Euplectin and Coneuplectin, New Naphthopyrones from the Lichen *Flavoparmelia euplecta*

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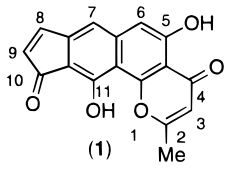
Received July 2, 1999

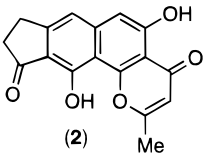
Two new naphthopyrones, euplectin (**1**) and coneuplectin (**2**), have been isolated from the lichen *Flavoparmelia euplecta* and their structures elucidated using multidimensional NMR spectroscopic methods, including highfield (600 MHz) gHMBC and gNOE experiments. Cytotoxicity of the more abundant naphthopyrone (**1**) against the murine P-815 mastocytoma cell line (IC_{50} ca. 1.67 μg/mL) has also been evaluated. These compounds are the first lichen metabolites known to contain indenone or indanone moieties in their structure. *F. euplecta* was also found to contain the known metabolites usnic acid, protocetraric acid, and skyrin.

Considerable interest has recently been shown in the potent pharmacological activities exhibited by lichen-derived metabolites.[1−6] As part of ongoing investigations into the identification of new pharmacologically active lichen compounds,[7,8] we have turned our attention to the foliose lichen *Flavoparmelia euplecta* (Stirton) Hale (Parmeliaceae).

F. euplecta is endemic to the eastern states of Australia and is characterized by an orange-red lower medullar, which turns an intense purple color on treatment with 10% aqueous KOH.[9] HPLC of the total acetone extract of this lichen confirmed the presence of the cortical pigment usnic acid[10] and the medullary depsidone, protocetraric acid[11] as major metabolic constituents. The lichen was also found to contain the anthraquinone skyrin[12] and two yellow-orange pigments of unknown structure. In this paper we report the isolation and structure elucidation of these two naphthopyrone-derived pigments, euplectin (5,11-dihydroxy-2-methylindeno[5,6-*h*]4H-chromene-4,10-dione) (**1**) and coneuplectin (5,11-dihydroxy-2-methylindano[5,6-*h*]4H-chromene-4,10-dione) (**2**) from the lichen *Flavoparmelia euplecta*.



(**1**)



(**2**)

The lichen material was collected from rock outcrops in pastoral land on the south coast of New South Wales, Australia. Purification of the ethereal extracts of the lichen by fractional crystallization, followed by preparative TLC of the subsequent mother liquors, gave a number of colored pigments, including usnic acid, skyrin, **1**, and **2**.

Euplectin (**1**) was isolated as fine orange-red needles, mp 320 °C (dec). The mass spectrum of **1** exhibited a molecular ion at *m/z* 294, and the corresponding high-resolution data suggested a molecular formula of $C_{17}H_{10}O_5$. The ^{1}H NMR spectrum contained signals characteristic of a strongly chelated phenolic hydroxy substituent at δ 13.05, a broad exchangeable singlet at δ 11.07, two aromatic hydrogens (δ 6.87 and 6.84), an olefinic proton (δ 6.27), and a downfield methyl group at δ 2.51. The spectrum also indicated the presence of two coupled "alkene" protons (J = 5.7 Hz) resonating as doublets centered at δ 7.49 and 6.19. The ^{13}C NMR data further supported the proposed molecular formula, exhibiting 17 discrete resonances. Two α,β-unsaturated carbonyl groups (δ 198 and 183) were readily apparent, as were five aromatic or olefinic methines at δ 147, 132, 116, 110.3, and 110.0, which were assigned by gHMQC data.

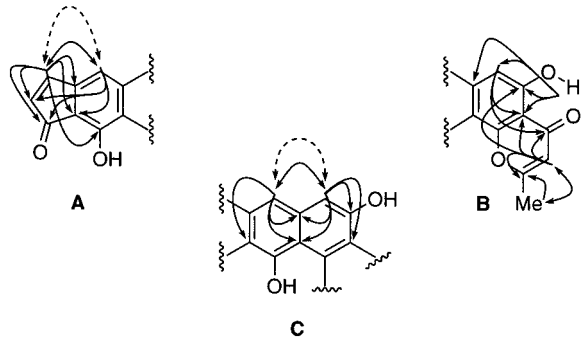


Figure 1. Key gHMBC (arrows) and gNOE (dotted arrows) correlations observed in the spectra of **1**.

The presence of the indenone moiety (partial structure A, Figure 1) was suggested from long-range H−C connectivities observed in the highfield (500 MHz) gHMBC spectrum of **1**. Of particular importance in establishing the substitution pattern of this fragment were the $^3J_{CH}$ correlations observed between H-8 and C-7 (δ 116) and also between H-7 and C-10a (δ 107). Further analysis of the gHMBC data revealed a second skeletal fragment, the chromone nucleus (partial structure B). $^3J_{CH}$ Correlations observed between H-3,H-6 and C-4a (δ 110.2) and also $^4J_{CH}$ coupling observed between H-3,C-5 (δ 160) and H-6,C-4 (δ 183) were particularly instructive in the identification of this moiety. The ^{1}H and ^{13}C NMR assignments of this fragment are in good agreement with those made for other compounds containing a 6-hydroxy-2-methylchromone moiety.[13] The remaining ambiguity, the substitution pattern of the central naphthalene core (partial structure C), was resolved by means of highfield (600 MHz) gHMBC and gNOE experiments, the latter revealing clear correlations between H-6 (δ 6.84) and H-7 (δ 6.87).

