Phytochemistry 95 (2013) 351–359

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

# Phenylphenalenones and oxabenzochrysenones from the Australian plant *Haemodorum simulans*

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ARTICLE INFO

*Article history:*
Received 7 December 2012
Received in revised form 19 July 2013
Available online 20 August 2013

*Keywords:*
*Haemodorum simulans*
Haemodoraceae
Phenylphenalenones
Oxabenzochrysenones
Natural products

ABSTRACT

Chemical investigation of the Australian plant *Haemodorum simulans* (Haemodoraceae) resulted in the isolation of two new phenylphenalenones, haemoxiphidone and haemodordioxolane from the bulbs together with the first report of an oxabenzochrysenone glycoside, haemodoroxychrysenose from the aerial parts of the plant. Also isolated were two previously described phenylphenalenones 5,6-dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione and haemodorone and two oxabenzochrysenones 5-hydroxyl-2-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one and 5-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one. The X-ray structure of the phenylphenalenone 5,6-dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione was secured for the first time. All compounds were deduced by detailed spectroscopic analyses. HPLC-NMR chemical profiling of an enriched fraction containing a mixture of haemodordioxolane and 5,6-dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione facilitated the partial identification of these secondary metabolites. The structure previously assigned as xiphidone in our initial studies of this plant was re-assigned as the new isomer haemoxiphidone.



## 1. Introduction

Phenylphenalenones (Fig. 1) are secondary metabolites of phenylpropanoid origin which have been reported exclusively from the plant families Haemodoraceae, Musaceae, Pontederiaceae and Strelitziaceae (Opitz et al., 2002a). The family Haemodoraceae is predominately found in the southern hemisphere, with genera such as *Haemodorum* being endemic to Australia and Papua New Guinea (Ruggiero et al., 2012). Tetraoxygenated phenylphenalenones and related compounds modified by the formation of an ether bridge between ring A and ring D result in the formation of the oxabenzochrysenone (naphthoxanthenones) type structures (Fig. 1). The oxabenzochrysenone structure class has also been shown to occur almost exclusively in the family Haemodoraceae (Cooke and Dagley, 1979; Fang et al., 2011; Opitz et al., 2002a,b, 2003; Opitz and Schneider, 2002; Weiss and Edwards, 1969). The occurrence of the phenylphenalenones and oxabenzochrysenones in families of plants such as Musaceae and Haemodoraceae illustrates their close structural relationship and their importance in being potential chemotaxonomic markers for these families of plants (Otalvaro et al., 2002) and (Opitz et al., 2002a). Variations amongst the phenylphenalenone structure class include the occurrence of anhydrides or esters in the B ring and in the position of substitution of the phenyl D ring (Fig. 1). When comparing the number of reported oxabenzochrysenones (**1**–**8**) to that of the phenylphenalenones (**9**, **10**, **12**) there have been fewer oxabenzochrysenones identified (Fig. 2). This is due to the fact that the additional fused ring in the oxabenzochrysenones limits the number of sites for possible substitution (Opitz et al., 2002b).

As part of the continuing efforts of the Marine And Terrestrial NAtural Product (MATNAP) research group at RMIT University that studies the chemical diversity and ethnopharmacology of Australian plants, we recently had the opportunity to study the chemistry of *Haemodorum simplex* (subsequently revised to *Haemodorum simulans*) (Dias et al., 2009b). In this investigation a number of new and previously reported phenylphenalenones and their corresponding glycosides were identified from the bulbs of this plant. Biological activity of the crude extract, as well as the presence of minor constituents detected in the initial study indicated that a re-collection of this plant would be necessary in order to provide sufficient quantities of the minor constituents to permit an unequivocal identification. An extraction of the re-collected bulbs of *H. simulans* was combined with all remaining material that could be extracted from the initial collection of the plant. Subsequent fractionation of the bulbs of the plant yielded two known oxabenzochrysenones (**2**–**3**), two previously described

*Abbreviation:* gHSQCAD, gradient heteronuclear single bond coherence adiabatic.

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http://dx.doi.org/10.1016/j.phytochem.2013.07.019

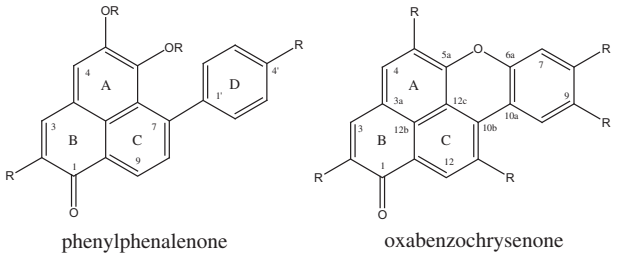



**Fig. 1.** General structure and numbering of the phenylphenalenone and oxabenzochrysenone structure classes.

phenylphenalenones (**9** and **12**) as well as two new phenylphenalenones (**11** and **13**). Investigation of the aerial components of the plant resulted in the identification of two oxabenzochrysenones, including a new oxabenzochrysenone glycoside (**14**).

## 2. Results and discussion

All fractions obtained from the silica flash chromatography of the dichloromethane extract (see Section 4.1.3) from the bulbs of *H. simulans* were analysed by analytical HPLC and $^1$H NMR spectroscopy. On the basis of these analyses one of these enriched column fractions (100% hexane fraction) was selected for further chemical profiling using HPLC-NMR. The on-flow and subsequent stop-flow HPLC-NMR analyses of this fraction indicated the presence of two principle compounds. The later eluting compound exhibited proton NMR resonances and a UV maxima at 348 nm, consistent with the phenylphenalenone structure class and the two methoxy resonances at $\delta_H$ 3.95, s and $\delta_H$ 3.27, s suggested that this compound could possibly be 5,6-dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (**12**). This partial identification could be substantiated by the fact that this compound had been previously isolated from this plant specimen and the off-line NMR data was available for comparison (Dias et al., 2009b). The earlier eluting compound also displayed a UV chromophore supportive of this compound being of the phenylphenalenone structure class at 348 nm, together with a diagnostic singlet at $\delta_H$ 6.03, integrating for two protons, confirming the presence of a highly deshielded methylene. A literature structure search of the phenylphenalenone structure class returned no possible matches for such a structural feature, suggesting a high probability of a new structural derivative. To unequivocally confirm the identity of this secondary metabolite off-line isolation and characterisation was undertaken.

