

**Austrocolorone B and austrocolorin B<sub>1</sub>, cytotoxic anthracenone dimers from the  
Tasmanian mushroom *Cortinarius vinosipes* Gasparini**

**Karren D. Beattie<sup>a,b\*1</sup>, Dion R. Thompson<sup>c</sup>, Evelin Tiralongo<sup>b</sup>, David Ratkowsky<sup>d</sup>, Tom  
W. May<sup>e</sup>, Melvyn Gill<sup>a2</sup>**

<sup>a</sup> *School of Chemistry, The University of Melbourne, Parkville, VIC 3010, Australia*

<sup>b</sup> *School of Pharmacy, Griffith University, Gold Coast Campus, QLD 4222, Australia*

<sup>c</sup> *Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, NSW 2480,  
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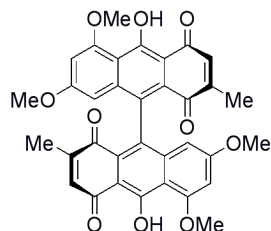
<sup>d</sup> *School of Agricultural Science, University of Tasmania, Hobart, TAS 7001, Australia*

<sup>e</sup> *Royal Botanic Gardens Melbourne, South Yarra, VIC 3141, Australia*

\*Corresponding author

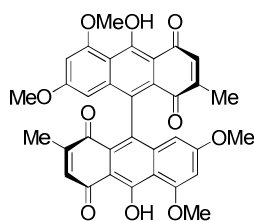
Dr Karren Beattie

School of Health and Human Sciences, Southern Cross University, Lismore Campus, NSW  
2480, Australia; *E-mail address:* karren.beattie@scu.edu.au; karren.beattie@hotmail.com  
Tel.: +61 2 66203358; fax: + 61 2 66203880.



<sup>1</sup> Present address: School of Health and Human Sciences, Southern Cross University, Lismore, NSW 2480, Australia.

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\*Corresponding author

Dr Karren Beattie

School of Health and Human Sciences, Southern Cross University, Lismore Campus, NSW 2480, Australia

*E-mail address:* karren.beattie@scu.edu.au; karren.beattie@hotmail.com

Tel.: +61 2 66203358; fax: + 61 2 66203880.

**Abstract**

<sup>†</sup> Present address: School of Health and Human Sciences, Southern Cross University, Lismore, NSW 2480, Australia.

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The yellow dihydroanthracenone dimer, austrocolorin B<sub>1</sub> and the unique violet-red 1,4-anthraquinone dimer, austrocolorone B, were isolated from the Tasmanian mushroom *Cortinarius vinosipes* and their structures and stereochemistry were determined from spectroscopic data. Austrocolorin B<sub>1</sub> and austrocolorone B were found to exhibit potent cytotoxic activity *in vitro* against the murine lymphoblast (P388D<sub>1</sub>) cell line with IC<sub>50</sub> values in the range 10–31 µg/mL.

**Keywords:** cytotoxic, anthraquinone, tricolorin, Australian fungi

Mushrooms of the genus *Cortinarius* are renowned for their vividly coloured fruiting bodies.<sup>1</sup> Chemical investigations have revealed that members of the genus metabolise a diverse range of anthraquinonoid pigments that have proved to be useful in the chemotaxonomic differentiation of species.<sup>1,2</sup> Whilst 9,10-anthraquinones and dihydroanthracenones, are widely reported from the genus, the occurrence of 1,4-anthraquinones and dimeric anthraquinones is less common.<sup>1</sup> At present the rarest of these dimers are members of the tricolorin group comprising of C10 to C10' coupled dihydroanthracenones, which are found only in the fungal genera *Leucopaxillus* and *Cortinarius*.<sup>1</sup> The tricolorins austrocolorin A<sub>1</sub> (**1**) and austrocolorin B<sub>1</sub> (**2**) were isolated from an undescribed Australian mushroom belonging to the subgenus *Dermocybe* of *Cortinarius*.<sup>3§\*\*</sup>

We report here the isolation, structural elucidation and *in vitro* cytotoxicity of a new member of the tricolorin group, namely the violet-red, 1,4 anthraquinone, austrocolorone B (**3**) together with the known, and closely related austrocolorin B<sub>1</sub> (**2**), from an Australian mushroom *Cortinarius vinosipes* Gasparini.<sup>7</sup>

Specimens of *C. vinosipes* were collected in damp gullies amongst wet sclerophyll forest in South-East Tasmania from several field trips between 1996–99.<sup>8</sup> Their beautiful 'rose-coloured' fruit bodies are very large (4.5–18 cm in diameter), with thick bulbous stipes, and vary in color from

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§ The first member of this group, tricolorin A, was isolated by Steglich and co-workers from *Leucopaxillus tricolor* and assigned the (3*S*,*M*,3*R*)-stereochemistry.<sup>4</sup>

\*\* *Dermocybe* has been variously treated as a distinct genus or as a subgenus of *Cortinarius*.<sup>5</sup> Phylogenetic reconstructions utilising molecular data show that all species of *Dermocybe* nest within *Cortinarius*.<sup>6</sup>

pink to reddish-brown to brick red.<sup>7</sup> The chopped, air-dried specimens (5.54 g) were macerated in EtOH and the filtered dried residue was partitioned between EtOAc and H<sub>2</sub>O. The organic phase was chromatographed on silica TLC plates [toluene-HCO<sub>2</sub>Et-HCO<sub>2</sub>H (50:49:1) as eluent] and revealed the presence of major yellow ( $R_f$  0.30) and violet-red ( $R_f$  0.63) zones.<sup>9</sup>

The major yellow zone (1.5 mg) was isolated by PTLC (as above) followed by gel permeation chromatography (Sephadex LH-20 suspended and eluted in MeOH) as an optically active yellow powder,  $[\alpha]_D -316$  ( $c$  0.045, CHCl<sub>3</sub>), mp 139–141 °C (dec., MeOH). Spectroscopic data indicated that this pigment, **2** was an isomer of austrocolorin. In a preceding article we established the absolute axial and central configuration of the austrocolorin A<sub>1</sub> and B<sub>1</sub> atropisomers from detailed analysis of the <sup>1</sup>H NMR data, the CD spectrum and by reductive cleavage of the biaryl bond and subsequent chiral HPLC analysis.<sup>3</sup> In this instance, the major yellow pigment from *C. vinosipes* was distinguished as the known (3*S*, *P*, 3'*S*)-austrocolorin B<sub>1</sub> atropisomer (**2**) from comparison of the published NMR and CD data.<sup>3</sup>

The violet-red zone (5.6 mg,  $R_f$  0.17) was subjected to further PTLC [silica, petrol -EtOAc-HCO<sub>2</sub>H (75:24:1)] in order to remove a colourless material, which was subsequently identified as ergosta-5,7,22*E*-trien-3 $\beta$ -ol ( $R_f$  0.38, 3.4 mg,  $6.9 \times 10^{-2}$  % yield / dry wt.) from spectroscopic analysis.<sup>10,11</sup> Crystallization of the violet-red pigment (1.8 mg,  $3.2 \times 10^{-4}$  % yield dry wt.) from EtOAc-petrol afforded optically active dark red needles,  $[\alpha]_D -419$  ( $c$  0.011, CHCl<sub>3</sub>).<sup>9</sup> The mass spectrum of the pigment showed a pseudo molecular ion  $[M+H]^+$  at  $m/z$  595 that, by high resolution mass measurement, corresponded to the molecular formula C<sub>34</sub>H<sub>26</sub>O<sub>10</sub>. This suggested a close structural relationship between this new compound and austrocolorin B<sub>1</sub> (C<sub>34</sub>H<sub>34</sub>O<sub>10</sub>). The <sup>1</sup>H NMR spectrum of the compound consisted of signals due to a chelated phenolic hydroxy group ( $\delta$  15.97), two *meta*-coupled aromatic protons ( $\delta$  6.12 and  $\delta$  6.64), two methoxy groups ( $\delta$  4.07 and  $\delta$  3.49), one C-methyl group ( $\delta$  1.93) and a quinonoid methine proton ( $\delta$  6.86). Significantly, the signal from the C-methyl group ( $\delta$  1.93) appeared as a doublet ( $J$  = 1.4 Hz) coupled to the quinonoid methine proton, which appeared as a doublet ( $J$  = 1.4 Hz) at  $\delta$  6.86. The UV spectrum exhibited a long

wavelength absorption at 522 nm consistent with a 1,4-anthraquinone chromophore.<sup>12</sup>

Of particular significance is the suggestion that the violet-red pigment possessed a high degree of symmetry, as the <sup>1</sup>H NMR spectrum (Table 1) contained signals for only one half of the protons indicated by its molecular formula. The identification of each half of the violet-red pigment as the partial structure **4**, followed from analysis of the 1D and 2D NMR data (Table 1), and from comparison with the corresponding data reported for the 7-methyl ester of viocristin (**5**).<sup>13</sup>

That the violet-red pigment consists of two units of the partial structure **4** coupled from C10 in one half to C10' in the other was evident from the <sup>1</sup>H NMR spectrum, in which there was no resonance that corresponded to a proton at C-10 ( $\delta_{\text{H-10}}$  in **5** is  $\delta$  7.87).<sup>13</sup> The 10,10' coupling is further supported by the upfield shift of the signals assigned to the aromatic protons at C-5/C-5' ( $\delta$  6.12) in comparison to their counterpart in the spectrum of **5** ( $\delta$  6.63)<sup>13</sup> due to the anisotropic shielding by the adjacent aromatic rings. Thus, the violet-red pigment, which we here call austrocolorone, must have the gross structural formula **3**, which is fully consistent with the results of HMBC and HMQC correlation experiments.

