

Pigments of fungi. Part 51.¹ Structure and stereochemistry of coupled pre-anthraquinones of the pseudophlegmacin type from Australian toadstools belonging to the genus *Dermocybe*

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(3'*R,P*)-Anhydropseudophlegmacin-9,10-quinone 6',8'-di-*O*-methyl ether **4** and the corresponding 1,6',8'-tri-*O*-methyl ether **5** are isolated together with the atropisomeric green pigments austroviridin **B 10** and austroviridin **A 11** from the fruiting bodies of indigenous Australian toadstools belonging to the genus *Dermocybe*. The structures of these pigments are determined by spectroscopic methods, while their absolute configurations are established from the respective CD spectra and by reductive cleavage of **5** to a mixture of (*R*)-torosachryson 8-*O*-methyl ether **12** and emodin 1-*O*-methyl ether **14**.

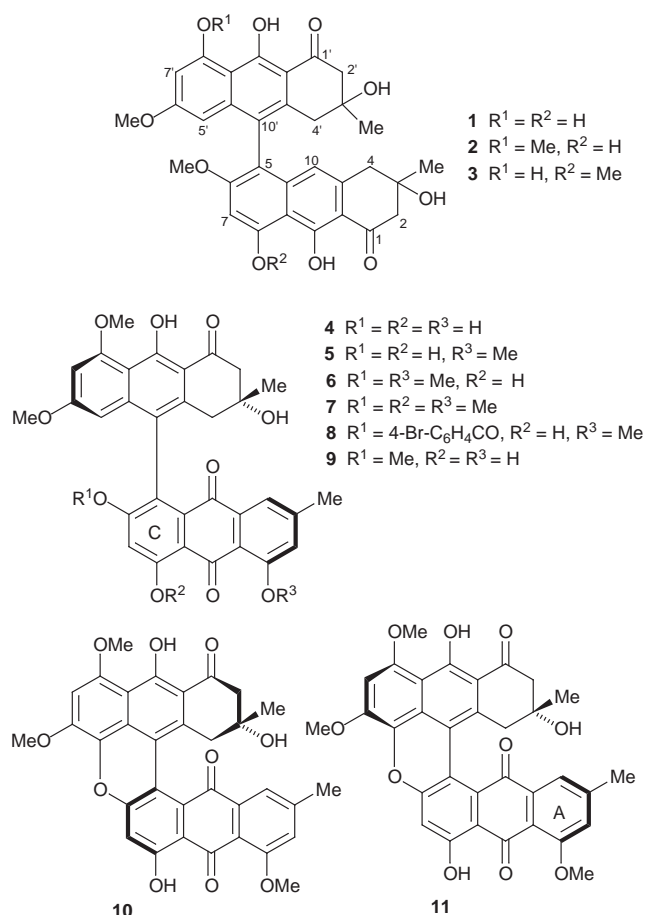
Introduction

Pigments formed by phenolic coupling between two 3,4-dihydroanthracen-1(2*H*)-one subunits dominate the chemistry of toadstools belonging to the genera *Cortinarius* and *Dermocybe*.² Of the many examples known, dimers linked by a biaryl bond between C-10 in one half of the molecule and C-5 in the other (the so-called pseudophlegmacin group)^{3,4} are rare and hitherto restricted to only three *Cortinarius* species. Interestingly, both atropisomers of the parent pseudophlegmacin **1** co-occur in *C. prasinus* while both atropisomeric forms of the two methyl ethers **2** and **3** are found in *C. russeoides*.² We report here the isolation and structural elucidation of four new members of this group in the form of the orange pseudophlegmacinquinone **4**, its methyl ether **5** and the green austroviridins **B 10** and **A 11**. The absolute configuration of the natural products **4**, **5**, **10** and **11** is established by spectroscopic and chemical methods.

Fruiting bodies of *Dermocybe* sp. WAT 26640[†] were collected from mixed *Eucalyptus* forest close to Melbourne, Australia. The diminutive toadstools are ochre with a distinctive bottle green zone on the stem immediately below the cap. The fresh fungus was extracted with ethanol and the deep green extracts were evaporated and partitioned between ethyl acetate and water. Chromatographic analysis of the organic phase revealed the presence of two green zones and five discrete yellow or yellow-orange zones. We describe here the structural elucidation, including the absolute axial and central stereochemistry where appropriate, of four of the five yellow-orange metabolites and the two green compounds, which we have called the austroviridins.

Results and discussion

The yellow pigment **5**, R_f 0.17, was obtained as an optically active yellow powder, mp 220–223 °C, $[a]_D^{25} +66.0$ (c 0.02 in MeOH), in a yield of $7 \times 10^{-3}\%$ of the fresh weight of the fungus. The mass spectrum exhibits a molecular ion at m/z 584 from which the molecular formula $C_{33}H_{28}O_{10}$ followed from the high resolution data. This was immediately suggestive of a dimeric octaketide structure and, coupled with long wavelength absorption at 438 nm in the electronic spectrum, suggested the



presence of an anthraquinone chromophore. The ¹H NMR spectrum of pigment **5** (Table 1) reveals the presence of two phenolic hydroxy groups (δ 15.36 and 13.78), three methoxy groups (δ 4.04, 3.68 and 3.56), aromatic and aliphatic methyl groups (δ 2.34 and 1.33, respectively), and two sets of methylene protons resonating as pairs of AB quartets with components centred at δ 2.67, 2.80, 2.85 and 2.93. The spectrum also reveals the presence of five aromatic protons. Two of these (δ 6.10 and 5.94) are *meta* coupled (J 2.2 Hz) while a second pair of doublets (δ 7.33 and 7.06) are broadened by allylic coupling. A sharp aromatic proton singlet at δ 7.02 and a broad, exchangeable

[†] The code refers to the accession number under which lyophilised voucher specimens are lodged in the herbarium of the Royal Botanic Garden, Edinburgh.

