Absolute stereochemistry of fungal metabolites: icterinoidins A1 and B1, and atrovirins B1 and B2[#]

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Dedicated, with respect and gratitude, to Professor Rodney W. Rickards in celebration of his 70th birthday

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Abstract

The absolute stereochemistry at the C 3 (and C 3', where appropriate) chiral centre(s) in the coupled dihydroanthracenones, the icterinoidins A_1 and B_1 and atrovirin B_2 (from *Dermocybe icterinoides*), is deduced by application of the '*syn-anti* rule', which relies on an empirical relationship between the sign of the Cotton effect couplet centred close to 275 nm in the CD spectrum and the chemical shift of the enantiotopic methylene protons at C 4 in the ^{1}H NMR spectrum of these pre-anthraquinones. The conclusions also allow assignment of central stereochemistry to atrovirin B_1 (from *Cortinarius atrovirens*). In addition, we have applied Steglich's kinetic resolution method to confirm the (*P*)-axial configuration of icterinoidin B_1, previously deduced by using Nakanishi's 'exiton chirality' method.

Keywords: Fungal pigments, dihydroanthracenone dimmers, icterinoidins A_1, B_1, atrovirin B_2, stereochemistry, atropisomers, *Dermocybe icterinoides*, chemotaxonomy

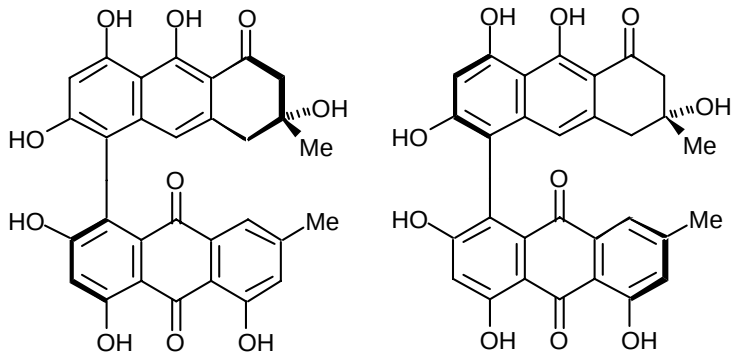
Introduction

In an earlier paper in this series[1] we described, *inter alia*, the isolation and structural elucidation of two new atropisomeric 5,5'-coupled dihydroanthracenone–anthraquinones, the icterinoidins A_1 (**1**) and B_1 (**2**), atrovirin B_2 (**3**), a diastereoisomer of the known atrovirin B_1 (**4**)[2] (no central stereochemistry yet implied), and the well known orange pigment (*P*)-(+)-skyrin (**5**)[3] from the

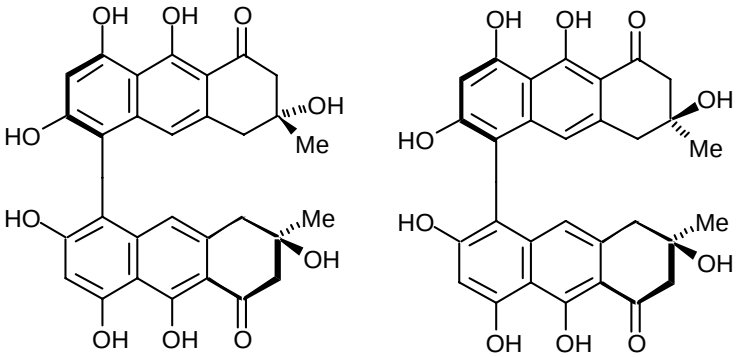
#Part 73 in the series 'Pigments of Fungi'; for Part 72 see: Donner, C. D.; Gill, M.; Tewierik, L. *Molecules*, **2004**, *9*, 498.

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ethanolic extracts of the pale-green capped toadstool *Dermocybe icterinoides*, first described by Horak[4] and examined chromatographically by Keller *et al.*,[5] which was gathered by us in native forest on the South Island of New Zealand. The axial stereochemistry of the natural products **1**, **2** and **3** was evident by inspection of the sign of the long and short wavelength maxima and minima of the intense Cotton effect doublet close to 275 nm resulting from 'exciton coupling' between the two extended naphthalene chromophores in molecules of this type.[2b,6-9] However, at that time, the stereochemistry at the C 3 and C 3' chiral centres in the icterinoidins A_1 (**1**) and B_1 (**2**) and in atrovirin B_2 (**3**) [and in the known atrovirin B_1 (**4**)][2] was not known. Nevertheless, the CD spectra of the icterinoidins **1** and **2** reveal that they are atropisomers, and consequently, that both compounds must have the same chirality at the C 3 and C 3' stereogenic centres. Similarly, while it was plain that the atrovirins B_2 (**3**) (from *D. icterinoides*)[1] and B_1 (**4**) (from *Cortinarius atrovirens*)[2,9] have near super imposable B-type CD curves,[1] distinct differences in the respective ^{1}H NMR spectra established that these pigments too must be diastereoisomers. We describe herein the application of the 'syn-anti rule',[2b,9] which exploits the empirical relationship between the respective CD and ^{1}H NMR spectra of individual pre-anthraquinones such as **1**, **2**, **3**, and **4**, which allows the determination of the absolute central stereochemistry in all four of these complex natural products.



