$C_8H_{10}O_2$: 138.0681); 138.0309 (calc. for $C_7H_6O_3$: 138.0317); 125.0235 (calc. for $C_6H_5O_3$: 125.0239).

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4-AMINOPHYSCION, AN ANTHRAQUINONE DERIVATIVE FROM DERMOCYBE (*AGARICALES*)

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Key Word Index—*Dermocybe*; Cortinariaceae; anthraquinones; erythroglaucin; physcion; 4-aminophyscion (4-amino-1,8-dihydroxy-6-methoxy-3-methyl-9,10-anthraquinone).

Abstract—The anthraquinones physcion, erythroglaucin and 4-aminophyscion (4-amino-1,8-dihydroxy-6-methoxy-3-methyl-9,10-anthraquinone) have been isolated from the fungal species *Dermocybe canaria* Horak (ined.). 4-Aminophyscion is reported for the first time as a natural product and represents the first fungal anthraquinone with an amino group.

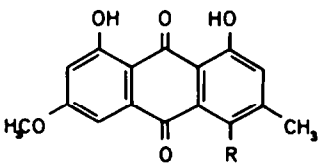
INTRODUCTION

Anthraquinone derivatives occur in a great variety in species of the genus *Dermocybe* where they are responsible for the bright colours of the fruit bodies [1]. Since their occurrence and distribution has proved particularly useful in differentiating infrageneric taxa [2–4] we have examined anthraquinone pigments of *Dermocybe canaria* Horak (ined.), which is a bright yellow to orange coloured species reported from New Zealand.

RESULTS AND DISCUSSION

Extraction of the dried carpophores of the fungus with methanol gave a deep red solution which was concentrated, diluted in hydrochloric acid and subsequently extracted with ethyl acetate to remove pigments from the aqueous phase. The anthraquinones **1** and **2** were separated by column chromatography. Compound **3** needed further isolation by preparative TLC.

The most abundant anthraquinone, yellow crystals, $C_{16}H_{12}O_5$, proved to be spectroscopically indistinguish-



1 R = H
2 R = OH
3 R = NH_2

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able from physcion (1) [5] and this identity was confirmed by direct comparison with an authentic reference sample [6]. The second anthraquinone, red crystals, $C_{16}H_{12}O_6$, was identified by spectroscopic and chromatographic comparison as erythroglaucin (2) [7–9].

Compound (3), red crystals, mp 216–217°, exhibits a mass spectrum with a molecular ion at m/z 299 and fragment ions at m/z 270, 256 and 228. High resolution mass measurement of the molecular ion established the molecular formula $C_{16}H_{13}O_5N$, notably indicating the presence of a nitrogen atom in the molecule. From the ^{1}H NMR spectrum the pigment contains one methyl, one methoxyl and two *peri*-hydroxyl groups, two *meta*-coupled protons (δ6.66 and 7.42, J = 2.6 Hz) and one isolated aromatic proton (δ7.07). A very broad signal in the range δ = 6.40–7.45 exchangeable with D_2O is ascribed to an amino group which is consistent with IR absorption at 3480 cm^{-1}. Strong carbonyl absorption at 1595 cm^{-1} and visible absorption at 418, 495, 519 and 566 nm indicate an anthraquinone chromophore bearing an amino group in the α-position. Smooth formation of physcion (1) on exposure to nitrous acid in aqueous THF establishes structure 3 in which the amino group is placed at position C-4.

Physcion (1) and erythroglaucin (2) have previously been reported from higher fungi. These pigments occur in various species of the genera *Dermocybe* and *Cortinarius* [10–12]. 4-Aminophyscion (3) is reported for the first time as a natural product and represents the first natural anthraquinone derivative with an amino group.

The compounds 1, 2 and 3 are isolated from dried carpophores in yields of 2.3×10^{-2}, 0.7×10^{-3} and 0.2×10^{-3} %, respectively. It is notable that physcion (1) is the main pigment in fruit bodies of *D. canaria* since previous studies [10–12] report it as only a minor compound in species of *Dermocybe* and *Cortinarius*. Furthermore, our isolation of anthraquinones 1, 2 and 3 and the absence of the other typical *Dermocybe* anthraquinones [10, 11, 13] suggest a mode of pigment formation which is unique in the *Dermocybe* group.

The anthraquinones 1, 2 and 3 are most likely derived in this organism from atrochrysone, a metabolite of *Cortinarius* species [1, 12] and probably the precursor of all anthraquinones of the emodin group isolated from *Dermocybe* and *Cortinarius* [1]. It is feasible that physcion (1) is the immediate precursor of erythroglaucin (2) which is derived by hydroxylation in position C-4. Following transamination of a keto form of erythroglaucin (2) may result in formation of 4-aminophyscion (3).

