Pigments of fungi. Part 43.[1,2] Cardinalins 1–6, novel pyranonaphthoquinones from the fungus *Dermocybe cardinalis* Horak

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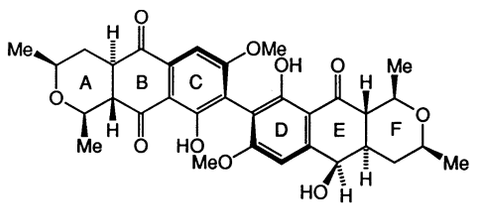
The cardinalins 1–6, a series of stereochemically complex, cytotoxic pyranonaphthoquinone dimers and their hydroquinone precursors, have been isolated from the New Zealand toadstool *Dermocybe cardinalis* and their structures established by spectroscopic methods. The cardinalins are the first pyranonaphthoquinones to be found in the Higher Fungi.

Quinones based on 3,4-dihydro-1*H*-naphtho[2,3-*c*]pyran that bear carbon substituents at C-1 and C-3 in the heterocyclic ring form an important class of biologically active natural products that includes the nanaomycins and griseusins, deoxyfrenolicin, kalafungin, granaticin and the actinorhodins. The simplest examples, in which both of the carbon substituents are methyl groups, include the eleutherins, protoaphins and the ventiloquinones. To date, quinones of this type have been restricted in the main to plants (particuarly those belonging to *Rhamnaceae*), soil bacteria (especially various *Streptomyces*) and insects.[3] Herein we disclose full details of the isolation from the New Zealand toadstool *Dermocybe cardinalis* of the first members of a new class of pyranonaphthoquinones, the cardinalins, which are the first quinones of this type to be discovered in the Higher Fungi.



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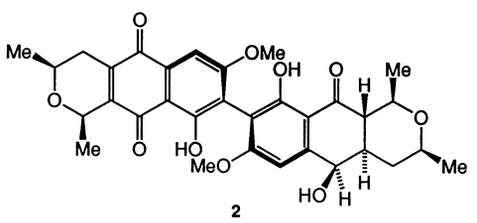

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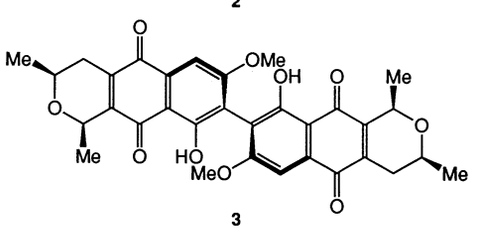

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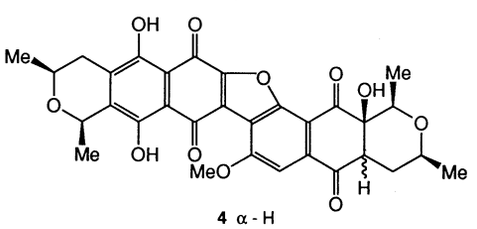
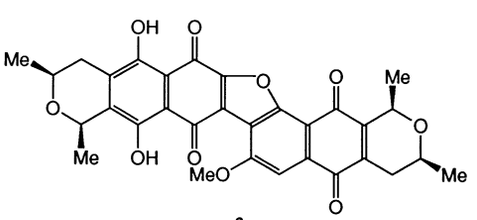


4 α - H
5 β - H



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Results and discussion

Dermocybe cardinalis produces distinctive purple and orange–yellow fruit bodies that are among the most spectacular toadstools found in New Zealand *Nothofagus* forests.[4] The ethanolic extracts of air-dried specimens of *D. cardinalis* were examined chromatographically by Keller *et al*. who correctly suggested[5] that the yellow and purple–red pigments present are new quinones that are unique to this organism. They also speculated, wrongly as it turns out, that the major colouring components are anthraquinones, as is indeed the case in the vast majority of *Dermocybe* species examined to date.[6,7]

As part of our continuing studies[8] on the chemistry of indigenous Australasian *Dermocybe* and *Cortinarius* species we have examined the constituents of *Dermocybe cardinalis*. The deep red ethanolic extracts of fresh fruit bodies, which were collected near Christchurch, New Zealand, showed significant cytotoxic activity (IC_{50} of 0.47 μg cm^{-3}) against a P388 murine leukaemia cell line and, as a consequence of this activity, the individual components of the complex mixture were examined separately. After repeated chromatography on silica gel and subsequent gel permeation through Sephadex LH-20, a colourless compound (cardinalin 1 **1**), two yellow quinones (the cardinalins 2 **2** and 3 **3**) and three red–purple pigments (the cardinalins 4 **4**, 5 **5** and 6 **6**) were isolated in pure form.

Cardinalin 1 **1** was obtained as an optically active colourless solid in a yield of 9.1×10^{-3}% based on the fresh weight of the fungus. The molecular formula $C_{32}H_{36}O_{10}$, which followed from the electron impact and fast-atom bombardment (FAB) mass spectra, immediately suggested that cardinalin 1 **1** is comprised of two octaketide subunits.[7] In the IR spectrum, cardinalin 1 **1** exhibits absorptions at 3420 and 1630 cm^{-1} typical of hydroxy and conjugated carbonyl groups, respectively. The ^{1}H NMR spectrum of **1** (Table 1) revealed the presence of two intramolecularly hydrogen bonded phenolic hydroxy groups (δ 12.04 and 12.71), two isolated aromatic protons (δ 6.56 and 7.19) and two methoxy groups (δ 3.84 and 3.88). Additionally, there are five methine protons attached to oxygenated carbons [δ 3.53 (2 H), 3.76, 3.88 and 4.62] and four secondary methyl groups [δ 1.26, 1.29, 1.56 and 1.59 (all d, *J ca.* 6 Hz)]. The ^{13}C NMR spectrum of **1** (Table 2) corroborated the assignments made above and, furthermore, identified three ketonic carbonyls (δ 195.9, 201.5 and 202.9), four methine carbons (δ 42.2, 47.2, 49.1

Table 1 ^{1}H NMR data (400 MHz; $CDCl_3$) for cardinalins 1 **1**, 2 **2** and 3 **3**[a]