1 **2**



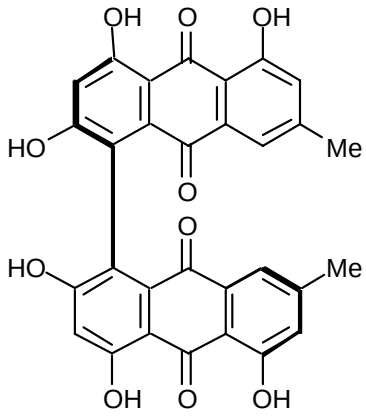
3 **4**

The axial stereochemistry of icterinoidin B_2 (**2**), previously defined by the 'exciton chirality' method,[1] is confirmed by application of Steglich's kinetic resolution method.[9]

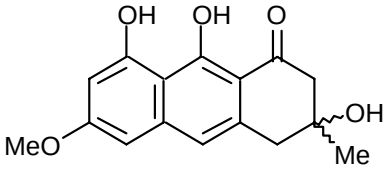
Results and Discussion

Details of the isolation and purification of the icterinoidins A_1 (**1**), B_1 (**2**) and atrovirin B_2 (**3**) from *Dermocybe icterinoides* are described in detail elsewhere[1] and need not be repeated here.

The axial chirality of dimeric pre-anthraquinones of the type discussed here is conveniently determined by inspection of the CD spectrum in which the sign of an intense ($\Delta\varepsilon \approx 100$) Cotton effect couplet centred near 275 nm can be directly correlated with the helical twist between the asymmetric chromophores.[6-9] Thus, a compound exhibiting a negative Cotton effect at longer wavelength and a positive one at shorter wavelength (an 'A-type' curve according to Steglich)[10] is consonant with 'negative chirality' (an anticlockwise twist between the aromatic chromophores),[6] while a compound showing the mirror image Cotton effect couplet (a 'B-type' curve)[10] corresponds to 'positive chirality' (a clockwise aromatic helical twist).[6] In the case of the icterinoidins and atrovirins this leads, according to the Prelog-Helmchen rules,[11] to the (*P*)-axial stereochemistry for icterinoidin B_1 (**2**) and atrovirin B_2 (**3**) and the (*M*)-axial chirality for icterinoidin A_1 (**1**).[1]



5



6

A far more demanding task in coupled pre-anthraquinones of this type is the determination of the absolute configuration at the chiral centres. Although chemical methods have been developed in certain cases,[9,12] (*vide infra*), an empirical relationship between the axial configuration, evident from the CD spectrum, and the chemical shift difference ($\Delta\delta$) in the ^{1}H NMR signals of the diastereotopic C 4 methylene protons can be reliably translated into the absolute stereochemistry at the adjacent C 3 chiral centre(s). In some cases, the emergent conclusions have been corroborated by chemical back-up.[13-15]

This relationship, which was pioneered by Oertel and Steglich,[2b] notes the difference in the magnitude of anisotropic influence of one half of the pre-anthraquinone dimer on the C 4 protons of the other. Thus, the *pseudo*-axial and *pseuedo*-equatorial protons at C 4 in the monomer system, torosachrysone (**6**) and its derivatives, resonate in the ^{1}H NMR spectrum, near coincidentally, between δ 3.02 and 3.10.[10,16] This is also the case in 7,7'-linked dimers belonging

to the flavommanin group of torosachrysone dimers,[10] in which the biaryl linkage is remote and therefore the C 4 protons are relatively unperturbed by any anisotropic influence from the second aromatic ring. However, in the spectra of 5,5'- (atrovirin),[2] 5,10'-(pseudophlegmacin),[17] 7,10'-(phlegmacin)[18] and 10,10'- (tricolorin)[19] dimers, in which the C 4 methylene protons are in the zone of influence of the adjacent biaryl ring system, differential shielding can be translated in streochemical terms.[20] The method is particularly effective in those cases where more than one diastereoisomer of a biaryl system is known as a natural product.[20]

A plausible rationale for these spectroscopic observations is illustrated here by using the simplified model systems that are shown in Figures 1 and 2. Thus, in a dimer with the (3*S*,*M*)-[or the (3*R*,*P*)]-stereochemistry [Figure 1, (a) and (b)], the C 4 methylene protons are shielded, more or less equally so, by the appended C 5 naphthalene ring system, and signals from both protons are near-coincident or show only a small $\Delta\delta$ that is typically ≤0.08 ppm. Since the hydroxyl group at C 3 in the dihydroanthracenone ring occupies an axial configuration, it follows that the relative stereochemistry between C 3 and the biaryl axis in this case must be (3*S**,*M**). This relative disposition of the naphthalene rings and the C 3 hydroxyl was termed 'anti' by Oertel.[2b,10]

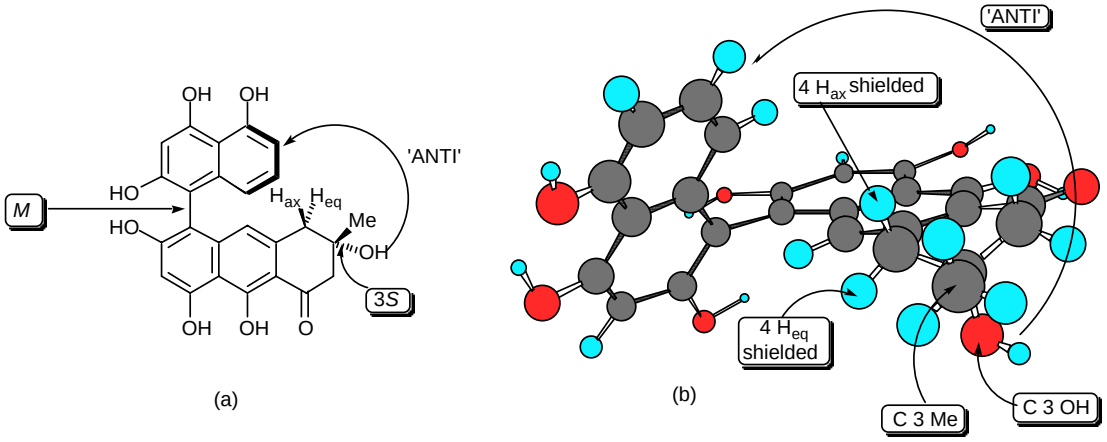


Figure 1. (a) Structure of a model coupled naphthalene-dihydroanthracenone system with (3*S**,*M**)-relative stereochemistry, and (b) Chem-3D MM2™ energy minimized structure of the structure shown in (a).

