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BIFURCARENONE, AN INHIBITOR OF MITOTIC CELL DIVISION FROM THE BROWN ALGA BIFURCARIA GALAPAGENSIS

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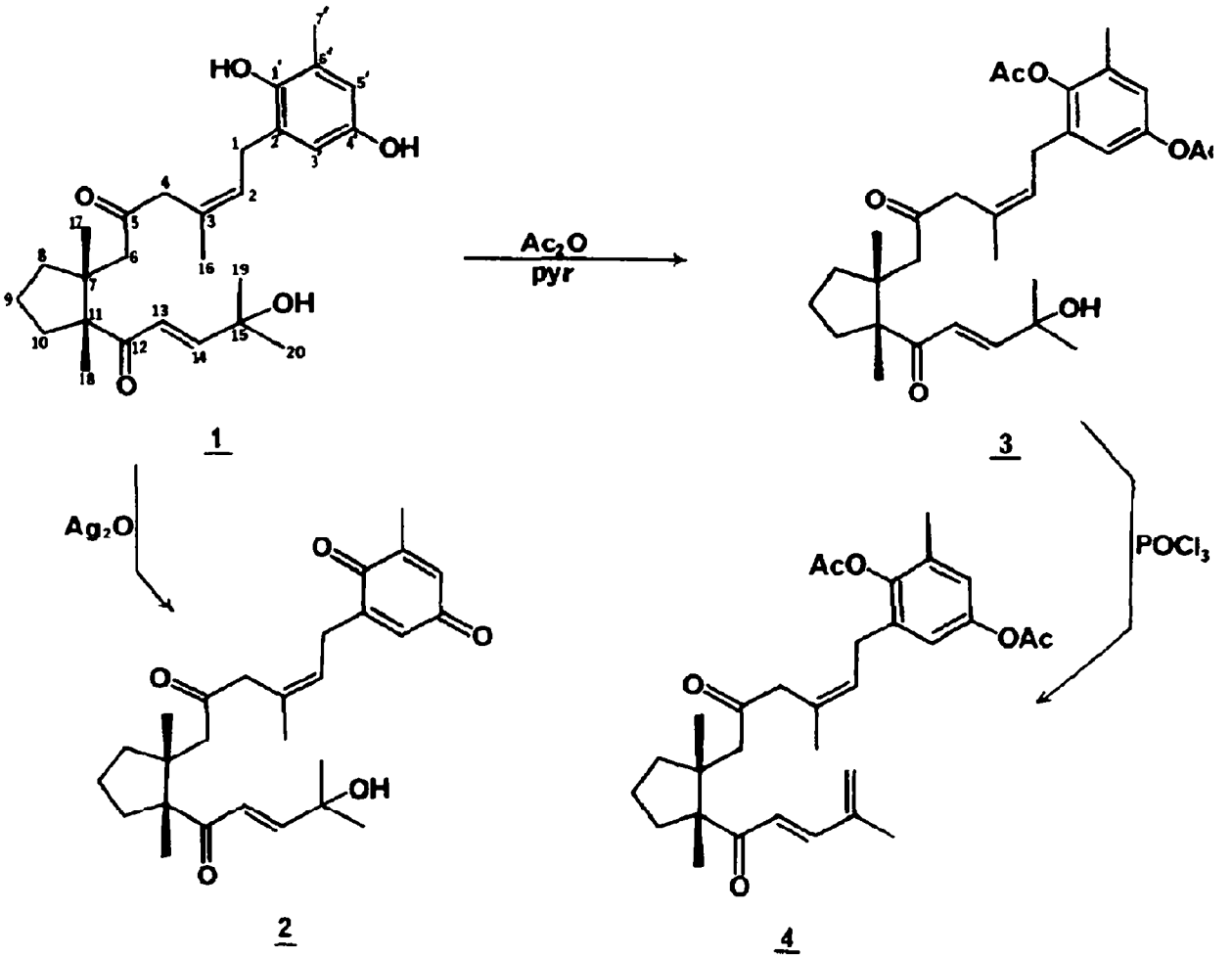
Summary: A C_{27} hydroquinone, bifurcarenone, apparently produced via mixed biosynthesis, has been isolated from the brown seaweed Bifurcaria galapagensis (Cystoseiraceae). The structure of bifurcarenone was determined by chemical and spectral methods, and this compound was found to exhibit antibacterial properties, as well as to inhibit cell cleavage of the fertilized egg from the urchin Strongylocentrotus purpuratus.

During a recent expedition to the Archipelago de Colon (Galapagos Islands), it became obvious that the voracious marine herbivore population of that island group systematically avoided several abundant seaweeds, and particularly the luxuriant growths of the brown alga Bifurcaria galapagensis (Piccone et Grunow in Piccone) Womersley (Cystoseiraceae). The infamous Galapagos marine iguana, Amblrhyncus cristatus, was no exception, as it was routinely found foraging for very small seaweeds while literally passing over massive quantities of B. galapagensis. We wish to report here that this alga is a rich source for unique biologically-active natural products, and to describe the structure of a major metabolite, bifurcarenone (1). Bifurcarenone possesses a structurally unprecedented monocyclic diterpenoid moiety in combination with a hydroquinone C_7 unit. Bifurcarenone was found to exhibit moderate in vitro antibacterial activity[1], and to inhibit mitotic cell division in the fertilized urchin (Strongylocentrotus purpuratus) egg assay, ED_{50} = 4.0 µg/ml[2].