Position	Chemical shift (δ), multiplicity and coupling constant (J/Hz)		
	1	**2**	**3**
1-H	3.88 (dq, 9.4, 6.0)	4.83 (qdd, 6.6, 4.0, 2.6)	4.84 (qdd, 6.6, 4.0, 2.6)
1′-H	3.76 (dq, 9.4, 6.0)	3.76 (dq, 9.4, 6.0)	
3-H	3.53 (m)	3.57 (dqd, 10.0, 6.1, 2.6)	3.58 (dqd, 10.2, 6.2, 2.6)
3′-H	3.53 (m)	3.53 (dqd, 12.5, 6.1, 2.0)	
4-H_α	2.17 (ddd, 13.8, 4.2, 1.8)	2.75 (dt, 18.5, 2.6)	2.76 (dt, 18.5, 2.6)
4′-H_α	1.67 (ddd, 12.6, 4.2, 2.1)	1.68 (ddd, 12.5, 4.2, 2.0)	
4-H_β	1.62 (td, 13.8, 11.5)	2.26 (ddd, 18.5, 10.0, 4.0)	2.27 (ddd, 18.5, 10.2, 4.0)
4′-H_β	1.79 (q, 12.6)	1.79 (q, 12.5)	
4a-H	3.09 (ddd, 13.2, 11.5, 4.2)		
4a′-H	2.36 (tdd, 12.6, 4.2, 2.4)	2.36 (tdd, 12.5, 4.2, 2.4)	
5′-H	4.62 (d, 2.4)	4.62 (d, 2.4)	
6-H	7.19 (s)	7.32 (s)	7.33 (s)
6′-H	6.56 (s)	6.56 (s)	
10a-H	2.69 (dd, 13.2, 9.4)		
10a′-H	2.80 (dd, 12.6, 9.4)	2.80 (dd, 12.5, 9.4)	
1-Me	1.56 (d, 6.0)	1.56 (d, 6.6)	1.57 (d, 6.6)
1′-Me	1.59 (d, 6.0)	1.60 (d, 6.0)	
3-Me	1.29 (d, 6.1)	1.37 (d, 6.1)	1.38 (d, 6.2)
3′-Me	1.26 (d, 6.1)	1.27 (d, 6.1)	
7-OMe	3.88 (s)	3.89 (s)	3.90 (s)
7′-OMe	3.84 (s)	3.83 (s)	
9-OH	12.04 (s)	12.34 (s)	12.36 (s)
9′-OH	12.71 (s)	12.72 (s)	

[a] Assignments confirmed by COSY, HMQC, HMBC and NOESY experiments.

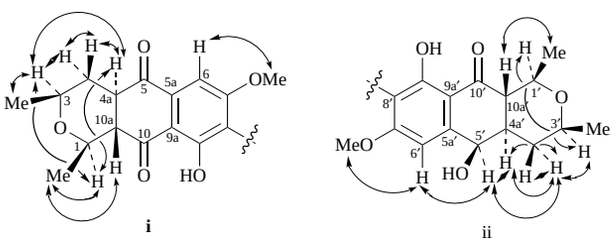


Fig. 1 Partial structures **i** (rings A, B and C) and **ii** (D, E and F) of cardinalin 1 **1** showing selected NOEs

and 53.3), two methylene groups (δ 32.6 and 35.2) and twelve sp^2-hybridised carbons; all together, resonances were observed from thirty two discrete carbon atoms.

Consideration of these NMR data, together with the results of ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY experiments performed on cardinalin 1 led to the construction of partial structures **i** and **ii** (Fig. 1), both of which contain a fused 3,4,5,6-tetrahydro-2,6-dimethyl-2*H*-pyran ring (rings A and F) that sits in a chair conformation. Thus, the connectivity and relative stereochemistry depicted in ring A of partial structure **i** emerged from analysis of the various geminal and vicinal couplings (Table 1) and the recognition that the coupling constants that sequentially link 1-H, 10a-H, 4a-H, 4-H_β and 3-H, all exceed 9 Hz. This fact pointed to a series of *trans*-diaxial relationships that must exist between these protons in partial formula **i** as ring A is preceeded. As a consequence of this, the 1-Me and 3-Me groups must both occupy an equatorial configuration and therefore be mutually *cis*-disposed, while the junction between rings A and B is necessarily *trans*-fused. These stereochemical relationships are fully supported by the results of a NOESY experiment that revealed the enhancements that are depicted diagramatically in Fig. 1.

The substitution pattern in rings B and C in **i** was deduced as follows. The long-range, inverse-detected ^{1}H–^{13}C spectrum (HMBC) of cardinalin 1 revealed correlations ($^2J_{CH}$ and $^3J_{CH}$) between the proton 10a-H, which occupies the A–B ring junction, and both of the carbonyl carbons C-10 (δ 201.5) and C-5 (δ 195.9), while the latter also correlated both with 4a-H and with the isolated aromatic proton 6-H (δ 7.19) in ring C. Furthermore, the proton 6-H and the methoxy group (δ 3.88) must be adjacent to one another in ring C since they exhibit a mutual NOE, and C-6 must be *para* disposed towards the chelated phenolic hydroxy group (δ 12.04) since both C-8 (δ 115.3) and C-9a (δ 113.1) display HMBC connectivity simultaneously to 6-H and the proton of the C-9 hydroxy group. It follows then that the quaternary sp^2-hybridised carbon, C-8 (δ 115.3), is the last site remaining for coupling of the fragment **i** to the rest of the cardinalin 1 molecule.

The B–C ring system in cardinalin 1 **1** represents a rare example of a naturally occurring β-hydronaphthoquinone, the presence of which is supported by an electronic absorption at 323 nm.[9]

It should be noted that the relative orientation between rings A and C in **i**, at least at this point in the discussion, remains equivocal. However, biogenetic considerations strongly suggest that the orientation shown in **i** is correct and this suggestion is supported (*vide infra*) by data derived from the closely related partial structure **ii** (Fig. 1).