In contrast, and as is evident from Figure 2, (a) and (b), the corresponding C 4 methylene protons in a dimer with the (3*R*,*M*)- [or the (3*S*,*P*)]-stereochemistry the H_{eq} 4 proton is differentially shielded with respect to its H_{ax} 4 counterpart and, consequently, in the ^{1}H NMR spectrum of a dimer with the (3*R**,*M**)-relative stereochemistry, the $\Delta\delta$ 0.15-0.25 ppm. The relative disposition of the naphthalene ring and the C 3 hydroxyl group, in this case, was referred to as 'syn' by Oertel.[2b,10]

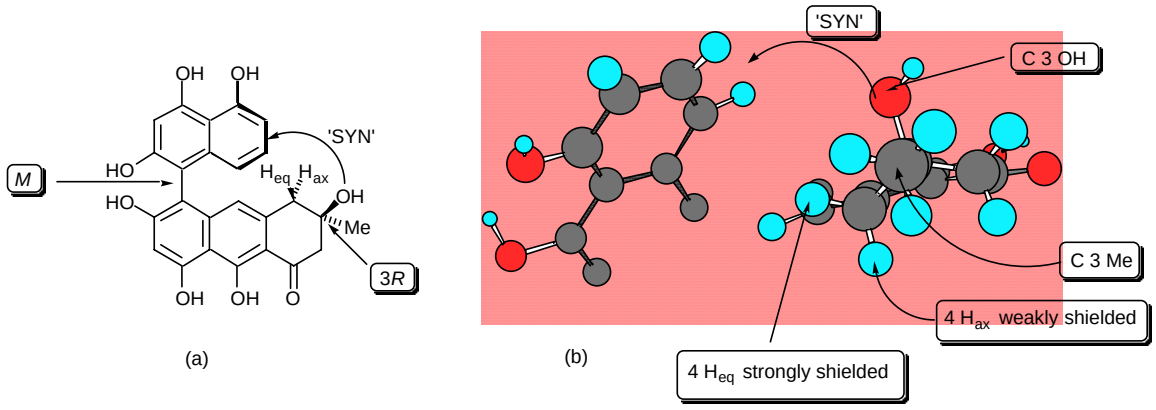


Figure 2. (a) Structure of a model coupled naphthalene-dihydroanthracenone system with (3*R**,*M**)-relative stereochemistry, and (b) Chem-3D MM2™ energy minimized of the structure shown in (a).

Turning now to the natural products **1**-**4**, the ^{1}H NMR spectroscopic data for the C 4 methylene protons of the icterinoidins A_1 (**1**) and B_1 (**2**), and the atrovirins B_2 (**3**)[1] and B_1 (**4**)[2] are collected in Table 1. The spectrum of icterinoidin A_1 (**1**) contains an AB quartet with components centred at δ 2.75 and 2.90 ($\Delta\delta$ = 0.15 ppm). This relatively large shift difference categorizes **1** as belonging in the *syn* model (Figure 2) and, since the pigment exhibits an A-type CD Cotton effect curve, it follows that the absolute stereochemistry of icterinoidin A_1 (**1**) is (3*R*,*M*). In contrast, the C 4 methylene protons in the ^{1}H NMR spectrum of icterinoidin B_1 (**2**) resonate closer together at δ 2.90 and 2.85 ($\Delta\delta$ = 0.05 ppm) corresponding to the *anti* model (Figure 1) and therefore **2** has the (3*R*,*P*)-absolute configuration.

The signals from H_{ax} 4,4' and H_{eq} 4,4' in the ^{1}H NMR spectrum of atrovirin B_2 (**3**) appear together as a broad, two-proton singlet at δ 2.87. This is in accord with the *anti* model (Figure 1) and, when coupled with a B-type CD curve, leads to the (3*R*,*P*,3'*R*) absolute configuration for **3**. It is likely that atrovirin B_2 is a biogenetic precursor of icterinoidin B_1 in *Dermocybe icterinoides* (see Scheme 3).

Finally, the ^{1}H NMR spectrum of atrovirin B_1 (**4**), a compound isolated by Steglich *et al*. from *Cortinarius atrovirens*[2] that, like **3**, has (*P*)-axial chirality according to the CD curve,[9] H_{eq} 4,4' and H_{ax} 4,4' appear as an AB quartet with components well separated (δ 2.73 and 2.95, respectively; $\Delta\delta$ = 0.22), which accords with the (3*S*,*P*,3'*S*)-absolute stereochemistry for atrovirin B_1.