Table 1 ^1H NMR data (CDCl_3 , 400 MHz) for the natural products **4** and **5**, the methyl ethers **6**, **7** and **9**, and the 6-*O*-(4-bromobenzoyl) ester **8**

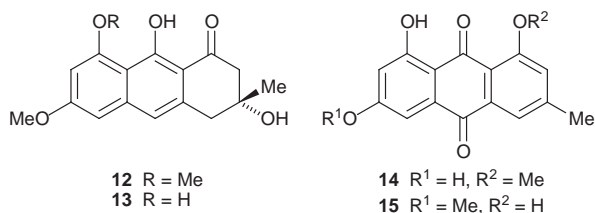
Proton	Chemical shift (δ), multiplicity and coupling constant (J/Hz)					
	4	5	6	7	8	9
2-H	7.04, br s	7.06, br s	7.08, br s	7.01, br s	7.13, br s	7.04, br s
4-H	7.27, br s	7.33, br s	7.38, br s	7.16, br s	7.45, br s	7.30, br s
7-H	7.01, s	7.02, s	6.86, s	6.89, s	7.41, s	6.86, s
3-Me	2.30, s	2.34, s	2.37, s	2.32, s	2.40, s	2.32, s
1-OH	12.01, s	—	—	—	—	12.02, s
1-OMe	—	4.04, s	4.06, s	3.99, s	4.09, s	—
6-OH	6.57, br s	6.78, br s	—	—	—	—
6-OMe	—	—	4.00, s	4.00, s	—	4.00, s
8-OH	12.92, s	13.78, s	14.04	—	13.75, s	13.16, s
8-OMe	—	—	—	3.98, s	—	—
2'-H _{ax}	2.79, d, 17.7	2.80, d, 17.6	2.78, d, 17.4	2.77, d, 17.2	2.71, d, 17.2	2.78, d, 17.4
2'-H _{eq}	2.87, dd, 17.7, 1.9	2.85, dd, 17.6, 1.9	2.90, dd, 17.4, 1.5	2.89, br d, 17.2	2.87, br d, 17.2	2.90, br d, 17.4
4'-H _{ax}	2.83, d, 15.9	2.93, d, 16.1	2.68, d, 15.6	2.66, br s	2.71, d, 16.9	2.65, d, 15.9
4'-H _{eq}	2.65, dd, 15.9, 1.9	2.67, dd, 16.1, 1.9	2.61, dd, 15.6, 1.5	—	2.64, br d, 16.9	2.58, br d, 15.9
5'-H	5.95, d, 2.4	5.94, d, 2.2	5.89, d, 2.4	5.85, d, 2.5	5.99, d, 2.2	5.89, d, 2.2
7'-H	6.23, d, 2.4	6.10, d, 2.2	6.41, d, 2.4	6.40, d, 2.5	6.41, d, 2.2	6.44, d, 2.2
3'-Me	1.31, s	1.33, s	1.26, s	1.26, s	1.21, s	1.26, s
6'-OMe	3.61, s	3.56, s	3.57, s	3.49, s	3.62, s	3.59, s
8'-OMe	3.86, s	3.68, s	3.72, s	3.75, s	3.98, s	3.75, s
9'-OH	15.46, s	15.36, s	15.44, s	15.43, s	15.43, s	15.45, s
4-BrC ₆ H ₄	—	—	—	—	7.25, 7.38, each d, 8.8	—

Table 2 ^{13}C NMR chemical shifts (δ) (100 MHz, CDCl_3) for the pseudophlegmacinquinones **4** and **5**

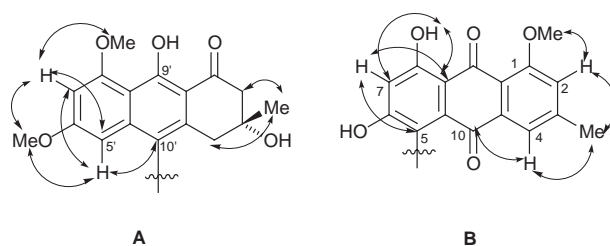
4^a				5^a			
Carbon	δ_{C}	Carbon	δ_{C}	Carbon	δ_{C}	Carbon	δ_{C}
1'	202.8	1	162.7	1'	202.7	1	160.5
2'	51.1	2	121.6	2'	51.2	2	121.2
3'	70.0	3	148.7	3'	70.1	3	147.1
4'	41.3	4	124.1	4'	41.5	4	121.7
4a'	132.8	4a	136.9	4a'	132.1	4a	137.2
5'	97.4	5	120.7	5'	97.7	5	118.7
6'	162.4	6	161.4	6'	161.7	6	160.9
7'	96.8	7	109.4	7'	96.7	7	109.6
8'	161.4	8	165.4	8'	160.9	8	165.2
8a'	110.7	8a	113.2	8a'	112.8	8a	112.8
9'	165.9	9	191.1	9'	166.1	9	187.7
9a'	109.7	9a	118.5	9a'	109.7	9a	118.4
10'	111.4	10	183.3	10'	110.9	10	184.0
10a'	140.7	10a	133.6	10a'	140.8	10a	135.8
3'-Me	28.7	3-Me	22.0	3'-Me	28.9	3-Me	22.2
6'-OMe	55.1	—	—	6'-OMe	55.1	1-OMe	55.8
8'-OMe	55.6	—	—	8'-OMe	55.5	—	—

^a Assignments are consistent with the results of HMQC and HMBC experiments.

singlet at δ 6.78 complete the spectrum. Comparison of these data with those recorded previously for torosachrynone 8-*O*-methyl ether **12**⁵ and emodin 1-*O*-methyl ether **14**^{6,7} reveals that



the new quinone corresponds to a combination of the sub-units **A** and **B** (Fig. 1) joined at the C-10' and C-5 positions, respectively. The substitution pattern within each of the partial structures **A** and **B** was evident from the HMBC and NOESY correlations that are summarised diagrammatically in Fig. 1. The substructures **A** and **B** are further consistent with the ^{13}C NMR data for **5** (Table 2), which shows thirty-three discrete reson-

**Fig. 1** HMBC and NOESY correlations in the dihydroanthracenone **A** and anthraquinone **B** subunits in the natural product **5**.

ances. Among other things, they confirm the presence of ketone and quinone carbonyl groups (δ 202.7, 187.7 and 184.0), methylene groups at C-2' and C-4' (δ 51.2 and 41.5, respectively) and the quaternary carbon C-3' (δ 70.1) of the dihydroaromatic ring in **A**.