The structure and relative stereochemistry of partial structure **ii** for cardinalin 1 followed from analysis of the remaining spectroscopic data (Tables 1 and 2). Thus, the chemical shifts and coupling constants of the protons in ring F of **ii** are similar to their counterparts in ring A in partial formula **i**, with the notable exception that 4a′-H (δ 2.36) appears shielded ($\Delta\delta$ 0.73 ppm) when compared to 4a-H and exhibits additional vicinal coupling (J 2.4 Hz) to the alcoholic methine proton at C-5′ in ring E (δ_H 4.62, δ_C 70.4). The magnitude of this vicinal coupling and the observation of NOEs between 5′-H and both 4a′-H and 4′-H_α are consistent only with the relative stereochemistry at C-4′, C-4a′ and C-5′ shown in **ii**, in which 5′-OH occupies a β-oriented pseudoaxial configuration. The relative orientation between rings D and F in **ii** was established unequivocally by the appearance of a mutual NOE between the alcoholic methine proton 5′-H and its aromatic neighbour 6′-H (δ 6.56) in ring D in addition to those between 5′-H, 4a′-H and 4′-H_α in ring F. Additional support arises from the observation of a strong correlation between 6′-H and the alcoholic carbon C-5′ in the HMBC spectrum of cardinalin 1. Other NOEs (Fig. 1) and HMBC correlations observed for rings D and E in **ii** correspond closely with those arising from rings B and C, respectively, in **i** and parallel conclusions regarding the substitution pattern in rings D and E and the location of the biaryl bond in

Table 2 ^{13}C NMR data (100 MHz; $CDCl_3$) for cardinalins 1 **1**, 2 **2**, 3 **3**, 4 **4** and 5 **5**[a]

	Chemical shift (δ) and multiplicity[b]				
Position	**1**	**2**	**3**	**4**	**5**
C-1	72.5d	69.8d	69.8d	70.7d	70.7d
C-1′	72.6d	72.6d		74.9d	76.7d
C-3	71.8d	68.7d	68.7d	68.7d	68.6d
C-3′	72.1d	72.1d		72.7d	71.2d
C-4	32.6t	30.5t	30.6t	30.8t	30.8t
C-4′	35.2t	35.2t		28.7t	28.3t
C-4a	49.1d	142.9s	143.0s	138.9s	139.3s
C-4a′	42.2d	42.2d		54.0d	53.8d
C-5	195.9s	183.3s	183.2s	163.7s	164.9s[d]
C-5′	70.4d	70.4d		194.4s	194.3s
C-5a	136.0s	132.9s	133.2s	110.3s	110.4s
C-5a′	145.4s	145.4s		137.1s	137.8s
C-6	100.9d	102.9d	102.7d	176.8s[d]	175.7s[e]
C-6′	103.1d	103.1d		103.2d	103.9d
C-7	163.6s	163.3s	163.2s	155.2s[e]	155.0s[f]
C-7′	163.2s	163.2s		159.5s	160.7s
C-8	115.3s	115.3s	115.2s[c]	123.4s	123.1s
C-8′	109.1s	109.0s		118.5s	119.0s
C-9	160.5s	161.1s	160.9s	170.0s[d]	168.7s[e]
C-9′	161.7s	161.7s		154.8s[e]	154.9s[f]
C-9a	113.1s	110.2s	110.2s	109.5s	109.5s
C-9a′	110.4s	110.4s		114.4s	112.8s
C-10	201.5s	188.0s	188.0s	163.7s	164.8s[d]
C-10′	202.9s	202.9s		191.7s	195.0s
C-10a	53.3d	146.7s	146.7s	144.0s	144.3s
C-10a′	47.2d	47.2d		76.7s[c]	78.2s
1-Me	21.6q	21.3q	21.3q	21.0q	21.0q
1′-Me	22.4q	22.4q		15.5q	15.4q
3-Me	21.9q	21.2q	21.2q	21.3q	21.3q
3′-Me	22.0q	22.0q		21.7q	21.6q
7-OMe	56.5q	56.6q	56.6q		
7′-OMe	56.2q	56.2q		57.1q	57.3q

[a] Assignments confirmed by HMQC and HMBC experiments. [b] Multiplicities determined by an HMQC experiment. [c] Chemical shift derived from HMBC experiment. [d,e,f] Values in the same vertical column that bear the same superscript letter may be interchanged.

ring D can be drawn. It follows then that cardinalin 1 can be assigned the coupled hydropyranoquinone structure **1**.

Cardinalin 1 **1** contains nine stereogenic centres and, although we do not yet know the absolute configuration at these centres, we are confident from the spectroscopic data discussed above and from the relationship between the A–B–C and D–E–F ring systems in cardinalin 3 **3** (*vide infra*) that the relative stereochemistry shown in formula **1** is correct. A tenth element of chirality is introduced into cardinalin 1 by virtue of the presence of the sterically encumbered C-8 to C-8′ biaryl bond. By analogy with other fungal and plant metabolites that contain hydroxy and methoxy groups in all four *ortho* positions surrounding the biaryl axis, cardinalin 1 **1** should be capable of existing in two atropisomeric forms at room temperature.[8,10] Consequently, the absence of any asymmetric doubling in the NMR spectra of the compound **1** is consistent with the natural product occurring in only one of these two stereochemical forms. Again, the argument in favour of the (*S*) axial configuration depicted in formula **1** for cardinalin 1 will be referred to in a later part of this paper.