Whilst **3** does not contain any stereogenic centers, optical activity arises by virtue of the restricted rotation about the biaryl axis. The *P* absolute configuration of the axis in **3**, as shown in the formula is suggested on biosynthetic grounds if it is assumed that austrocolorone is derived by dehydration and oxidation of austrocolorin B<sub>1</sub> (**2**). Nevertheless, it has been reported that the axial configuration of asymmetric bianthrils can be confirmed from the shape of the CD spectrum (Fig. 1).<sup>14-16</sup>

Exciton coupling between the asymmetrically disposed long axes of the two bianthryl chromophores in such systems manifests itself as a bisignate Cotton effect couplet centred in the vicinity of 260 nm.<sup>15,16</sup> According to the theory, a biaryl in which there is a clockwise helical twist between the two aromatic chromophores (positive chirality) gives rise to a CD spectrum that exhibits a positive Cotton effect to longer wavelengths and a negative one to shorter wavelengths (an A type curve).<sup>14-16</sup> Alternatively, molecules in which there is an anticlockwise helical twist

between the chromophores (negative chirality), give rise to a CD spectrum in which, at longer wavelengths, the Cotton effect is negative and at shorter wavelengths it is positive (a B type curve).<sup>14-16</sup> In the case of austrocolorone, the Cotton effect couplet centered at 254 nm exhibits a positive chirality (B type curve). On this basis, the stereochemistry at the C-10 / C-10' biaryl axis in austrocolorone **3** is designated as *P* according to the Prelog-Helmchen rules,<sup>17</sup> and reflects a clockwise helical twist between the two anthraquinone ring systems as shown in structure **3**. Whilst 10,10'-bianthraces (or 9,9'-bianthraces) are described in the literature, such as the rheidins A-C, emodinbianthrone and palmidin A-D,<sup>18</sup> these arise as dimers of a 9,10-anthracenedione moiety. Austrocolorone B is the first naturally occurring 10,10'-coupled (or 9,9'-coupled) 1,4-anthracenedione dimer reported in the scientific literature.

The occurrence of austrocolorin B<sub>1</sub> in both *C. vinosipes* and *Cortinarius* sp. WAT26641, a species examined previously by our group,<sup>3</sup> suggests that they may be closely related. On the basis of morphology, *C. vinosipes* has been assigned to the *Cortinarius* subgenus *Telamonia*<sup>7</sup> whereas *Cortinarius* sp. WAT26641 has been assigned to the subgenus *Dermocybe*.<sup>3</sup> From a chemotaxonomic standpoint, anthraquinones are predominantly metabolised by species of *Cortinarius* belonging to the subgenus *Dermocybe*, however anthraquinones have also been reported from some species of the subgenus *Telamonia*.<sup>1</sup> Molecular data have proved useful for elucidating evolutionary relationships within *Cortinarius*, and morphology is not always congruent with DNA sequence data.<sup>6</sup> At present, there is no molecular data to confirm the subgeneric placement of either *C. vinosipes* or *Cortinarius* sp. WAT 26641, nor the relationship of the two species to each other.

It is noted that these pigments are structurally related to the anthracycline antibiotics such as doxorubicin (**6**), which are amongst the most widely prescribed and effective chemotherapeutic agents.<sup>19-21</sup> Anthracyclines have been found to exert their cytotoxic effects by binding intercalatively with DNA, and in some cases RNA, or *via* the inhibition of topoisomerase II.<sup>20</sup> More

recently, there has been renewed interest in the structurally related quinonoids, hypericin (**7**) and hypocrellins, as photosensitizing agents for utilization in photodynamic cancer therapy.<sup>22,23</sup>

Previous investigations into the antibacterial activity of a large collection of *Cortinarius* species have found that the lipophilic extract *C. vinosipes* was active against *Staphylococcus aureus* (IC<sub>50</sub> 0.06–0.13 mg/mL) and *Pseudomonas aeruginosa* (IC<sub>50</sub> 0.54–0.96 mg/mL).<sup>24††</sup> In this study, austrocolorin B<sub>1</sub> and austrocolorin B were evaluated for cytotoxic activity via a cell proliferation assay against murine lymphoblast (P388D<sub>1</sub>) cells. Austrocolorin B<sub>1</sub> and austrocolorin B exhibited notable cytotoxic activity (with IC<sub>50</sub> values of 10 µg/mL and 31 µg/mL, respectively) relative to the positive controls emodin, curcumin and chlorambucil (with IC<sub>50</sub> values of 31, 22 and 215 µg/mL, respectively). Austrocolorin B<sub>1</sub> exhibited a distinctly biphasic dose response curve, whereupon at concentrations between ca. 1–20 µg/mL cell proliferation appeared to have ceased while cytotoxicity only became relevant above this concentration range. Consequently, **2** was evaluated in a cell cycle analysis assay however, this did not give an indication of cell cycle arrest.

In conclusion, this is the first report of the major pigments from the Tasmanian mushroom *C. vinosipes* and the first report of a naturally occurring 10,10'-coupled, 1,4-anthraquinone, being only the third tricolorin known from the genus *Cortinarius*.

## Acknowledgments

The Australian Research Council (ARC) is acknowledged for their long-standing financial support (to M.G.). We would like to thank Ms. Genevieve Gates and the late Mrs. Ann Ratkowsky for their assistance with the collection of mushroom specimens. K.B. is grateful to the Centre for Phytochemistry and Pharmacology at Southern Cross University for access to their NMR facility.

## Supplementary data

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<sup>††</sup> Due to the limited quantity of material we were unable to undertake antibacterial screening of these pigments.

Supplementary data (1D and 2D NMR spectra for austrocolorin B<sub>1</sub> and austrocolorone B, cytotoxic dose response curves, general experimental, fungi collection, pigment extraction and isolation details, and mammalian cell assay procedures) associated with this article can be found in the online version, at doi:

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9. (*P*)-Austrocolorone B (**3**). Dark red needles; mp >320 °C (EtOAc-petrol); R<sub>f</sub> 0.63, 1.8 mg, 3.2 × 10<sup>-4</sup>% yield dry wt.; [α]<sub>D</sub> -419 (c 0.011, CHCl<sub>3</sub>); UV (EtOH) λ<sub>max</sub> 522.0 (log ε, 3.78), 336.5 (3.72), 293.0 sh (3.78) 254.0 (4.62), 203.5 (4.39) nm ; CD (Figure 1, MeOH) 398 nm (Δε, 0.0), 312 (+2.0), 304 (0.0), 292 (-4.2), 275 (0.0), 262 (+12.5), 254 (0.0), 249 (-8.2), 241 (0.0), 237 (+1.8), 229 (0.0), 223 (-1.2), 216 (0.0), 208 (+1.6), 203 (0.0), 200 (-5.5);

- IR (KBr)  $\nu_{\max}$  3447, 2916, 1638 and 1596  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data ( $\text{CDCl}_3$ ) see Table 1; (+)-LRESIMS (30 eV)  $m/z$  (rel. int.), 595 (20), 428 (59), 370 (25), 354 (97), 316 (38), 288 (100), 283 (83), 280 (60), 227 (39), 220 (91); HREIMS  $m/z$  594.1504 [ $\text{M}^+$ ] ( $\text{C}_{34}\text{H}_{26}\text{O}_{10}$  requires 594.1526).
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## Tables

Table 1. NMR (CDCl<sub>3</sub>, 500 MHz) data for austrocolorone B (**3**)

## Legends

none

## Figures

Figure 1. Circular dichroism spectrum of austrocolorone (**3**) in methanol

Table 1. NMR (CDCl<sub>3</sub>, 500 MHz) data for austrocolorone B (**3**)

Position	<sup>13</sup> C	<sup>1</sup> H (mult., <i>J</i> in Hz, int.)	gCOSY	gHMBC
1,1'	188.3			
2,2'	136.8	6.86 (d, 1.4, 2H)	3,3'	9a,9a', 4,4'
3,3'	149.7			
3,3'-Me	17.0	1.93 (d, 1.4, 6H)	2,2'	2,2', 3,3', 4,4'
4,4'	184.6			
4a,4a'	134.8			
5,5'	100.9	6.12 (d, 2.2, 2H)	7,7'	7,7', 8a,8a', 4a,4a', 6,6', 8,8'
6,6'	163.1			
6,6'-OMe	55.5	3.49 (s, 6H)		8,8'
7,7'	101.1	6.64 (d, 2.2, 2H)	5,5', 8,8'-OMe <sup>a</sup>	5,5', 8a,8a', 6,6', 8,8'
8,8'	162.3			
8,8'-OMe	56.6	4.07 (s, 6H)	7,7' <sup>a</sup>	8,8'
8a,8a'	113.9			
9,9'-OH	166.5	15.97 (s, 2H)		
9a,9a'	108.9			
10,10'	125.9			
10a,10a'	140.9			

<sup>a</sup> Weak correlation

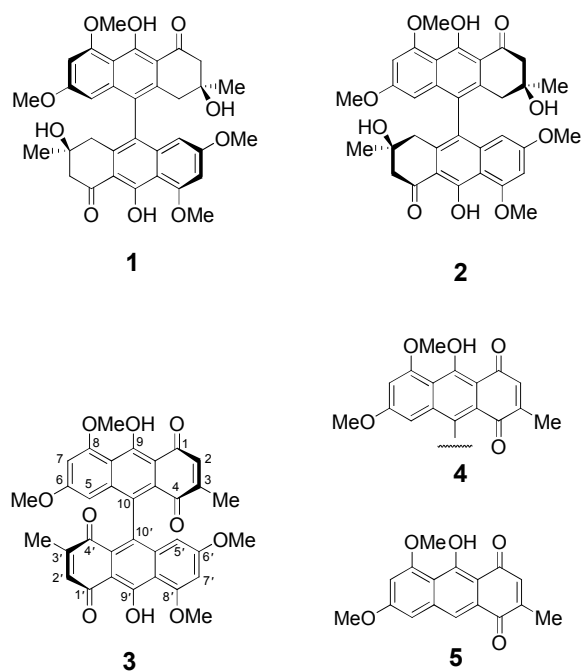


Figure 1, for color reproduction on the web.