The location of the biaryl bond between C-10' in **A** and C-5 in **B** follows from the absence in the spectrum of **5** of any signal attributable to 10'-H (*cf.* $\delta_{10\text{-H}}$ 6.87 in the spectrum of **12**),⁵ the

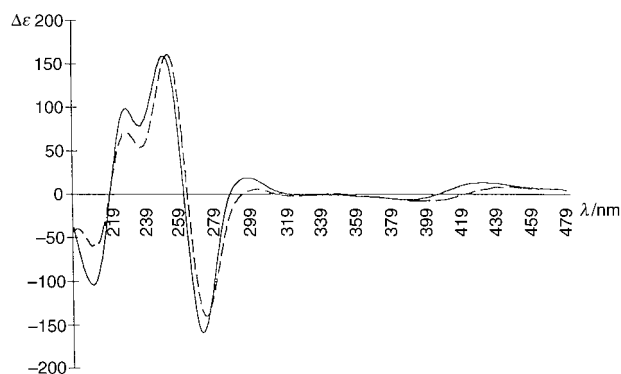


Fig. 2 CD spectra (MeOH) of the pseudophlegmacinquinones **4** (-----) and **5** (—).

chemical shift of 5'-H (δ 5.94 compared to δ 6.56 in **12**)⁵ and the appearance of 7-H as a singlet at δ 7.02. This chemical shift is consistent with the presence of an aryl substituent at C-5 (*cf.* δ_{7-H} 6.67 in the spectrum of **14**)^{6,7} while the multiplicity proves that there is no proton at C-5. The biaryl connectivity is fully consistent with the results of the HMBC experiment shown in Fig. 1. For example, the C-10' terminus of the biaryl bond (δ 110.9) correlates over three bonds with 5'-H (δ 5.94) while C-5 (at the other end of the biaryl bond) correlates with 7-H (δ 7.02). It is notable that the quinonoid carbon, C-10, correlates only with 4-H (δ 7.33); had the biaryl bond stemmed from C-7 in **B** then this carbon would have been coupled to 4-H and 5-H. All of the data discussed so far support the pseudophlegmacinquinone structure **5** for the new yellow pigment, albeit as yet without stereochemical detail.

With dimethyl sulfate **5** affords a mixture of the 6-*O*-methyl ether **6** and the 6,8-di-*O*-methyl ether **7**. The methyl ethers **6** and **7** are easily separated by chromatography and identified by ¹H NMR spectroscopy (Table 1). It is interesting to note from the data in Table 1 that as the phenolic ring in the anthraquinone portion of **5** is progressively methylated, the signal due to the axial proton at C-4' is increasingly shielded by the C-10' aromatic substituent. This point has stereochemical implications that will be discussed later in the paper. Treatment of **5** with 4-bromobenzoyl chloride gave the benzoate **8** that, unfortunately, did not form crystals suitable for X-ray analysis. Nevertheless, the absolute stereochemistry of the pigment **5** could be determined from the CD spectrum and chemical degradation as will be demonstrated below.

The second yellow pigment, R_f 0.21, from *Dermocybe* sp. WAT 26640, C₃₂H₂₆O₁₀ (mass spec.), mp 215–218 °C, [α]_D +76.0 (*c* 0.04 in CHCl₃) was obtained in 3 × 10⁻³% yield. The molecular formula differs from that of **5** only by the elements CH₂ and it was quickly identified as the phenolic analogue **4** of the ether **5** from the ¹H NMR spectrum (Table 1). Thus, while the spectrum of **4** is very similar in most respects with that of **5** it lacks the resonance due to the 1-*O*-methyl group of **5** (δ 4.04) and contains instead an additional hydrogen bonded phenolic hydroxy resonance (δ 12.01). Treatment of the pigment **4** with diazomethane gave the 6-*O*-methyl ether **9** (Table 1).

The absolute configuration at the chiral axis in the new pseudophlegmacinquinones **4** and **5** was determined from the CD spectra, which are shown in Fig. 2. The spectra show that both compounds exhibit a strong negative Cotton effect at longer wavelength and a strong positive one at shorter wavelength close to 260 nm. This is in accord with an anticlockwise helical twist between the long axes of the aromatic chromophores, as is depicted in the structures **4** and **5**.⁸ Both pigments are therefore designated (*P*) according to the Prelog-Helmchen rules.⁹

The (*R*)-stereochemistry at C-3' in the natural product **5**, as shown, was determined by chemical degradation as follows. Treatment of a solution of **5** in dilute aqueous sodium hydrox-

ide with solid sodium dithionite brought about a change in the colour of the solution from orange to pale yellow (approximately 3 min). The mixture was immediately quenched by the addition of dilute hydrochloric acid and the products were extracted into ethyl acetate. The products were separated from unchanged **5** by gel filtration and from each other by PLC and HPLC over silica gel. Analysis of the products led to the following results. The anthraquinone fragment was identified as emodin 1-*O*-methyl ether **14** by isolation and direct comparison of the mass and ¹H NMR spectra with those of an authentic sample.⁶ This confirmed that the structure and substitution pattern deduced from the spectroscopic data for the anthraquinone moiety in the natural product **5** are correct. In turn, the dihydroanthracenone fragment was identified as (*R*)-torosachrysonone 8-*O*-methyl ether **12** by chiral HPLC comparison with an authentic sample of anisochiral **12**.^{5,10,11} HPLC also established that the enantiomeric excess of the sample of **12** obtained from **5** is greater than 99.8% and consequently that the natural product itself is enantiomerically pure. The structures of both halves of **5** are therefore confirmed and the stereochemistry of the pseudophlegmacinquinone **5** is defined as (3'*R,P*). We propose that because of the close biosynthetic relationship between the pigments **4** and **5** and the marked similarity of their respective ¹H and ¹³C NMR spectra that the pigment **4** also possesses the (3'*R,P*) absolute stereochemistry.

The (3'*R,P*) stereochemistry for **4** and **5** and hence for their methyl ethers **6**, **7** and **9** places 4'-H_{ax} in all of these molecules on the same face (the top face as drawn) as the bulk of the tricyclic C-10' substituent. The shift to increasingly higher field of the ¹H NMR signal due to 4'-H_{ax} as the electron density in the anthraquinone C ring is increased during progressive methylation is therefore entirely consistent with the axial and central stereochemistry deduced above.

