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Hybocarpone, a novel cytotoxic naphthazarin derivative from mycobiont cultures of the lichen *Lecanora hybocarpa*

Michael A. Ernst-Russell,[a] John A. Elix,[a,*] Christina L. L. Chai,[a] Anthony C. Willis,[b] Nobuo Hamada[c] and Thomas H. Nash III[d]

[a]*Department of Chemistry, The Faculties, Australian National University, Canberra, ACT, 0200, Australia*
[b]*Research School of Chemistry, Institute of Advanced Studies, Australian National University, Canberra, ACT, 0200, Australia*
[c]*Osaka City Institute of Public Health and Environmental Sciences, 8-34 Tojo Cho, Tennouji-ku, Osaka, 543, Japan*
[d]*Department of Botany, Arizona State University, Tempe, Arizona, 85287, USA*

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Abstract

Hybocarpone (**1**), a novel pentacyclic naphthazarin-derived dimer has been isolated from mycobiont cultures derived from the lichen *Lecanora hybocarpa*. The structure of hybocarpone (**1**) was established on the basis of 1D and 2D NMR spectroscopy. Its relative stereochemistry was predicted with the use of molecular modelling and confirmed by X-ray crystallographic analysis. Hybocarpone (**1**) was found to be a potent cytotoxin (IC_{50} *c* 0.27 μM), active against the murine P815 mastocytoma cell line.

Lichens produce a multitude of secondary metabolites which are either exclusive to or characteristic of these organisms.[1] A number of such compounds have been found to exhibit potent biological activities, including antimicrobial,[2] antiproliferative or cytotoxic effects,[3–5] antiviral[6] and enzymic inhibitory behaviour e.g. activity against HIV-1 integrase[7] and HIV-1 reverse transcriptase.[8]

Recent efforts to identify new bioactive lichen compounds have included the cultivation of spore-derived lichen mycobionts. These cultures have been shown to be capable of producing metabolites with both known and unprecedented structures in large amounts under osmotically stressed conditions.[9] We now report the isolation of hybocarpone (**1**), a naphthazarin derived pentacycle, from the cultured lichen mycobiont of *Lecanora hybocarpa* (Tuck.) Brodo and the structural elucidation of this metabolite using both spectroscopic and crystallographic methods.

Specimens of the lichen were collected from woodland in Louisiana, USA and cultures of the mycobiont were prepared from spores discharged from apothecia on the lichen thallus. The acetone extracts of the cultural broth were purified by repeated fractional crystallisation to yield hybocarpone (**1**) as the major product.[10] In addition, the crude broth extract was also found to contain the known

* Corresponding author. Tel: +61 2 62492937; fax: +61 2 62490760; e-mail: john.elix@anu.edu.au

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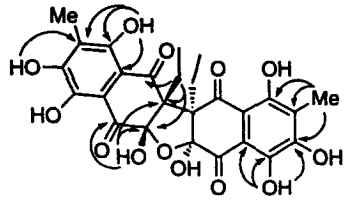
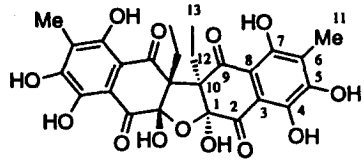
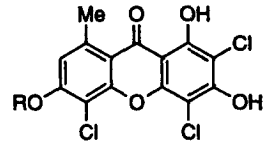


Figure 1. Diagnostic ^{1}H and ^{13}C long range correlations observed in the gHMBC spectrum of **1**

xanthones arthothelin (**2**) and 6-*O*-methylarthothelin (**3**) which were identified by comparison with authentic, synthetic material (HPLC, UV and MS).[11,12]



(1)



R = H (2) R = Me (3)

The UV spectrum of hybocarpone (**1**) was suggestive of a naphthazarin system and IR absorption bands at 3382 and at 1652, 1632 and 1596 cm^{-1} indicated the presence of hydroxy groups and conjugated carbonyl moieties, respectively.

Further support for a naphthazarin skeleton was provided by the ^{1}H NMR spectrum with two strongly chelated phenolic protons at δ 10.94 and 13.13 ppm. Two additional hydroxyl protons were evident as broader singlets at δ 4.90 and 6.57 and a singlet consistent with an aromatic methyl group was observed at δ 2.28. A triplet at δ 0.68 and two multiplets at δ 2.28 and 2.31 indicated the presence of an ethyl functionality, however the chemical shift of the triplet attributed to the ethyl CH_3 protons (δ 0.68) was uncharacteristically upfield.[1]

High resolution mass spectrometric analysis of **1** showed a molecular ion at 544.1218, corresponding to a molecular formula of $C_{26}H_{24}O_{13}$. The EIMS showed little fragmentation apart from the base peak at *m/z* 264 (corresponding to $C_{13}H_{12}O_6$). This evidence, coupled with the low number of signals observed in the ^{1}H NMR spectrum indicated that hybocarpone (**1**) was an oxo-bridged dimer with a high degree of symmetry.