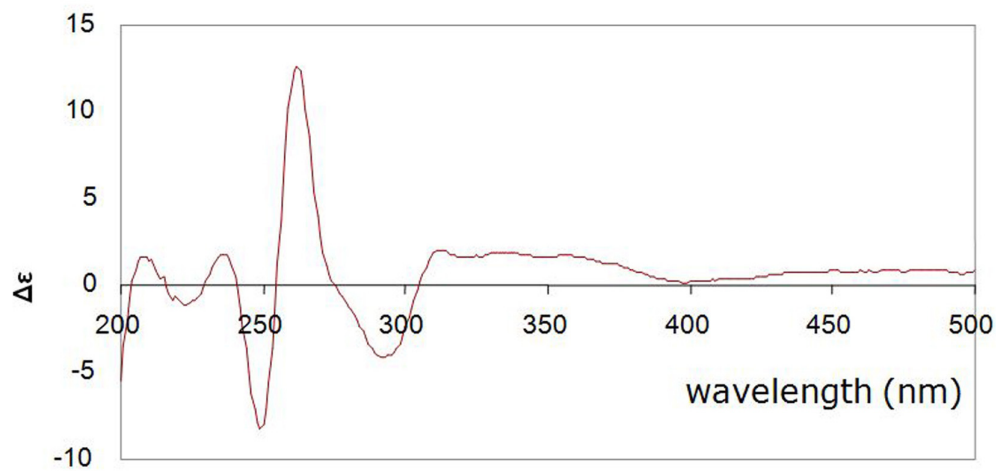


Figure 1. Circular dichroism spectrum of austrocolorone (**3**) in methanol

Figure 1, for black and white reproduction in print.

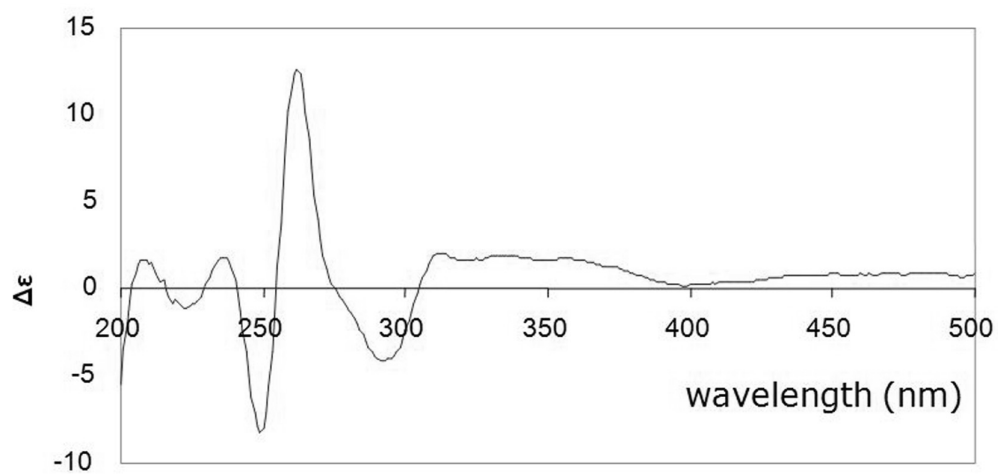
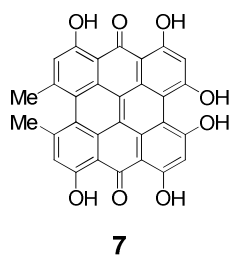
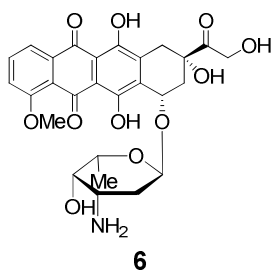


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## S1 Supplementary data contents page

**Title:** Austrocolorone B and austrocolorin B<sub>1</sub>, cytotoxic anthracenone dimers from the Tasmanian mushroom *Cortinarius vinosipes*

**Authors:** Karren D. Beattie<sup>a,b,\*†</sup>, Dion R. Thompson<sup>c</sup>, Evelin Tiralongo<sup>b</sup>, David Ratkowsky<sup>d</sup>, Tom W. May<sup>e</sup>, Melvyn Gill<sup>a</sup>

**Addresses:** <sup>a</sup> School of Chemistry, The University of Melbourne, Parkville, VIC 3010, Australia

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<sup>c</sup> Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, NSW 2480, Australia

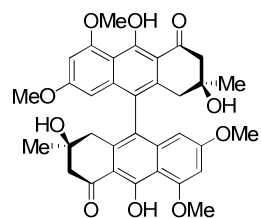
<sup>d</sup> School of Agricultural Science, University of Tasmania, Hobart, TAS 7001, Australia

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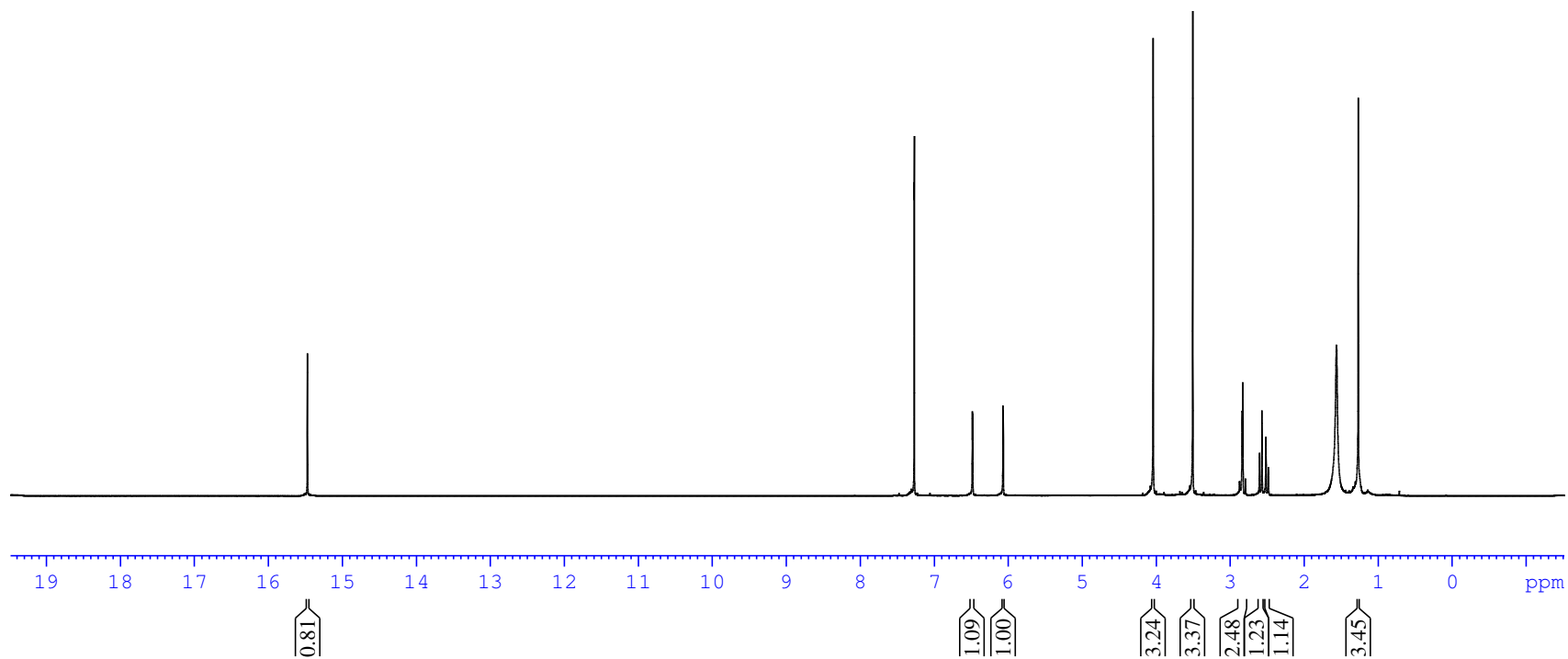
**\*Corresponding author contact details:** Tel: + 61 2 66203358; fax: + 61 2 66203880; E-mail address: karren.beattie@scu.edu.au; karren.beattie@hotmail.com; <sup>†</sup> Present address: School of Health and Human Sciences, Southern Cross University, Lismore, NSW 2480, Australia

- S2** <sup>1</sup>H NMR for austrocolorin B<sub>1</sub> (**2**) in CD<sub>3</sub>OD
- S3** gCOSY for austrocolorin B<sub>1</sub> (**2**) in CD<sub>3</sub>OD
- S4** HSQC for austrocolorin B<sub>1</sub> (**2**) in CD<sub>3</sub>OD
- S5** gHMBC for austrocolorin B<sub>1</sub> (**2**) in CD<sub>3</sub>OD
- S6** <sup>13</sup>C JMOD for austrocolorin B<sub>1</sub> (**2**) in CD<sub>3</sub>OD
- S7** <sup>1</sup>H NMR for austrocolorone B (**3**) in CD<sub>3</sub>OD
- S8** gCOSY for austrocolorone B (**3**) in CD<sub>3</sub>OD
- S9** HSQC for austrocolorone B (**3**) in CD<sub>3</sub>OD
- S10** gHMBC for austrocolorone B (**3**) in CD<sub>3</sub>OD
- S11** <sup>13</sup>C JMOD for austrocolorone B (**3**) in CD<sub>3</sub>OD
- S12** Cytotoxic activity of compounds against mouse lymphoblast cells
- S13** General experimental details
- S14** Fungi material
- S15** Extraction and isolation of pigments from *C. vinosipes*
- S16** Mammalian cell assays