The second pigment (**2**), isolated as fine yellow needles, exhibited a molecular ion two mass units higher than its congenor (**1**). The ^{1}H NMR spectrum revealed two meth-

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10.1021/np9903245 CCC: $19.00
Published on Web 11/24/1999

ylene groups resonating as multiplets at δ 2.78−2.82 and δ 3.16−3.20, suggesting that **2** was the 8,9-dihydro derivative of **1**. This conclusion was further supported by examination of the ^{13}C NMR spectrum, which revealed an α,β-unsaturated carbonyl group at δ 209 in addition to two methylene carbons at δ 25 and 36. The complete assignment of the ^{1}H and ^{13}C NMR chemical shifts of **2** was confirmed by gHMBC and gNOE spectral data.

Euplectin (**1**) exhibited moderate cytotoxicity toward the growth of murine P-815 mastocytoma cells, with an IC_{50} of 1.67 μg/mL. Unfortunately the paucity of the minor naphthopyrone (**2**) precluded the evaluation of its cytotoxic activity.

Compounds containing indenone or indanone moieties have not previously been observed in lichenized fungi and are rarely found in nature. Indeed, naturally occurring compounds containing a cyclopenta-naphthopyran core are extremely rare. It is intriguing to note that the only other metabolites known to possess this framework are the closely related ligustrones, three metabolites isolated from the pathogenic rust *Cercospora ligustrina* Boerema.[14]

Experimental Section

General Experimental Procedures. The general procedures have been described previously.[7] ^{1}H and ^{13}C NMR spectra were recorded on the following spectrometers: Varian Gemini 300, Varian Inova 500, or Varian Inova 600.

Lichen Material. The material of *Flavoparmelia euplecta* (Stirt.) Hale was collected on exposed rocks in pasture, Brigadoon farm, Bunga, 18 km south of Bermagui, New South Wales, Australia. A voucher specimen, J. A. Elix 40363, has been lodged in the Australian National Herbarium, Canberra.

Chromatography. The lichen fragments were freed as far as possible from obvious organic substrate material and extracted either with warm acetone (for TLC) or with warm methanol (for HPLC). Compounds were identified by TLC using the methods standardized for lichen products[15−18] and by HPLC with retention index values (RI) calculated from benzoic acid and solorinic acid controls.[19,20] For TLC, standard R_f values were determined in four independent TLC solvent systems: (A) toluene−dioxan−acetic acid (180:45:5); (B) hexane−*tert*-butyl methyl ether−formic acid (140:72:18); (C) toluene−acetic acid (170:30), and (D) cyclohexane−ethyl acetate (75:25). For HPLC, a Spectra System, a Phenomenex Hypersil 5C_{18} column (250 by 4.6 mm), and a spectrometric detector operating at 254 nm with a flow rate of 1 mL/min were used. Two solvent systems were used: 1% aqueous orthophosphoric acid and methanol in the ratio 3:7 (A) and methanol (B). The run started with 100% A and was raised to 58% B within 15 min, then to 100% B within a further 15 min, followed by isocratic elution in 100% B for a further 10 min. The HPLC was coupled to a photodiode array detector for UV spectroscopic comparisons.

Extraction and Isolation. The dried lichen thallus (21.8 g) was extracted in a Soxhlet extractor with anhydrous diethyl ether for 26 h. The ethereal solution was then concentrated and permitted to cool, whereupon most of the unwanted protocetraric acid precipitated. The solution was filtered, and the filtrate was subjected to radial chromatography over Si gel using ethyl acetate as eluent. The faster moving yellow band afforded a mixture of usnic acid and skyrin (68 mg), the composition of which was confirmed by HPLC and UV spectroscopy. The slower moving orange band contained a mixture of the naphthopyrones that were separated by preparative layer chromatography over Si gel using 15% acetic acid−toluene as eluent. Two major yellow-orange bands developed. The faster moving band yielded a mixture of **1** and **2** (94 mg, 0.4%) as a yellow-orange solid. Repeated crystallization from $CHCl_3$ afforded **1** (10.5 mg). The mother liquors thus enriched in **2** were concentrated and the residue repeatedly crystallized from $CHCl_3$ to afford the minor naphthopyrone (**2**) (3.1 mg).