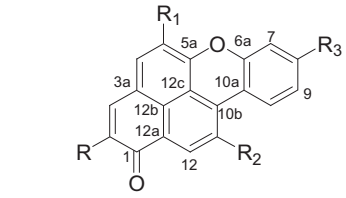
Fractionation of the crude dichloromethane extract of the bulbs of the *H. simulans* was carried out as described in Section 4.4 Semi-preparative reversed phased HPLC analysis of the 100% hexane silica column fraction resulted in the isolation of 5,6-dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (**12**) and haemodordioxolane (**13**). The 20:80 hexane:dichloromethane and 100% dichloromethane silica column fractions were combined and further purified by semi-preparative reversed phased HPLC to yield an isomer of xiphidone which we named haemoxiphidone **(11)**, while the 80:20 and 60:40 dichloromethane:ethyl acetate silica column fractions were combined and subjected to semi-preparative reversed phased HPLC analysis to yield the previously reported compounds 5-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**3**) and haemodorone (**9**). On the basis of the similarities observed in the analytical HPLC chromatograms and $^1$H NMR analyses of the 40:60 and 20:80 dichloromethane:ethyl acetate silica column fractions, these fractions were combined and subjected to semi-preparative reversed phased HPLC analysis to yield 5-hydroxyl-2-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**2**).

Flash silica chromatography was also carried out on the dichloromethane extract of the aerial parts of *H. simulans* as described in Section 4.4 to yield 15 fractions. On the basis of the subsequent analytical HPLC and $^1$H NMR analyses, the 80:20 dichloromethane:ethyl acetate silica column fraction was subjected to reversed phased semi-preparative HPLC to yield 5-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**3**), which was also present in the bulbs of the plant. The methanol extract of the aerial parts of *H. simulans* was subjected to $C_{18}$ Vacuum Liquid Chromatography (VLC) as detailed in Section 4.4, and the 100% methanol and 80:20 methanol:water fractions were subjected to reversed phased semi-preparative HPLC to afford haemodoroxychrysenose (**14**).

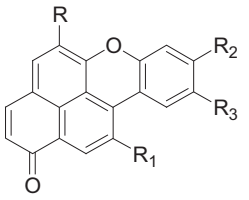
The structure of haemoxiphidone (**11**) was established to be an isomer of xiphidone on the basis of 1D and 2D NMR spectroscopy and was supported by mass spectrometry. The HR-ESIMS of (**11**) displayed an *m*/*z* at 333.1125 $[M+H]^+$ (calcd. for $C_{21}H_{17}O_4$: *m*/*z* 333.1049) consistent with 14 degrees of unsaturation and a molecular formula $C_{21}H_{16}O_4$. A characteristic UV at 375 nm was consistent with the phenylphenalenone structure class and the IR stretches at 3246 and 1730 $cm^{-1}$ supported the presence of a hydroxyl and carbonyl functionalities. Our initial investigations of the *H. simulans* specimen had incorrectly concluded the structure of this compound to be xiphidone (**10**) (Cremona and Edwards, 1974; Dias et al., 2009b). The structure of xiphidone (**10**) is reported as having the phenyl moiety at the 7 position (although the alternative 9 position was also considered), the methoxy at position 5 and the hydroxyl group at position 6. In our re-isolation of this compound, we noted that there were several anomalies with the NMR data acquired compared to the literature NMR data for xiphidone (**10**) (Cremona and Edwards, 1974; Dias et al., 2009b). The MATNAP research group is currently investigating a range of different *Haemodorum* species. In one of these separate studies[1] we have also independently re-isolated this isomer of xiphidone. These two separate isolations and independent analyses have provided the opportunity to re-evaluate the structure we initially assigned as being xiphidone (**10**) (Dias et al., 2009b). On the basis of 1D and 2D NMR analysis (acquired in $d_6$-DMSO) of the compound obtained from *H. simulans* (Table 1) key gHMBC NMR correlations (Fig. 3) were observed from $\delta_H$ 8.37, d, $J$ = 9.0 Hz (H-7), $\delta_H$ 7.55, s (H-4) and $\delta_H$ 3.95, s (6-$OCH_3$) to $\delta_C$ 141.2 (C6), thereby placing an aromatic proton at position 7 and the methoxy moiety at position 6. Further correlations from $\delta_H$ 8.37, d, $J$ = 9.0 Hz (H-7) and $\delta_H$ 7.49, d, $J$ = 9.0 Hz (H-8) to $\delta_C$ 144.1 (C-9), supported the presence of the phenyl moiety being located at position 9. In addition gHMBC NMR correlations from the exchangeable hydroxyl proton at $\delta_H$ 10.05, bs (5-OH) to $\delta_C$ 122.9 (C-4) and $\delta_C$ 141.2 (C-6) allowed for the positioning of the hydroxyl group at position 5.

On the basis of an independent and co-current isolation from a separate species of *Haemodorum*[1] the 1D and 2D NMR analysis (acquired in $CDCl_3$) of the compound, once again, supported an isomer of xiphidone (**10**) whereby the phenyl moiety was substituted at position 9 and the methoxy and hydroxyl substituents were located at positions 6 and 5 respectively (Table 1). Most of the key HMBC NMR correlations, just like those observed in $d_6$-DMSO were also observed in $CDCl_3$, except those from the exchangeable hydroxyl proton, which was not observed in this solvent. Additional single irradiation 1D NOE NMR studies were conducted in $CDCl_3$ to unequivocally confirm that the phenyl D ring was located at position 9. The key NOE NMR enhancement observed was from $\delta_H$ 8.32, d, $J$ = 8.5 Hz (H-7) to the position 6 methoxy at $\delta_H$ 4.06, s. This confirmed that the phenyl D ring could not be located at the 7 position, as is the case for many of the phenylphenalenones. Other important NOE NMR enhancements were those observed between

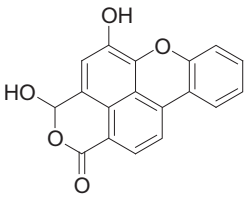
[1] Unpublished results.



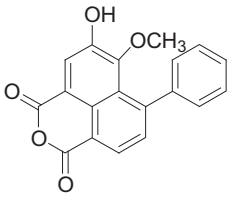
(**1**) R = OH $R_1$ = OH $R_2$ = H $R_3$ = H
lachnanthofluorone (incorrect structure)
(**2**) R = $OCH_3$ $R_1$ = OH $R_2$ = H $R_3$ = H
(**3**) R = H $R_1$ = $OCH_3$ $R_2$ = H $R_3$ = H
(**4**) R = H $R_1$ = OH $R_2$ = OH $R_3$ = H
(**5**) R = H $R_1$ = H $R_2$ = H $R_3$ = $OCH_3$



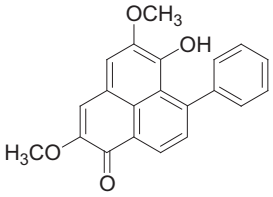
(**6**) R = H $R_1$ = OH $R_2$ = OH $R_3$ = OH
haemofluorone B (incorrect structure)
(**7**) R = $OCH_3$ $R_1$ = H $R_2$ = $OCH_3$ $R_3$ = $OCH_3$