Cardinalin 2 **2**, $C_{32}H_{34}O_{10}$ (*m/z* 578), was obtained as fine yellow needles in a yield of 10.6×10^{-3}% of the weight of the fresh fungus. The molecular formula immediately suggested a very close relationship between the cardinalins 1 and 2. Comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 2, respectively) obtained for the two compounds supported a close relationship and, further, established that cardinalin 2 contains the partial structure **ii** previously assigned to the D–E–F segment of the cardinalin **1**. All of the differences between the cardinalins 1 and 2 must therefore be confined to the A–B–C ring system. The NMR spectra of cardinalin 2 lack those resonances previously observed in the ^{1}H NMR spectrum of **1** that were assigned to the methine protons 4a-H and 10a-H, while the signals assigned to the corresponding carbons (δ 49.1 and 53.3, respectively) in the ^{13}C NMR spectrum of **1** are replaced in the spectrum of **2** by resonances characteristic of two sp^2-hybridised carbons (δ 142.9 and 146.7). This implied that ring B in the pigment **2** is quinonoid and this was confirmed by the ^{13}C chemical shifts of the C-5 (δ 183.3) and C-10 (δ 188.0) carbonyl carbons in the NMR spectrum and by UV–VIS absorption at 423 nm, characteristic of a juglone chromophore.[11]

The A–B–C ring system of cardinalin 2 **2** closely resembles less elaborate pyranonaphthoquinones of the eleutherin and ventiloquinone types.[3] These latter quinones are known with both *cis* and *trans* relative stereochemistry between the methyl groups at C-1 and C-3 in the dihydropyran ring and consistent trends in the ^{1}H NMR spectra of several pairs of diastereoisomers of this type have been recognised, analysed and subsequently applied effectively to further structure elucidation in related systems.[12,13] Particularly diagnostic are the chemical shifts of the secondary methine protons 1-H and 3-H, and the magnitude of the homoallylic coupling constants observed between 1-H and each of the two diastereotopic protons at C-4. In the spectra of the *trans* isomers the chemical shift of 1-H is typically between δ 5.0 and 5.3 while 3-H usually resonates below δ 4.[12] In such cases $J_{1,4}$ is characteristically small (J <2 Hz). The $J_{1,4}$ couplings are much larger (J 2.5–4.0 Hz) in quinones belonging to the *cis* series, while the chemical shifts of 1-H and 3-H are usually less than δ 5.0 and 3.7, respectively. In the ^{1}H NMR spectrum of **2**, 3-H resonates at δ 3.57 while 1-H (δ 4.83) is coupled to 4-H$_\alpha$ (δ 2.75) and to 4-H$_\beta$ (δ 2.26) with coupling constants of 2.6 and 4.0 Hz, respectively. These data are consistent only with the *cis* disposition of methyl groups in ring A of **2**. That both 1-Me and 3-Me in **2** occupy pseudoequatorial configurations is also evident from the NOESY spectrum of cardinalin 2, which reveals (*inter alia*) the close spacial proximity of 1-H and 3-H.

Cardinalin 3 **3**, a yellow powder that was obtained in only 0.9×10^{-3}% yield, exhibited a molecular ion at *m/z* 574 in the mass spectrum from which the molecular formula $C_{32}H_{30}O_{10}$ was obtained by high resolution mass measurement. Significantly, the ^{1}H and ^{13}C NMR spectra of **3** (Tables 1 and 2, respectively) show signals from only one half of the protons and carbons indicated by the molecular formula. Furthermore, the chemical shifts of the protons and carbons in the NMR spectra of **3** are either coincident or very nearly so with the corresponding signals from rings A, B and C of cardinalin 2 **2**. Cardinalin 3 is therefore assigned the binaphthoquinone structure **3**.

At this point it is appropriate to return to the question of the stereochemistry of the cardinalins 1–3, about which brief mention has already been made. Cardinalin 3 **3**, like its cometabolites **1** and **2**, shows no evidence whatsoever of asymmetric doubling in either the ^{1}H or ^{13}C NMR spectra. It may be confidently assumed therefore that the cardinalins 1–3 occur in *D. cardinalis* as discrete atropisomers, at least within the limits of spectroscopic detection. In the case of cardinalin 3 **3** the appearance of signals from only one half of the protons and carbons in the molecule assumes extra significance in that it reveals the presence in **3** of a twofold axis of symmetry and thereby proves that rings A and F in **3** must have the same absolute configuration. In the light of the close biogenetic relationship that must exist between cardinalin 3 and the other coupled pyranoquinones discussed here we feel confident in assuming that the stereochemistry at C-1 and C-3 in ring A is the same as that at C-1′ and C-3′, respectively, in ring F in all cases.

The CD spectra of the cardinalins 2 and 3 are shown in Fig. 2. They both display a bisignate Cotton effect couplet centred close to 275 nm that is characteristic of exciton coupling between two aromatic chromophores.[14] A similar couplet, in

Table 3 ^{1}H NMR data (400 MHz; $CDCl_3$) for cardinalins 4 **4**, 5 **5** and 6 **6**[a]

Position	Chemical shift (δ), multiplicity and coupling constant (J/Hz)		
	4	**5**	**6**
1-H	5.05 (qdd, 6.4, 2.9, 1.9)	5.04 (qdd, 6.6, 2.9, 2.2)	5.05 (qdd, 6.4, 2.8, 2.2)
1′-H	4.09 (q, 6.3)	3.62 (q, 6.0)	4.90 (qdd, 6.4, 4.0, 2.6)
3-H	3.66 (m)	3.66 (dqd, 10.3, 6.1, 2.2)	3.65 (m)
3′-H	3.66 (m)	3.71 (dqd, 11.7, 6.0, 2.2)	3.64 (m)
4-H_α	2.93 (dt, 17.9, 1.9)	2.93 (dt, 17.8, 2.2)	2.93 (dt, 18.0, 2.1)
4′-H_α	2.18 (ddd, 14.3, 4.6, 1.7)	2.55 (dt, 13.7, 2.2)	2.79 (dt, 18.0, 2.6)
4-H_β	2.43 (ddd, 17.9, 10.1, 2.9)	2.43 (ddd, 17.8, 10.3, 2.9)	2.43 (ddd, 18.0, 10.4, 2.8)
4′-H_β	1.86 (dt, 14.3, 12.0)	1.97 (ddd, 13.7, 11.7, 5.0)	2.29 (ddd, 18.0, 10.4, 4.0)
4a′-H	3.25 (dd, 12.0, 4.6)	3.29 (dd, 5.0, 2.2)	
6′-H	7.53 (s)	7.66 (s)	7.60 (s)
1-Me	1.66 (d, 6.4)	1.66 (d, 6.6)	1.65 (d, 6.4)
1′-Me	1.51 (d, 6.3)	1.06 (d, 6.0)	1.62 (d, 6.4)
3-Me	1.41 (d, 6.3)	1.41 (d, 6.1)	1.41 (d, 6.4)
3′-Me	1.33 (d, 6.1)	1.32 (d, 6.0)	1.39 (d, 6.4)
7′-OMe	4.22 (s)	4.27 (s)	4.25 (s)
5-OH	13.11 (s)	13.10 (s)	13.12 (s)
10-OH	13.96 (s)	13.98 (s)	13.98 (s)

[a] Assignments confirmed by COSY, HMQC, HMBC and NOESY experiments.