The green compounds (R_f 0.19 and 0.22) present in the extracts of *Dermocybe* sp. WAT 26640 were separated from the yellow and yellow-orange constituents by gel filtration through Sephadex LH-20 and isolated in a combined yield of 3 × 10⁻²% based on the fresh weight of the fungus. They were obtained initially as a mixture in which the more mobile pigment **10** predominated over the more polar pigment **11** to the extent of 83:17. The two compounds could be separated from each other only with difficulty and, in the first instance, the spectra of the mixture were obtained. The presence in the mass spectrum of a single molecular ion at *m/z* 582 suggested that these green pigments are isomers with the molecular formula C₃₃H₂₆O₁₀ (high resolution mass measurement). This formula is two hydrogen atoms less than the composition of the pseudophlegmacinquinone **5** and immediately suggests a close relationship between the three pigments. Comparison of the ¹H and ¹³C NMR spectra of **10** (Table 3) and **11** (Experimental) shows that all signals in the spectra of the mixture are either closely paired or, in some cases coincident, a situation that suggested that the green compounds are diastereoisomers. The pigments could be separated, albeit incompletely, by repeated preparative thin layer chromatography or by HPLC. In this way a sample of **10** was obtained that contained only 9% of **11** and, conversely, a sample of **11** was obtained that contained only 10% of **10**. It was not possible to improve on these ratios using the methods employed here since, among other things, the compounds **10** and **11** are in equilibrium at room temperature (*vide infra*). Nevertheless, by working with the biased samples it was possible to assign all of the ¹H and ¹³C NMR data to individual molecules and to deduce the structures and absolute configuration of both green pigments.

The green pigment **10** (R_f 0.22) exhibits an ¹H NMR spectrum (Table 3) that bears all the hallmarks of a coupled dihydroanthracenone of the pseudophlegmacin type.² Thus, by comparison with the corresponding data for the pigments **4** and **5** (Table 1) it is possible to identify the presence in **10** of the same dihydroanthracenone and anthraquinone segments that

Table 3 NMR data (400 MHz ^1H , 100 MHz ^{13}C , CDCl_3) for austroviridin B **10**

Position	Chemical shift (δ), multiplicity and coupling constant (J/Hz)		HMBC Correlated ^{13}C	NOESY Correlated ^1H
	δ_{H}	$\delta_{\text{C}}^{a,b}$		
1'	—	201.5, s		
2' ax	2.79, d, 18.1	50.9, s	C-1'	
eq	2.86, dd, 18.1, 2.4		C-1'	
3'	—	70.1, s		
4' ax	2.87, d, 15.6	42.0, t	C-2', 4a', 9a', 10'	
eq	2.71, dd, 15.6, 2.4		C-4a', 10'	
4a'	—	132.2, s		
5'	—	130.8, s		
6'	—	147.6, s		
7'	6.64, s	95.6, d	C-5', 6', 8', 8a'	
8'	—	156.4, s		
8a'	—	109.1, s		
9'	—	166.5, s		
9a'	—	110.7, s		
10'	—	113.2, s		
10a'	—	130.3, s		
3'-Me	1.19, s	29.8, s	C-2', 3', 4'	4'-H _{ax} , 4'-H _{eq}
6'-OMe	4.05, ^c s	56.8, ^c q	C-6'	H-7'
8'-OMe	4.04, ^c s	56.6, ^c q	C-8'	9'-OH
9'-OH	15.91, s	—	C-8a', 9', 9a'	
1	—	160.5, s		
2	7.15, br s	118.3, d	3-Me, C-4, 9a	1-OMe
3	—	147.3, s		
4	7.47, br s	120.3, d	3-Me, C-2, 9a, 10	4'-H _{eq}
4a	—	136.4, s		
5	—	114.8, s		
6	—	161.6, s		
7	6.90, s	108.0, d	C-5, 6, 8, 8a	
8	—	164.3, s		
8a	—	114.5, s		
9	—	186.9, s		
9a	—	117.8, s		
10	—	186.5, s		
10a	—	134.2, s		
3-Me	2.52, s	22.4, q	C-2, 3, 4, 4a	H-2, H-4
1-OMe	4.08, s	57.0, ^c q	C-1	
8-OH	13.68, s	—	C-7, 8, 8a	

^a Multiplicity here refers to one-bond couplings only. ^b Assignments are in accord with the results of HMQC experiments. ^c Assignments in the same column that bear the same superscript letter may be interchanged.

are found in the yellow pigment **5** and to conclude that these fragments are joined in **10** by a biaryl bond between C-5 and C-10'. The substitution pattern and connectivity in **10** were further supported by the results of HMBC and NOESY experiments that are summarised in Table 3. In fact, the only significant differences between the NMR data from **5** and **10** are (i) the absence in the ^1H NMR spectrum of **10** of a resonance due to a proton at C-5' (δ_{H} 5.94 in the spectrum of **5**), (ii) the appearance of the signal due to 7'-H in **10** as a singlet rather than a doublet, and (iii) the ^{13}C chemical shift and coupling of C-5' in **10**. C-5' is deshielded by 34 ppm compared to its counterpart in **5** and is coupled (only) through three bonds to H-7'. These observations are readily explained and the molecular formula of the green pigment **10** is accounted for if the C-6 hydroxy group in **5** is phenolically coupled to C-5' leading to the heptacyclic structure shown. The extended chromophore in the pigment **10**, which we have called austroviridin B, would account for long wavelength absorption at 604 nm in the electronic spectrum (Fig. 3).¹²

The CD spectrum of austroviridin B **10**, shown in Fig. 4, has a positive Cotton effect to longer and a negative Cotton effect to shorter wavelength.† This corresponds to a clockwise helical

† A CD spectrum like this one that contains a bisignate Cotton effect with a maximum at longer wavelength and a minimum at shorter wavelength has traditionally been referred to as a 'B-type' curve and the names of compounds that exhibit such a curve have been given the suffix 'B'.¹³