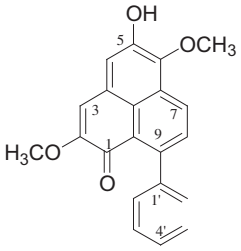
(**8**)



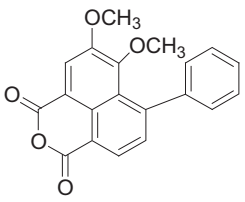
(**9**)



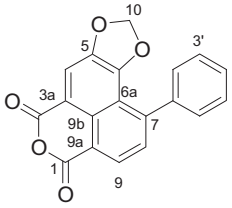
(**10**)
xiphidone



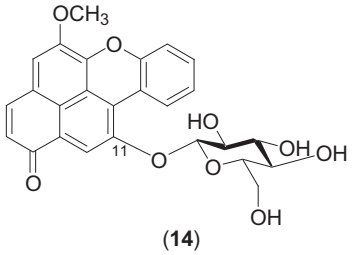
(**11**)
haemoxiphidone



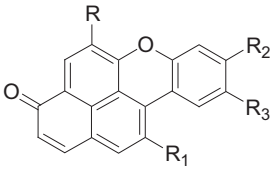
(**12**)



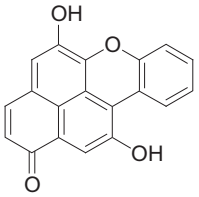
(**13**)
haemodordioxolane



(**14**)
haemodoroxychrysenose



(**15**) R = OH $R_1$ = H $R_2$ = OH $R_3$ = OH
haemofluorone B (revised structure)
(**16**) R = *O*-β-D-glucopyranosyl $R_1$ = $OCH_3$ $R_2$ = H $R_3$ = H
(**17**) R = $OCH_3$ $R_1$ = H $R_2$ = H $R_3$ = H



(**18**) lachnanthofluorone (revised structure)

**Fig. 2.** Selection of previously reported as well as new phenylphenalenones and oxabenzochrysenones pertinent to this study.

$\delta_H$ 6.75, s (H-3) and $\delta_H$ 7.43, s (H-4) as well as to the methoxy protons at $\delta_H$ 3.87, s (2-$OCH_3$). These NOE NMR enhancements confirmed the location of the 2-methoxy moiety to be next to the C-1 carbonyl and the position 4 methine to be adjacent to the 5-hydroxyl substituent and allowed us to unequivocally confirm the structure as haemoxiphidone (**11**), a new isomer of xiphidone (**10**).

The structure of haemodordioxolane (**13**) was determined on the basis of 1D and 2D NMR spectroscopy (Table 2), and mass spectrometry. The HR-ESIMS of (**13**) displayed an *m*/*z* at 319.0603 $[M+H]^+$ (calcd. for $C_{19}H_{11}O_5$: *m*/*z* 319.0528) consistent with 15 degrees of unsaturation and a molecular formula $C_{19}H_{10}O_5$. The $^1H$ and gCOSY NMR spectra identified the presence of a set of overlapped aromatic proton signals [$\delta_H$ 7.50, m (H-2′/H-6′) and $\delta_H$ 7.48, m (H-3′/H-4′/H-5′)], one singlet aromatic proton [$\delta_H$ 8.25, s (H-4)] and two *ortho* aromatic protons [$\delta_H$ 7.65, d, *J* = 7.5 Hz (H-8) and $\delta_H$ 8.44, d, *J* = 7.5 Hz (H-9)], along with a singlet proton [$\delta_H$ 6.20, s (H-10)], which were consistent with that of the phenylphenalenone structure class, as supported by the characteristic UV maxima at 368 nm (Dias et al., 2009b).

In combination with the $^{13}C$ NMR spectrum, the 2D gHSQCAD and gHMBC NMR experiments allowed for the complete assignment of haemodordioxolone (**13**). It was established via the gHSQCAD NMR experiment that the singlet proton at $\delta_H$ 6.20, s (H-10) was a deshielded methylene with a corresponding carbon resonance at $\delta_C$ 103.2 (C-10), suggestive of a dioxolane moiety and

**Table 1**
$^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data for haemoxiphidone (**11**) in $d_6$-DMSO and in $CDCl_3$.

| | $d_6$-DMSO | | | | $CDCl_3$ | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Position | $\delta_C$[a], mult | $\delta_H$ ($J$ in Hz) | gCOSY | gHMBC | $\delta_C$[a], mult | $\delta_H$ ($J$ in Hz) | gCOSY | gHMBC | NOE |
| 1 | 178.9, s | – | | | 180.0, s | | | | |
| 2 | 152.3, s | – | | | 153.1, s | | | | |
| 3 | 111.7, d | 7.10, s | | 1, 2, 4, 9b | 110.9, d | 6.75, s | 4, 2-OC$\underline{H}_3$ | 1, 2, 4, 3a, 9b | 4, 2-OC$\underline{H}_3$ |
| 3a | 124.8, s | – | | | 121.1, s | | | | |
| 4 | 122.9, d | 7.55, s | | 3, 3a (*w*), 5, 6, 9b | 120.7, d | 7.43, s | 3 | 3, 5, 6, 3a, 9b | |
| 5 | 146.6, s | – | | | 146.2, s | | | | |
| 6 | 141.2, s | – | | | 141.2, s | | | | |
| 6a | 125.4, s | – | | | 126.9, s* | | | | |
| 7 | 127.2, d | 8.37, d (9.0) | 8 | 6, 9, 6a (*w*), 9b | 126.8, d | 8.32, d (8.5) | 8 | 5 (*w*), 6, 9, 9b | 6-OC$\underline{H}_3$ |
| 8 | 131.2, d | 7.49, d (9.0) | 7 | 7 (*w*), 6a, 9a, 1′ | 131.7, d | 7.59, d (8.5) | 7 | 7, 6a, 9a, 1′ | 7 |
| 9 | 144.1, s | – | | | 145.8, s | | | | |
| 9a | 127.6, s | – | | | 126.1, s* | | | | |
| 9b | 119.8, s | – | | | 121.3, s | | | | |
| 1′ | 142.9, s | – | | | 142.7, s | | | | |
| 2′ | 128.1, d* | 7.27, dd (1.5, 8.5) | 3′ | 9, 3′, 4′, 6′ | 127.9, d | 7.35, m | | 9, 3′, 4′ | |
| 3′ | 128.0, d* | 7.38, dd (6.5, 7.5) | 2′ | 1′, 2′, 5′ | 128.2, d | 7.44, m | | 1′, 2′, 5′ | |
| 4′ | 126.6, d | 7.34, dd (7.0, 7.5) | | 2′, 3′, 5′, 6′ | 126.9, d | 7.39, m | | 2′, 3′, 5′, 6′ | |
| 5′ | 128.0, d* | 7.38, dd (6.5, 7.5) | 6′ | 1′, 3′, 6′ | 128.2, d | 7.44, m | | 1′, 3′, 6′ | |
| 6′ | 128.1, d* | 7.27, dd (1.5, 8.5) | 5′ | 9, 2′, 4′, 5′ | 127.9, d | 7.35, m | | 9, 2′, 4′, 5′ | |
| 2-$OCH_3$ | 55.3, q | 3.76, s | | 2 | 55.5, q | 3.87, s | 3 | 2 | 3 |
| 5-OH | – | 10.05, bs | | 4, 5, 6 | - | ND | | | |
| 6-$OCH_3$ | 61.2, q | 3.95, s | | 6 | 62.9, q | 4.06, s | | 6 | 7 |

[a]Carbon assignments based on gHSQCAD and gHMBC NMR experiments.
*Signals may be interchanged.
ND signal not detected.
*w*Weak correlation.