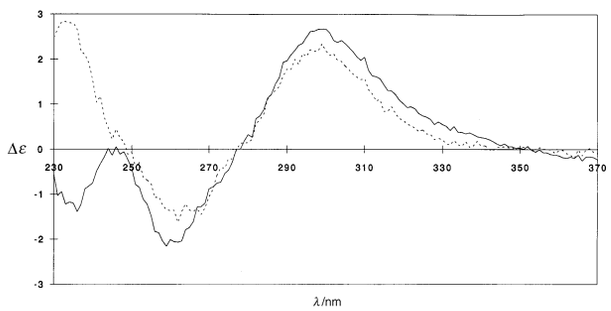
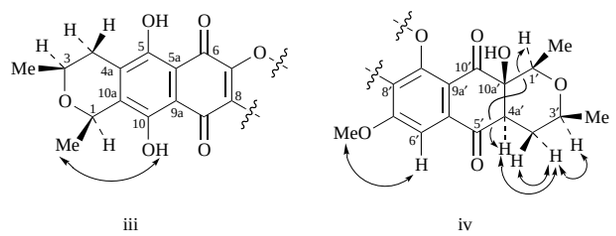


Fig. 2 CD spectra of cardinalins 2 **2** (———) and 3 **3** (------)



iii iv

Fig. 3 Partial structures **iii** and **iv** of cardinalin 4 **4** showing selected NOEs; the oxygen atom at C-7 in **iii** and C-9′ in **iv** is shared

which the first Cotton effect (the one to longer wavelength) is positive while the second Cotton effect is negative ('positive chirality'), is also observed in the CD spectrum of cardinalin 1 (Experimental section), and strongly suggests that the cardinalins 1–3 share the same axial configuration. Furthermore, if an analogy is drawn between the shapes of the CD spectra depicted in Fig. 2 and the corresponding spectra of coupled binaphthyls of the flavomannin type,[15] in the case of which the stereochemistry at the biaryl linkage is known,[10] then it follows that the cardinalins 1–3, **1**–**3**, possess (*S*) chirality at the stereogenic axis.

Cardinalin 4 **4** is a deep red microcrystalline solid (0.9×10^{-3}% yield) that owes its colour to the presence of a naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) chromophore [λ_{max} 514 (3.93), 552 (4.00) and 594 (3.81) nm].[11] The molecular formula $C_{31}H_{28}O_{11}$ for cardinalin 4 followed from the high resolution mass measurement of the molecular ion at *m/z 576* in the mass spectrum. The ^{1}H NMR spectrum of the pigment **4** (Table 3) shows signals due to two intramolecularly hydrogen bonded phenolic hydroxy groups (δ 13.11 and 13.96), one isolated aromatic proton (δ 7.53) and one methoxy group (δ 4.22). While these data reveal a distinct difference between the B–C and D–E ring systems in cardinalin 4 compared to the corresponding parts of the cardinalins 1–3, the continued appearance of four secondary methyl resonances (δ 1.33, 1.41, 1.51 and 1.66) together with their accompanying methine multiplets [δ 3.66 (2 H), 4.09 and 5.05, respectively] indicated that cardinalin 4, like its co-metabolites **1**–**3**, contains two fused 3,6-dihydro-2,6-dimethyl-2*H*-pyran rings (rings A and F). That the pyran ring A in **4** abuts the naphthazarin nucleus (rings B and C) was evident from the appearance in the HMBC spectrum of **4** of correlations between C-4a (δ 138.9) and both of the protons 4-H_α (δ 2.93) and 5-OH (δ 13.11), while C-10a (δ 144.0) correlated both with 1-Me (δ 1.66) and 10-OH (δ 13.96) (see partial structure **iii** in Fig. 3). The relative stereochemistry in ring A of **4** was deduced from the chemical shifts of the secondary methine protons 1-H (δ 5.05) and 3-H (δ 3.66) and the large homoallylic coupling constants linking 1-H with the protons of the C-4 methylene group (J 1.9 Hz to 4-H_α; 2.9 Hz to 4-H_β).[12]

The ^{13}C NMR spectrum of cardinalin 4 (Table 2) fully supports the presence of the partial structure **iii** in the molecule. Furthermore, since the C-7 resonance appears as a singlet close to δ 150 this carbon must be bonded to an oxygen atom but, as later evidence will attest, this oxygen atom cannot be part of the methoxy group (δ_H 4.22, δ_C 57.1). Partial structure **iii** therefore accounts for the elements $C_{15}H_{12}O_6$ of the molecular formula $C_{31}H_{28}O_{11}$ of **4**, and includes the naphthazarin chromophore which itself includes both of the chelated hydroxy groups that are present in cardinalin 4.

The remaining signals in the ^{13}C and ^{1}H NMR spectra of cardinalin 4 (Tables 2 and 3, respectively) together with the results of HMBC and NOESY experiments (Fig. 3) led unequivocally to partial structure **iv** for the D–E–F rings of this pigment. Thus, among the as yet unassigned signals in the ^{13}C NMR spectrum of **4** there are two conjugated ketone carbonyls (δ 191.7 and 194.4), six other sp^2-hybridised carbon atoms (δ 159.5*, 154.8*, 137.1, 118.5, 114.4 and 103.2), including two (*) that are oxygenated and three oxygenated sp^3-hybridised carbons (δ 76.7, 74.9 and 72.7), one of which (δ 76.7) occupies a tertiary centre (HMBC).

