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AN ANTIBACTERIAL PIGMENT FROM THE SPONGE *DENDRILLA MEMBRANOSA*.

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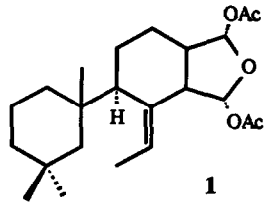
Abstract: The antimicrobial component of the Antarctic sponge,*Dendrilla membranosa*, was identified as 4,5,8-trihydroxyquinoline-2-carboxylic acid (3) by interpretation of spectral data and confirmed by synthesis.

Recently, we reported[2] the anti-microbial properties of an extract from *Dendrilla membranosa*, a bright yellow Antarctic sponge which may be immune to predation by nudibranchs and sea-stars due to the presence of defensive chemicals.[3] The non-polar partition of this extract provided two new mildly antimicrobial terpenes, 9,11-dihydrogracilin A (1) and membranolide (2),[2] however, the majority of the antibacterial activity against the marine bacteria *Vibrio anguillarum* and *Beneckea harveyii* B-392 resided in the aqueous partition. We describe, here, the isolation of the antibacterial yellow pigment, 4,5,8 trihydroxyquinoline-2-carboxylic acid (3) from *D. membranosa* and the confirmation of it's structure by spectroscopic and synthetic methods.



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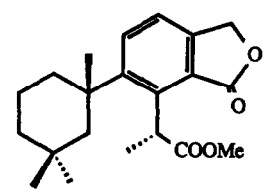
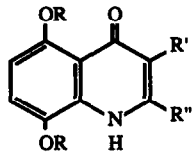


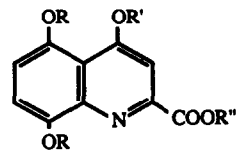
2

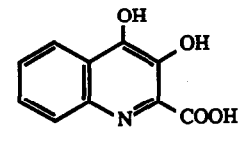
Carboxylic acid 3[4,5] was obtained as a water soluble yellow solid after Sephadex LH 20 chromatography (methanol eluent) of the *n* -butanol soluble fraction of the aqueous extract from *D. membranosa*.. The FAB mass spectrum of 3 showed parent ions at m/z 222 (MH^+) and 244 ($M+Na^+$) and the formula $C_{10}H_7NO_5$ was obtained by accurate mass measurement (m/z 244.0218, Δ -0.4 mmu). The UV, IR ^1H NMR and ^{13}C NMR spectra, together with a positive ferric chloride test, suggested a trihydroxy substituted quinoline-2-carboxylic acid but the substitution pattern was not immediately apparent from this data.

The correct isomer of 3 was chosen from several possibilities on the basis of nuclear Overhauser difference spectroscopic (NOEDS) measurements on the dimethoxy derivative 4 in $CDCl_3$.[6] Irradiation of the carbomethoxyl group proton signal (δ 4.05 s, 3H) induced a weak nOe to the aromatic proton singlet H-3 (7.51 s, 1H) while irradiation of the 4-OMe signal (4.20 s, 3H) gave nOe's to both H-3 (28%) and the 5-OH signal (7.97 s, 1H, D_2O exchangeable, 2%). Most significant was the observation of nOe's *to both* of the mutually coupled aromatic doublets (6.96 d, J = 8.5 Hz; 7.15, d, J = 8.5 Hz, ca. 6%) upon irradiation of the 5-OH signal. This excludes the 5, 6 dihydroxy isomer of 4 and can only be explained by saturation transfer between the two chemically exchanging phenolic protons and simultaneous nOe's to the ortho disposed H-6 and H-7

protons which they flank.[7]The structure **3** was unambiguously verified by synthesis,*via* ester **5**, according Butenandt.[8] Synthetic **3** was identical to the natural product by ^1H NMR, ^{13}C NMR and TLC.







3 R = R' = H, R" = COOH
6 R = R" = H, R' = OH

4 R = H, R' = R" = Me
5 R = Me, R' = H, R" = Et
8 R = Me, R' = H, R" = H

7

This is the first report of the natural occurence of **3**. As some sponges are known to harbor symbiotic bacteria it is noteworthy that **3**, together with secondary metabolites, uranidine (**6**),[9] and the quinoline, **7**[10], from sponges of the genus *Verongia*, resemble several xanthurenic acid metabolites that have been shown to be involved in tryptophan catabolism in *Pseudomonas* spp.[11]

Both synthetic and natural **3** inhibited the growth of *Staphyllococcus aureus, V. anguillarum, B. harveyi* B-392 at 100 μg/disk but the synthetic intermediates **5** and **8**[12] were inactive. Quinolone-2-carboxylic acids are known to possess anti-allergic properties,[13] however, quinolone **3** did not inhibit phospholipase A_2.[14]

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4 Obtained from the larger sample 85-102A (see ref 2.) *4,5,8-trihydroxyquinoline-2-carboxylic acid* (**3**)(0.7% of dry weight after extraction): mp. 295-300° dec.; UV (H_2O) 347 nm (ε 3080), 331 (3900), 270 (sh, 6550), 250 (14600), 232 (17100); (pH 10) 353 (4250), 280 (sh, 6550), 258 (13300), 236 (16300); IR (KBr) 3700-2500, 3340, 1630 (br), 1600, 1540, 1450, 1390, 1255, 1180, 1050, 815 cm^{-1}; ^1H NMR (d_6 DMSO) 6.41 (d, 1H, J = 8.4 Hz), 6.53 (s, 1H), 7.04 (d, 1H, J = 8.4 Hz), 10.50 (bs, 3H), 13.46 (s); ^{13}C NMR (d_6 DMSO) 106.0d, 106.3d, 113.3s, 117.4d, 129.1s, 137.1s, 146.5s, 152.4s, 162.0s, 183.6s; FABMS m/z 266 ($M\text{-}H^+\text{+}2Na^+$), 244 ($M\text{+}Na^+$), 222 ($MH^+$). Exact mass 244.0218, $C_{10}H_7NO_5$+Na requires 244.0222.

5 Compound **3** is depicted as the quinolone tautomer which probably predominates in solution.See ref. 11.

6 Treatment of **3** with silver oxide and an excess of methyl iodide in DMF (3h) gave *methyl 5,8-dihydroxy-4-methoxyquinoline-2-carboxlate* (**4**); IR ($CHCl_3$) 3490, 3010, 2950, 1725, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) 4.05 (s, 3H), 4.20 (s, 3H), 6.96 (d, 1H, J = 8.5 Hz), 7.15 (d, 1H, J = 8.5 Hz), 7.51 (s, 1H), 7.80 (bs, 1H, D_2O exch.), 7.97 (s, 1H, D_2O exch.); Exact mass EIMS m/z 249.0634 (M^+), $C_{12}H_{11}NO_5$ requires 249.0637.

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12 Prepared by hydrolysis of **5** (10% KOH, MeOH/ H_2O; HCl).

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14 We thank Prof. R. Jacobs of the University of California at Santa Barbara for this result.

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