Table 1. Selected ^{1}H NMR and CD data from the natural products **1**, **2**, **3**[1] and **4**[2] that are involved in the determination of their absolute axial and central stereochemistry

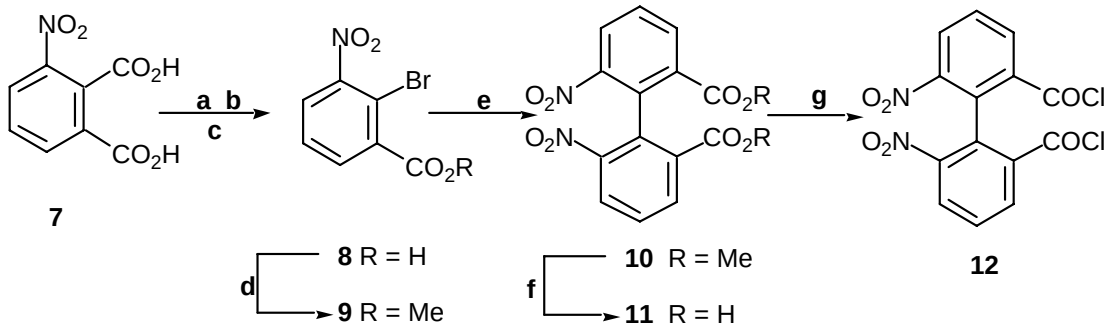
Pigment	Chemical shift (δ), multiplicity and coupling constants (J, Hz) for H_2 4 in δ_6-acetone		$\Delta\delta$ H_{ax}–H_{eq}	CD type	Absolute configuration
	H_{ax} 4	H_{eq} 4			
Icterinoidin A_1 (**1**)	2.90, d, 17.6	2.75, d, 17.6	0.15	A	3*R, M*
Icterinoidin B_1 (**2**)	2.90, d, 16.0	2.85, d, 16.0	0.05	B	3*R, P*
Atrovirin B_2 (**3**)	2.87, 2H, br. s	—	≤0.01	B	3*R, P,* 3'*R*
Atrovirin B_1 (**4**)[2b]	2.95, d, 16.5	2.73, d, 16.5	0.22	B	3*S, P,* 3'*S*

Chemical verification of the (M)- and (P)-absolute axial stereochemistry of the icterinoidins A_1 (1) and B_1 (2), respectively

Steglich and coworkers developed a chemical method for the determination of the axial configuration of the dimeric pre-anthraquinones flavomannin A_1 and atrovirin B_1 (**4**).[9] The method relies for its effectiveness on the kinetic resolution of (±)-6,6'-dinitrodiphenic acid dichloride (**12**) by the various axially chiral natural products. The method was first calibrated using the individual (*M*)- and (*P*)-atropisomers (**13a**) and (**13b**), respectively, of 2,2'-dihydroxy-1,1'-binaphthol, the absolute stereochemistry of which was already known. Thus, cyclic diester formation between the (*M*)-binaphthol (**13a**) and (±)-(**12**) gave residual excess of the (*P*)-(–)-atropisomer (**14**) of the diphenic acid. Conversely, when the (*P*)-binaphthol (**13b**) was reacted with (±)-(**12**) a residual excess of the (*M*)-(+)-diphenic acid (**15**) was obtained. The method was subsequently applied successfully to flavomannin A_1 and to atrovirin B_1 (**4**).[9]

We elected to apply this method to icterinoidin B_1 (**2**) [and therefore, by default, to icterinoidin A_1 (**1**)] in order to confirm the conclusions drawn previously from the CD method.[1]

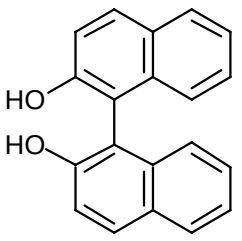
Consequently, (±)-6,6'-dinitrodiphenic acid dichloride (**12**) was first prepared according to literature methods (Scheme 1).[21-24] Treatment of 3-nitrophthalic acid (**7**) with mercuric acetate followed by bromination of the intermediate organomercurate, gave 2-bromo-3-nitrobenzoic acid (**8**). Esterification of (**8**) followed by reductively coupling of the bromo-ester (**9)** using copper powder at high temperature gave the biaryl ester (**10),** hydrolysis of which gave 6,6'-dinitrodiphenic acid (**11**). Exposure of (**11**) to thionyl chloride afforded 6,6'-dinitrodiphenic acid dichloride (**12**).



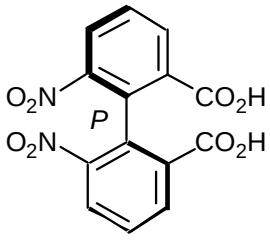
Scheme 1. (a) $Hg(OAc)_2$, NaOH (2.5 M), reflux, 90 h; (b) NaBr, Br_2, NaOH (2.5 M), reflux, 5 min; (c) HCl (conc.); (d) MeOH, HCl, reflux, 12 h; (e) Cu, 170 °C, 1 h; (f) NaOH (s), EtOH (aq), reflux, 3 h, then HCl (conc.); (g) $SOCl_2$, reflux, 24 h.

To test the efficacy of the method in our hands, Steglich's methodology was first repeated using commercially available (*M*)-2,2'-dihydroxy-1,1'-binaphthalene (**13a**) and the (±)-acid chloride (**12**) (Experimental). This gave a residual excess of the (*P*)-(–)-diphenic acid (**14**) (Table 2) in an excess close to that observed by Steglich.[9] Similarly, reaction between (±)-6,6'-dinitrodiphenic acid dichloride (**12**) and (*P*)-(+)-skyrin (**5**), from *D. icterinoides*, gave a residual excess of the (*M*)-(+)-diphenic acid (**15**), identical in specific rotation with Steglich's result with material isolated from *Cortinarius odoratus* (Table 2).[25] Both results are consistent with a (*P*)-axial stereochemistry for (+)-skyrin (**5**).