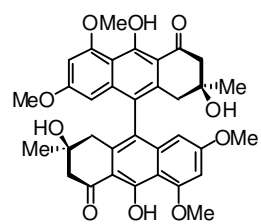
## S2 $^1\text{H}$ NMR for austrocolorin B<sub>1</sub> (2) in CD<sub>3</sub>OD



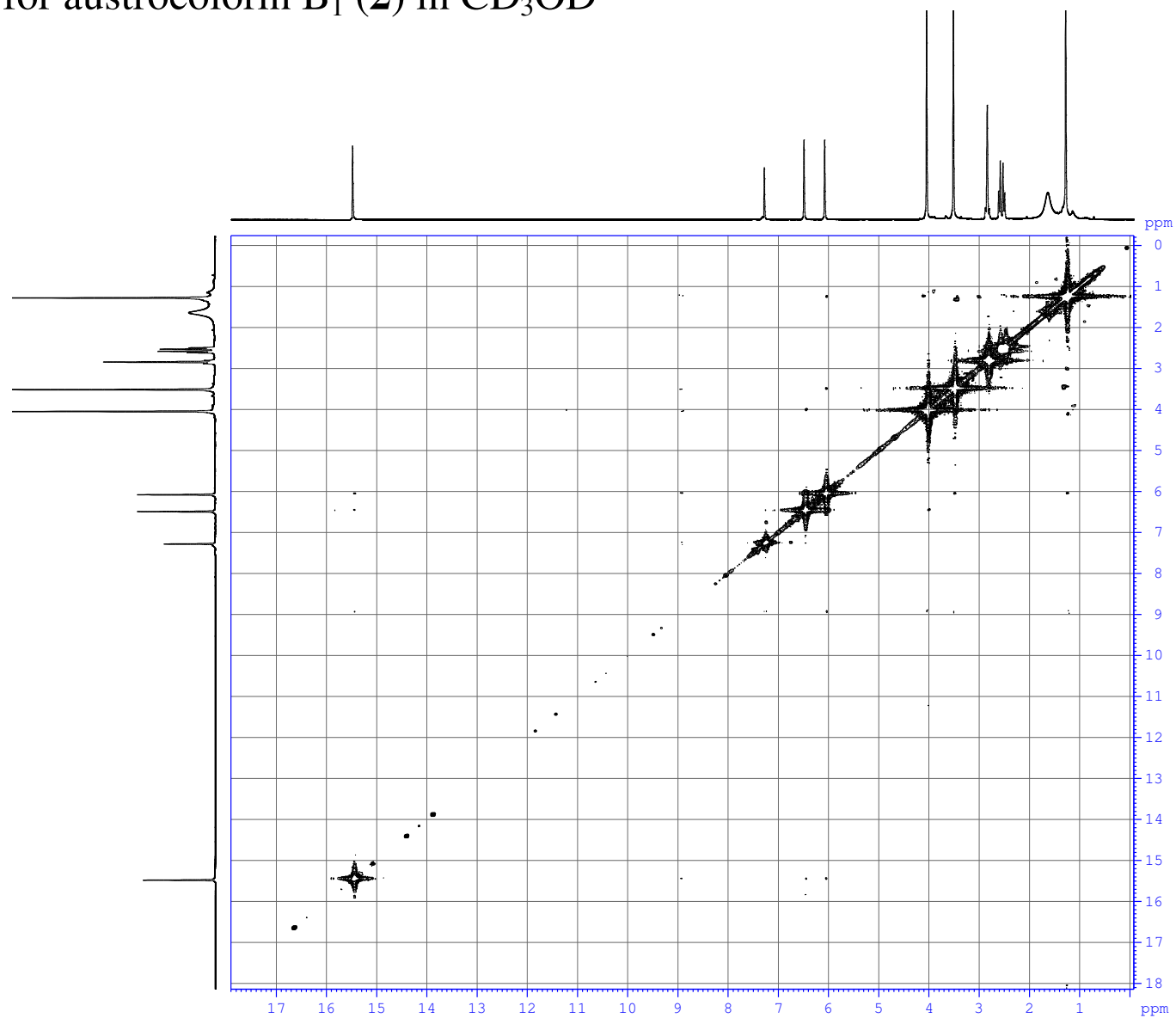
**2**



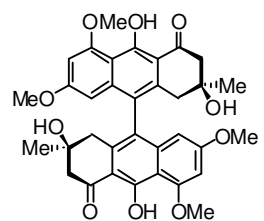
### S3 gCOSY for austrocolorin B<sub>1</sub> (2) in CD<sub>3</sub>OD



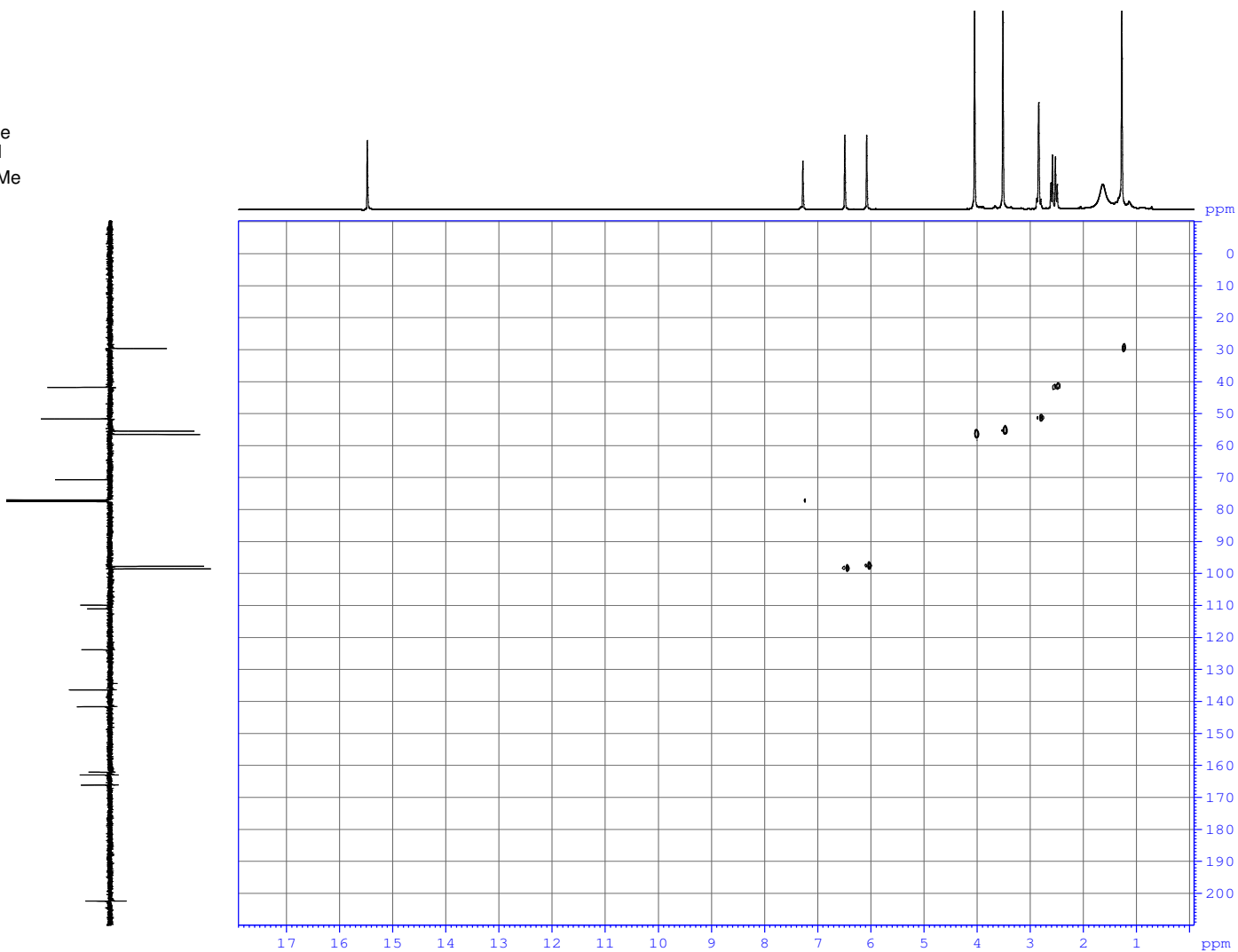
2



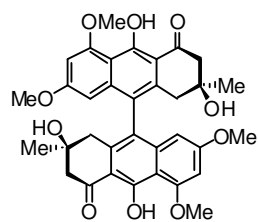
# S4 HSQC for austrocolorin B<sub>1</sub> (2) in CD<sub>3</sub>OD