The substitution pattern in the aromatic ring D of **iv** followed from several HMBC correlations that all involve the isolated proton 6′-H. Thus, this proton correlates not only with the carbonyl carbon C-5′ but also with the tertiary sp^2 carbons C-5a′, C-7′, C-8′ and C-9a′. Since 6′-H also exhibits an NOE with the protons of the methoxy group (δ 4.22) the latter must be located at C-7′, which itself resonates at δ 159.5. Since the oxygen atom connected to the last remaining low field aromatic carbon, C-9′,

may be neither protonated nor methylated it must be part of an ether linkage.

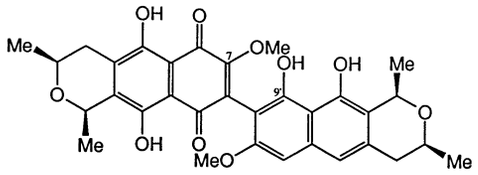
In the HMBC spectrum of cardinalin 4 the carbonyl carbon at C-5′ in **iv** correlated not only with the aromatic proton 6′-H in ring D but also with the methine proton 4a′-H, which lies at the junction of rings E and F, thereby establishing that the relative orientation of the rings D and F in cardinalin 4 is as shown in **iv**. The substitution pattern and stereochemistry in ring F in **iv** was deduced from the ^{1}H NMR spectrum of cardinalin 4 as follows. The proton 4a′-H appears at δ 3.25 as a doublet of doublets (J 12.0 and 4.6 Hz). The larger of these couplings reflects a strong *trans*-diaxial interaction with 4′-H_β and, together with NOE correlations between 4a′-H and both of the protons 4′-H_α and 1′-H, placed 4a′-H in an axial configuration as part of a *trans*-fused tetrahydropyranyl ring. The absence of further coupling in the signal for 4a′-H and the sharp quartet multiplicity of 1′-H combine to preclude the presence of a proton at C-10a′ in **iv**. Indeed, the fact that 1′-H exhibits $^2J_{CH}$ coupling with the tertiary oxygenated centre (δ 76.7) serves to locate a hydroxy group at that position.

The molecular formula of cardinalin 4 is now fully accounted for by the partial structures **iii** and **iv** (Fig. 3). Provided that the oxygen atom depicted at C-7 in **iii** and at C-9′ in **iv** is common to both parts and, together with the C-8 to C-8′ biaryl bond, constitutes a benzofuranoid bridge, then the sub-units **iii** and **iv** can be brought together as structure **4**.

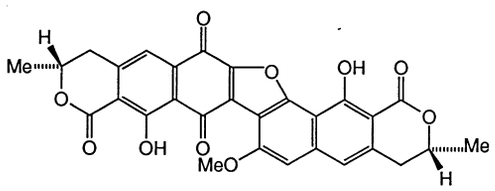
Cardinalin 5 **5** (21.5×10^{-3}%) is the C-4a′ epimer of cardinalin 4 **4**. A comparison of the ^{13}C and ^{1}H NMR data (Tables 2 and 3, respectively) demonstrates that cardinalin 5 contains the same substructure **iii** as is present in cardinalin 4. Indeed, the only significant differences between the NMR data of the cardinalins 4 and 5 arise in those regions of the spectrum that are associated with the protons (and to a lesser extent, the corresponding carbons) belonging to the dimethylpyran ring F. Thus, in the ^{1}H NMR spectrum of cardinalin 5, 4a′-H appears at δ 3.29 as a doublet of doublets with coupling constants of 5.0 and 2.2 Hz (to 4′-H_β and 4′-H_α, respectively). The magnitudes of these couplings reflect, in turn, equatorial–axial and diequatorial dihedral angles between 4a′-H and each of its vicinal neighbours, an arrangement which places 4a′-H in an equatorial configuration and establishes that the E–F ring junction in cardinalin 5 must be *cis* fused. This conclusion was corroborated by the downfield shifts of 1′-H ($\Delta\delta$ 0.47 ppm) and the proton of the C-1′ methyl group ($\Delta\delta$ 0.45 ppm) in the ^{1}H NMR spectrum of cardinalin 5 when compared with their counterparts in the spectrum of cardinalin 4 **4**. These changes in chemical shift reflect the anisotropic shielding of the C-1′ substituents by the combined effect of the C-10′ carbonyl group and the aromatic ring D. Similar shifts occur among *cis* fused members of the nanaomycin series.[16]

The final cardinalin to be described here, cardinalin 6 **6**, was isolated as a red powder in sub-milligram quantities (0.2×10^{-3}% yield). High resolution mass measurement of the molecular ion at *m/z* 558 led to the molecular formula $C_{31}H_{26}O_{10}$ which, together with the ^{1}H NMR data (Table 3) and long wavelength absorption at 556 and 600 nm in the UV–VIS spectrum, indicated a close relationship between cardinalin 6 and the cardinalins 4 and 5. The difference between the molecular formula of cardinalin 6 and that of its co-metabolites **4** and **5** corresponds to the elements of water. This fact, together with the absence of a signal in the ^{1}H NMR spectrum of cardinalin 6 that could be assigned to a proton at C-4a′, suggested that ring E in this pigment is quinonoid. This suggestion was supported by the similarity in chemical shift and couplings of the ring F protons in the spectrum of cardinalin 6 when these are compared with the analogous protons in the spectrum of cardinalin 3 **3**. Consequently, the extended, heptacyclic bisquinone structure **6** emerged for cardinalin 6.