The $CHCl_3$/MeOH extract (1/1) of the frozen alga was fractionated by open-column silica gel chromatography, using isooctane/CH_2Cl_2/EtOAc solvents, to yield 1 and several other unknown compounds as complex mixtures. Final purification was achieved by HPLC on µ-porasil (35% EtOAc/isooctane) to yield 1 as a colorless mobile oil (0.26% dry wt. alga). Bifurcarenone (1) showed $[\alpha]_D$ - 5.7° (c 0.34, $CHCl_3$), and analysed for $C_{27}H_{38}O_5$ by high resolution mass spectrometry M^+ m/e obs. 442.2712; calc. 442.2719. The infrared spectrum of this compound ($CHCl_3$) showed absorptions for hydroxyl (ν_{O-H} 3450 cm^{-1}), unstrained ketone ($\nu_{C=O}$ 1710 cm^{-1}), α,β-unsaturated ketone ($\nu_{C=O}$ 1680 cm^{-1}), and olefin ($\nu_{C=C}$ 1620 cm^{-1}) functionalities. The UV spectrum consisted of absorption at 215 and 292 nm (ε = 15,600, 3,400), indicative of a hydroquinone chromophore, and an absorption at 225 nm (ε = 16,200), characteristice of the α,β-unsaturated ketone moiety.



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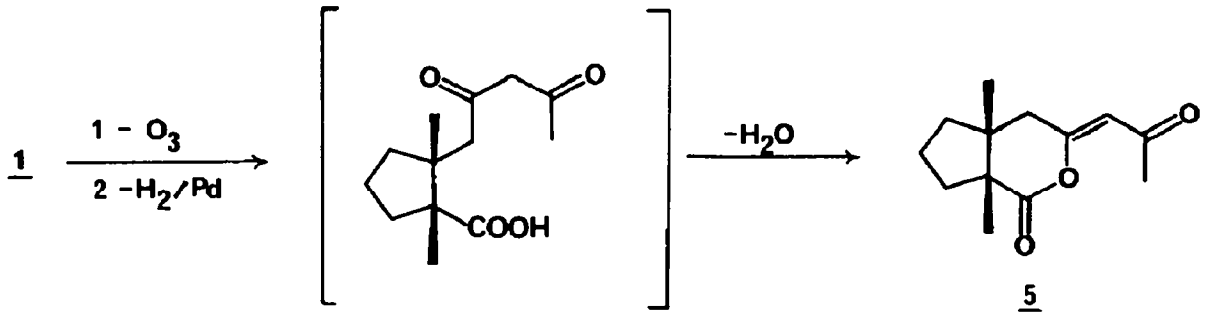
The ^{13}C NMR spectral features of bifurcarenone, in conjunction with the molecular formula data and aforementioned infrared and ultraviolet characteristics, illustrated the unsaturated ketone (δ206.2 s, 122.7 d, 153.8 d)[3], the unstrained ketone (δ209.9 s), a non-conjugated trisubstituted olefin (128.0 d, 125.8 s), a tertiary alcohol (71.2 s), and a dialkylated p-hydroquinone ring (δ149.9 s, 131.0 s, 113.4 d, 145.2 s, 115.5 d, 128.0 s)[4]. In addition, two quaternary carbons (60.3 s, 46.6 s), six methylene carbons (δ56.5 t, 47.0 t, 36.7 t, 34.2 t, 29.3 t, 29.3 and six methyl groups (δ29.3 q, 28.8 q, 21.2 q, 20.2 q, 20.1 q, 16.3 q) were observed. Consideration of these data showed bifurcarenone to be composed of a monocyclic diterpene unit coupled at C-1 with a methyl-substituted p-benzohydroquinone.

An initial formulation of structure 1 for bifurcarenone could be made by analysis of its 2 MHz ^1H NMR features, including the results of spin-decoupling experiments in $CDCl_3$. The 1, 2, 5-substitution pattern on the hydroquinone ring was readily assessed by the presence of two meta coupled (J = 3Hz) protons at δ6.55 and 6.45 (C-3',5'). The C-1 benzylic methylene was observed at δ3.34 (d, J = 8 Hz), and it was coupled to an adjacent olefin (δ 5.43, t, J = 8 Hz). A broadened two-proton singlet at δ3.04 was assigned as the C-4 methylene, since it was noticeably sharpened by irradiation of the C-2 olefin proton. The C-6 methylene was observed as an AB pattern at δ2.47 and 2.34, J = -17 Hz. The protons of the α, β-unsaturated ketone, C-13, 14, w