**Table 2**
$^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data for haemodordioxolane (**13**) in $d_6$-DMSO and in $CDCl_3$.

| | $d_6$-DMSO | | | | $CDCl_3$ | | | |
|---|---|---|---|---|---|---|---|---|
| Position | $\delta_C$[a], mult | $\delta_H$ ($J$ in Hz) | gCOSY | gHMBC | $\delta_C$[a], mult | $\delta_H$ ($J$ in Hz) | gCOSY | gHMBC |
| 1 | 160.8, s | – | – | – | 160.9, s | – | – | – |
| 2 | – | – | – | – | – | – | – | – |
| 3 | 160.0, s | – | – | – | 160.1, s | – | – | – |
| 3a | 112.4, s | – | – | | 112.4, s | – | – | |
| 4 | 115.1, d | 8.25, s | – | 3, 5, 6, 9b | 116.1, d | 8.26, s | - | 3, 5, 6, 9b |
| 5 | 145.9, s* | – | – | – | 146.2, s* | – | – | – |
| 6 | 149.1, s* | – | – | – | 149.5, s* | – | – | – |
| 6a | 118.4, s | – | – | – | 118.1, s | – | – | – |
| 7 | 143.7, s | – | – | – | 144.1, s | – | – | – |
| 8 | 129.6, d | 7.65, d (7.5) | 9 | 6a, 9a, 1′ | 129.1, d | 7.57, d (7.5) | 9 | 6a, 9a, 1′ |
| 9 | 131.4, d | 8.44, d (7.5) | 8 | 1, 7, 9b | 132.3, d | 8.54, d (7.5) | 8 | 1, 7, 9b |
| 9a | 115.4, s | – | – | – | 116.4, s | – | – | – |
| 9b | 128.4, s | – | – | – | 129.5 s | – | – | – |
| 10 | 103.2, t | 6.20, s | – | 5, 6 | 102.9, t | 6.09, s | – | 5, 6 |
| 1′ | 138.9, s | – | – | – | 139.1, s | – | – | – |
| 2′ | 129.3, d | 7.50, m** | – | 3′, 4′ | 128.5, d | 7.43, m** | – | 7, 6′ |
| 3′ | 127.7, d** | 7.48, m** | – | 1′, 2′ | 127.8, d** | 7.48, m** | – | 4′, 5′ |
| 4′ | 127.7, d** | 7.48, m** | – | 2′, 6′ | 127.8, d** | 7.48, m** | – | 3′, 5′ |
| 5′ | 127.7, d** | 7.48, m** | – | 1′, 6′ | 127.8, d** | 7.48, m** | – | 3′, 4′ |
| 6′ | 129.3, d | 7.50, m** | – | 4′, 5′ | 128.5, d | 7.43, m** | – | 7, 2′ |

[a]Carbon assignments based on gHSQCAD and gHMBC NMR experiments.
*Signals may be interchanged.
**Signals overlapped.

confirmed by the HMBC correlations of $\delta_H$ 6.20, s (H-10) to the aromatic carbons at $\delta_C$ 145.9 and $\delta_C$ 149.1 (C-5 and C-6). The remaining aromatic protons indicated the presence of an anhydride (Dias et al., 2009b), with $\delta_H$ 8.25, s (H-4) and $\delta_H$ 8.44, d, $J$ = 7.5 Hz (H-9) displaying correlations to $\delta_C$ 160.0 (C-3) and $\delta_C$ 160.8 (C-1) respectively. The IR stretches at 1770 and 1728 cm$^{-1}$ further supported the presence of the anhydride functionality. The coupling of the *ortho* aromatic protons, along with the key HMBC correlation (Fig. 3) of $\delta_H$ 8.44, d, $J$ = 7.5 Hz (H-9) to $\delta_C$ 160.8 (C-1), allowed for the positioning of the phenyl ring at position 7, which was also confirmed by the correlations observed from $\delta_H$ 7.65, d, $J$ = 7.5 Hz (H-8) to $\delta_C$ 138.9 (C-1′) and correlations from $\delta_H$ 8.44, d, $J$ = 7.5 Hz (H-9) and $\delta_H$ 7.50, m (H-2′/H-6′) to $\delta_C$ 143.7 (C-7). This allowed for the unequivocal assignment as haemodordioxolone (**13**).

Haemodoroxychrysenose (**14**) was determined on the basis of 1D and 2D NMR spectroscopy (Table 3) and mass spectrometry. The HRESIMS of **14** displayed an $m/z$ at 479.1337 $[M+H]^+$ (calcd. for $C_{26}H_{23}O_9$: $m/z$ 479.1264), consistent with 16 degrees of unsaturation and a molecular formula $C_{26}H_{22}O_9$, while the UV/Vis spectrum displayed a maxima at $\lambda$ 537 nm, consistent with the