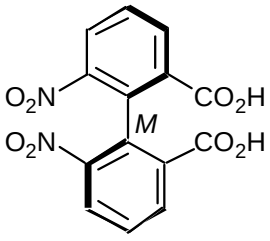
Confident that our techniques are reproducible, the method was next applied to icterinoidin B_1 (**2**). After exposure to (±)-6,6'-dinitrodiphenic acid dichloride (**12**), work up gave a residual excess of (*M*)-(+)-6,6'-dinitrodiphenic acid (**15**) (Table 2). Icterinoidin B_1 (**2**) must therefore have reacted faster with the (*P*)-(–)-6,6'-dinitrodiphenic acid dichloride to form the cyclic diester **16** (Scheme 2). This is in full accord with the conclusion drawn from the CD spectrum, i.e., that icterinoidin B_1 (**2**) has the (*P*)-axial configuration. Icterinoidin A_1 (**1**) can therefore be assigned the complementary (*M*)-configuration at the chiral axis.



13a (*M*)-chirality
13b (*P*)-chirality



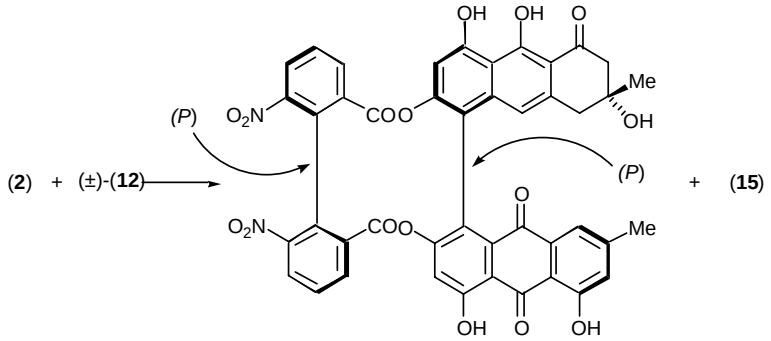
14



15

Table 2. Specific rotation of recovered 6,6'-dinitrodiphenic acid (**14**) or (**15**) from kinetic resolution of (±)-6,6'-dinitrodiphenic acid dichloride (**12**) by (*M*)-binaphthol (**13a**), (*P*)-skyrin (**5**) and icterinoidin B_1 (**2**)

Compound	Specific rotation $[\alpha]_D$ of residual dinitrodiphenic acid	$[\alpha]_D$ of residual dinitro-diphenic acid according to Steglich[9]
M)-2,2'-Dihydroxy-1,1'-binaphthyl **13**	–19.8 (1.2 g/100 mL)	–21.4
(*P*)-Skyrin (**5**)	+20.4 (1.6 g/100 mL)	+20.4
Icterinoidin B_1 (**2**)	+18.0 (1.4 g/100 mL)	—



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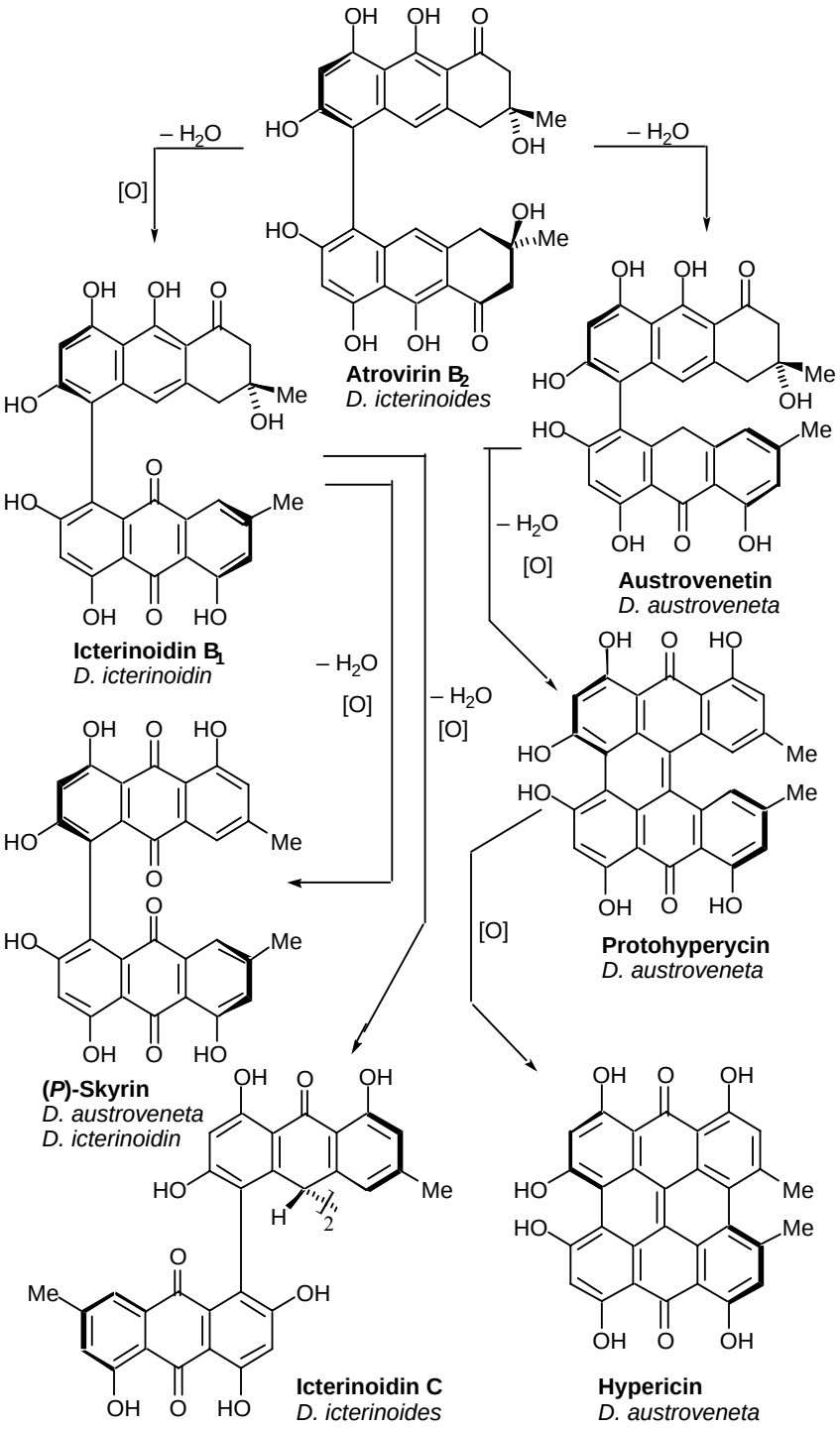
Scheme 2. Kinetic resolution of (±)-6,6'-dinitrodiphenic acic dichloride (**12**) by icterinoidin B_1 (**2**).