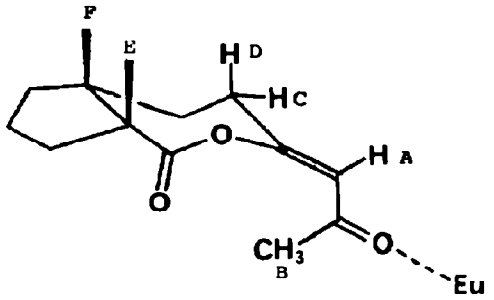
found at δ6.66 and δ6.88 as an AB pattern with J = 16 Hz. The magnitude of the coupling consta allowed a trans olefin geometry to be assigned, and the lack of further coupling of the β-proto (C-14), suggested that C-15 was a quaternary carbon. Other bands in the ^{1}H NMR spectrum were t C-7' singlet methyl group at δ2.22, the C-16 singlet methyl at δ1.62 (sharpens upon irradiation of the δ5.43 olefin proton), two bridgehead methyl singlets at δ1.19 and 1.18 (C-17, 18), and two deshielded singlet methyl resonances at δ1.32 and 1.30 (C-19, 20). Two D_2O exchangeable ba were also observed at δ7.45 and 4.82, respectively. The C-2 - C-3 olefin geometry was assigned Z based upon the C-16 methyl resonance of greater than 20 ppm in the ^{13}C NMR spectrum reflectin diminished gamma-shielding effect. The only shielded methyl group (16.3 ppm) was readily assig to the C-7' aromatic methyl[4].

Silver oxide oxidation of 1 gave the corresponding p-benzoquinone 2, which illustrated cla ical infrared quinone carbonyl absorptions ($\gamma_{C=O}$ 1680 cm^{-1}). Acetylation of 1 (Ac_2O/py/RT) gave the diacetate 3 which still possessed infrared hydroxyl absorptions (γ_{O-H} 3600 cm^{-1}). Subseque dehydration of 3 with $POCl_3$ at 0° in pyridine produced a terminal dieneone which showed UV absorptions at 271 nm (ε = 13,000) and ^{1}H NMR bands (δ5.38 and 5.41 for C-20 terminal methylene, and a new olefin methyl singlet at δ 1.89) in support of structure 4.

The final structure assignment for this new metabolite was secured by reductive ozonolysis 1 to yield the keto-lactone 5, which analysed for $C_{13}H_{18}O_3$ by mass spectrometry. The infrared spectrum of 5 showed lactone carbonyl absorption (1765 cm^{-1}), and the UV spectrum showed λ_{max} nm (ε= 18,200). The ^{1}H NMR spectrum of 5 showed that the two bridgehead methyls were intact i this fragment (δ1.32 s, 1.07 s), as well as an AB methylene group (δ2.48, 2.31, J = 15 Hz) and methyl ketone group (δ2.46 s). A singlet olefin proton at δ5.23 was observed, and using relate



model compounds recently reported, the stereochemistry of the olefin in 5 could be securely assigned as Z[5]. Analysis of Eu(fod)$_3$-induced ^{1}H NMR shifts for 5 gave convincing evidence to assign stereochemistry to this structure and, therefore, to complete the structure of bifurcare none. The simplified form of the pseudocontact shift equation using angle terms was employed, and the results for pertinent protons are summarized below:



H's	Δδ	θ	r(meas)	r(calc)	% erro
A	8.88	25	4.8°A	4.8°A	0
B	8.09	19	5.4	5.2	3.7
C	2.45	25	7.0	7.3	4.3
D	2.41	16	7.7	7.8	1.3
E	1.50	2	9.7	9.6	1.0
F	1.13	13	10.1	10.2	1.0

The alternative structure with E and F methyls trans was eliminated since an error of 36% for F methyl was observed.

The structure of bifurcarenone is unique among the diterpenoids, particularly with refere to the anti-Markovnikoff cyclization between C-7 and C-11. B. galapagensis contains a series related metabolites which will be the subject of a subsequent complete paper.

ACKNOWLEDGEMENTS

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REFERENCES AND NOTES

1. Bifurcarenone showed in vitro antibacterial properties against five test organisms as determined by the agar plate assay disc method (0.5 mg/disc). The organisms assayed and results were: E. coli (13 mm total zone of inhibition), S. aureus (8.5 mm), P. aeruginosa (17 mm), E. aerogenes (9mm) and B. subtilis (11 mm).

2. The pharamacological properties of this cell line have been recently summarized, see R. S Jacobs, S. White and L. Wilson, Federation Proc. in press (1980).

3. The polarized olefin of this enone group was readily recognized by additional long range coupling.

4. Assignments for these aromatic carbons and the C-7' methyl were made based upon a compari with calculated valves by methods found in J. B. Stothers, Carbon-13 NMR Spectroscopy, Academic Press, New York, 1972.

5. T. Kitamura, T. Imagawa, and M. Kawanisi Tetrahedron Lett. 3443 (1978).

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