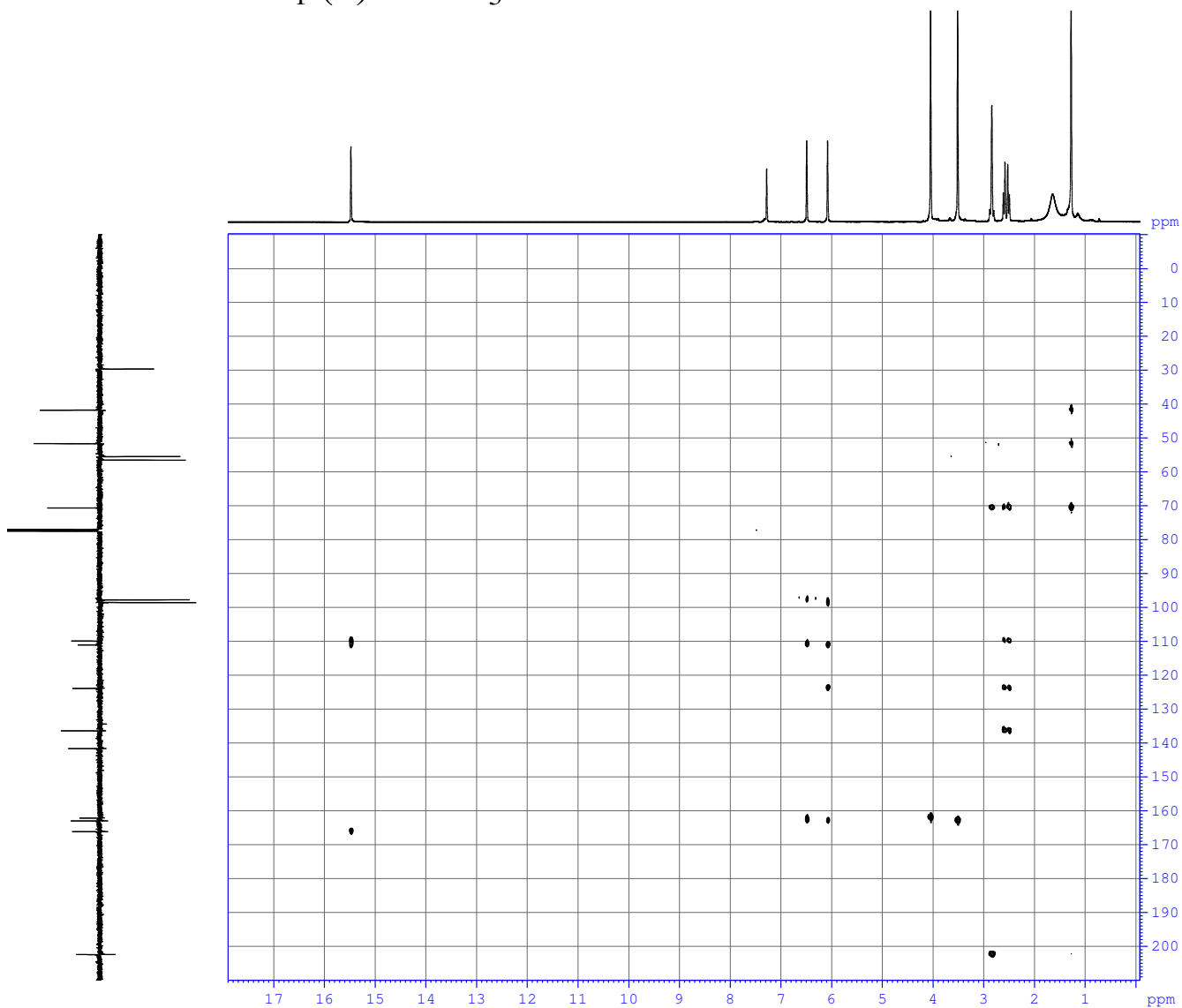
2



# S5 gHMBC for austrocolorin B<sub>1</sub> (**2**) in CD<sub>3</sub>OD

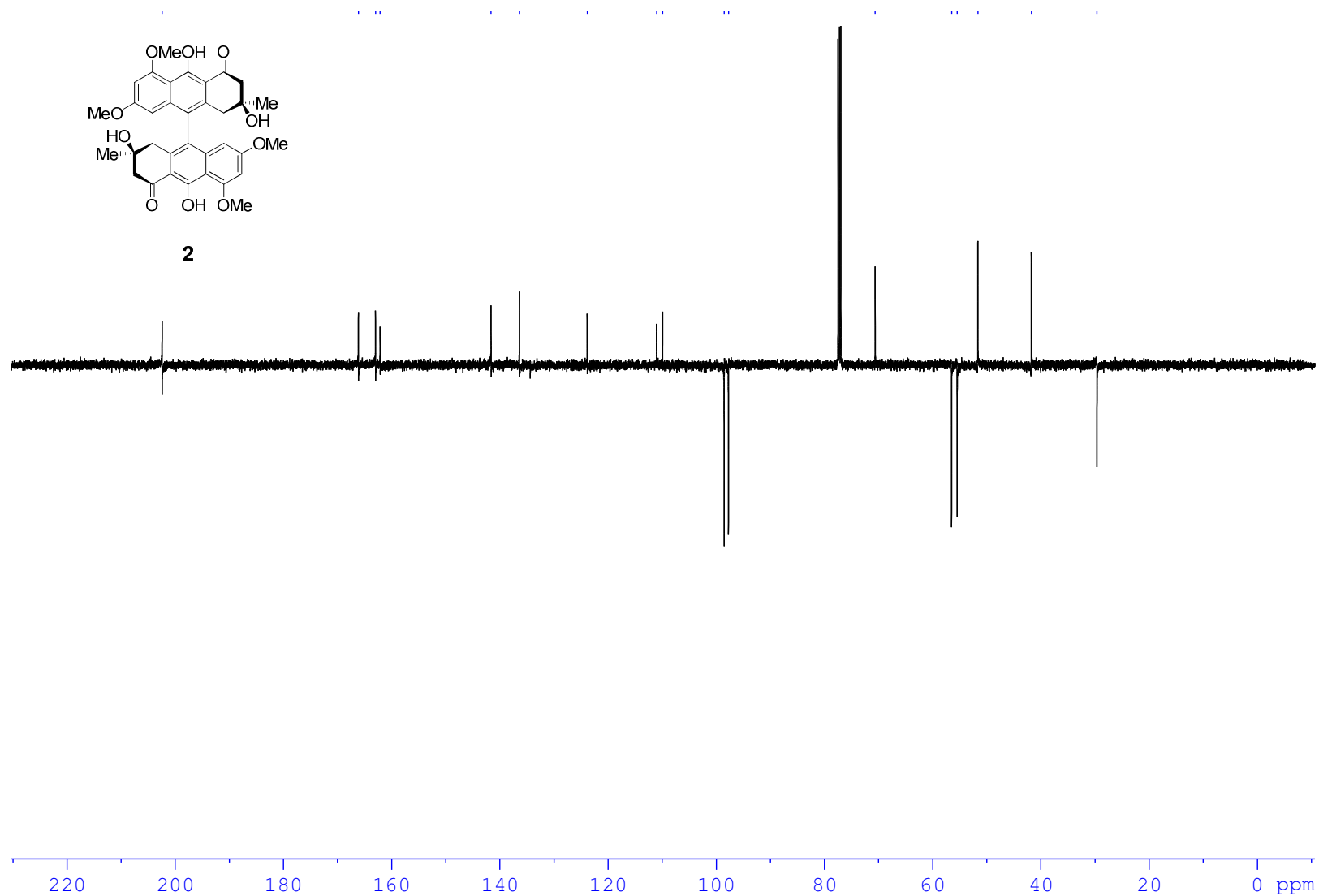


**2**

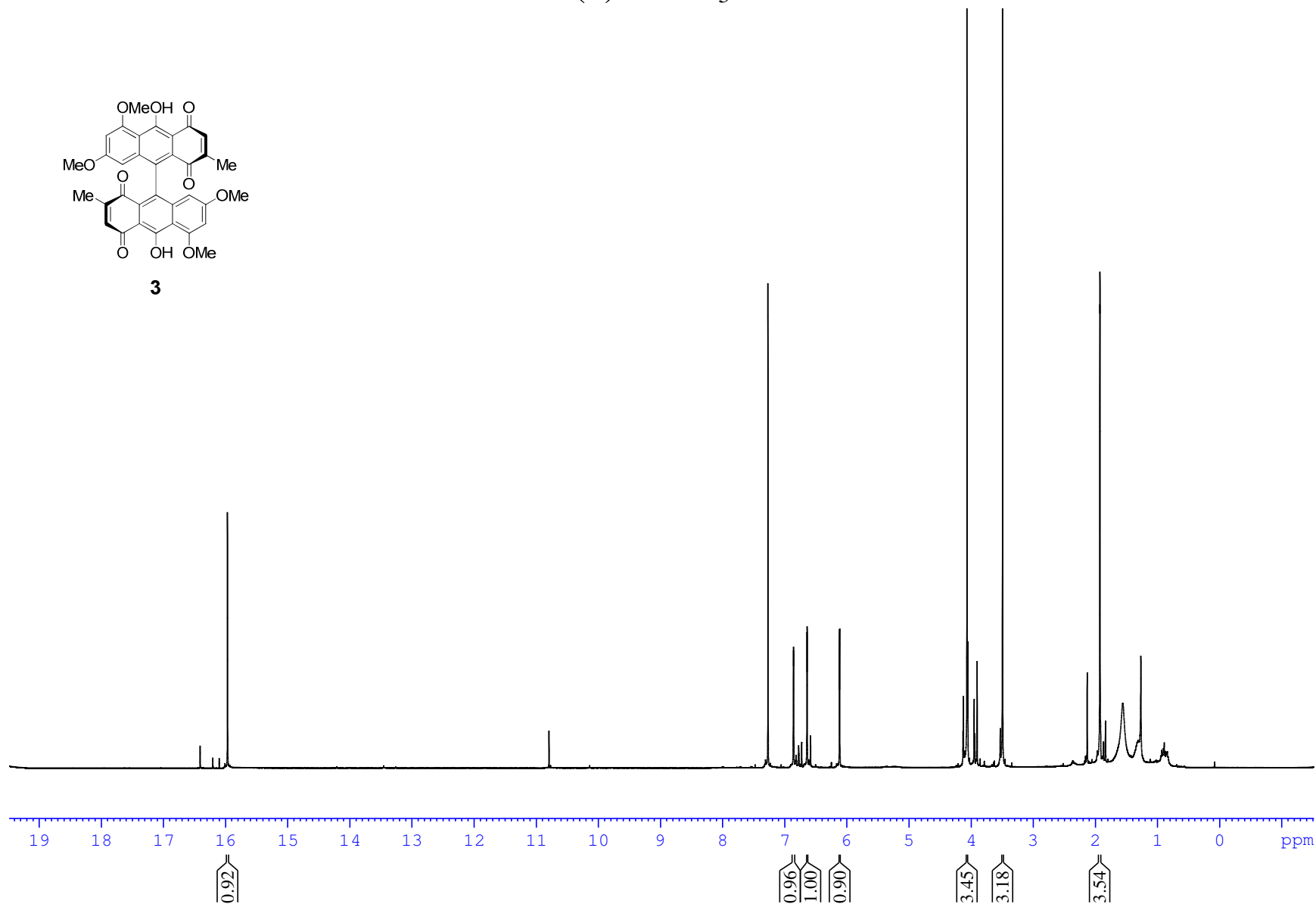
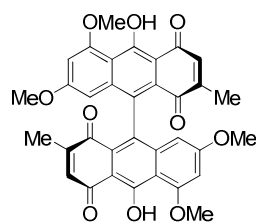