The biosynthesis pathway described above for this *Dermocybe* from New Zealand is biogenetically close to those of *Dermocybe* species from the Northern Hemisphere. An alternative pathway of pigment formation is evaluated in an Australian *Dermocybe* species [14, 15]. This indicates the diversity of *Dermocybe* in Southern Hemisphere. This accords with a TLC survey of several species from this region (G. Keller, ined.).

EXPERIMENTAL

General. ^{1}H NMR spectra were recorded at 90 MHz (WH 90 Bruker) and 400 MHz (WM 400 Bruker) for solns in $CDCl_3$ with TMS as internal standard. Mass spectra were run on MS 30 and MS 50 (70 eV, 300 μA) with data system DS 50 instruments (A. E. I.). IR spectra were measured on a Perkin–Elmer integrated ratio and a SP 1100 (Pye Unicam) spectrophotometer. UV spectra were run on a Cary 17 (Varian) spectrophotometer. Sephadex LH-20 (Pharmacia) and silica gel (Mallinckrodt, Serva) was used for CC. Preparative TLC was performed on silica gel 60 G (Merck) layers (0.1 × 20 × 20 cm) on glass plates.

Fungal material. Carpophores of *D. canaria* were collected in 1982 from Te Anau, South Island, New Zealand. The species was collected and identified by Egon Horak, Geobotanisches Institut, ETH Zürich. Voucher specimens are kept in the herbarium ZT.

Extraction and isolation of anthraquinones. Finely ground air dried carpophores of *D. canaria* (5.23 g) were extracted with MeOH (250 ml) by stirring for 10 hr, at 40°. The extraction was repeated and the combined extracts were evaporated under red. pres. The resulting crude extract was allowed to stand in an excess of 2 M HCl (100 ml) for acid hydrolysis of the glycosidic portion of the anthraquinones. The deep red soln was exhaustively extracted with EtOAc and the organic phase was dried (Na_2SO_4) and evaporated. The resulting deep red solid (0.556 g) was chromatographed on a column (5.5 × 28 cm) of Sephadex LH-20 in MeOH and the eluate was collected in 20 ml fractions. The later fractions containing the anthraquinones were combined and evaporated to dryness under red. pres. The anthraquinone residue (181 mg) was chromatographed on a column (6 × 15 cm) of silica gel (Mallinckrodt). Toluene–CCl_4 (5:2) eluted chromatographically and spectroscopically homogeneous erythroglaucin (2) and physcion (1). A slower moving anthraquinone 3 was eluted with toluene and subsequently purified by preparative TLC with toluene–EtOAc–HOAc (4:4:1).

Erythroglaucin (2). Red needles (EtOAc); mp 204° (3.8 mg); UV λ_{max} ($CHCl_3$) nm (log ε): 233 (4.28), 256 (4.05), 277 (4.05), 308 (3.85), 466 (sh) (3.89), 478 (sh) (3.95), 493 (4.00), 513 (sh) (3.88), 527 (3.83); ^{1}H NMR (90 MHz, $CDCl_3$); δ2.36 (*d*, J = 0.6 Hz, Me), 3.95 (*s*, OMe), 6.71 (*d*, J = 2.6 Hz, 7-H), 7.14 (*s* (br), 2-H), 7.42 (*d*, J = 2.6 Hz, 5-H), 12.35 (*s*, OH), 12.44 (*s*, OH), 13.35 (*d*, J = 0.6 Hz, OH); MS (220°) m/z (rel. int.): 300 $[M]^+$ (100), 257 (9), 229 (6).

4-Aminophyscion (4-amino-1,8-dihydroxy-6-methoxy-3-methyl-9,10-anthracenedione) (3). Red needles (EtOAc); mp 216–217° (1.1 mg); UV λ_{max} (EtOH) nm (log ε): 231 (4.16), 266 (3.87), 311 (sh) (3.54), 398 (3.35), 418 (3.32), 495 (sh) (3.64), 519 (3.79), 566 (3.74); IR ν_{max}^{KBr} cm^{-1}: 3480 (*m*), 2980 (*m*), 2940 (*s*), 2875 (*m*), 1655 (*w*), 1610 (sh) (*m*), 1595 (*ss*), 1545 (*m*), 1450 (*m*), 1380 (*m*), 1335 (*m*), 1285 (sh) (*m*), 1260 (*ss*), 1235 (sh) (*m*), 1190 (w), 1165 (*m*), 1105 (*s*), 1035 (*s*), 915 (w), 860 (*s*), 800 (*s*), 740 (w), 695 (w); ^{1}H NMR (90 MHz, $CDCl_3$); δ2.29 (*d*, J = 0.6 Hz, Me), 3.93 (*s*, OMe), 6.66 (*d*, J = 2.6 Hz, 7-H), 7.07 (*s* (br), 2-H), 7.42 (*d*, J = 2.6 Hz, 5-H), 6.40–7.45 (NH_2), 12.53 (*s*, OH), 12.95 (*s*, OH); MS (180°) m/z (rel. int.): $C_{16}H_{13}O_5N$, calc. 299.0794, found 299.0803 $[M]^+$ (100.00), $C_{15}H_{12}O_4N$, calc. 270.0766, found 270.0743 (6.20), $C_{14}H_{10}O_4N$, calc. 256.0610, found 256.0612 (13.27), $C_{13}H_{10}O_3N$, calc. 228.0661, found 228.0659 (6.71).