Taxonomic notes and possible biogenetic relationships

Dermocybe icterinoides has been placed taxonomically close to another green capped Australasian species, *D. austroveneta* by Keller.[26] Some time ago, we studied the chemistry of *D. austroveneta*,[27,28] Fruit bodies of *D. austroveneta*, when attacked by predators or upon decay turn to a rich, red-purple colour. From the fresh fruit bodies of *D. austroveneta* extracted under 'normal conditions' we isolated (*P*)-(+)-skyrin (**5**) and the purple pigment hypericin (Scheme 3).[27] When the fungus was frozen in liquid N_2 in the field and subsequentially extracted under N_2 in the absence of air and light we were able to isolate the purple protohypericin and the labile green pigment austrovenetin (Scheme 3).[28] The absolute stereochemistry at C 3 and C 3' in atrovirin pigment B_2 (**2**) and in the other coupled pigments from *Dermocybe icterinoides* and *D. austroveneta* that are shown in Scheme 3 is (*R*) and is (*P*) at the axis. This points to a close biogenetic relationship between these members of, what we here dub, 'the atrovirin B_2 cascade'.

The pigments of *D. icterinoides* and *D. austroveneta* fall logically into the biogenetic pattern shown in Scheme 3. Chemically, they have affinities, either actual or artefactual,[28] not only with other members of the section *Pauperae* of the subgenus *Icterinula* but also to subsection *Atrovirantes* of section *Scauri* Fr. of subgenus *Phlegmacium*, which is characterized by the

presence of the atrovirins (**3**) and/or (**4**), skyrin (**5**), and probably hypericin.[26] Our results[29] provide further support for the suggestion that section *Pauperae* of subgenus *Icterinula* should be grouped with subsection *Atrovirentes,* section *Scauri* Fr. of subgenus *Icterinoides*.[5]



Scheme 3. Possible biosynthetic relationships between members of the 'atrovirin B_2 cascade'.

Experimental Section

General Procedures. All reactions were carried out under atmosphere of dry N_2. PTLC was performed on 20 x 20 cm glass plates coated with 1.0 mm of silica gel (Merck Kieselgel GF_{254} applied as a suspension in water) Plates were activated at 110 °C for 1.5 h prior to use. TLC was performed on Macherey-Nagel precoated aluminium plates (0.25 mm, Macherey-Nagel SIL G-25 UV_{254}) and visualised both in daylight and under short (254 nm) and long (360 nm) wavelength UV light. Commercial deuteriochloroform (Cambridge Isotope Laboratories) was washed with water, dried (K_2CO_3), distilled, and stored in the dark. All other solvents and reagents were purified before use by published procedures.

Equipment ^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL JNM GX-400 spectrometer operating at 399.65 MHz (^{1}H) and 100.4 MHz (^{13}C) using Varian Unity Plus Version 5.1 software. Chemical shifts (δ) are quoted in ppm from tetramethylsilane as internal standard and using deuteriochloroform as the solvent unless stated otherwise. UV-vis. spectra were recorded on a Varian SuperScan 3 spectrophotometer using ethanol as the solvent; log ε is quoted in parentheses after each absorption maximum. Electron impact (EI) mass spectra were recorded with either VG Micromass 7070F or JEOL JMS-AX505HF instruments operating at 70 eV unless stated otherwise. The results of accurate mass measurements are presented as a molecular formula in parentheses. Specific rotations were measured on a Perkin-Elmer 241 MC polarimeter at the room temperature; the solvent used is quoted in parenthesise with concentration (*c*) measured in g/100 mL. Melting points were determined on a Köfler micro hot stage apparatus and are uncorrected. Combustion analysis was performed by Chemical and Micro Analytical Services Pty Ltd, North Essendon, Victoria.