**Table 3**
$^{1}$H (500 MHz) and $^{13}$C (125 MHz) NMR data for haemodoroxychrysenose (**14**) in $d_6$-DMSO.

| Position | $\delta_C$[a], mult | $\delta_H$ (*J* in Hz) | gCOSY | gHMBC | NOESY |
|---|---|---|---|---|---|
| 1 | 182.3, s | – | – | – | |
| 2 | 126.2, d | 6.72, d (9.5) | 3 | 3a, 12a | 3 |
| 3 | 140.9, d | 8.06, d (9.5) | 2 | 1, 3a, 4, 12b, 12c (*w*) | 2 |
| 3a | 119.4, s* | – | – | | |
| 4 | 119.2, d | 8.10, s | – | 3, 3a, 5, 5a, 12b | 5-OC<u>H</u>$_3$ |
| 5 | 142.2, s | – | – | – | – |
| 5a | 140.8, s | – | – | – | – |
| 6 | – | – | – | – | – |
| 6a | 151.2, s | – | – | – | – |
| 7 | 117.0, d | 7.49, dd (1.5, 8.5) | 8 | 6a, 9, 10a, 10b (*w*) | 8 |
| 8 | 131.5, d | 7.59, ddd (1.5, 8.0, 8.5) | 7, 9 | 6a, 10 | 7, 9 |
| 9 | 124.6, d | 7.37, ddd (1.5, 8.0, 8.5) | 8, 10 | 7, 10 (*w*), 10a | 8, 10 |
| 10 | 130.0, d | 9.24, dd (1.5, 8.5) | 9 | 6a, 8, 10b | 9, 2′-O<u>H</u> |
| 10a | 118.3, s | – | – | – | – |
| 10b | 119.4, s* | – | – | – | – |
| 11 | 150.6, s | – | – | – | – |
| 12 | 119.4, d* | 8.48, s | – | 1, 3a (*w*), 10a (*w*), 10b, 11, 12a, 12b | 1′ |
| 12a | 127.9, s | – | – | – | – |
| 12b | 117.5, s | – | – | – | – |
| 12c | 121.1, s | – | – | – | – |
| 5-OCH$_3$ | 56.8, q | 4.08, s | – | 5 | 4 |
| 1′ | 101.3, d | 5.39, d (8.0) | 2′ | 11, 2′, 5′ | 12 |
| 2′ | 73.4, d | 3.58, m | 1′, 3′, 2′O<u>H</u> | 1′ | 3′ |
| 3′ | 77.3, d | 3.46, m | 2′, 4′, 3′O<u>H</u> | – | 2′ |
| 4′ | 69.4, d | 3.32, m | 3′, 5′, 4′O<u>H</u> | 3′ | - |
| 5′ | 76.6, d | 3.42, m | 4′ | – | 6a′ |
| 6a′ and 6b′ | 60.4, t | 3.58, m | 6b′, 6′O<u>H</u> | 5′ - | 5′, 6b′ 6a′ |
| | | 3.70, m | 6a′, 6′O<u>H</u> | | |
| 2′OH | – | 5.61, d (5.5) | 2′ | 1′ | 10, 3′O<u>H</u> |
| 3′OH | – | 5.26, d (4.5) | 3′ | – | 2′O<u>H</u>, 4′O<u>H</u> |
| 4′OH | – | 5.10, d (5.5) | 4′ | 4′ | 3′O<u>H</u>, 6′O<u>H</u> |
| 6′OH | – | 4.56, t (5.5) | 6a′, 6b′ | – | 4′O<u>H</u> |

[a]Carbon assignments based on $^{13}$C, DEPT, gHSQCAD and gHMBC NMR experiments.
*Signals overlapped.
*w*Weak correlation.

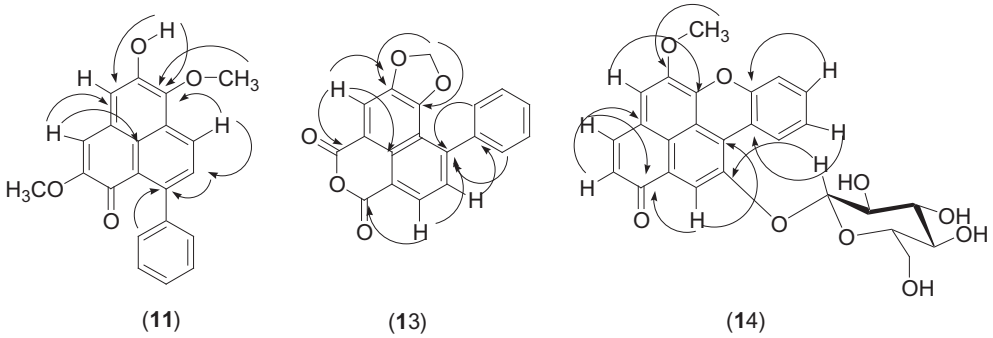



**Fig. 3.** Key gHMBC NMR correlations observed for haemoxiphidone (**11**), haemodordioxolane (**13**) and haemodoroxychrysenose (**14**).

oxabenzochrysenone structure class (Opitz et al., 2002a). In addition the IR stretches at 3383 and 1731 cm$^{-1}$ supported the presence of hydroxyl and carbonyl functionalities. The $^{1}$H NMR and gCOSY spectra identified the presence of two singlet aromatic proton signals [$\delta_H$ 8.10, s (H-4)] and [$\delta_H$ 8.48, s (H-12)], a pair of *ortho* aromatic proton signals [$\delta_H$ 6.72, d, $J$ = 9.5 Hz (H-2) and $\delta_H$ 8.06, d, $J$ = 9.5 Hz (H-3)], along with four aromatic proton signals [$\delta_H$ 7.37, ddd, $J$ = 1.5, 8.0, 8.5 Hz (H-9), $\delta_H$ 7.49, dd,$J$ = 1.5, 8.5 Hz (H-7), $\delta_H$ 7.59, ddd, $J$ = 1.5, 8.0, 8.5 Hz (H-8) and $\delta_H$ 9.24, dd,$J$ = 1.5, 8.5 Hz (H-10)], a methoxy group [$\delta_H$ 4.08, s (5-OCH$_3$)] and resonances consistent with a sugar moiety including four methines at [$\delta_H$ 5.39, d,$J$ = 8 Hz (H-1′) and those occurring between $\delta_H$ 3.32 and $\delta_H$ 3.57 (H-2″, H-3″, H-4″, H-5″) and one methylene [$\delta_H$ 3.58, m (H-6a′) and $\delta_H$ 3.70, m (H-6b′)]. Also observed were the exchangeable hydroxyl moieties of the sugar unit at $\delta_H$ 4.56, t, $J$ = 5.5 Hz (6′-OH) and those at $\delta_H$ 5.61, d, $J$ = 5.5 Hz (2′-OH), $\delta_H$ 5.26, d, $J$ = 4.5 Hz (3′-OH) and $\delta_H$ 5.10, d, $J$ = 5.5 Hz (4′-OH).

Studies of model oxabenzochrysenones and structurally similar phenylphenalenones indicated that the two doublets at $\delta_H$ 6.72 and $\delta_H$ 8.06, with a coupling constant of 9.5 Hz, are characteristic of protons α and β to the carbonyl group in such systems (Cooke and Dagley, 1979; Opitz et al., 2002b). This provided evidence that eliminated substitution at the 2 position of the oxabenzochrysenone as a possible structure. However, in light of the structural revision of haemofluorone B from (**6**) to (**15**) (Cooke and Dagley, 1979; Chaffee et al., 1981; Feutrill and Whitelaw, 1981), there were

two possible isomeric structures, namely (**14**) and (**16**), to be considered as possibilities for the structure of haemodoroxychrysenose. Isomeric oxabenzochyrsenononones such as (**3**) and (**17**) have been reported from *Musa acumina* and *Wachendorfia thyrsiflora* (Opitz et al., 2002b). These compounds display different NMR data but importantly they are also distinguishable by means of the differences displayed in their UV absorption spectra with compound (**17**) displaying an additional absorption band around 500 nm (Opitz et al., 2002b). The UV absorption spectrum of haemodoroxychrysenose (**14**) was compared to literature oxabenzochrysenones and this provided support for the 11-glycosyl substituted oxabenzochrysenone isomer.

