Draculone, a New Anthraquinone Pigment from the Tropical Lichen *Melanotheca cruenta*

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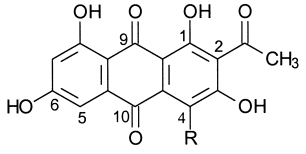
Melanotheca cruenta = *Pyrenula cruenta*, Trypetheliaceae, Anthraquinones, Draculone

A new red anthraquinone, draculone, has been isolated from the corticolous tropical lichen *Melanotheca cruenta* (= *Trypethelium cruentum* = *Pyrenula cruenta*) together with minor quantities of the known anthraquinone pigment haematommone. The structure of draculone was determined as 2-acetyl-1,3,4,6,8-pentahydroxyanthraquinone by spectroscopic methods.

Introduction

In continuing our studies on the chemistry of the tropical lichen family Trypetheliaceae, we report here the analysis of the species *Melanotheca cruenta* (Mont.) Fée, formerly named *Trypethelium cruentum* Mont. *M. cruenta* forms blood-red patches on sun-exposed branches of trees growing on the shores of lakes and marshes in Florida. The lichen was collected from the bark of *Myrica cerifera* and *Ilex cassini.* In this communication we report on the structure of draculone (**1**), the pigment responsible for the red colour. It is accompanied by haematommone (**2**), which has been previously isolated from *Haematomma puniceum* by Huneck *et al.* (1991).



R = OH draculone (**1**)
R = H haematommone (**2**)

Results and Discussion

The lichen was removed from the bark with a scalpel, washed with light petroleum and extracted with ethyl acetate. HPLC of the extract on reversed phase (RP-18) yielded two main fractions, which were analysed by UV/Vis, ^{1}H NMR and ^{13}C NMR spectroscopy as well as mass spectrometry. The yellow and major pigment was identified as haematommone (**2**) by comparison of its UV/Vis, EI-MS and ^{1}H NMR data with those reported in the literature (Huneck *et. al.*, 1991). The red main pigment exhibits absorption maxima in the UV/Vis spectrum at λ_{max} = 210, 232, 276, 306, 512, and 546 nm, suggesting an anthraquinone chromophore substituted with three α-OH groups (Thomson, 1971). The HR EI-MS shows a molecular ion at *m/z* 330, corresponding to the molecular formula $C_{16}H_{10}O_8$. On silylation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) a pentatrimethylsilyl derivative, **3**, was formed suggesting the presence of five hydroxy groups. In the ^{1}H NMR spectrum ([D$_6$]DMSO) a methyl singlet at δ_H 2.52 and two doublets for *meta*-protons at δ_H 6.63 and 7.19 (^4J = 2.0 Hz) are visible. The methyl protons exhibit HMBC correlations to a carbonyl group (δ_C 200.3) indicating the presence of a $COCH_3$ group. This leads to structure **1** for the pigment, which was confirmed by ^{1}H,^{1}H-COSY, HSQC and HMBC experiments (Fig. 1). Due to the low solubility of **1** in most organic solvents, a complete set of ^{13}C NMR data could only be obtained with the pertrimethylsilyl derivative **3**. By virtue of its blood red colour we propose the name draculone for this pigment (Stoker, 1897).

The isolation of draculone (**1**) and haematommone (**2**) from *Melanotheca cruenta* correlates with the known occurrence of quinone pigments in the Trypetheliaceae family. Physcione and seca-

lonic acid have been found in *Trypethelium eluteriae* whilst trypethelone and derivatives are observed in cultures of the mycosymbiont of this species (Mathey *et al.*, 1980). Physcion, endocrocin, isohypocrellin and related perylenequinones occur in *Laurera sanguinaria* and xanthorin in *Laurera purpurina* (Mathey, 1987; Mathey *et al.*, 1987; 1994; Van Roy *et al.*, 1995). Another feature of *Melanotheca cruenta* in common with the Trypetheliaceae family is the fluorescence of the four cells spores (Mathey and Hoder, 1978; Hoder and Mathey, 1980). On this basis it seems more reasonable to use the old name *Melanotheca cruenta* rather than *Pyrenula cruenta*.

Experimental

General

Preparative HPLC: Column: Nucleosil RP-18 (Macherey-Nagel, 250 × 20 mm; 7 μm, UV detection at 310 nm). UV: Perkin-Elmer Lambda spectrophotometer. NMR: Bruker AMX-600 spectrometer (^{1}H at 600.1, ^{13}C at 150.9 MHz), chemical shifts in δ rel. to $CDCl_3$ (δ_H 7.26, δ_C 77.7) or [D_6]DMSO (δ_H 2.49, δ_C 39.5) as internal standard. HR EI-MS: Finnigan MAT 90 instrument using EI at 70 eV.

Lichen material

M. cruenta was removed from the bark of sun-exposed branches of *Ilex cassini* and *Myrica cerifera*, collected on the shores of lakes and swamps in Florida in March 2000 (leg. et det. A. Mathey, D. Griffin).

