SHORT REPORT

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Pigment pattern of the Chilean mushroom *Dermocybe nahuelbutensis* Garrido & E. Horak

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Abstract: Fruiting bodies of the Chilean mushroom *Dermocybe nahuelbutenis* Garrido & E. Horak (syn.: *Cortinarius nahuelbutensis* (Garrido & E. Horak) E. Valenz. & G. Moreno) were chemically investigated for the first time and afforded the new dimeric anthraqinone 7,7'-emodinphyscion (**1**) beside the know anthraquinones dermolutein (**2**), endocrocin (**3**), skyrin (**4**) and the dimeric pre-anthraquinone derivative flavomannin C (**5**). The chemotaxonomic significance of the pigments is discussed.

Keywords: *Dermocybe nahuelbutensis;* fungal fruiting bodies; pigments; chemotaxonomic significance.

1. Fungal Source

Fruiting bodies of the mushroom *Dermocybe nahuelbutenis* Garrido & E. Horak (syn.: *Cortinarius nahuelbutensis* (Garrido & E. Horak) E. Valenz. & G. Moreno) were chemically investigated for the first time. The herein investigated specimens were collected in Chile, Región del Bio Bío, Concepción, 36° 50′ 13′′/ 73° 01′ 28′′ s. l., 160 m.a.s.l., on soil beneath *Nothofagus obliqua* (Mirb.) Oerst. in a remnant of mixed native forest dominated by *N. obliqua* and *Aextoxicon punctatum* Ruiz & Pav., in May 2012 (collection CONC-F0874, leg. et det. G. Palfner & N. Arnold). Voucher specimens are deposited in the Fungarium of Concepción University (CONC-F). A duplicate is deposited at Leibniz Institute of Plant Biochemistry.

2. Previous Studies

The occurrence of neutral anthraquinones, anthraquinone carboxylic acids as well as mono- and dimeric pre-anthraquinones has proved as a valuable aid in the taxonomy of the genus *Dermocybe*[1]. The

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isolation and structure elucidation on the pigments of European *Dermocybe* was mainly carried out in the past by the group of Steglich and for some Australasian species by the group of Gill [5-7]. Comparative paper- and thin layer chromatographic studies were so far performed on European *Dermocybe s*pecies [8-16] as well as on taxa from North America [17], Australia [18] and New Zealand [19]. Only two South American species (*D. amoena, D. icterina)* occurring in Argentinia [19,20] and Chile [21] has been investigated to date.

3. Present Study

Air-dried and powdered fruiting bodies (20 g) were extracted exhaustively with acidified acetone. After evaporation of the solvent, the resulting crude extract (2.9 gr) was dissolved in water, partitioned with ethyl acetate and evaporated to dryness. Separation of the ethyl acetate extract by column chromatography on acetylated polyamide (Polyamid MN SC 6-AC, Macherey, Nagel & Co., Düren) using a series of solvents with increasing polarity (benzene, ethyl acetate, acetone, methanol) yielded 11 fractions. Fraction 2 was chromatographed over silicagel 60 (70-230 mesh, Merck) using the isocratic solvent system toluene : ethyl acetate : chloroform 2:1:2 and yielded compound **1** (10 mg).