Synthesis of (±)-6,6'-dinitrodiphenic acid dichloride (12)

2-Bromo-3-nitrobenzoic acid (8). 3-Nitrophthalic acid (7) (14.1 g, 0.073 mol) was dissolved in an aqueous solution of NaOH (2.5 M, 53 mL) 40 °C and the mixture was filtered. To the filtrate was added a solution of mercuric acetate (23.3 g, 0.073 mol) in AcOH (3.3 mL) and H_2O (47 mL) and the suspension was heated and filtered while hot. The filtrate was heated at reflux for 90 h and filtered. On cooling, anhydro-2-hydroxymercuri-3-nitrobenzoic acid (22.0 g, 82%) was obtained as a cream powder: m.p. >340 °C, EI-MS *m/z* 367 ([M (^{202}Hg)]$^+$, 2), 366 ([M (^{201}Hg)]$^+$, 2), 365 ([M (^{200}Hg)]$^+$, 3), 364 ([M ^{199}Hg)]$^+$, 2), 149 (38), 94 (42), 69 (60), 56 (100). Anhydro-2-hydroxymercuri-3-nitrobenzoic acid was dissolved in aqueous NaOH (2.5 M, 90 mL) at 100 °C. Conc. HCl (5.15 mL) was added over 5 min followed by AcOH (1.8 mL) as the mixture was cooled to room temperature. A solution of NaBr (7.51 g, 0.073 mol) and a solution of Br_2 (11.70 g, 0.073 mol) in H_2O (9.0 mL) was added and the mixture was heated at reflux for 5 min. Solid NaOH (1.24 g) was added and the mixture was filtered. The filtrate was acidified with conc. HCl (9.2 mL) and the resulting precipitate was filtered off and crystallized from aqueous EtOH to give 2-bromo-3-nitrobenzoic acid (**8**) (6.05 g, 42%) as colourless needles: m.p. 186-187 °C (lit.[22] m.p. 185-187 °C); δ_H 7.76 (1H, m, H 5), 8.03 (2 x 1H, m, H 4 and H 6); δ_C 112.0 (C 2),

127.3 (C 5), 130.0 (C 4), 133.7 (C 6), 137.2 (C 1), 152.4 (C 3), 166.4 (CO_2H); EI-MS *m/z* 247 ([M (^{81}Br)]$^+$, 73), 245 ([M ^{79}Br)]$^+$, 75), 217 (14), 215 (16), 145 (35), 143 (41), 92 (100), 75 (86), 62 (60).

Methyl 2-bromo-3-nitrobenzoate (9). A solution of 2-bromo-3-nitrobenzoic acid (**8**) (5 g, 0.02 mol) in MeOH (150 mL) was saturated with HCl and the solution was heated at reflux for 12 h. On cooling, the *product* was filtered off and crystallized from MeOH to give methyl 2-bromo-3-nitrobenzoate (**9**) (5.00 g, 95%) as colourless plates: m.p. 77-78 °C (lit.[22] m.p. 78-78.5 °C); δ_H 3.97 (3H, s, 1-CO_2Me), 7.52 (1H, t, *J* 7.8 Hz, H 5), 7.76 (1H, d, *J* 7.8 Hz, H 6), 7.85 (1H, d, *J* 7.8 Hz, H 4); δ_C 53.1 (1-CO_2CH_3), 112.9 (C 2), 126.7 (C 5), 128.1 (C 4), 133.0 (C 6), 135.8 (C 1), 152.0 (C 3), 165.6 (1-CO_2CH_3); EI-MS *m/z* 260 ([M–1 (^{81}Br)]$^+$, 43), 258 ([M-1 (^{79}Br)]$^+$, 44), 229 (98), 227 (100), 183 (27), 181 (27), 119 (25), 75 (95).

Dimethyl (±)-6,6'-dinitrodiphenate (10). Methyl 2-bromo-3-nitrobenzoate (**9**) (5 g, 0.019 mol) was maintained at 172 °C before Cu powder (3.2 g, 0.05 mol) was added over 15 min. The mixture was stirred for 45 min at 177 °C, cooled to rt, and toluene was added. The solution was filtered and the filtrate was evaporated to dryness and the residue was crystallized from aqueous EtOH (95%) to give dimethyl (±)-6,6'-dinitrodiphenate (**10**) (2.95 g, 85%) as colourless needles: m.p. 126-127 °C (lit.[23] m.p. 129 °C);δ_H 3.65 (6H, s, 1,1'-CO_2Me), 7.68 (2H, t, *J* 7.9 Hz, H 4,4'), 8.32 (2H, d, *J* 7.9 Hz, H 3,3'), 8.34 (2H, d, *J* 7.9 Hz, H 5,5'); δ_C 52.6 (1-CO_2CH_3), 127.9 (C 4,4'), 128.8 (C 5,5'), 130.9 (C 2,2'), 133.1 (C 1,1'), 135.0 (C 3,3'), 148.9 (C 6,6'), 165.0 (1,1'-CO_2CH_3); EI-MS *m/z* 358 (M$^+$, 3), 286 (100), 56 (81).

(±)-6,6'-Dinitrodiphenic acid (11). To a solution of dimethyl (±)-6,6'-dinitrodiphenate (**10**) (1.8 g, 5 mmol) in aqueous EtOH (50%, 40 mL) was added solid NaOH (0.9 g) and the resulting solution was heated at reflux for 3 h. The mixture was acidified with conc. HCl and the solid so formed was filtered off. The product was crystallized from AcOH to give (±)-6,6'-dinitrodiphenic acid (**11**) (1.2 g, 72%) as colourless needles: m.p. 261-263 °C (lit.[23] m.p. 259 °C); δ_H 7.82 (2H, t, *J* 8.0 Hz, H 4,4'), 8.37 (2H, d, *J* 8.0 Hz, H 3,3'), 8.38 (2H, d, *J* 8.0 Hz, H 5,5'); δ_C 128.4 (C 4,4'), 130.0 (C 5,5'), 132.6 (C 2,2'), 133.7 (C 1,1'), 135.8 (C 3,3'), 150.2 (C 6,6'), 166.1 (1,1'-CO_2H); EI-MS *m/z* 332 (M$^+$, 3), 286 (100), 240 (43), 196 (26), 149 (22), 123 (35), 97 (36), 83 (53), 69 (95), 56 (81).