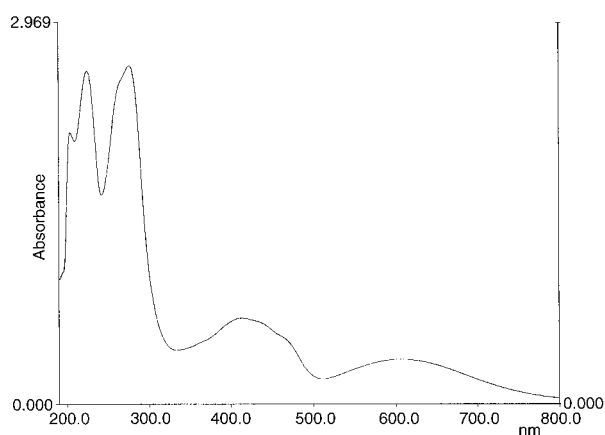


Fig. 3 UV/vis spectrum (EtOH) of a mixture of the austroviridins B **10** and A **11**.

twist between the long axes of the aromatic chromophores in accord with the axial stereochemistry shown in formula **10**. Austroviridin B **10** can therefore be assigned the (*M*) configuration at the biaryl axis.

The slower moving green pigment **11** (R_f 0.19), which we have called austroviridin A, exhibits ^1H and ^{13}C NMR spectra (Experimental section) that only differ in their fine detail from those recorded for **10**, suggesting that the two compounds are diastereoisomers. The CD spectrum of austroviridin A **11** is also

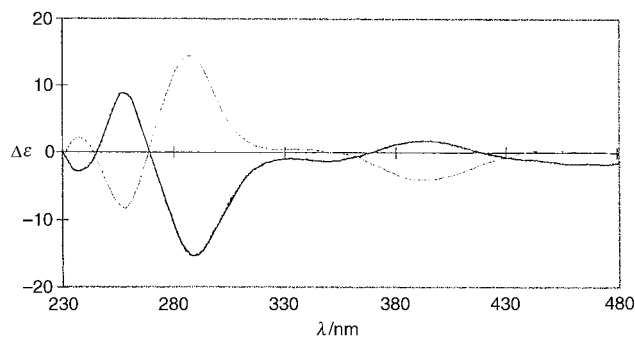


Fig. 4 CD spectra (CHCl_3) of the austroviridins **10** (····) and **11** (—).

shown in Fig. 4 from which it becomes clear that the pigments **10** and **11** are atropisomers and therefore, they must have the same chirality at C-3'. The absolute stereochemistry at the biaryl axis in austroviridin A must be (*P*), as shown in formula **11**.

It seems likely that the green pigments **10** and **11** are formed biosynthetically from pseudophlegmacin precursors, e.g. **4** and/or **5**, by phenolic coupling between the C-6 hydroxy group in one half of the untethered molecule to C-5 in the other. If this is indeed the case then it would be expected that the stereochemistry at C-3' in the austroviridins **B 10** and **A 11** would be (*R*) as in the parent **5**. On this basis, it follows that the austroviridins **10** and **11** from *Dermocybe* sp. WAT 26640 have the (3'*R,M*) and (3'*R,P*) absolute configuration, respectively. Support for this suggestion comes from the difference in the chemical shift of the aliphatic methyl protons (3'-Me) in the ^1H NMR spectra of **10** and **11**. In the spectrum of **10** these protons resonate at δ 1.19, which is close to the range (δ 1.2–1.3) observed for an unperturbed C-3' methyl group. The same protons in the spectrum of **11**, however, are shielded and appear at δ 1.03. This is consistent with the configuration shown in structure **11** in which the C-3' methyl group is on the same β -face of the molecule as the C-10' anthraquinone substituent and might be expected to suffer increased anisotropic shielding by the quinone A ring.

The austroviridins **B 10** and **A 11** have also been isolated from the fruiting bodies of the Australian *Dermocybe* sp. WAT 24274 in which they are accompanied by compounds of the flavomannin¹⁴ and dermocanarin types.¹⁵

During repeated attempts to obtain each of the austroviridins **B 10** and **A 11** in pure form it became clear that the two compounds were undergoing atropisomerisation during chromatography and in solution. Thus, when a sample of **10** + **11** (91:9 by integration of the phenolic hydroxy proton signals at δ 15.91 and 15.85, respectively) in deuteriochloroform was stored at 4 °C and the composition of the mixture was monitored periodically by ^1H NMR spectroscopy, a gradual increase in the proportion of **11** was observed. After 15 days the ratio of **10**:**11** was 70:30 but it did not change further over the following month. Similarly, when a mixture of **10** and **11** biased 37:63 in favour of **11** was monitored under the same conditions, the proportion of **10** gradually increased over 15 days to a constant ratio of 70:30. It is clear from these experiments that the presence of the dibenzopyran structure in the austroviridins **10** and **11** and the absence of the 6-*O*-methyl group and the C-5' proton must, respectively, restrict the dihedral angle and offer less steric hindrance to rotation between the dihydroanthracenone and anthraquinone rings in these molecules so that slow atropisomerisation is feasible. This is the first example that we have encountered in which a coupled pre-anthraquinone is capable of rotation about the biaryl axis at room temperature. As mentioned earlier, when the pigments **10** and **11** are isolated in admixture from the fungus with the least possible delay the composition is 83:17 and this changes

over two weeks at 4 °C to 70:30. Apparently, the austroviridins **B** and **A** are not present in their equilibrium proportions in the fresh ethanolic extract and it is possible that, in the intact toadstool, the green colour is due mainly, if not entirely, to the presence of austroviridin **B 10**.

The last two yellow pigments (R_f 0.40 and 0.80) from *Dermocybe* sp. WAT 26640 were identified as torosachryson **13** ($9 \times 10^{-3}\%$) and physcion **15** ($6 \times 10^{-3}\%$) by spectroscopic comparison with authentic materials.¹⁶ The stereochemistry of **13** was determined by chiral HPLC,¹¹ which showed that this compound occurs in *Dermocybe* sp. WAT 26640 as an anisochiral mixture in which the (*S*)-enantiomer ent-**13** predominates to the extent of 44% ee.