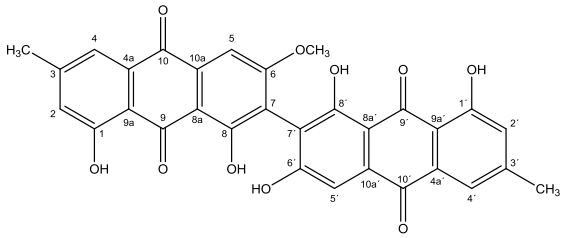
*7,7'-Emodinphyscion (**1**):* Yellow amorphous powder; R_f = 0.64 (solvent toluene : ethylformiate : formic acid 10/5/3 v/v); UV (MeOH): λ_{max} 200, 285, 440, 461. ^{1}H NMR (600 MHz, DMSO-*d6*): δ (ppm) = 2.49 (3H, s, 3'-CH_3), 2.51 (3H, s, 3-CH_3), 4.00 (3H, s, 6-OMe), 7.24 (1H, br s-like, H-2'), 7.28 (1H, br s-like, H-2), 7.34 (1H, very br s, H-5'), 7.53 (1H, br s, H-5), 7.58 (1H, br s-like, H-4'), 7.64 (1H, br s-like, H-4), 11.99 (1H, br s, OH), 12.18 (1H, br s, OH), 12.5 (1H, br s, OH). ^{13}C NMR (125 MHz, DMSO-*d6*): δ (ppm) = 21.5 (2 x CH_3), 56.5 (CH_3, 6-OMe), 103.0 (CH, C-5), 107,8 (C, C-8a'), 110.4 (C, C-8a), 112.6 (C, C-7'), 113.5 (C, C-9a'), 113.7 (C, C-9a), 115.5 (C, C-7), 120.3 (CH, C-4'), 120.6 (CH, C-4), 124.0 (CH, C-2'), 124.2 (CH, C-2), 132.8 (C, C-4a'), 132.9 (C, C-4a), 134.1 (C, C-10a'), 134.5 (C, C-10a), 147.8 (C, C-3'), 148.6 (C, C-3), 161.2 (C, C-8), 161.3 (C, C-1'), 161.5 (CH, C-1), 162.4 (C, C-8'), 164.0 (C, C-6), 181.2 (C, C-10), 181.6 (C, C-10'), 190.5 (C, C-9). HR-ESI-MS : *m/z* 551.09876 $[M-H]^-$ (calc. 551.09837 for $C_{31}H_{19}O_{10}^-$).

Compound **1**, 7,7'-emodinphyscion, was isolated as a yellow amorphous powder, for which the UV spectrum showed λ_{max} at 200, 285, 440, 461 nm and in combination with the bathochromic shift after adding NaOH to λ_{max} at 287, 522 nm indicating its anthraquinonic nature. Its molecular formula was determined as $C_{31}H_{20}O_{10}$ by the HRESIMS $[M-H]^-$ ion at *m/z* 551.09876 (calc. for $C_{31}H_{19}O_{10}^-$ 551.09837). ^{1}H NMR signals corresponding to aromatic rings of the dimeric anthraquinone skeleton were observed at δ_H 7.64 (br s-like), 7.58 (br s-like), 7.53 (br s), 7.34 (very br s), 7.28 (br s-like), and 7.24 (br s-like). Three of the five OH protons show relatively sharp singlets at δ_H 12.50, 12.18, and 11.99. The remaining two OH signals are too broad to be detected. Three singlets corresponding to three protons each belong to two methyl (δ_H 2. 51, 2.49) and one *O*-methyl group (δ_H 4.00). Based on the HMBC and NOE correlations as well as comparison with literature data of the structural related compounds **5** [22] and **6** [23], compound **1** was established as a new bisanthraquinone named 7,7'-emodinphyscion (Fig. 1). Some of the NMR signals could not be detected, presumably due to a hindered rotation around the biaryl axis resulting in severe signal broadening. Therefore, the proposed structure was verified by dehydration and alkaline oxidation of flavomannin C (**5**) according literature [23], which yielded 7,7'-emodinphyscion (**1**).

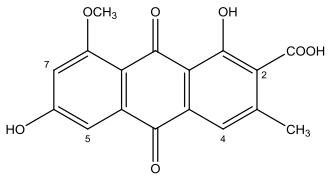
Repeated column chromatography of fraction 8 and 11 on Sephadex LH 20 (solvents methanol and acetone: methanol 4:1 v/v) resulted in detection of three known compounds **2-5**. The structures of the isolated compounds **2-5** were determined based on their spectroscopic data (^{1}H-NMR, ^{13}C-NMR, ESI-FTICR-MS, UV/Vis) and comparison with published data as dermolutein (**2**) (13.6 mg) [24, 25], endocrocin (**3**) (8.1 mg) [24, 25, 26], skyrin (**4**) (4.3 mg) [27] and the recently described dimeric pre-

[1] The taxonomic rank of *Dermocybe* is still object of discussion. Some mycologist regard *Dermocybe* as a separate genus [1,2], others insert *Dermocybe* as a subgenus in genus *Cortinarius* [3,4]. In accordance with chemical literature [5, 6] we treat here *Dermocybe* as a genus.