The 2D gHSQCAD and gHMBC NMR experiments allowed for the complete assignment of haemodoroxychrysenose (**14**). In particular both gHSQCAD and gHMBC NMR correlations (Fig. 3) observed from $\delta_H$ 8.48, s (H-12) allowed for the positioning of the carbonyl moiety at position C-1 ($\delta_C$ 182.3). Additional correlations to the quaternary carbons at $\delta_C$ 119.4 (C-10b) and $\delta_C$ 150.6 (C-11) indicated that further substitution of the C ring was present. The *ortho* coupled aromatic proton at $\delta_H$ 8.06, d, $J$ = 9.5 Hz (H-3) displayed a correlation to $\delta_C$ 182.3 (C-1) placing the *ortho* coupled aromatic protons in ring B. Correlations from the singlet methine at $\delta_H$ 8.10, s (H-4) to $\delta_C$ 140.9 (C-3) and $\delta_C$ 140.8 (C-5a), along with the methoxy at $\delta_H$ 4.08, s (5-$OCH_3$) to $\delta_C$ 142.2 (C-5), allowed for the positioning around the A ring. The remaining coupled aromatic protons [$\delta_H$ 7.49, dd, $J$ = 1.5, 8.5 Hz (H-7), $\delta_H$ 7.59, ddd, $J$ = 1.5, 8.0, 8.5 Hz (H-8), $\delta_H$ 7.37, ddd, $J$ = 1.5, 8.0, 8.5 Hz (H-9) and $\delta_H$ 9.24, dd, $J$ = 1.5, 8.5 Hz (H-10)] were due to the D ring of the oxabenzochrysenone type structure. The gHMBC NMR correlation from $\delta_H$ 5.39, d, $J$ = 8.0 Hz (H-1′) to $\delta_C$ 150.6 (C-11) allowed for the sugar moiety to be linked to the C ring at position 11.

The positioning of the methoxy and sugar moieties was substantiated by NOESY NMR enhancements observed between $\delta_H$ 5.39, d, $J$ = 8.0 Hz (H-1′) and $\delta_H$ 8.48, s (H-12) as well as between $\delta_H$ 8.10, s (H-4) and $\delta_H$ 4.08, s (5-$OCH_3$). A further NOESY correlation was observed from $\delta_H$ 9.24, dd, $J$ = 1.5, 8.5 Hz (H-10) to $\delta_H$ 5.61, d, $J$ = 5.5 Hz (2′-OH) which unequivocally positioned the sugar moiety at position 11. The aromatic proton at position 10 is clearly influenced by the close proximity of the sugar moiety as it experiences a further downfield shift compared to closely related compounds (Cooke and Dagley, 1979; Chaffee et al., 1981). An important TOCSY NMR correlation observed between $\delta_H$ 8.10, s (H-4) and $\delta_H$ 8.06, d,$J$ = 9.5 Hz (H-3) provided further confirmation that the sugar moiety was not located at the alternative 2 position of the oxybenzochrysenose. In consideration of the NOESY and TOCSY NMR data the unequivocal structure of haemodoroxychrysenose (**14**) was deduced as 11-*O*-β-D-glucopyranosyloxy-5-methoxy-6-oxabenzochrysen-1-one.

In reviewing the literature on the oxabenzochrysenones, we can now also unequivocally revise the structure of lachnanthofluorone from (**1**) to (**18**) as suggested by Cooke and re-address the NMR assignments made in 1974 (Cooke and Dagley, 1979; Feutrill and Whitelaw, 1981). In particular the proton signal at $\delta_H$ 9.10 with 8.0 Hz coupling had been tentatively assigned to position 7, but this can now be definitively assigned to position 10 of the oxabenzochrysenone. Also the aromatic proton resonances at $\delta_H$ 7.92 and $\delta_H$ 6.64 with a coupling of 9.5 Hz should be reassigned to the protons α and β to the carbonyl group. Confirmation that the original structure for lachnanthofluorone was incorrect is supported by the isolation of (**1**) (Opitz et al., 2002a). This compound was reported to be lachnanthofluorone despite the fact that the NMR data for these compounds were very different (Weiss and Edwards, 1969; Edwards and Weiss, 1974).

The $^1$H and 2D NMR, along with ESI-MS data for 5-hydroxyl-2-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**2**) (Opitz et al., 2002a), 5-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**3**) (Opitz et al., 2002b), haemodorone (**9**) and 5,6-dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (**12**) (Dias et al., 2009b), were all found to be consistent with the literature data (Dias et al., 2009b; Opitz et al., 2002a,b).

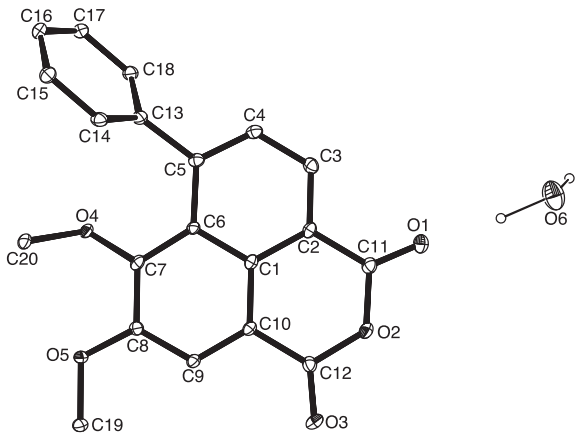



**Fig. 4.** Single crystal X-ray structure (ORTEP) of 5,6-dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (**12**).

5,6-Dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (**12**) was isolated from the 100% hexane silica column fraction that also contained **13**. Upon drying fine fluorescent yellow crystals were obtained, allowing for the first single crystal X-ray diffraction study of **12** to be carried out. Compound **12** crystallises as a hemihydrate, with the water oxygen situated on a 2-fold axis of symmetry, forming a hydrogen bond with carbonyl oxygen O1: O(6)-H(6′) 0.823(5)(9) Å, O1...H6, 2.174(14) Å, O1...O6 2.982(4) Å and O1...H6...O6 167(6)°. A thermal ellipsoid plot of **12** depicting 20% elliposoids as determined by X-ray crystallographic analysis is given in Fig. 4. Full details are provided in the Section 4.