This is the first report of the occurrence of pigments of the pseudophlegmacin class from *Dermocybe* and the first time that the absolute configuration of a member of this group has been determined unequivocally. The stereochemistry of the pseudophlegmacins **A** and **B** from *Cortinarius prasinus* was tentatively proposed to be (3'*S,P*) and (3'*S,M*), respectively, from the CD and ^1H NMR data.³

The deep bottle green colour of the austroviridins **10** and **11** is exceptional since only a relatively small number of natural compounds are green. Of course, the chlorophylls are the most important and widespread; others include the aphinins (from insects such as greenfly),¹² xylindein (from the wood-staining fungus *Chlorosplenium aeruginascens*),¹⁷ 'Lo-ka'o' (an ancient plant dyestuff),¹⁸ tecomaquinone (from teak wood),¹⁹ hypoxylxerone **A** (from the green mycelium of the fungus *Hypoxylon fragiforme*)²⁰ and austrovenetin, which we have reported from the toadstool *Dermocybe austroveneta*.²¹ A blue-green pigment, prasinone, for which an extended pseudophlegmacinquinone structure has been proposed, has been isolated from *Cortinarius prasinus*.³

Experimental

General

Melting points were determined on a hot-stage apparatus and are uncorrected. IR spectra were recorded using a Perkin-Elmer 983 G spectrophotometer for samples as potassium bromide discs. Electronic spectra were recorded on a Varian SuperScan 3 spectrophotometer using ethanolic solutions in a 10 mm quartz cell. NMR spectra were recorded with a JEOL JNM-GX-400 spectrometer (^1H at 399.65 MHz and ^{13}C at 100.4 MHz) for solutions in CDCl_3 . Mass spectra were recorded on V. G. Micromass 7070F and JEOL JMS AX505H spectrometers at 70 eV (probe). Specific rotations were measured for methanol solutions (unless otherwise stated) using Perkin-Elmer 241MC and JASCO DIP-1000 polarimeters and are given in units of 10^{-1} deg $\text{cm}^2 \text{g}^{-1}$. CD spectra were obtained using an AVIV 62DS spectrometer for solutions in methanol except where stated otherwise.

Materials

Thin layer chromatography (TLC) and preparative TLC (PLC) were performed on Merck precoated silica gel 60 F_{254} and Merck Kieselgel 60 GF_{254} (20 g silica gel spread on 20×20 cm glass plates), respectively. Visualisation was under UV light (254 or 366 nm). R_f -values quoted for pure compounds were measured using toluene–ethyl formate–formic acid (50:49:1) as eluent. Gel permeation chromatography employed a column (40×3.5 cm) of Sephadex LH-20 suspended in and eluted with methanol, unless stated otherwise.

Dermocybe sp. WAT 26640 was collected in the Murrindindi Nature Park, Victoria, Australia from under mixed *Eucalyptus* and *Leptospermum* during June 1995 and in subsequent years. *Dermocybe* sp. WAT 24274 was collected from the same location and from parts of the King Lake State Park, Victoria in June–July 1990 and in subsequent years. Voucher specimens

are lodged in the herbarium of the Royal Botanic Garden, Edinburgh, under accession numbers WAT 26640 and WAT 24274, respectively. They were placed in *Dermocybe* by Dr R. Watling, MBE (Edinburgh).

Isolation of metabolites from *Dermocybe* sp. WAT 26640

Fresh fruit bodies (115 g) were macerated in ethanol (1 l) at room temperature overnight. The deep green extract was concentrated and the aqueous slurry was partitioned, in several portions, between ethyl acetate (400 ml) and water (400 ml). The organic phases were combined, dried and evaporated to afford a green residue (820 mg) that was separated by gel permeation through a column (55 × 3.5 cm) of Sephadex LH-20 to afford a faster moving yellow zone and a slower moving green zone. These two fractions were collected and purified separately.

The yellow zone was separated by PLC using toluene–ethyl formate–formic acid (50:49:1) as eluent and each fraction was passed through a column of Sephadex LH-20 to give the following compounds in order of decreasing polarity on silica gel: (i) (*3'R,P*)-anhydropseudophlegmacin-9,10-quinone 1,6',8'-tri-*O*-methyl ether **5** (R_f 0.17) (8.5 mg, $7 \times 10^{-3}\%$ fresh weight) as yellow crystals, mp 218–220 °C (from CH_2Cl_2) (Found: M^+ , 584.1655. $\text{C}_{33}\text{H}_{28}\text{O}_{10}$ requires: M , 584.1622; $[\alpha]_D +66.0$ (c 0.02); CD λ_{max} 298 ($\Delta\epsilon$, +18.3), 272 (–159.3), 251 (+158.8), 237 (+78.3), 230 (+98.5) and 211 nm (–104.6); ν_{max} 3410, 1648 and 1611 cm^{-1} ; λ_{max} (log ϵ) 222 (4.42), 274 (4.50), 332 (3.62), 407 (3.96) and 438 nm (3.78); λ_{max} (EtOH + OH^-) 275 (4.45), 322 (4.11) and 515 nm (3.67); m/z 584 (M^+ , 87%), 566 (100), 549 (35), 284 (27), 270 (26) and 56 (31); δ_{H} Table 1; δ_{C} Table 2, (ii) (*3'R,P*)-anhydropseudophlegmacin-9,10-quinone 6',8'-di-*O*-methyl ether **4** (R_f 0.21) (3.0 mg, $3 \times 10^{-3}\%$ fresh weight) as yellow crystals, mp 217–219 °C (from CH_2Cl_2) (Found: M^+ , 570. $\text{C}_{32}\text{H}_{26}\text{O}_{10}$ requires: M , 570); $[\alpha]_D +76.0$ (c 0.04, CHCl_3); CD λ_{max} 304 ($\Delta\epsilon$, +4.9), 274 (–140.8), 253 (+160.6), 238 (+53.9), 230 (+71.4) and 210 nm (–59.8); ν_{max} 3437 and 1623 cm^{-1} ; λ_{max} (log ϵ) 220 (4.43), 273 (4.57), 320 (3.92), 404 (3.85) and 460 nm (3.37); λ_{max} (EtOH + OH^-) 237 (4.61), 318 (4.24), 404 (3.95) and 537 nm (3.81); m/z 570 (M^+ , 100%), 552 (91) and 56 (19); δ_{H} Table 1; δ_{C} Table 2, (iii) torosachryson **13** (R_f 0.40) (10.5 mg, $9 \times 10^{-3}\%$ fresh weight) as yellow-green crystals identical with an authentic sample,¹⁶ and (iv) physcion **15** (R_f 0.80) (6.5 mg, $6 \times 10^{-3}\%$ fresh weight) as yellow crystals identical with an authentic sample.¹⁶