Euplectin (1): red-orange needles, mp 320 °C (dec); UV (MeOH) λ_{max} 219, 246, 280, 328, 433 nm; ^{1}H NMR ($CDCl_3$) δ 2.53 (3H, s, 2-Me), 6.19 (1H, d, J = 5.7 Hz, 9-H), 6.27 (1H, s, 3-H), 6.84 (1H, s, 7-H), 6.87 (1H, s, 6-H), 7.49 (1H, d, J = 5.7 Hz, 8-H), 9.60 (1H, br s, 11-OH), 13.09 (1H, br s, 5-OH); ^{13}C NMR ($CDCl_3$) δ 198.4 (C-10), 182.7 (C-4), 168.0 (C-2), 160.3 (C-5), 156.8 (C-11b), 155.6 (C-11), 147.2 (C-8), 144.1 (C-6a), 142.5 (C-7a), 132.3 (C-9), 115.6 (C-7), 110.3 (C-3), 110.2 (C-4a), 110.1 (C-6), 108.3 (C-11a), 107.7 (C-10a), 20.6 (C-12); gHMBC: 3-H to (C-2, -2), (C-4, -2*w*), (C-4a, -3), (C-5, -4*w*), (C-12, -3); 5-OH to (C-4a, -3), (C-5, -2), (C-6, -3), (C-6a, -4*w*); 6-H to (C-4, -4), (C-4a, -3), (C-5, -2), (C-6a, -2*w*), (C-7, -3), (C-11a, -3); 7-H to (C-6, -2), (C-6a, -2), (C-8, -3), (C-9, -5*w*), (C-10, -4), (C-10a, -3), (C-11a, -3); 8-H to (C-7, -3), (C-7a, -2), (C-9, -2), (C-10, -3), (C-10a, -3), (C-11, -4*w*); 9-H to (C-7a, -3), (C-8, -2), (C-10, -2), (C-10a, -3), (C-11a, -4*w*); 12-H to (C-2, -2), (C-3, -3); EIMS *m/z* 295 (41%), 294 (M, 100) 266 (21), 254 (26), 170 (17), 115 (14), 113 (12); HREIMS *m/z* [M^+] 294.0535 (calcd for $C_{17}H_{10}O_5$ 294.0528); standard TLC R_f values:[15−18] R_f (A) 0.53; (B) 0.17; (C) 0.48; (E) 0.15; standard HPLC:[19,20] t_R 26.13 min; RI 0.26.

Coneuplectin (2): yellow needles, mp 295 °C (lit.[14] 283 °C); ^{1}H NMR ($CDCl_3$) δ 2.56 (3H, s, 2-Me), 2.78−2.82 (2H, m, 9-H), 3.16−3.20 (2H, m, 8-H), 6.31 (1H, s, 3-H), 6.82 (1H, s, 6-H), 7.05 (1H, s, 7-H), 11.14 (1H, br s, 11-OH), 12.93 (1H, br s, 5-OH); ^{13}C NMR ($CDCl_3$) δ 209.6 (C-10), 182.4 (C-4), 167.5 (C-2), 159.0 (C-5), 158.7 (C-11b), 157.5 (C-11), 152.0 (C-7a), 144.6 (C-6a), 110.9 (C-3), 113.9 (C-7), 116.4 (C-10a), 109.8 (C-4a), 106.5 (C-6), 106.2 (C-11a), 36.1 (C-9), 25.4 (C-8), 20.6 (C-12); gHMBC: 3-H to (C-2, -2), (C-4, -2*w*), (C-4a, -3), (C-5, -4*w*), (C-12, -3); 5-OH to (C-4a, -3), (C-5, -2), (C-6, -3); 6-H to (C-4a, -3), (C-5, -2), (C-7, -3), (C-11a, -3); 7-H to (C-6, -2), (C-6a, -2), (C-8, -3), (C-10a, -3), (C-11a, -3); 8-H to (C-7, -3), (C-7a, -2), (C-9, -2), (C-10, -3), (C-10a, -3); 9-H to (C-7a, -3), (C-8, -2), (C-10, -2), (C-10a, -3*w*); 12-H to (C-2, -2), (C-3, -3); EIMS *m/z* 297 (31%), 296 (M, 100); HREIMS *m/z* [M^+] 296.0682 (calcd for $C_{17}H_{12}O_5$ 296.0685); standard TLC R_f values:[15−18] R_f (A) 0.55; (B) 0.17; (C) 0.47; (E) 0.11; standard HPLC:[19,20] t_R 25.56 min; RI 0.25.

Cytotoxic Activity. To determine the antiproliferative capacity of euplectin (**1**), 100 μL of P-815 cells in minimum Eagle's essential medium (F15) and 10% fetal bovine serum at a final cell concentration of 0.5×10^6 per mL were pipetted into 96-well plates containing the serially diluted (1:2 dilution, starting concentration of 100 μM) naphthopyrone (**1**), with gliotoxin as a reference compound. The cells were incubated for 18 h at 37 °C before being pulsed with tritiated thymidine (1 μCi/ well) for a further 6 h. Cells were harvested using a Pharmacia Betaplate Liquid Scintillation counter. Three replicate wells were used to determine each data point.

Acknowledgment. We thank the Australian Research Council for generous financial support. M.E.R. is the grateful recipient of an ANU scholarship. We also express our thanks to Dr. Paul Waring (John Curtin School of Medical Research, ANU) for assistance with the cytotoxicity assays. Thanks are extended to Dr. Max Keniry (NMR Centre, ANU) for helpful discussions and Peta Simmonds (NMR Centre, ANU) for technical assistance.

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NP9903245