Deamination of compound 3. 0.81 mg of 1 were added to 0.20 ml of 2.5 M HCl, 0.4 ml of 2.5 M $NaNO_2$ and 0.25 ml of THF. The soln was kept under argon and stirred at room temp. for 30 min. Reaction was observed by means of TLC. The solvent was removed under red. pres. and the remaining solid was diluted in 20 ml H_2O. Repeated extraction with EtOAc and evaporation of the dried extract (Na_2SO_4) resulted in a yellow pigment (0.56 mg) which is identical to physcion (1) by comparison of its UV, ^{1}H NMR (400 MHz, $CDCl_3$) and mass spectra. Identity was further proved by TLC with a reference sample [7] in three different solvent systems.

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MOSCATILIN, A BIBENZYL DERIVATIVE FROM THE ORCHID *DENDROBIUM MOSCATUM*

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Key Word Index—*Dendrobium moscatum*; Moscatilin; bibenzyl derivative; Orchidaceae.

Abstract—Moscatilin, a new bibenzyl derivative isolated from the orchid *Dendrobium moscatum*, was shown to have the structure 4,4′-dihydroxy-3,3′,5-trimethoxybibenzyl.

INTRODUCTION

In our previous communications [1–14] we reported the structure elucidations of a number of compounds isolated from a series of Indian orchids. These compounds represent several structural types, *viz.*, 9,10-dihydrophenanthrenes [1], 9,10-dihydrophenanthropyrans [2–6] and pyrones [2–4, 7], phenanthrenes [8, 9], bibenzyl derivatives [10], fluorenones [11], triterpenoids [12, 13] and steroids [14]. One of the above phenanthrene derivatives, moscatin (**1**) was earlier isolated [9] from the orchid *Dendrobium moscatum* which also showed the presence of another compound forming a difficultly separable mixture with **1**. Repeated chromatography of this mixture has now resulted in the isolation of this compound in pure and crystalline state. The structure of this compound, designated as moscatilin, has been established as **2a** from the following spectral and chemical evidence.

RESULTS AND DISCUSSION

Moscatilin, $C_{17}H_{20}O_5$ *m/z*: 304 $[M]^+$, mp 84°, showed UV absorptions, λ_{max} 211, 234 sh and 281.5 nm (log ε 4.51, 4.10 and 3.61), which are strikingly similar to those of erianin (**2e**) [10], a bibenzyl derivative of the orchid *Eria carinata*. The phenolic nature of the compound was indicated by its characteristic colour reactions, alkali-induced bathochromic shift of the UV maxima [$\lambda_{max}^{0.1N\ NaOH-EtOH}$ 221, 252.5 and 293.5 nm (log ε 4.28, 4.21 and 3.84)] and IR spectrum showing a band at 3428 cm^{-1}. The presence of two phenolic hydroxyl groups in moscatilin was confirmed by the formation of a diacetyl derivative, $C_{21}H_{24}O_7$ *m/z*: 388 $[M]^+$, mp 112°, with acetic anhydride and pyridine.

The ^1H NMR spectrum of moscatilin showed a nine-proton singlet at δ3.81 for three aromatic methoxyl groups, a four-portion singlet at 2.79 for the four benzylic methylene protons of a bibenzyl derivative, and two one-proton singlets at 5.30 and 5.39 (both disappeared on deuterium exchange) for two phenolic hydroxyl groups. The spectrum also revealed signals for five aromatic protons, two of which appeared as a two-proton singlet at δ6.30. The relatively upfield position of these protons corresponds to the two *ortho*-protons of a 3,4,5-trioxygenated benzyl moiety [10]. The remaining three aromatic protons of moscatilin resonated at δ6.60 (*d*, J = 2 Hz), 6.77 (*d*, J = 8 Hz) and 6.74 (*dd*, J_1 = 8 Hz, J_2 = 2 Hz). The chemical shifts and the splitting patterns of these protons are typical of H-2, H-5 and H-6, respectively, of a 3,4-dioxygenated benzyl system. The foregoing spectral data thus suggest a 3,3′,4,4′,5-pentaoxygenated bibenzyl structure for moscatilin.