The stereochemistry of torosachryson **13** from *Dermocybe* sp. WAT 26640 was determined by HPLC [Daicel Chiralpak AD column (10 μm ; 0.46×25 cm)] with ethanol containing trifluoroacetic acid (0.05%) (0.5 ml min^{-1}) that gave peaks at retention times 11.5 min (72%) and 15.9 min (28%) corresponding to (*S*)- and (*R*)-torosachryson, respectively.¹¹

The green zone from the Sephadex column was evaporated to dryness to afford a mixture of the *austroviridins* **B 10** (R_f 0.22) and **A 11** (R_f 0.19) (35 mg, 83:17 by ^1H NMR spectroscopy, $3 \times 10^{-2}\%$ fresh weight) as a green powder from CHCl_3 –petrol (Found: M^+ , 582.1513. $\text{C}_{33}\text{H}_{26}\text{O}_{10}$ requires: M , 582.1526). Repeated PLC using toluene–ethyl formate–formic acid (50:49:1) and chloroform–ethyl acetate (1:1) gave (i) austroviridin **B 10** (containing 9% of **11**) as green needles from CHCl_3 –petrol, mp 278–281 °C, $[\alpha]_D -62$ (c 0.01), -47 (c 0.02 in CHCl_3); CD (CHCl_3) λ_{max} 394 ($\Delta\epsilon$, -4.0), 326 (+0.4), 286 (+14.4), 257 (–8.2) and 237 nm (2.2); ν_{max} 3431, 1643 and 1613 cm^{-1} ; λ_{max} (log ϵ) 227 (4.36), 265 (4.35), 278 (4.36), 417 (3.75), 457sh (3.68) and 604 nm (3.44); λ_{max} (EtOH + OH^-) 259 (3.63), 281 (3.46), 347 (3.61), 458 (3.08) and 684 nm (2.85); m/z 582 (M^+ , 42%), 564 (48), 546 (66), 284 (16), 149 (16), 91 (30) and 57 (100); δ_{H} and δ_{C} Table 3, and (ii) austroviridin **A 11** (containing 10% of **10**) as green needles from CHCl_3 –petrol, mp 277–281 °C, $[\alpha]_D -95$ (c 0.01), -120 (c 0.02 in CHCl_3); CD (CHCl_3) λ_{max} 392 ($\Delta\epsilon$, +1.7), 351 (–1.4), 289 (–15.3), 256 (+8.8) and 237 nm (–2.83); ν_{max} 3441, 1655 and 1619 cm^{-1} ; λ_{max} (log ϵ) 226 (4.47), 265

(4.35), 278 (4.36), 416 (3.77), 463 (3.15) and 603 nm (3.07); λ_{max} (EtOH + OH^-) 266 (4.57), 361 (3.97), 449 (3.98) and 537 nm (3.81); m/z as for **10** above; δ_{H} 1.03 (3H, s, 3'-Me), 2.52 (3H, s, 3-Me), 2.76 (1H, dd, J 14.8, 2.4 Hz, 4'- H_{eq}), 2.79 (1H, d, J 18.4 Hz, 2'- H_{ax}), 2.92 (1H, dd, J 18.4, 2.4 Hz, 2'- H_{eq}), 2.93 (1H, d, J 14.8 Hz, 4'- H_{ax}), 4.04, 4.06 and 4.07 (each 3H, s, 1, 6' and 8'-OMe), 6.64 (1H, s, 7'-H), 6.91 (1H, s, 7-H), 7.14 (1H, s, 2-H), 7.53 (1H, br s, 4-H), 13.75 (1H, s, 8-OH), 15.85 (1H, s, 9'-OH); δ_{C} 22.5 (q, 3-Me), 26.7 (q, 3'-Me), 43.1 (t, C-4'), 52.4 (t, C-2'), 56.6, 56.9 and 57.0 (each q, 1, 6' and 8'-OMe), 70.2 (s, C-3'), 95.6 (d, C-7'), 108.3 (d, C-7), 109.1 (s, C-8a'), 110.4 (s, C-9a'), 112.7 (s, C-10'), 114.6 (s, C-8a), 114.9 (s, C-5), 117.7 (s, C-9a), 118.4 (d, C-2), 121.0 (d, C-4), 130 (s, C-10a'), 130.9 (s, C-5'), 133.3 (s, C-4a'), 133.7 (s, C-10a), 136.3 (s, C-4a), 147.5 and 147.6 (each s, C-3 and C-6'), 156.4 (s, C-8'), 160.6 (s, C-1), 161.6 (s, C-6), 164.3 (s, C-8), 166.6 (s, C-9'), 186.1 (s, C-10), 187.0 (s, C-9), 202.3 (s, C-1').

(*3'R,P*)-Anhydropseudophlegmacin-9,10-quinone 1,6,6',8'-tetra-*O*-methyl ether **6** and 1,6,6',8,8'-penta-*O*-methyl ether **7**