(±)-6,6'-Dinitrodiphenic acid dichloride (12). A solution of (±)-6,6'-dinitrodiphenic acid (**11**) (1.0 g, 3 mmol) in thionyl chloride (3.6 g, 0.03 mol) was heated at reflux for 24 h. Excess thionyl chloride was removed by distillation under reduced pressure and the residue was crystallized from $CHCl_3$ to give (±)-6,6'-dinitrodiphenic acid dichloride (**12**) (0.75 g, 68%) as colourless needles: m.p. 154-155 °C (lit.[24] m.p. 155-157 °C); δ_H 7.80 (2H, t, *J* 7.9 Hz, H 4,4'), 8.41 (2H, d, *J* 7.9 Hz, H 3,3'), 8.44 (2H, d, *J* 7.9 Hz, H 5,5'); EI-MS *m/z* 370 ([M (^{37}Cl)]$^+$, 1), 368 ([M (^{35}Cl)]$^+$, 3), 106 (100), 79 (27).

Kinetic resolution experiments

(*M*)-(+)-Binaphthol (13a). To a solution of (*M*)-(+)-binaphthol (**13a**) (12 mg, 0.042 mmol) in benzene (5 mL) containing pyridine (50 mL) was added (±)-6,6'-dinitrodiphenic acid dichloride

(**12**) (30 mg, 0.081 mmol). The solution was stirred for 12 h at room temperature, extracted with aqueous $NaHCO_3$ (0.1 M, 10 mL) and the aqueous phase was acidified with aqueous AcOH (1 M). The products were extracted into $CHCl_3$ and the solvent was dried and evaporated. The residue was purified by PLC using chloroform-ethyl formate (1:1) as the eluant to give 6,6'-dinitrodiphenic acid (8 mg) in which the (*P*)-(–)-atropisomer (**14**) predominated: m.p. 229-232 °C (lit.[23] m.p. 229 °C); $[\alpha]_D$ –19.8 (MeOH, *c* 1.2) (lit.[23] $[\alpha]_D$ –126.0 [MeOH, *c* 2.9]); δ_H 7.82 (2H, t, *J* 8.0 Hz, H 4,4'), 8.37 (2H, d, *J* 8.0 Hz, H 3,3'), 8.38 (2H, d, *J* 8.0 Hz, H 5,5').

(*P*)(+)-Skyrin (5). To a solution of (*P*)-(+)-skyrin (**5**) (30 mg, 0.056 mmol) in THF (5 mL) containing pyridine (50 mL) was added (±)-6,6'-dinitrodiphenic acid dichloride (**12**) (40 mg, 0.11 mmol). The solution was stirred for 12 h at r.t. after which the THF was evaporated. The residue was dissolved in $CHCl_3$ (5 mL) and extracted with aqueous NaOH (0.1 M, 10 mL). The aqueous phase was acidified with aqueous AcOH (1 M) and extracted with $CHCl_3$. The solvent was removed under reduced pressure and the residue was purified by PLC using toluene-HCO_2Et-HCO_2H (50:49:1) as the eluant to give a mixture of 6,6'-dinitrodiphenic acid atropisomers (6 mg) in which the (*M*)-(+)-stereoisomer (**15**) was predominant: m.p. 230-232 °C (lit.[20] m.p. 230-231 °C); $[\alpha]_D$ +20.4 (MeOH, *c* 1.6) (lit.[23] $[\alpha]_D$ +127.0 [MeOH, *c* 2.0]); δ_H 7.82 (2H, t, *J* 8.0 Hz, H 4,4'), 8.37 (2H, d, *J* 8.0 Hz, H 3,3'), 8.38 (2H, d, *J* 8.0 Hz, H 5,5').

(+)-Icterinoidin B_1 (2). To a solution of (+)-icterinoidin B_1 (**2**) (15 mg, 0.028 mmol) in THF (5 mL) containing pyridine (50 mL) was added (±)-6,6'-dinitrodiphenic acid dichloride (**12**) (22 mg, 0.06 mmol). The solution was stirred for 12 h at room temperature and the THF was evaporated. The residue was dissolved in $CHCl_3$ (5 mL) and extracted with aqueous NaOH (0.1 M, 10 mL). The aqueous phase was acidified with aqueous AcOH (1 M) and extracted with $CHCl_3$. The solvent was removed under reduced pressure and the residue was purified by PLC using toluene-HCO_2Et-HCO_2H (50:49:1) as the eluant to give 6,6'-dinitrodiphenic acid (**12**) (4 mg) enriched in the (*M*)-(+)-atropisomer (**15**), m.p. 230-232 °C (lit.[23] m.p. 230-231 °C); $[\alpha]_D$ +18.0 (MeOH, *c* 1.4) (lit.[23] $[\alpha]_D$ +127.0 [MeOH, *c* 2.0]); δ_H 7.82 (2H, t, *J* 8.0 Hz, H 4,4'), 8.37 (2H, d, *J* 8.0 Hz, H 3,3'), 8.38 (2H, d, *J* 8.0 Hz, H 5,5').

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