

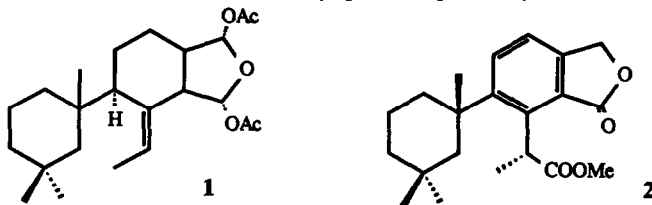
AN ANTIBACTERIAL PIGMENT FROM THE SPONGE *DENDRILLA MEMBRANOSA*.

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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 reported² 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 membranolid (2),² however, the majority of the antibacterial activity against the marine bacteria *Vibrio anguillarum* and *Beneckea harveyi* B-302 resided in the aqueous partition. We describe

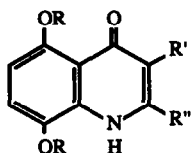
here, the isolation of the antibacterial yellow pigment, 4,5,8-trihydroxyquinoline-2-carboxylic acid (3) from *D. membranosa* and the confirmation of its structure by spectroscopic and synthetic methods.



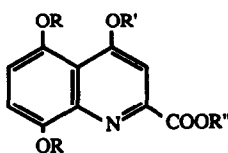
Carboxylic acid 3^{4,5} was obtained as a water soluble yellow solid after Sephadex LH 20 chromatography (methanol eluent) of the *n*-butanol soluble fraction of the aqueous extract from *D. membranosa*. The FAB mass spectrum of 3 showed parent ions at m/z 222 (MH^+) and 244 ($M+Na^+$) and the formula $C_{10}H_7NO_5$ was obtained by accurate mass measurement (m/z 244.0218, Δ -0.4 mmu). The UV, IR ¹H NMR and ¹³C 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

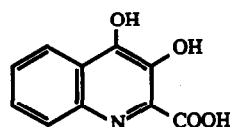
protons which they flank.⁷The structure 3 was unambiguously verified by synthesis, via ester 5, according Butenandt.⁸ Synthetic 3 was identical to the natural product by ¹H NMR, ¹³C NMR and TLC.



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



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This is the first report of the natural occurrence 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, ⁷¹⁰, 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 *Staphylococcus aureus*, *V. anguillarum*, *B. harveyi* B-392 at 100 µg/disk but the synthetic intermediates 5 and 8¹² were inactive. Quinolone-2-carboxylic acids are known to possess anti-allergic properties,¹³ however, quinolone 3 did not inhibit phospholipase A₂.¹⁴

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- 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 (H₂O) 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 (d₆ 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); ¹³C NMR (d₆ 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-H⁺+2Na⁺), 244 (M+Na⁺), 222 (MH⁺). Exact mass 244.0218, C₁₀H₇NO₅+Na requires 244.0222.
- Compound 3 is depicted as the quinolone tautomer which probably predominates in solution. See ref. 11.
- Treatment of 3 with silver oxide and an excess of methyl iodide in DMF (3h) gave methyl 5,8-dihydroxy-4-methoxyquinoline-2-carboxylate (4); IR (CHCl₃) 3490, 3010, 2950, 1725, 1605 cm⁻¹; ¹H NMR (CDCl₃) 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, D₂O exch.), 7.97 (s, 1H, D₂O exch.); Exact mass EIMS m/z 249.0634 (M⁺), C₁₂H₁₁NO₅ requires 249.0637.
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- We thank Prof. R. Jacobs of the University of California at Santa Barbara for this result.