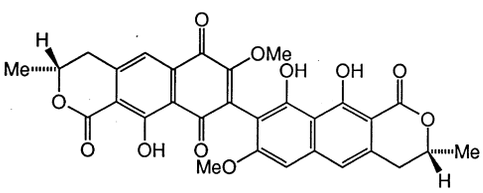
The benzofuran bridge linking the A–B–C and D–E–F rings in the cardinalins 4 **4**, 5 **5** and 6 **6** is presumably formed by an intramolecular conjugate addition of the C-9′ hydroxy group to C-7 in an incipient 7-methoxynaphthazarin such as **7**, followed by loss of the elements of methanol. A series of such transformations finds precedence in another area of benzoisochromanquinone chemistry. Thus, rubrosulfin **8** co-occurs with viomellein **9** in cultures of *Aspergillus sulfureus*[17] and *Penicillium viridicatum*,[18] and the latter can be transformed chemically into the former by treatment with potassium carbonate in acetone.[17]



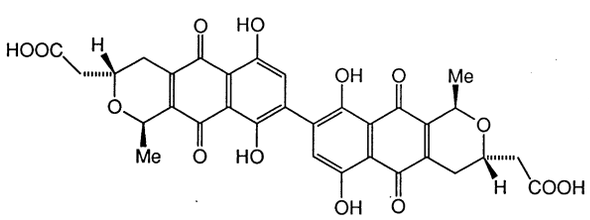
7



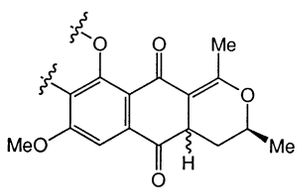
8



9



10



11

Hitherto, the chemistry of *Dermocybe* has been dominated by monomeric and dimeric anthraquinones, dihydroanthracenones and tetrahydroanthraquinones as the principal colouring matters.[6–8] Indeed, the original partition of *Dermocybe* from *Cortinarius* and its elevation from the rank of subgenus to genus was made principally on the presence of anthraquinonoid colouring components.[19] The presence of the cardinalins **1**–**6** in *Dermocybe cardinalis* is therefore doubly significant for not only does it identify the only known example of a toadstool placed in the genus *Dermocybe* that is not dominated by anthraquinonoid chemistry, but also represents the first report to date of the natural occurrence of pyranonaphthoquinones in Basidiomycotina.

Although 1,3-disubstituted 3,4-dihydro-1*H*-pyranonaphthoquinones are known in nature with a range of substitution patterns and stereochemistries, the only other pyranonaphthoquinone 'dimers' known prior to our work are a series of bacterial pigments related to actinorhodin **10**.[3] These quinones, which were originally isolated from cultures of *Streptomyces coelicolor*

Table 4 *In vivo* cytotoxicities of the cardinalins[a]

Compound	P388 IC_{50} (μg cm^{-3})
Cardinalin 1 **1**	>12.50
Cardinalin 2 **2**	>12.50
Cardinalin 3 **3**	>12.50
Cardinalin 4 **4**	0.28
Cardinalin 5 **5**	0.40
Cardinalin 6 **6**	>12.50

[a] IC_{50} values refer to the concentration of the compound that produces, during a twofold dilution series, a 50% reduction in the number of viable P388 murine leukaemia cells over a 72 h incubation period.

and *S. lividans*, have engendered considerable synthetic[20] and biosynthetic[21] interest.

The antibiotic range and potency of quinones based on 3,4-dihydro-1*H*-naphtho[2,3-*c*]pyran is now well established and their potential to act as bioreductive alkylating agents[22,23] has stimulated considerable interest in the synthesis[24] of these predominantly plant, insect and bacterial pigments. As mentioned at the start of this paper, the crude ethanolic extract of *Dermocybe cardinalis* is a potent inhibitor of the growth of P388 murine leukaemia cells (IC_{50} 0.47 μg cm^{-3}). We have also evaluated the cytotoxicity of the individual cardinalins **1**–**6** in the same assay and the results are collected in Table 4. From these data it is clear that the activity associated with the crude extract is confined to the red–purple quinones **4** and **5**, an observation that prompts us to suggest that these two compounds may be capable of transformation, *in vivo*, to an α-methylene ketone of the type **11** that could subsequently act as a trap for bionucleophiles. Notably, both **4** and **5** possess a proton at C-1 and a hydroxy group at C-10a that are stereoelectronically suitably aligned for elimination of the elements of water to generate **11** without the need for a reduction step.

Experimental

General

Melting points were determined on a Kofler 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 10 mm quartz cells and ethanolic solutions, NMR Spectra were recorded with a JEOL JNM-GX-400 spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz) for solutions in $CDCl_3$. Mass spectra were recorded on V. G. Micromass 7070F and JEOL JMS AX505H spectrometers at 70 eV (probe) for EI or as a thioglycerol matrix using FAB ionisation. Specific rotations were measured using a Perkin-Elmer 241MC polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. CD Spectra were obtained using an AVIV 62DS spectrometer for solutions in methanol.

Materials

Thin layer chromatography (TLC) and preparative TLC (PLC) were performed on Merck pre-coated 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 (GPC) employed a column (40 × 3.5 cm) of Pharmacia Sephadex LH-20 suspended in and eluted with methanol–dichloromethane–formic acid (49:49:2).

Dermocybe cardinalis was collected in the Cragieburn State Park, Canterbury, New Zealand from under *Nothofagus solandri* var. *cliffortioides* during April 1991 and April 1995. Voucher specimens are lodged in the herbarium of the Royal Botanic Garden, Edinburgh, under accession number WAT 22916 and were identified by Dr R. Watling.

Isolation of metabolites from *D. cardinalis*

Fresh material (211 g) was macerated and extracted first in ethanol (500 cm^3) and then in ethanol (500 cm^3) containing hydrochloric acid (2 M, 2%). The extracts were combined and evaporated and the residue was partitioned between ethyl acetate and water. The organic phase was separated, dried and evaporated to afford a deep red residue (1.75 g) that was separated into zones by PLC using toluene–ethyl formate–formic acid (50:49:1) as eluent. Each zone was freed from aliphatic material by PLC using dichloromethane–formic acid (19:1) and dichloromethane–ethyl acetate (3:2) as eluents. Further purification by PLC [hexane–toluene–ethyl formate–formic acid (10:5:5:0.1)] and GPC afforded the following pure compounds.