The dimeric nature of the molecule was also apparent in the ^{13}C NMR spectrum of **1**. The 13 signals observed were consistent with two pairs of carbonyl carbons, 12 aromatic carbons, two pairs of quaternary carbons at δ 99 and 67, two methyl groups and a pair of ethyl substituents. ^{13}C chemical shift data were assigned on the basis of APT, gHMQC and gHMBC experiments.

Fig. 1 shows the HMBC correlations supporting the proposed structure for hybocarpone (**1**). The substituents on the aromatic moieties of the molecule were readily established from the observed two and three bond connectivites. In addition, the chemical shifts of the two carbonyl carbons (δ 193, 198) are consistent with a partially saturated quinonoid derivative (typical quinone carbonyls resonate at δ 180–190 ppm). The signal at δ 99 is typical for a hemiacetal moiety. Further support for the substitution pattern of the central tetrahydrofuran ring was provided by connectivities observed in the gHMBC spectrum.

The isochronicity observed in the ^{1}H and ^{13}C NMR spectra of **1** can be attributed to the molecule possessing either a twofold axis (C_2) or a mirror plane (σ) of symmetry. However, the observed optical activity of $[\alpha]_D$ −19.2 is not compatible with the existence of a mirror plane in hybocarpone (**1**),[13] therefore the two carbon atoms (δ 67 ppm) which link the two portions of the dimer must have the same relative configuration.

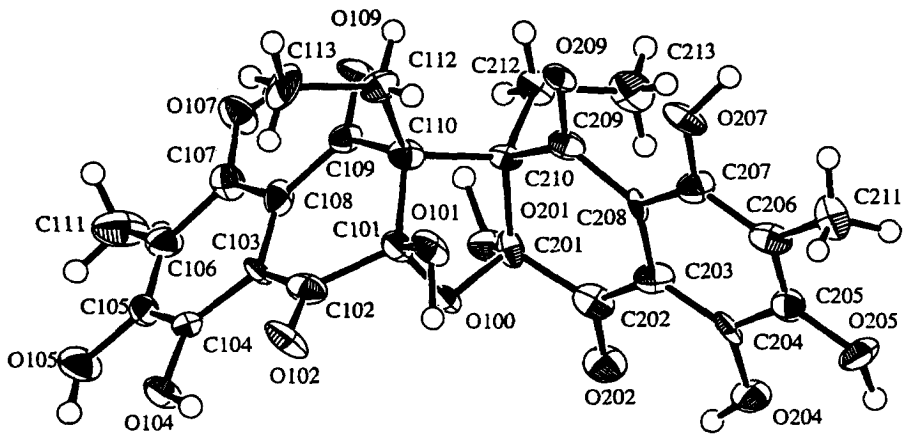


Figure 2. Thermal ellipsoid diagram of **1** with selected atom labelling. Ellipsoids show 50% probability levels. Hydrogen atoms are drawn as circles with small radii

Minimal energy calculations were carried out using the MM2* force field (Macromodel 5.5) in an attempt to differentiate between the two remaining diastereomeric configurations. These calculations predicted that the hemiacetal structure with $R^*R^*R^*R^*$ configuration (adjacent hydroxy and ethyl substituents are *syn*) was *c* 12 kcal mol^{-1} more stable than the alternative diastereomer (adjacent hydroxy and ethyl substituents are *anti*).

The structure and proposed relative configuration of hybocarpone (**1**) was confirmed by a single crystal X-ray diffraction study (Fig. 2). A crystal suitable for X-ray work was obtained by slow evaporation of a solution of aqueous methanol, crystal data: monoclinic, space group $P2_1$ (#4), *a* 10.903(3), *b* 12.431(6), *c* 19.457(3) Å, β 98.25(2)°, *V* 2610(2) $Å^3$; *Z* 2, D_c 1.467 g cm^{-3}, *F* (000) 1208, μ (CuKα) 10.31 cm^{-1}, λ 1.54178 Å, crystal size 0.14×0.07×0.04 mm.

Hybocarpone (**1**) was found to possess potent cytotoxicity against the murine mastocytoma P815 cell line, yielding an IC_{50} value of 0.15 μg/ml. Further investigations into the activity of **1** are currently in progress.

A number of monomeric and simple, dimeric naphthazarins have been isolated as pigments from plants and marine invertebrate organisms though they are rare in lichen sources.[1,14–16] The dinaphtho[2,3-*b*:2,3-*d*]furan-tetraone carbon skeleton of hybocarpone (**1**) is highly unusual and has not previously been observed in nature.