Extraction and isolation

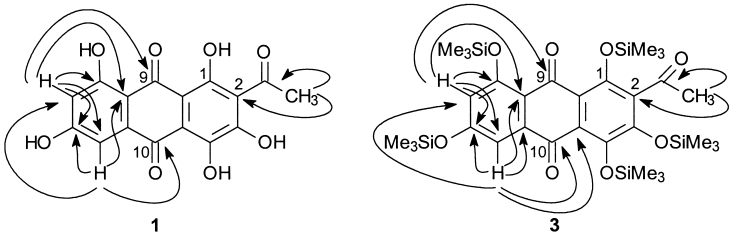
Approximately 200 mg of lichen material from *Melanotheca cruenta* was defatted by washing with light petroleum (b.p. 60–80 °C). The residue was then extracted with ethyl acetate yielding the crude red pigments. They were separated by preparative HPLC on a RP-18 column [solvent A: H_2O/MeCN (9:1 v/v), solvent B: CH_3CN; gradient: 70% A/30% B → 100% B (60 min)] to yield draculone (**1**), R_T = 33.4 min (red solution) and haematommone (**2**), R_T = 35.3 min (yellow solution). The two fractions were each stored in the HPLC solvent mixture overnight at 4 °C to effect crystallisation.

Haematommone (**2**)

Yield 0.3 mg, orange-red needles; UV/Vis: $\lambda_{max}^{CH_3OH}$ (nm): 205, 235, 270, 281, 310, 464; ^{1}H NMR (600 MHz, [D_6]DMSO): δ (ppm): 2.53 (s, 3H, CH_3), 6.58 (d, 1H, H-8), 7.06 (s, 1H, H-4), 7.09 (d, 1H, H-6), 11.19 (s, br, 1H, OH), 11.95 (s, br, 1H, OH), 12.37 (s, br, 1H, OH), 12.96 (s, br, 1H, OH). EI-MS: *m/z* (%): 314 (37), 299 (100), 281 (4), 215 (8), 187 (4), 169 (3).

Draculone (**1**)

Yield 3 mg, dark-red needles, m.p. 242 °C (decomp.). The pink solution in acetonitrile exhibits a spectacular orange-red fluorescence. **1** dissolves in conc. H_2SO_4 with violet colour which turns azure-blue on addition of boric acid. UV/Vis: $\lambda_{max}^{CH_3OH}$ (nm, lg ε) = 210 (4.04), 232 (3.95), 276 (3.93), 306 (3.73), 512 (3.60), 546 (sh, 3.47). ^{1}H NMR (600 MHz, [D_6]DMSO, 292 K): δ (ppm): (s, 3H, CH_3), 6.63 (d, $^4J_{HH}$ = 2.0 Hz, 1H, H-7), 7.19 (d, $^4J_{HH}$ = 2.0 Hz, 1H, H-5), 8.21 (s, 1H, OH), 11.47 (s, 1H, OH), 12.18 (s, 1H, OH), 13.15 (s, 1H, OH) (one of the OH signals could not be observed). ^{13}C-NMR (151 MHz, [D_6]DMSO, 292 K): δ (ppm): 32.1 ($CO\mathit{C}H_3$), 108.3 (C-5), 108.7 (C-7), 109.3 (C-8a), 122.4 (C-2), 164.3 (C-8), 165.1 (C-6), 184.4 (C-9), 186.5 (C-10), 200.3 ($\mathit{C}OCH_3$) (the missing signals could not be detected due to solubility problems). EI-MS: *m/z* (%): 330 (100), 315 (19), 312 (9), 302



1 **3**

Fig. 1. HMBC correlations in **1** and its pertrimethylsilyl derivative **3**.

(15), 287 (24), 284 (16), 260 (22), 245 (6), 242 (4), 241 (8), 231 (4), 228 (4), 213 (7), 189 (6), 161 (4), 137 (4), 134 (2), 91 (1), 77 (2), 43 (5). HR EI-MS: found 330.0377 $[M]^+$ (calc. for $C_{16}H_{10}O_8$ 330.0376).

Pertrimethylsilyl derivative **3**

N-Methyl-*N*-trimethylsilyltrifluoroacetamide (50 µl) was added to 2 mg of **1** and the resulting solution kept for 2 h at 40 °C. Then excess MSTFA was removed under reduced pressure.

^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm): 0.21 [s, 9H, $Si(CH_3)_3$], 0.23 [s, 9H, $Si(CH_3)_3$], 0.24 [s, 9H, $Si(CH_3)_3$], 0.32 [s, 9H, $Si(CH_3)_3$], 0.34 [s, 9H, $Si(CH_3)_3$], 2.47 (s, 3H, $COCH_3$), 6.54 (d, 1H, H-7), 7.19 (d, 1H, H-5). ^{13}C-NMR (151 MHz, $CDCl_3$): δ (ppm): 1.0 [$Si(CH_3)_3$], 1.1 [$Si(CH_3)_3$], 1.3 [$Si(CH_3)_3$], 1.45 [$Si(CH_3)_3$], 1.48 [$Si(CH_3)_3$], 32.7 ($COCH_3$), 112.3 (C-5), 118.6 (C-7), 121.1 (C-8a), 122.0 (C-4a), 125.6 (C-1a), 135.9 (C-2), 138.8 (C-5a), 143.6 (C-4), 146.8 (C-3), 149.8 (C-1), 157.3 (C-8), 160.3 (C-6), 182.4 (C-9), 184.3 (C-10), 202.4 ($COCH_3$).

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