S6

$^{13}\text{C}$  JMOD for austrocolorin B<sub>1</sub> (**2**) in CD<sub>3</sub>OD

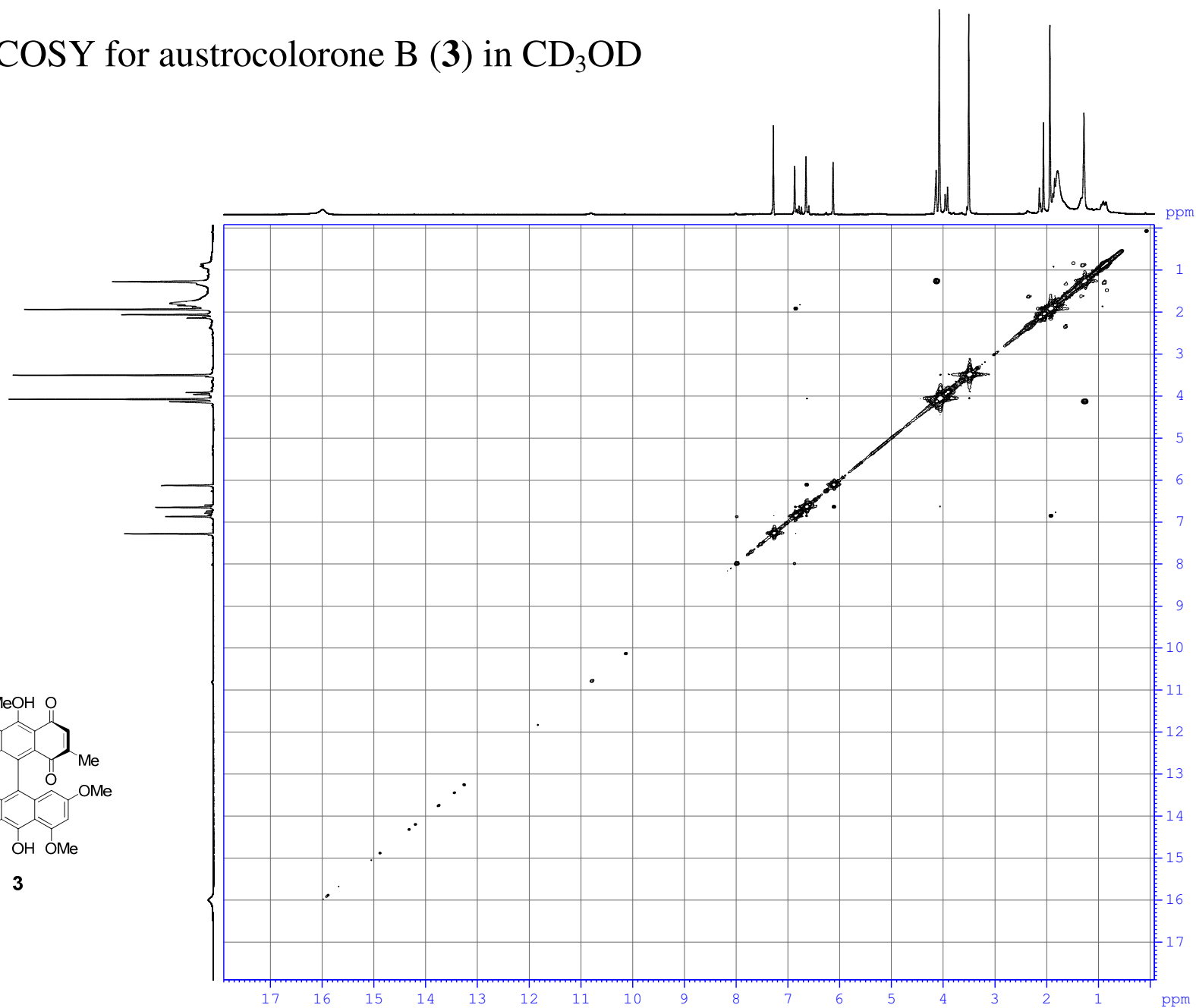
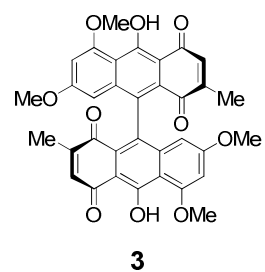


**S7**  $^1\text{H}$  NMR for austrocolorone B (**3**) in  $\text{CD}_3\text{OD}$

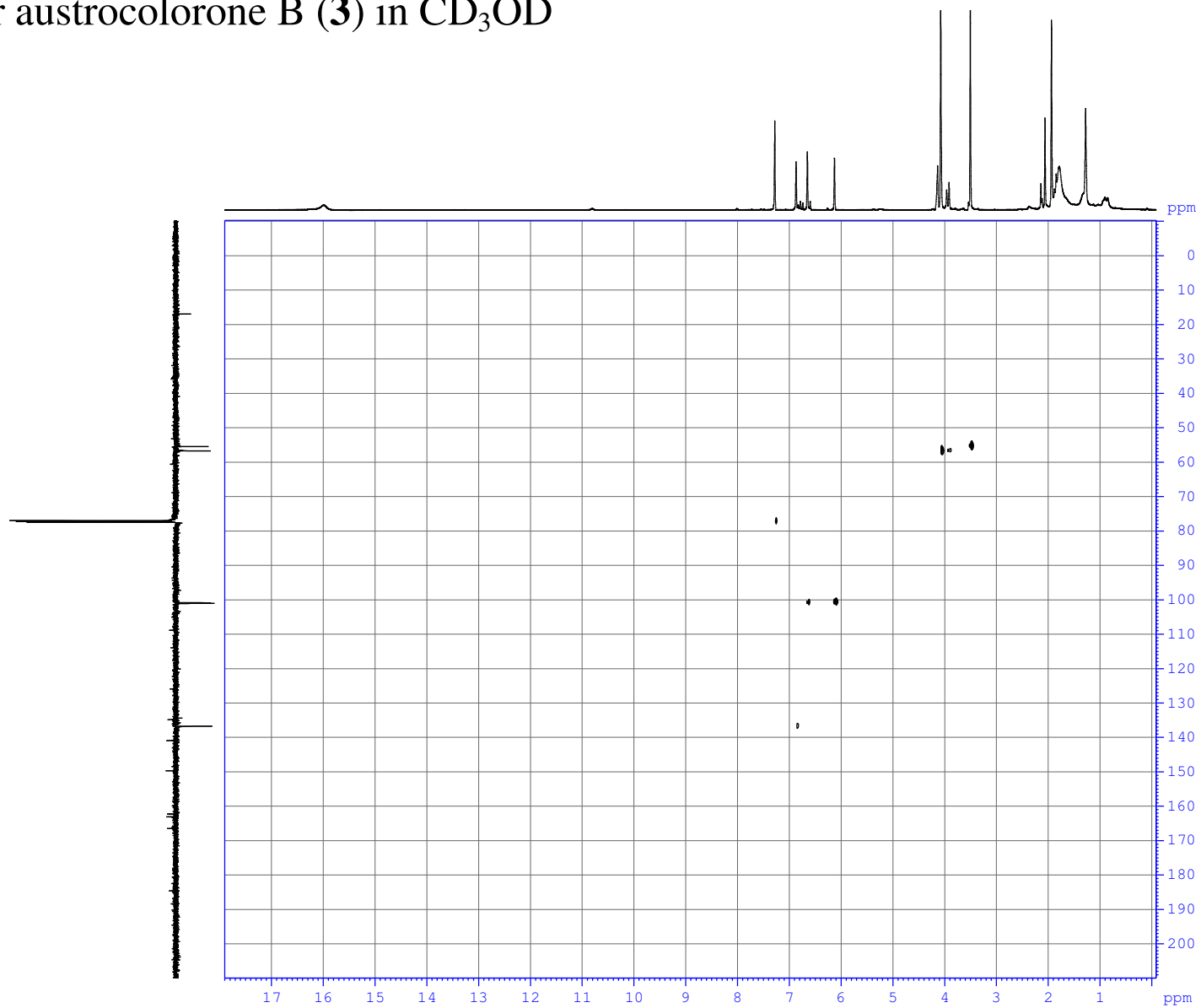
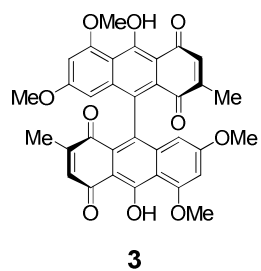