The pseudophlegmacinquinone **5** (9.5 mg) and dimethyl sulfate (5 drops) were heated under reflux in acetone in the presence of an excess of anhydrous potassium carbonate. After 2 h the mixture was cooled in ice, filtered and the filtrate was concentrated, diluted with water (20 ml) and the products were extracted into ethyl acetate (3 × 10 ml). The combined extracts were dried (MgSO_4) and evaporated and the residue was separated by PLC using toluene–ethyl formate–formic acid (50:49:1) to give, in order of decreasing polarity, the *title compounds* **6** (5.8 mg, 60%) (R_f 0.35), as orange needles, mp 210–214 °C (from CH_2Cl_2) (Found: M^+ , 598.1834. $\text{C}_{34}\text{H}_{30}\text{O}_{10}$ requires: M , 598.1839); CD λ_{max} 297 ($\Delta\epsilon$, +24.8), 271 (–225.3), 251 (+225.8), 237 (+92.9), 230 (+137.7) and 212 nm (–132.0); ν_{max} 3471, 1648 and 1611 cm^{-1} ; λ_{max} (log ϵ) 225 (4.53), 273 (4.56) and 408 nm (4.06); λ_{max} (EtOH + OH^-) 257 (4.58), 272 (3.94), 411 (4.77) and 646 nm (3.30); m/z 598 (M^+ , 13%), 580 (100), 298 (23), 284 (22), 252 (13) and 78 (21); δ_{H} Table 1, and **7** (3.5 mg, 35%) (R_f 0.15), as red needles, mp 216–219 °C (from CH_2Cl_2) (Found: M^+ , 612.1995. $\text{C}_{35}\text{H}_{32}\text{O}_{10}$ requires: M , 612.1995); CD λ_{max} 289 ($\Delta\epsilon$, +26.2), 269 (–206.4), 248 (+194.8), 239 (+131.3), 231 (+179.8) and 213 (–173.8); ν_{max} 3453, 1648 and 1611 cm^{-1} ; λ_{max} (log ϵ) 226 (4.66), 274 (4.56) and 402 nm (4.07); m/z 612 (M^+ , 26%), 594 (60), 370 (63), 313 (43), 310 (61), 295 (38), 273 (22), 253 (100), 243 (24), 226 (29), 215 (36), 211 (22), 203 (22), 199 (70) and 92 (90); δ_{H} Table 1.

(*3'R,P*)-Anhydropseudophlegmacin-9,10-quinone 6-(4-bromo)-benzoate 1,6',8'-tri-*O*-methyl ether **8**

To a solution of the pseudophlegmacinquinone **5** (5 mg) in dry pyridine (0.5 ml) was added 4-bromobenzoyl chloride (4.6 mg) in ether (0.5 ml) and the mixture was stirred at room temperature for 3 days. Ether (30 ml) was added and the solution was washed with water (20 ml). The aqueous phase was extracted with ether (3 × 10 ml) and the organic phases were combined, washed with saturated copper(II) sulfate solution (20 ml), dried (MgSO_4) and evaporated to dryness. The residue (7.2 mg) was purified by PLC using toluene–ethyl formate–formic acid (50:49:1) as eluent to afford the *title compound* **8** (5 mg, 76%) (R_f 0.34), as an orange powder (Found: M^+ , 766.1047. $\text{C}_{40}\text{H}_{31}\text{O}_{11}\text{Br}$ requires: M , 766.1050); λ_{max} (log ϵ) 226 (4.66), 274 (4.56) and 406 nm (2.55); m/z 768/766 (M^+ , 30%), 750/748 (43), 566 (21), 185/183 (34), 105 (23), 91 (100), 85 (22), 83 (37), 77 (28), 75 (38), 71 (39), 69 (32), 57 (55) and 55 (44); δ_{H} Table 1.

(*3'R,P*)-Anhydropseudophlegmacin-9,10-quinone 6,6',8'-tri-*O*-methyl ether **9**

The pseudophlegmacinquinone **4** (1 mg) was exposed to an excess of ethereal diazomethane for 4 h. The excess reagent was

destroyed by dropwise addition of acetic acid and the solvents were removed under reduced pressure. Purification of the residue by PLC using toluene–ethyl formate–formic acid (50:49:1) as eluent gave the *title compound* (0.6 mg) as an orange film; ν_{\max} 3446 and 1610 cm^{-1} ; δ_{H} Table 1.

Isolation of the austroviridins 10 and 11 from *Dermocybe* sp. WAT 24274

Fresh fruiting bodies (330 g) were macerated in ethanol (2×500 ml) at room temperature overnight. The deep green extract was concentrated and the aqueous slurry was partitioned, in several portions, between ethyl acetate (250 ml) and water (250 ml). The organic phases were combined, dried and evaporated to afford a green residue that was passed through a column (55×3.5 cm) containing Sephadex LH-20 using methanol–dichloromethane–formic acid (49:49:1) as eluent. The green zone that eluted was purified further by PLC as described above to give a mixture of the austroviridins **B 10** and **A 11** (26 mg, $7.9 \times 10^{-3}\%$ fresh wt) identical in all respects with the materials described above.

Reductive cleavage of pseudophlegmacinquinone 5

To the dark yellow solution of the pseudophlegmacinquinone **5** (3 mg) in aqueous sodium hydroxide (1 M, 1.5 ml) was added solid sodium dithionite (30 mg). The colour of the solution changed immediately to dark yellow and after 1 min further portions of sodium dithionite (60 mg in total) were added. After 3 min the pale yellow solution was cooled in ice, neutralised with dilute hydrochloric acid (10%, *ca.* 0.5 ml) and the products were extracted into ethyl acetate (3×20 ml). The extracts were dried and evaporated and the residue was passed through a column containing Sephadex LH-20 (40×2 cm) using methanol–dichloromethane (1:1) as eluent. The first band to elute contained unchanged **5**, the second and third bands contained cleavage products that were further purified and analysed as follows.

The second band was analysed by chiral HPLC [Daicel Chiralpak-AD (10 μm ; 0.46×25 cm)] with ethanol–hexane (2:3) as eluant (0.5 ml min^{-1}). (*R*)-Torosachryson 8'-*O*-methyl ether **12** eluted after a retention time of 67.2 min. There was no trace of a peak at retention time 15.8 min corresponding to its enantiomer. The chromatogram was calibrated with a sample of 66% ee (*S*)-torosachryson 8'-*O*-methyl ether **12**,^{1,5} the individual enantiomers of which were separated by chiral HPLC (conditions as described above) and identified by comparison of their CD spectra with published data.²²

The third band was purified by PLC using toluene–ethyl formate–formic acid (50:49:1) to afford emodin 1-*O*-methyl ether **14** as an orange powder, δ_{H} 2.51 (3H, s, 3-Me), 4.06 (3H, s, 1-OMe), 6.67 (1H, d, *J* 2.6 Hz, 7-H), 7.16 (1H, br s, 2-H), 7.22 (1H, d, *J* 2.6 Hz, 5-H), 7.76 (1H, br s, 4-H), 13.28 (1H, s, 8-OH); *m/z* 284 (M^+ , 1%), 201 (24), 199 (54), 166 (50), 151 (92), 149 (34), 138 (39), 91 (100), 77 (25) and 65 (22), identical with an authentic sample.⁶

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