## 3. Concluding remarks

This study describes the use of HPLC-NMR to conduct initial online chemical profiling of an enriched fraction. Subsequent off-line methodologies led to the isolation of the new phenylphenalenones haemoxiphidone (**11**) and haemodordioxolane (**13**) from the bulbs of *H. simulans* as well as the new oxabenzochrysenone glycoside haemodoroxychrysenose (**14**) from the aerial parts of the plant. The biological activity of all compounds was evaluated in the P388 anti-tumour assay and all compounds were found to be inactive. The bioactive components present in the crude extracts of the bulbs remain unidentified.

## 4. Experimental

### 4.1. General experimental procedures

For detailed information on the general experimental procedures, optical rotation measurements, analytical HPLC analyses, IR and UV/Vis Spectra, please see references (Dias et al., 2009a; Dias and Urban, 2009; Reddy and Urban, 2008). Electrospray (ESI) and HR-ESI mass spectra were obtained on an Agilent 6220 Accurate mass LC-TOF system connected to an Agilent 1200 series HPLC. The reference compound for all accurate masses was a purine/HP0921 mix. The eluant was 100% $CH_3CN$ operating at a flow rate of 0.3 mL/min, scanning from 100 to 2000 $m/z$. The ESI conditions were 8 L/min $N_2$, 325 °C drying gas temperature and the capillary voltage was 3500 V. All semi-preparative reversed phased HPLC analyses were performed on a Varian Prostar 210 (Solvent Delivery Module) equipped with a Varian Prostar 335 PDA detector

(monitored at $\lambda_{max}$ 254 and 300 nm) and STAR LC WS Version 6.0 software and a Phenomenex Prodigy ODS (3) 100 Å $C_{18}$ 250 × 10 mm (5 μm) column with a flow rate of 3.5 mL/min. NMR spectra were acquired on a 500 MHz Varian INOVA NMR spectrometer in $d_6$-DMSO, $CDCl_3$ or $CD_3OD$ with referencing to solvent signals (2.50 ppm and 39.5 ppm, 7.26 ppm and 77.0 ppm and 3.30 ppm and 49.0 ppm respectively). Crystallographic diffraction data were collected with an Oxford SuperNova diffractometer using Cu radiation ($\lambda$ = 1.54184 Å). Data were reduced using the CrysalisPRO software. The temperature of the data collection was maintained at 130 K, using an Oxford Cryostream cooling device. The structure was solved by direct methods, and difference Fourier synthesis and was refined on $F^2$ (SHELXL-97) (Sheldrick, 2008). Thermal ellipsoid plot was generated using program ORTEP-3 (Farrugia, 1997) integrated within the WINGX program suite (Farrugia, 1999).

In the acid hydrolysis of haemodoroxychrysenose (**14**), approximately 0.5 mg of **14** was treated with 10 mL of 15% aqueous HCl. The reaction mixture was heated to 90 °C and stirred for 6 h. The solution was neutralised by drop-wise addition of 5% aqueous NaOH to which ethyl acetate was then added. The aqueous partition was evaporated under reduced pressure and the residue spotted on aluminum backed silica gel TLC plates together with standard samples of β-D-glucopyranose, β-D-galactose, β-D-rhamnose, and β-D-xylose. The TLC plate was developed using the solvent system consisting of *n*-butanol:HOAc:$H_2O$ (3:1:1) and was then air dried, sprayed with 10% aqueous $H_2SO_4$ and then heated in an oven at 110 °C. The hydrolysis products from compound **14** exhibited a characteristic coloured spot ($R_f$ = 0.63) that was identical with that observed for β-D-glucopyranose.

HPLC-NMR analysis was performed using a 500 MHz Varian Unity INOVA Spectrometer equipped with a Varian $^1H[^{13}C]$ pulsed field gradient flow probe with a flow cell of 60 μL (active volume) coupled to a Varian Prostar 230 Solvent Delivery Module with a Varian Prostar 335 PDA detector and a Prostar 430 Autosampler. Both on-flow and stop-flow HPLC-NMR analysis was performed via the CORBA communication between the HPLC and NMR (version VNMRJ 2.1b software) using an isocratic 80% $CH_3CN/D_2O$ HPLC solvent system on a Varian Microsorb-MV $C_{18}$ 150 × 4.6 mm (5 μm) column at 1.0 mL/min with detection at $\lambda_{max}$ 254 and at 315 nm. The 2H resonance of $D_2O$ provided the field-frequency lock, and the spectra were cantered on the methyl resonance of acetonitrile. Suppression of resonances from HOD and the methyl of acetonitrile was accomplished using the WET solvent suppression (Smallcombe et al., 1995). For convenience, the residual methyl resonance of acetonitrile was referenced to 1.96 ppm. For both the on-flow and stop-flow HPLC-NMR experiments, 50 μL injections (4120 μg) of the enriched fraction were used after dissolving in a mixture of 80% $CH_3CN/D_2O$.

### 4.2. Biological evaluation

For detailed information on the biological assays please see reference (Reddy and Urban, 2008). A 2 g portion of the initial collection of the *H. simulans* specimen (aerial parts and bulbs) were extracted separately with 3:1 methanol:dichloromethane (40 mL) and evaluated in several assays (anti-tumour, anti-viral and anti-microbial) at a concentration of 50 mg/mL at the University of Canterbury, Christchurch, New Zealand. The crude extract of the aerial parts displayed moderate cytotoxicity towards P388 with an $IC_{50}$ of 104.7 μg/mL, while the bulbs displayed potent cytotoxicity with an $IC_{50}$ of <4.8 μg/mL. Both the aerial parts and the bulbs displayed similar anti-viral activity, with cytotoxicity towards the BSC-1 cells, indicating that it could not be concluded if the *Herpes simplex* or *Polio* virus were inhibited. Similar levels of anti-microbial activity were also observed between the aerial parts and the bulbs of the plant. Moderate anti-fungal activity towards *Trichophyton mentagrophytes* was observed for both crude extracts, with the bulbs being more active of the two, with a zone of inhibition of 5 mm, while a zone of inhibition of 1 mm was observed for the aerial parts. In addition both the aerial parts and bulbs displayed a zone of inhibition of 3 mm against *Bacillus subtilis*. Both extracts showed no activity towards *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* or *Cladosporium resinae*. All isolated compounds (**2**, **3**, **10**, **11**, **13** and **14**) were evaluated for P388 activity and all exhibited little to no cytotoxicity. These compounds were not further evaluated in the anti-viral or anti-microbial assays as access to these assays was no longer possible.