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References

1. Huneck, S.; Yoshimura, I. *Identification of Lichen Substances*; Springer: Berlin, 1996.
2. Lauterwein, M.; Oethinger, M.; Belsner, K.; Peters, T.; Marre, R. *Antimicrob. Agents Chemother.* **1995**, *39*, 2541.

3. Ernst-Russell, M. A.; Chai, C. L. L.; Hurne, A. M.; Waring, P.; Hockless, D. C. R.; Elix, J. A. *Aust. J. Chem.* **1999**, *52*, 279.
4. Ogmundsdottir, H. M.; Zoega, G. M.; Gissurarson, S. R.; Ingolfsdottir, K. *J. Pharm. Pharmacol.* **1998**, *50*, 107.
5. Leao, A. M. A. C.; Buchi, D. F.; Iacomini, M.; Gorin, P. A. J.; Oliveira, M. B. M. *J. Submicro. Cytol. Pathol.* **1997**, *29*, 503.
6. Yamamoto, Y.; Miura, Y.; Kinoshita, Y.; Higuchi, M.; Yamada, Y.; Murakami, A.; Ohigashi, H.; Koshimizu, K. *Chem. Pharm. Bull.* **1995**, *43*, 1388.
7. Neamati, N.; Hong, H. X.; Mazumder, A.; Wang, S. M.; Sunder, S.; Nicklaus, M. C.; Milne, G. W. A.; Proksa, B.; Pommier, Y. *J. Med. Chem.* **1997**, *40*, 942.
8. Pengsuparp, T.; Cai, L.; Constant, H.; Fong, H. H. S.; Lin, L. Z.; Kinghorn, A. D.; Pezzuto, J. M.; Cordell, G. A.; Ingolfsdottir, K.; Wagner, H.; Hughes, S. H. *J. Nat. Prod.* **1995**, *58*, 1024.
9. (a) Hamada, N.; Ueno, T. *Phytochemistry* **1990**, *29*, 678; (b) Tanahashi, T.; Kuroishi, M.; Kuwahara, A.; Nagakura, N.; Hamada, N. *Chem. Pharm. Bull.* **1997**, *45*, 1183.
10. Hybocarpone (**1**): Orange prisms, mp 167–168°C; $[\alpha]_D$ −19.2 (c 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (4.41), 258 (4.51), 320 (4.14), 405 (4.16), 4.19 sh (4.11) nm; IR (KBr) ν_{max} 3382, 2925, 1652, 1632, 1596, 1457, 1437, 1420, 1382, 1282, 1207, 1137, 1074, 1040, 1011, 944 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (3H, t, *J*=7.4 Hz, CH_3CH_2), 2.28 (3H, s, $ArCH_3$), 2.31 (1H, m, CH_3CH_2), 2.62 (1H, m, CH_3CH_2), 4.90 (1H, s, 2-OH), 6.57 (1H, s, 5-OH), 10.94 (1H, s, 4-OH), 13.13 (1H, s, 7-OH); ^{13}C NMR ($CDCl_3$) δ 198.50 (9), 193.41 (2), 157.70 (7), 150.15 (5), 143.47 (4), 123.31 (6), 108.60 (8), 107.72 (3), 99.57 (1), 67.30 (10), 25.15 (12), 10.48 (13), 8.90 (11); EIMS *m/z* [M^+] 544 (38), 264 (100), 221 (30); HREIMS *m/z* [M^+] 544.1218 (calcd for $C_{26}H_{24}O_{13}$ 544.1217).
11. Huneck, S.; Höfle, G. *Tetrahedron* **1978**, *34*, 2491.
12. Elix, J. A.; Bennett, S. A. *Aust. J. Chem.* **1990**, *43*, 1587.
13. Eliel, E. L.; Wilen, S. H.; Mander, L. N. *Stereochemistry of Organic Compounds*; Wiley & Sons: New York, 1994.
14. (a) Thomson, R. H. *Naturally Occurring Quinones*; 2nd ed.; Academic Press: New York, 1971; (b) Thomson, R. H. *Naturally Occurring Quinones III, Recent Advances*; Chapman and Hall: London, 1987.
15. Yamamoto, Y.; Matsubara, H.; Kinoshita, Y.; Kinoshita, K.; Koyama, K.; Takahashi, K.; Ahmidjiam, V.; Kurokawa, T.; Yoshimura, I. *Phytochemistry* **1996**, *43*, 1239.
16. Stepanenko, L. S.; Krivoshchekova, P. S.; Dmitrenok, P. S.; Maximov, O. B. *Phytochemistry* **1997**, *46*, 565.