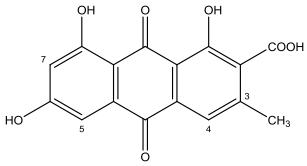
anthraquinone derivative flavomannin C (**5**) (9.7 mg) [22]. The obtained spectral data of the isolated compounds **2**-**5** were in agreement with the relevant references.



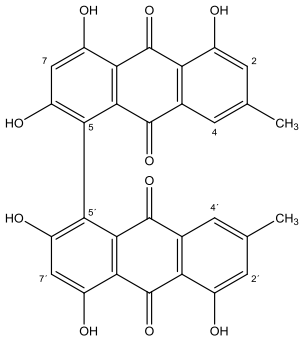
7,7'-Emodinphyscion (**1**)



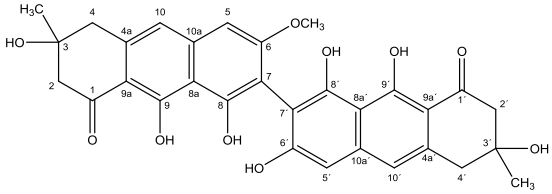
Dermolutein (**2)**



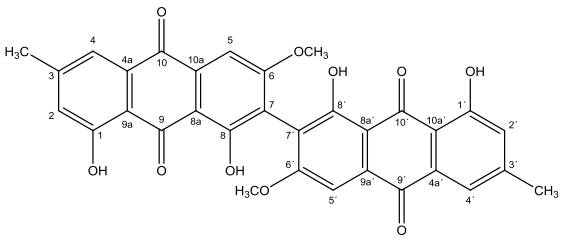
Endocrocin (**3**)



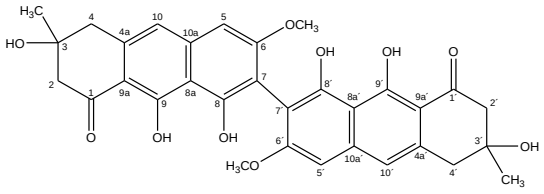
Skyrin (**4**)



Flavomannin C (**5**)



7,7'-Biphyscion (**6**)



Flavomannin-6,6'-di-*O*-methylether (**7**)

Figure 1. Isolated compounds from *Dermocybe nahuelbutensis.*

This study reported for the first time the chemical investigation of fruiting bodies of *D. nahuelbutensis*, a mushroom so far only known from Chile. Around 18 *Dermocybe* species are named for South America (Argentinia, Chile), among them 10 species for Chile [21]. Two naturally occurring anthraquinones, dermolutein (**2**) and endocrocin (**3**), and the bisanthraquinone skyrin (**4**), well known from different *Dermocybe* species [5] as well as the dimeric pre-anthraquinone flavommanin C (**5**), so far only known from *Talaromyces wortmannii*, an endophyte of *Aloe vera* [22], were isolated from fruiting bodies of the Chilean mushroom *D. nahuelbutensis* (Fig. 1). Additionally, the structure of the new compound 7,7'-emodinphysion (**1**), was determined according the spectroscopic data (Fig. 1). The occurrence of **1** may be an artefact formed during the isolation procedure of the dimeric pre-anthraquinone precursor flavomannin C (**5**). A similar origin was suggested for 7,7'-biphyscion (**6**), detectable in many European *Dermocybe*-species. In this case, flavomannin-6,6'-di-*O*-methylether (**7**), formed by oxidative coupling of two torosachrysone units, was suggested as potential precursor of **6** [5]. In European and North American taxa of *Dermocybe*, (pre-) anthraquinonoid pigments are important characters in species delimitation and in circumscription of sections within the genus [8, 15, 28]. Through the occurrence of dermolutein (**2**), endocrocin (**3**) and skyrin (**4**), *D. nahuelbutensis* shows a similar pigmentation pattern like *Dermocybe* species from the Northern hemisphere, but differs from Australasian and European species by the occurrence of 7,7'-emodinphyscion (**1**) and the mono-*O*-methyl dihydroanthracenone derivative flavomannin C (**5**). In opposite, oxidative 7,7' coupled di-*O*-methyl dihydroanthracenone derivatives like flavomannin-6,6'-di-*O*-methylether (**7**) are widespread in *Dermocybe*-species from the Northern hemisphere, but very rare in Australasian taxa and could so far only be detected in two species [6]. Therefore, we suggest from the chemotaxonomical point of view through the occurrence of the mono-*O*-methylated pigments such as 7,7'-emodinphyscion (**1**) and flavomannin C (**5**) in *D. nahuelbutensis*, that Chilean *Dermocybe* species present a separate linage in evolution of *Dermocybe*, which is more related to species of the Northern hemisphere than to Australasian species. This suggestion, based on pigment-chemical characters is meanwhile also supported on DNA level by the use of DNA sequences for recent phylogenetic studies in genus *Cortinarius* including *Dermocybe* [29].