S8

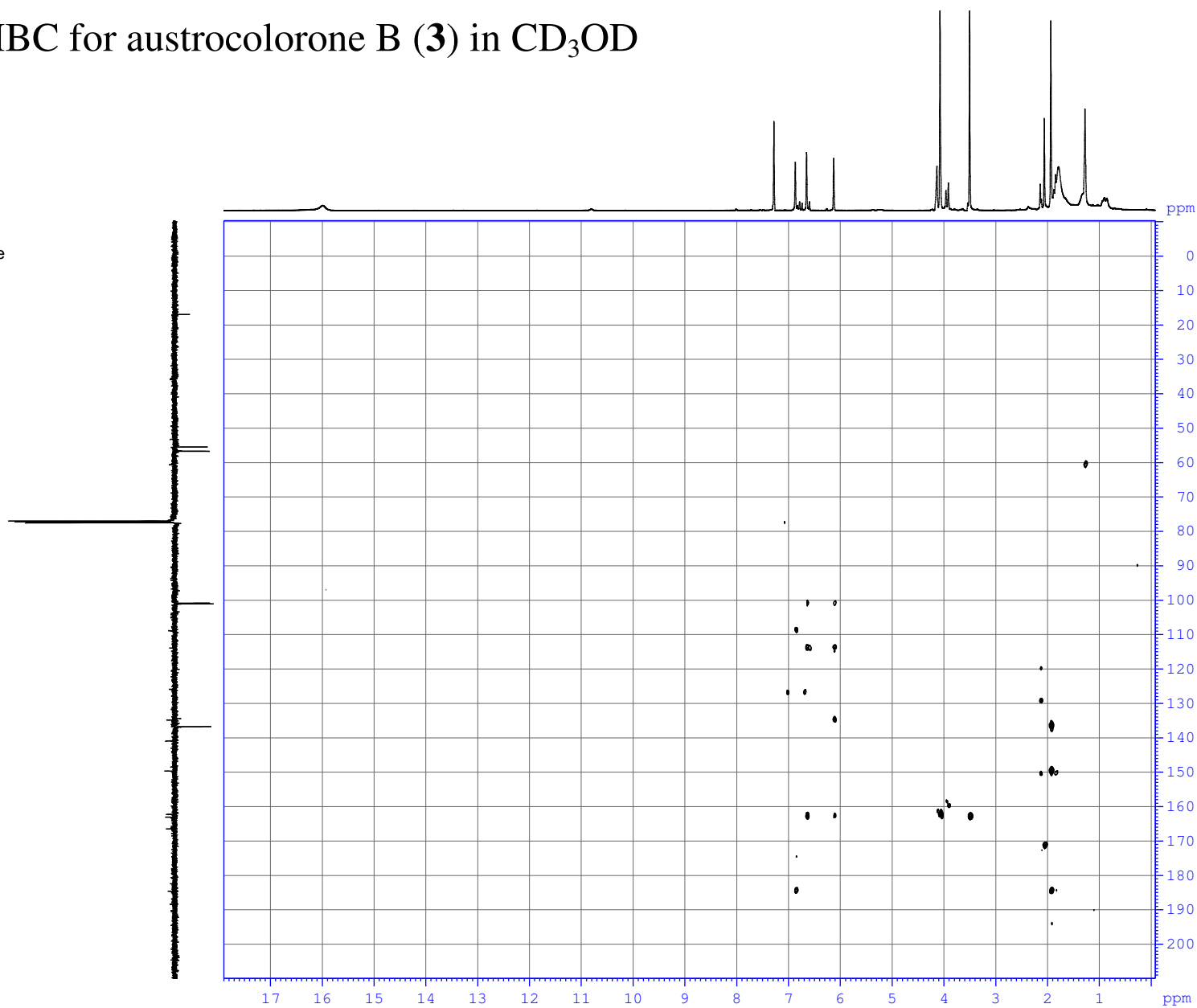
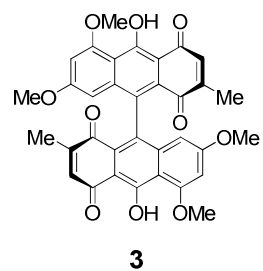
gCOSY for austrocolorone B (**3**) in CD<sub>3</sub>OD



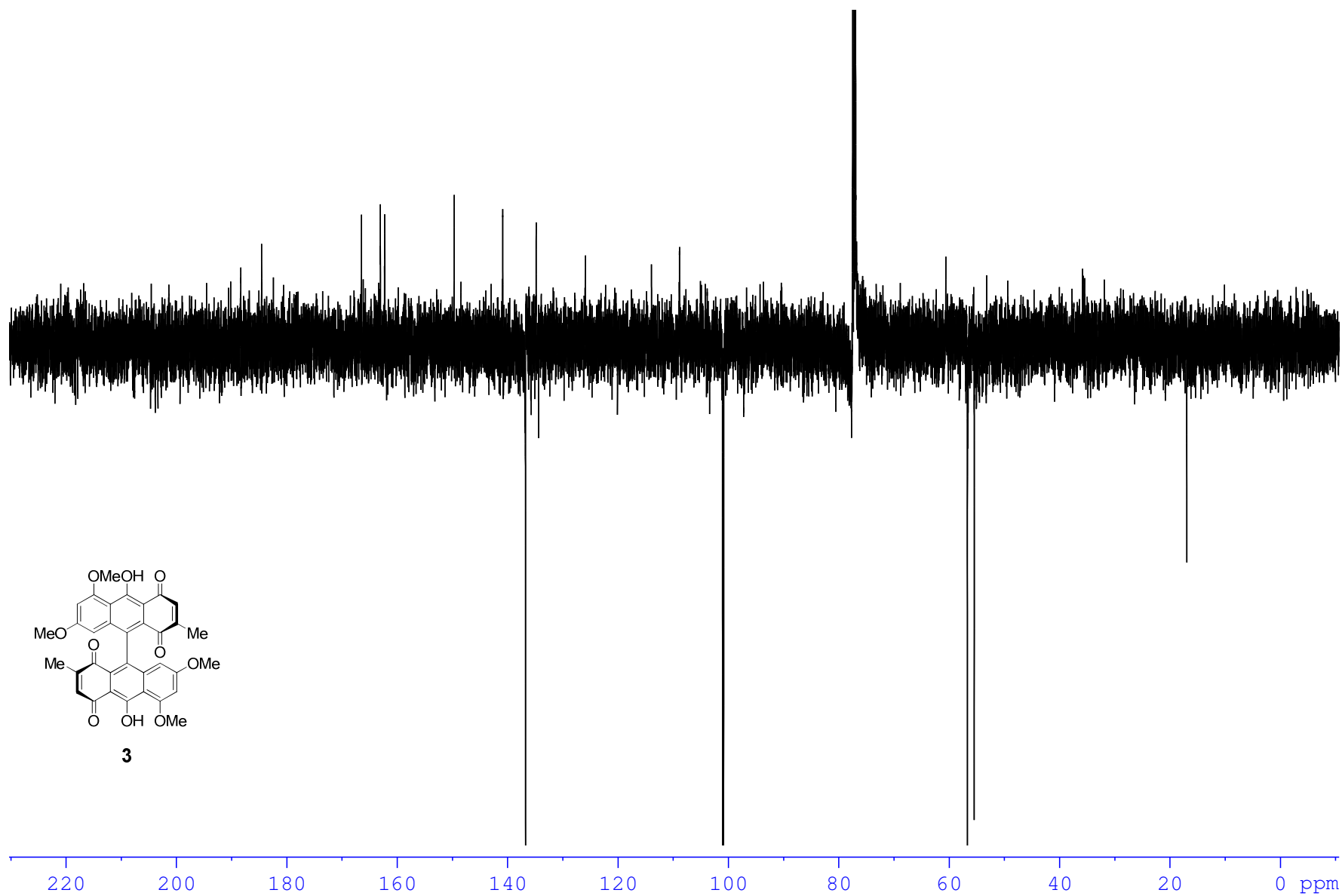
## S9 HSQC for austrocolorone B (**3**) in CD<sub>3</sub>OD