Cardinalin 1 **1** (9.1 mg) as a colourless solid (from CH_2Cl_2), R_f 0.48, mp 119–124 °C, $[\alpha]_D$ +3.4 ($CHCl_3$, *c* 0.16) (Found: M^+, 580.2300. $C_{32}H_{36}O_{10}$ requires *M*, 580.2308); *m/z* (EI) 580 (49%, M^+), 565 (100), 537 (10), 496 (5), 467 (8), 245 (10), 83 (43); *m/z* (FAB) 581 $(M + H)^+$; λ_{max}(EtOH)/nm 256 (log ε 3.98), 280 (3.80), 323 (3.63); ν_{max}(KBr)/cm^{-1} 3420 (OH), 1630 (C=O); CD λ_{max}(MeOH)/nm (Δε) 400 (0.0), 361 (−2.0), 343 (0), 330 (+2.0), 318 (0.0), 307 (−1.9), 298 (0.0), 288 (+2.5), 277 (0.0), 259 (−6.0); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Cardinalin 2 **2** (22.4 mg) as yellow needles (from CH_2Cl_2), R_f 0.49, mp 171–175 °C, $[\alpha]_D$ −25.9 ($CHCl_3$, *c* 1.20) (Found: M^+, 578.2173. $C_{32}H_{34}O_{10}$ requires *M*, 578.2152); *m/z* (EI) 578 (3%, M^+), 563 (4), 149 (12), 125 (11), 111 (22), 97 (40), 83 (41), 71 (61), 57 (100); *m/z* (FAB) 579 $(M + H)^+$, 601 $(M + Na)^+$; λ_{max}(EtOH)/nm 260 (log ε 4.28), 277 (sh, 4.03), 423 (3.44); ν_{max}(KBr)/cm^{-1} 3437 (OH), 1631 (C=O); CD λ_{max}(MeOH)/nm (Δε) 355 (0.0), 298 (+2.7), 277 (0), 261 (−2.2), 246 (0.0); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Cardinalin 3 **3** (1.8 mg) as a yellow powder (from CH_2Cl_2), R_f 0.86, mp 213–218 °C, $[\alpha]_D$ −26.9 ($CHCl_3$, *c* 0.52) (Found: M^+, 574.1817. $C_{32}H_{30}O_{10}$ requires *M*, 574.1839); *m/z* (EI) 574 (1%, M^+), 573 (3), 279 (3), 149 (13), 111 (14), 97 (27), 83 (31), 71 (48), 56 (100); *m/z* (FAB) 575 $(M + H)^+$; λ_{max}(EtOH)/nm 267 (log ε 4.06), 282 (sh, 3.80), 426 (3.46); ν_{max}(KBr)/cm^{-1} 3417 (OH), 1631, 1599 (C=O); CD λ_{max}(MeOH)/nm (Δε) 348 (0.0), 300 (+2.3), 277 (0.0), 263 (−1.5), 248 (0.0); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Cardinalin 4 **4** (2.0 mg) as deep red needles (from CH_2Cl_2–MeOH), R_f 0.65, mp 184–189 °C, $[\alpha]_D$ +48.9 ($CHCl_3$, *c* 1.02) (Found: M^+, 576.1649. $C_{31}H_{28}O_{11}$ requires *M*, 576.1631); *m/z* (EI) 576 (2%, M^+), 550 (3), 536 (2), 522 (2), 367 (14), 277 (19), 237 (18), 158 (91), 69 (93), 53 (100); *m/z* (FAB) 577 $(M + H)^+$, 599 $(M + Na)^+$; λ_{max}(EtOH)/nm 262 (log ε 4.65), 306 (4.31), 514 (3.93), 552 (4.00), 594 (3.81); ν_{max}(KBr)/cm^{-1} 3432 (OH), 1630 (C=O); CD λ_{max}(MeOH)/nm (Δε) 326 (0.0), 314 (−0.7), 296 (−0.3), 280 (−2.6), 273 (0.0), 261 (+9.7), 241 (0.0); ^{1}H NMR: Table 3; ^{13}C NMR: Table 2.

Cardinalin 5 **5** (45.3 mg) as deep red needles (from EtOAc–HCO_2H), R_f 0.59, mp 200–204 °C, $[\alpha]_D$ −3.58 ($CHCl_3$, *c* 2.24) (Found: M^+, 576.1626. $C_{31}H_{28}O_{11}$ requires *M*, 576.1631); *m/z* (EI) 576 (3%, M^+), 558 (22), 556 (16), 543 (9), 532 (100), 516 (8), 490 (13), 475 (12), 462 (15), 446 (10), 83 (21), 71 (31), 57 (58); *m/z* (FAB) 577 $(M + H)^+$, 599 $(M + Na)^+$; λ_{max}(EtOH)/nm 267 (log ε 4.62), 311 (4.26), 515 (3.98), 554 (3.88); ν_{max}(KBr)/cm^{-1} 3429 (OH), 1692, 1617, 1584 (C=O); CD λ_{max}(MeOH)/nm (Δε) 371 (0.0), 347 (−1.6), 326 (0), 313 (+1.5), 297 (+0.5), 286 (+0.7), 278 (0.0), 263 (−2.4), 248 (0.0); ^{1}H NMR: Table 3; ^{13}C NMR: Table 2.

Cardinalin 6 **6** (0.5 mg) as a red powder (from CH_2Cl_2–MeOH), R_f 0.83, mp >250 °C (decomp.), $[\alpha]_D$ +240.0 ($CHCl_3$, *c* 0.03) (Found: M^+, 558.1508. $C_{31}H_{26}O_{10}$ requires *M*, 558.1526); *m/z* (EI) 558 (6%, M^+), 331 (28), 289 (9), 229 (11), 159 (100), 127 (22), 109 (58), 97 (24), 85 (30), 71 (47), 57 (80); *m/z* (FAB) 559 $(M + H)^+$; λ_{max}(EtOH)/nm 270 (log ε 4.32), 556 (3.75), 600 (3.65); ν_{max}(KBr)/cm^{-1} 3432 (OH), 1590 (C=O); ^{1}H NMR: Table 3.

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