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Supporting information

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References

[1] M. Moser (1983). Kleine Kryptogamenflora, Band II b/2, Basidiomyceten: Die Röhrlinge und Blätterpilze, Kleine Kryptogamenflora. Gustav Fischer Verlag, Stuttgart, New York, pp. 1–533.
[2] Y. J. Liu, S. O. Rogers and J. F. Ammirati (1997). Phylogenetic relationships in *Dermocybe* and related *Cortinarius* taxa based on nuclear ribosomal DNA internal transcribed spacer, *Can. J. Bot*. **75**, 519-532.
[3] U. Peintner, J. M. Moncalvo and R. Vilgalys (2004). Toward a better understanding of the infrageneric relationships in *Cortinarius* (Agaricales, Basidiomycota), *Mycologia* **96**, 1042-1058.
[4] S. Garnica, M. Weiss and F. Oberwinkler (2003). Morphological and molecular phylogenetic studies in South American *Cortinarius* species, *Mycol Res.* **107**, 1143-1156.
[5] M. Gill and W. Steglich (1987). Pigments of fungi, *Progr. Chem. Org. Chem. Nat. Prod*. **51**, 1-297.
[6] M. Gill (1995). Pigments of Australasian *Dermocybe* toadstools, *Aust. J. Chem.* **48**, 1-26.
[7] M. Gill (2001). The biosynthesis of pigments in Basidiomycetes, *Aust. J. Chem.* **54**, 721-734.
[8] N. Arnold, H. Besl, A. Bresinsky and H. Kemmer (1987). Notizen zur Chemotaxonomie der Gattung *Dermocybe* (Agaricales) und zu ihrem Vorkommen in Bayern. *Z. Mykol*. **53**, 187-194.
[9] M. Gabriel (1960). IV. Deuxième contribution à la connaissance de la pigmentation des Cortinaires des groupes Sanguinei et Cinnamomei, *Ann. Univ. Sc. Nat. Lyon* **11-12**, 67-76.
[10] M. Gabriel (1960). Recherches sur les pigments des Agaricales. III. - Pigments des Cortinaires des groupes Cinnamomei et Sanguinei, *Bull. Soc. Mycol. France* **76**, 208-215.