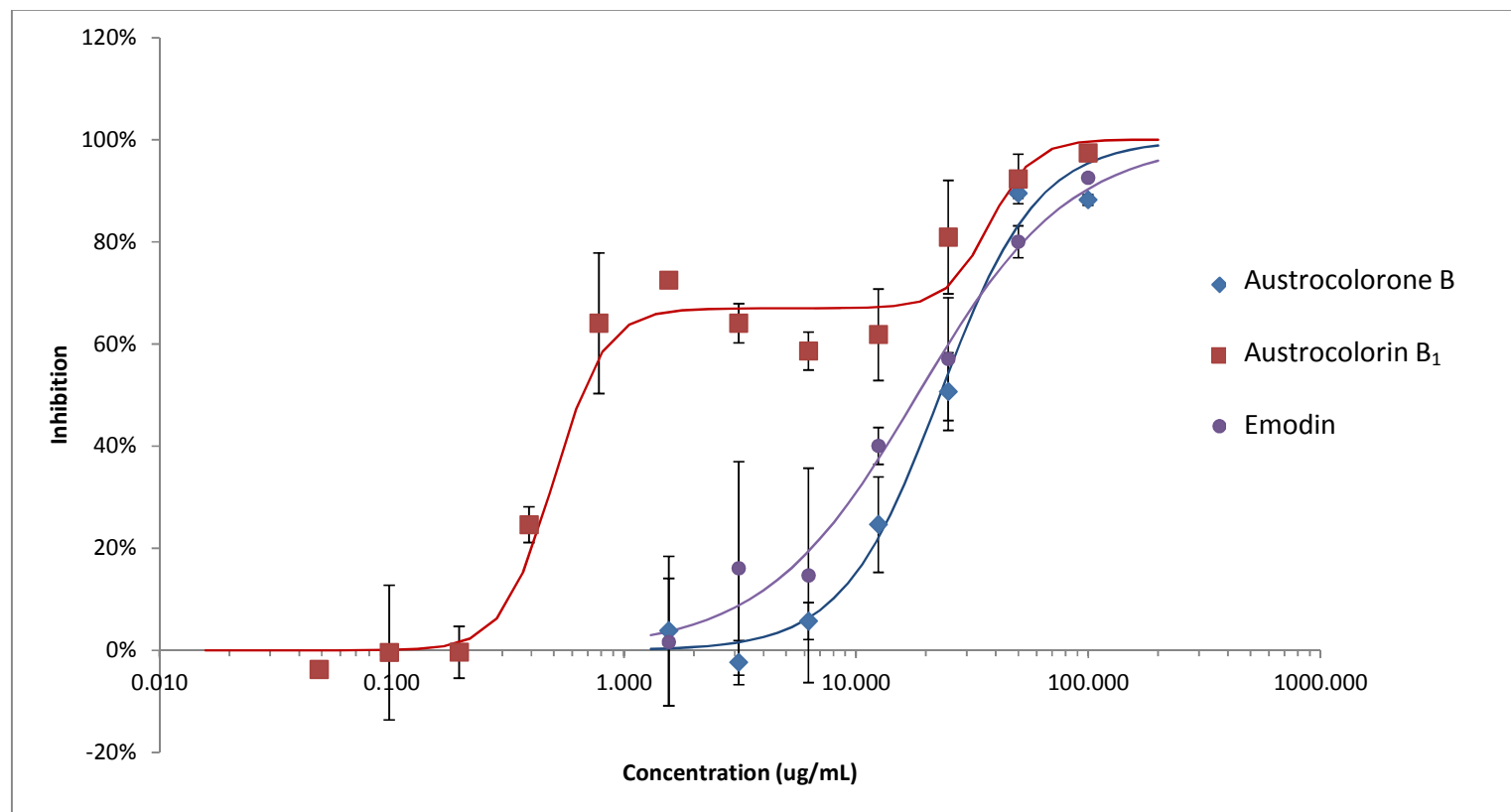
# S10 gHMBC for austrocolorone B (3) in CD<sub>3</sub>OD



**S11**  $^{13}\text{C}$  JMOD for austrocolorone B (**3**) in  $\text{CD}_3\text{OD}$



## S12 Cytotoxic activity of compounds against mouse lymphoblast cells



## S13 General experimental details

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Avance DRX-500 operating at 500 MHz ( $^1\text{H}$ ) and 126 MHz ( $^{13}\text{C}$ ) for solutions in deuteriochloroform (Cambridge Isotope Laboratories). Resonances are recorded on the  $\delta$  (ppm) scale, followed by multiplicity (s, d, t, q, m), coupling constants and integration. Mass spectra were recorded with Micromass Quattro II and Shimadzu GC-MS-QP5050A instruments at either 30 or 70 eV (probe). High resolution mass spectra were recorded on a Bruker 47E Bioapex FTMS (90 eV). Optical rotations were measured on a Perkin Elmer 241 MC polarimeter at 20 °C, with 10 cm path length, units are given in  $10^{-1}$  deg  $\text{cm}^2 \text{g}^{-1}$ , concentrations refer to grams per 100 mL for solutions in  $\text{CHCl}_3$ . Melting points were recorded on a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were recorded as potassium bromide discs using a Perkin Elmer 983G spectrophotometer. Ultraviolet spectra were recorded on a Varian Superscan 3 spectrophotometer for EtOH solutions. Log  $\epsilon$  is quoted in brackets after each absorption maximum. CD spectra were recorded on an AVIV 60DS Circular Dichroism spectrometer, sodium lamp, for solutions in MeOH. HPLC grade solvents were utilized for all experiments. Petrol refers to the fraction having the boiling range of 40–60 °C.

Analytical TLC and preparative TLC were performed on pre-coated plastic plates (Merck Kieselgel 60 GF<sub>254</sub>), and hand-made plates (Merck Kieselgel 60 F<sub>254</sub>, 20 g gel spread on 20 × 20 cm glass plates, 1.0 mm thickness) respectively. Visualisation was under UV light (254 and 366 nm). Steroids and terpenoids were visualized by dipping in phosphomolybdic acid (5% w/v in ethanol) and heating to 100 °C for development. Preparative TLC and TLC employed toluene- $\text{HCO}_2\text{Et}$ - $\text{HCO}_2\text{H}$  (50:49:1) as the mobile phase unless otherwise stated.

## S14 Fungal material

Three collections of *Cortinarius vinosipes* were provided by Dr David Ratkowsky and Genevieve Gates from the School of Agricultural Science, The University of Tasmania, Hobart, from several field trips between 1996-99. The codes refer to the accession numbers under which specimens are held in the National Herbarium of Victoria. Collection numbers and corresponding collection locations are: MEL2305426, Old Farm Road, Mt Wellington, Tasmania, February 1996; MEL2305425, Myrtle Gully (lower portion), Tasmania, March 1999; MEL2121970, Old Farm Trail, Tasmania, April 1999. The collections had been air dried, and stored in the dark at room temperature prior to our chemical investigation. Duplicates of these three collections were all examined by Bruno Gasparini when he described *Cortinarius vinosipes*.<sup>†</sup>

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<sup>†</sup> Gasparini, B. *Australasian Mycologist* **2001**, 20, 87-91.

## S15 Extraction and isolation of pigments from *Cortinarius vinosipes*

The dried fruit bodies (5.54 g) were macerated in ethanol, and extracted at room temperature, in the dark, for 24 hrs. The extract was filtered and then evaporated to dryness under reduced pressure. The dark red residue was then partitioned between EtOAc (3 × 15 mL) and H<sub>2</sub>O (30 mL). The yellow aqueous phase was evaporated to dryness and stored at -20 °C. The deep red coloured organic phase was also evaporated to dryness and fractionated by PTLC [toluene-HCO<sub>2</sub>Et-HCO<sub>2</sub>H (50:49:1) as eluent] to afford a more mobile purple band (*R<sub>f</sub>* 0.63) and a more polar yellow band (*R<sub>f</sub>* 0.30).

The crude purple residue (5.6 mg,  $1.0 \times 10^{-3}$  % yield / dry wt.) was eluted from the silica gel using EtOAc, and contained a mixture of ergosterol and austrocolorone (**3**). Preparative TLC using petrol-EtOAc-HCO<sub>2</sub>H (75:24:1) as the mobile phase, enabled adequate resolution of these metabolites. Elution of the purple band (*R<sub>f</sub>* 0.17) from the silica gel with EtOAc afforded the pure, violet-red austrocolorone (**3**, 1.8 mg,  $3.2 \times 10^{-2}$  % yield / dry wt.) and visualization with phosphomolybdic acid reagent indicated the second band (*R<sub>f</sub>* 0.38;  $6.9 \times 10^{-2}$  % yield / dry wt.), ergosterol (3.4 mg) which was also eluted with EtOAc.

The crude yellow residue (1.5 mg,  $2.7 \times 10^{-2}$  % yield / dry wt.) was eluted from the silica gel with EtOAc. The organic phase was evaporated to dryness under reduced pressure, redissolved in MeOH (ca. 1 mL), and purified further by gel permeation chromatography utilizing Sephadex LH-20 suspended and eluted in MeOH (40 × 3.5 cm diameter column) to afford austrocolorin B<sub>1</sub> (**2**).

## S16 Mammalian cell assays

**Cytotoxicity assay:** Cytotoxicity in P388D<sub>1</sub> murine lymphoblasts, (American Type Culture Collection) was assayed in 96-well plates using the ATPLite kit (PerkinElmer, Waltham, MA) with emodin (Sigma E7881) chlorambucil (Sigma C-0253) and curcumin (Sigma C-1386) as reference compounds. Cells were cultivated in DMEM media supplemented with 10% horse serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin and 100 µg/mL streptomycin. Test and reference compounds were added at seven different concentrations and incubated for 24 hours. The ATPLite assay was carried out according to the manufacturer's instructions and luminescence was measured on a Wallac Microbeta scintillation and luminescence counter (PerkinElmer, Waltham, MA). Half-maximal inhibitory concentration (IC<sub>50</sub>) values were calculated using Excel 2003 (Microsoft Corporation, Redmond, WA) and GraphPad Prism 4 (GraphPad Software, La Jolla, CA).

**Cell cycle analysis:** P388D<sub>1</sub> cells were incubated with 2, 4 and 10 µg/mL austrocolorin B<sub>1</sub> for 24 hours in the medium described above. Cells were extracted by centrifugation and resuspended in ice cold EtOH:H<sub>2</sub>O (70:30) and stored at -20 °C. For analysis, the cells were resuspended in phosphate buffered saline solution and stained with propidium iodide in the presence of RNase A. Cell staining was recorded on a FACS Canto II flow cytometer (BD Biosciences, Franklin Lakes, NJ) and analysed using ModFit (Verity Software House, Topsham, ME).