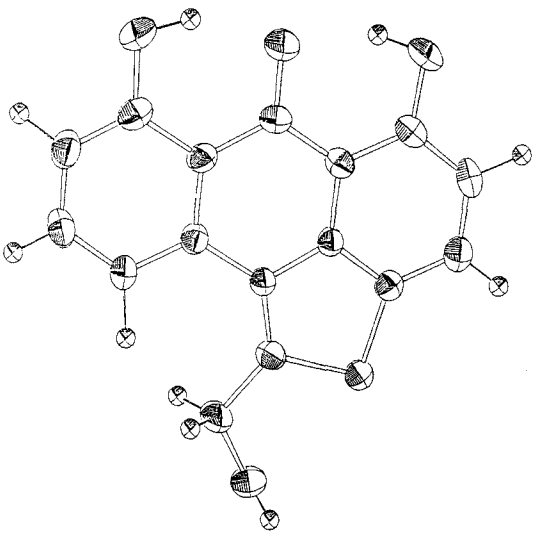
The compound (**1a**), m.p. > 260 °C was analyzed for $C_{16}H_{10}O_4S$ (m/z 298.0285) by high resolution EI mass spectrometry. The ^1H NMR (d_6-DMSO) indicated the presence of an ABC-[δ 7.02 (1H, d, J = 8.3 Hz), 7.30 (1H, d, J = 8.3 Hz), 7.78 (1H, t, J = 8.3 Hz)] and AB-type [δ 7.15 (1H, d, J = 8.8 Hz), 8.35 (1H, d, J = 8.8 Hz)] aromatic protons, a hydroxymethyl [δ 5.23 (2H, d, J = 5.4 Hz), 6.54 (1H, t, J = 5.4 Hz, exchangeable with D_2O)], and two phenolic protons [δ 11.81 (1H, s), 13.19 (1H, s)]. The formation of a triacetate (**1b**) confirmed the presence of three hydroxyls[3]. UV [λ_{max} (ε, EtOH): 434 nm (9800)] indicated the presence of a chromophore closely related to that of (**2**).

Since these spectral features could not complete the structural characterization of **1a**, X-ray crystallographic analysis was attempted. The structure was solved by the direct method (MULTAN 78) refined by block-diagonal least-squares to R = 0.058[5]. The computer-generated perspective drawing shown in the figure revealed that **1a** could be 5,7-dihydroxy-1-hydroxymethyl-6-oxo-6H-anthra[1,9-bc]thiophene.



Computer-generated perspective drawing of **1a**.

The minor compound (**1c**), m.p. 230–235 °C was analyzed for $C_{17}H_{10}O_5S$ (M^+m/z 326.0238). The UV [λ_{max} (ε, EtOH): 459 nm (8400)] was compatible with a chromophore closely related to that of **1a**. In addition, the ^{1}H NMR [δ 4.05 (3H, s)] and IR (ν_{max}: 1720 cm^{-1}) indicated the presence of a methoxycarbonyl. Therefore, **1c** could be depicted as 5,7-dihydroxy-1-methoxycarbonyl-6-oxo-6H-anthra[1,9-bc]thiophene.

The 6H-anthra[1,9-bc]thiophene ring system has never been found in nature yet, though it has been prepared as a dye[6].

The antioxidant activity of **1a** was in inverse proportion to its purity in the separation process, and it finally became inactive even at 100 μg/ml. This was presumably the result of its insolubility in the test medium, because the more soluble **1b** showed 99.5% inhibition at 10 μg/ml. Since the test medium also had an esterase activity, **1b** may have been enzymatically hydrolyzed to **1a**, to show potent activity. **2** showed 100% inhibition at <1 μm/ml. **1c** was not tested.

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1 Sato, A., Shindo, T., Kasanuki, N., and Hasegawa, K., J. nat. Prod. *52* (1989) 975.

2 **1a**: ^{13}C NMR [67.7 MHz, δ (d_5 – Py)] 61.1 (t), 111.2 (s), 115.0 (s), 115.4 (s), 115.8 (d), 117.6 (d), 120.7 (s), 126.6 (s), 131.9 (d), 136.5 (d), 138.8 (s), 160.2 (s), 164.5 (s), 191.2 (s). UV [λ_{max} nm (ε, EtOH)] 220 (24700), 231 (23100), 244 (sh) (13800), 285 (8400), 327 (4500), 366 (4500), 392 (sh) (7200), 420 (9000), 434 (9800). IR [ν_{max} cm^{-1} (nujol)] 3480, 1630, 1605, 1220, 1050, 830, 780. EIMS [m/z (%)] 298 (100), 281 (31), 265 (55).

3 **1b**: ^{1}H NMR [270 MHz, δ ($CDCl_3$)] 2.24 (3H, s), 2.48 (3H, s), 2.49 (3H, s), 5.79 (2H, s), 7.19 (1H, dd, J = 1.9, 7.8 Hz), 7.27 (1H, d, J = 8.3 Hz), 7.73 (1H, t, J = 7.8 Hz), 7.79 (1H, dd, J = 1.9, 7.8 Hz), 8.07 (1H, d, J = 8.3 Hz). ^{13}C NMR [67.7 MHz, δ ($CDCl_3$)] 20.7 (q), 21.25 (q), 21.31 (q), 61.2 (t), 119.8 (s), 121.9 (d), 123.8 (2 × d), 127.9 (d), 133.8 (s), 134.6 (2 × d), 139.1 (s), 143.2 (2 × s), 149.2 (2 × s), 152.3 (s), 169.6 (s), 170.1 (s), 180.7 (s). UV [λ_{max} nm (ε, EtOH)] 213 (42800), 232 (sh) (25700), 240 (sh) (21700), 262 (sh) (13800), 282 (15300), 302 (sh) (9200), 352 (sh) (6700), 390 (12200). IR [ν_{max} cm^{-1} ($CHCl_3$)]1752, 1640, 1600, 1580, 1360, 1200. EIMS [m/z (%)] 427 (27), 382 (60), 340 (100), 298 (50), 281 (60), 269 (30).

4 **1c**: ^{1}H NMR [270 MHz, δ ($CDCl_3$)] 4.05 (3H, s), 7.12 (1H, dd, J = 1.0, 8.3 Hz), 7.28 (1H, d, J = 8.8 Hz), 7.68 (1H, t, J = 8.3 Hz), 8.02 (1H, d, J = 8.8 Hz), 8.88 (1H, dd, J = 1.0, 8.3 Hz), 12.35 (1H, s), 13.21 (1H, s). IR [ν_{max} cm^{-1} ($CHCl_3$)] 1720, 1626, 1605, 1575, 1480, 1440, 1378, 840. EIMS [m/z (%)] 326 (100), 295 (33), 268 (18), 267 (13). UV [λ_{max} nm (ε, EtOH)] 228 (24000), 336 (6400), 377 (sh) (4000), 395 (6400), 443 (8600), 459 (sh) (8400).

5 Crystal data of **1a**: monoclinic, space group $P2_1/a$, Z = 4, lattice constants a = 19.043 (1), b = 9.9226 (7), c = 6.6988 (9) Å, β = 104.31 (1)° (1), Dc = 1.62 g/cm^3, V = 1226.5 (2) Å^3.

6 Krollpfeiffer F., Schneider, K. L., and Wissner, A., Ann. *566*, 139.