[11] M. Gabriel (1961). V. Troisième contribution à l'ètude des pigments des Cortinaires des groupes Sanguinei et Cinnamomei (Elegantes Fries), *Bull. Soc. Mycol. France* **77**, 262-272.
[12] M. Gabriel and D. Lamoure (1965). *Cortinarius (Dermocybe) uliginosus* Berk. var. *luteus* n. var., *Bull. Soc. Mycol. France* **81**, 258-263.
[13] I. Gruber (1970). Anthrachinonfarbstoffe in der Gattung *Dermocybe* und Versuch ihrer Auswertung für die systematik, *Z. Pilzk.* **36**, 95-112.
[14] K. Høiland (1981). Kanel-slørhattene (Cortinarius, underslægten Dermocybe) i Norden, *Svampe* **4**, 63-73.
[15] K. Høiland (1983). Cortinarius subgenus Dermocybe, *Opera Botanica* **71**, 1-113.
[16] G. Keller (1982). Pigmentationsuntersuchungen bei europäischen Arten aus der Gattung *Dermocybe* (Fr.) Wünsche, *Sydowia* **35**, 110-126.
[17] G. Keller and J. F. Ammirati (1983). Chemotaxonomic significance of anthraquinone derivatives in North American species of *Dermocybe*, sect. *Sanguineae*, *Mycotaxon* **18**, 357-377.
[18] R. H. Jones and T. W. May (2008). Pigment chemistry and morphology support recognition of *Cortinarius austrocinnabarinus* sp. nov. (Fungi: Cortinariaceae) from Australia, *Muelleria* **26**, 77-87.
[19] G. Keller, M. Moser, E. Horak and W. Steglich (1987). Chemotaxonomic investigation of species of *Dermocybe* (Fr.) Wünsche (Agaricales) from New Zealand, Papua New Guinea and Argentina, *Sydowia* **40**, 168-187.
[20] I. Gruber (1975). Papierchromatographische Pigmentanalyse von südamerikanischen Dermocyben und Cortinarien. In: M. Moser and E. Horak. *Cortinarius* Fr. und naheverwandte Gattungen in Südamerika, *Beih. Nova Hewigia* **52**, 524-540.
[21] N. Garrido (1988). Agaricales s.l. und ihre Mykorrhizen in den *Nothofagus*-Wäldern Mittelchiles, *Bibl. Mycol.* **120**, 1-528.
[22] R. Bara, I. Zerfass, A. H. Aly, H. Goldbach-Gecke, V. Raghavan, P. Sass, A. Mándi, V. Wray, P. L. Polavarapu, A. Pretsch, W. H. Lin, T. Kurtán, A. Debbab, H. Brötz-Oesterhelt and P. Proksch (2013). Atropisomeric dihydroanthracenones as inhibitors of multiresistant *Staphylococcus aureus*, *J. Med. Chem.* **56**, 3257-3272.
[23] W. Steglich, E. Töpfer-Petersen, W. Reininger, K. Gluchoff and N. Arpin (1972). Isolation of flavomannin-6,6′-dimethyl ether and one of its racemates from higher fungi, *Phytochemistry* **11**, 3299-3304.
[24] W. Steglich, W. Lösel and V. Austel (1969). Pilzpigmente, IV. Anthrachinon-Pigmente aus *Dermocybe sanguinea (*Wulf. ex Fr.) Wünsche und *D. semisanguinea* (Fr.), *Chem. Ber.* **102**, 4104-4118.
[25] M. Gill, P. M. Morgan, J. M. White and J. Yu (1998). Pigments of fungi. XLVII. Cardinalic acid, a new anthraquinone carboxylic acid from the New Zealand toadstool *Dermocybe cardinalis* and the synthesis and X-ray crystal structure of methyl 1,7,8-tri-*O*-methylcardinalate, *Aust. J. Chem.* **51**, 213-218.
[26] Y. Asahina and F. Fuzikawa (1935). Untersuchungen über Flechtenstoffe, LV. Mitteil.: über Endocrocin, ein neues Oxy-anthrachinon-Derivat, *Ber. dtsch. chem. Ges.* **68**, 1558-1565
[27] H. J. Banks, D. W. Cameron and W. D. Raverty (1976). Chemistry of the *Coccoidea*. II. Condensed polycyclic pigments from two Australian pseudococcids (Hemiptera), *Aust. J. Chem.* **29**, 1509-1521.
[28] G. Keller and J. F. Ammirati (1995). Chemotaxonomic studies on the pigmentation of North American species of *Dermocybe* (Fr.) Wünsche, section Dermocybe and related species, *Beih. Sydowia* **10**, 127-136.
[29] S. Garnica, M. E. Schön, K. Abarenkov, K. Riess, K. Liimatainen, T. Niskanen, B. Dima, K. Soop, T. G. Frøslev, T. S. Jeppesen, U. Peintner, R. Kuhnert-Finkernagel, T. E. Brandrud, G. Saar, B. Oertel and J. F. Ammirati (2016). Determining threshold values for barcoding fungi: lessons from *Cortinarius* (Basidiomycota), a highly diverse and widespread ectomycorrhizal genus, *FEMS Microbiol. Ecol.* **92**, 1-16.