### 4.3. Plant material

The plant specimens were collected by Mr. Allan Tinker from the Western Flora Caravan Park, 22 km north of Eneabba (Irwin botanical province), Western Australia (plant license SW008335). The first collection (aerial parts and bulbs) was conducted on the 20th of January 2005 and initially identified as *H. simplex*. The taxonomic classification was subsequently revised to *H. simulans*. A second collection of the bulbs of this plant was conducted on the 20th of February 2007. Voucher specimens designated the codes 2005-01a (bulbs), 2005-01b (aerial parts) and 2007-01 (bulbs) respectively are deposited at the School of Applied Sciences (Discipline of Applied Chemistry), RMIT University. The purpose of the specimen re-collection was to provide sufficient mass to undertake extraction, isolation and identification of the minor components present in the plant specimen.

### 4.4. Extraction and isolation

The first collection of the *H. simulans* bulbs 2005-01a (22 g) was extracted with 3:1 methanol:dichloromethane (400 mL). This extract was decanted and concentrated under reduced pressure and then subjected to sequential solvent partitioning (trituation) into dichloromethane (97.7 mg) and methanol (2.24 g) soluble extracts respectively. The remaining bulbs of the first collection were extracted with 1:1 methanol:dichloromethane (400 mL) and then combined with the initial extract. The second collection of the *H. simulans* bulbs, 2007-01 (36 g) were subjected to the same extraction procedure used for the first collection. Analysis of the subsequent dichloromethane and methanol extracts generated from this second collection of the bulbs, indicated that both fractions displayed very similar analytical HPLC and $^1H$ NMR spectra to those obtained from the first collection of the bulbs. On this basis, the dichloromethane and methanol soluble extracts from the 2007 specimen bulbs were combined with the dichloromethane and methanol soluble extracts of the 2005 specimen bulbs. These combined extracts were evaporated to dryness and stored at 4 °C. The dichloromethane soluble extract was subjected to silica flash chromatography using a 20% stepwise elution from hexane to dichloromethane to ethyl acetate and finally to methanol, resulting in 15 fractions. The 100% hexane fraction afforded 5,6-dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (**12**) (21.0 mg, 0.10%) and haemodordioxolane (**13**) (13.5 mg, 0.06%), while the 80:20 dichloromethane:ethyl acetate fraction resulted in the isolation of haemodorone (**9**) (7.5 mg, 0.03%) and 5-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**3**) (5.3 mg, 0.02%), which was also isolated from the aerial parts of the plant. The 40:60 and 20:80 dichloromethane:ethyl acetate fractions resulted in the isolation of 5-hydroxyl-2-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**2**) (8.3 mg, 0.04%). Finally the 40:60 hexane:dichloromethane fraction yielded haemoxiphidone (**11**) (7.8 mg, 0.03%). The aerial parts of the first collection of *H. simulans*, 2005-01b (26 g) were subjected to the same extraction procedure as described for the bulbs, and the dichloromethane soluble fraction was subjected to

flash silica chromatography using the same 20% stepwise elution profile adopted for the fractionation of the bulbs, resulting in 15 fractions. Subsequent semi-preparative reversed phased HPLC of the 80:20 dichloromethane: ethyl acetate fraction yielded 5-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**3**) (8.6 mg, 0.11%), which was also present in the dichloromethane soluble extract of the bulbs of the *H. simulans*. The methanol soluble extract was subjected to $C_{18}$ Vacuum Liquid Chromatography (VLC) with a 25% stepwise elution form water to methanol to ethyl acetate and finally to dichloromethane resulting in a total of 10 fractions. The 100% methanol and 80:20 methanol:water fractions were subjected to semi-preparative reversed phased HPLC to afford haemodoroxychrysenose (**14**) (23.5 mg, 0.31%).

### 4.5. Crystallography

Crystallographic diffraction data were collected with an Oxford SuperNova diffractometer using Cu radiation ($\lambda$ = 1.54184 Å). Data were reduced using the CrysalisPRO software. The temperature of the data collection was maintained at 130 K, using an Oxford Cryostream cooling device. The structure was solved by direct methods, and difference Fourier synthesis and was refined on $F^2$ (SHELXL-97) (Sheldrick, 2008) Thermal ellipsoid plot was generated using program ORTEP-3 (Farrugia, 1997) integrated within the WINGX program suite (Farrugia, 1999).

### 4.6. On-flow and stop-flow HPLC-NMR profiling

Fractions obtained from silica flash chromatography of the bulbs of *H. simulans* as detailed in the Section 4.4, were analysed by analytical HPLC and $^1$H NMR spectroscopy. This led to the selection of an enriched fraction (100% hexane fraction, 51 mg) for HPLC-NMR chemical profiling. This fraction was re-solubilised in 1 mL of HPLC-NMR grade $CH_3CN$ and filtered through a 0.45 μm PTFE membrane filter (HP045 Advantec, Japan). For the on-flow and stop-flow HPLC-NMR experiments 50 μL (2550 μg) of the fraction was injected and monitored at $\lambda_{max}$ 254 and 315 nm. The HPLC-NMR analyses were performed using the conditions described in Section 4.1.

#### 4.6.1. HPLC-NMR characterisation of compounds *12* and *13*

HPLC-NMR assignment of **12** from stop-flow HPLC-NMR: HPLC-NMR (500 MHz, 80:20 $CH_3CN:D_2O$): 8.39 (1H, d, $J$ = 7.5 Hz), 8.38 (1H, s), 7.48 (1H, d, $J$ = 7.5 Hz), 7.38 (3H, m), 7.29 (2H, m), 3.95 (3H, s), 3.27 (3H, s).

HPLC-NMR assignment of **13** from stop-flow HPLC-NMR: HPLC-NMR (500 MHz, 80:20 $CH_3CN:D_2O$): 8.43 (1H, d, $J$ = 7.5 Hz), 8.17 (1H, s), 7.55 (1H, d, $J$ = 7.5 Hz), 7.43 (5H, m), 6.03 (2H, s).

#### 4.6.2. Off-line characterisation of *2*, *3*, *11*, *12*, *13*, *14*

5-Hydroxyl-2-methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**2**): isolated as a purple powder. NMR and mass spectrometric data was identical to that reported in the literature (Opitz et al., 2002a).

5-Methoxy-1*H*-naphtho[2,1,8-*mna*]xanthen-1-one (**3**): isolated as an orange powder. NMR and mass spectrometric data was identical to that reported in the literature (Opitz et al., 2002b).

5-Hydroxyl-2,6-dimethoxy-9-phenyl-phenalen-1-one [haemoxiphidone (**11**)]: isolated as a brown powder; UV $\lambda$ (log $\varepsilon$) 276 (4.1), 375 (3.7) and 468 (3.5) nm; IR (film) $\nu$ cm$^{-1}$: 3246, 2928, 2854, 1730, 1627, 1556, 1466, 1359, 1212; $^1$H NMR (500 MHz, $d_6$-DMSO and $CDCl_3$) and $^{13}$C (125 MHz, $d_6$-DMSO and $CDCl_3$) details as well as the 2-D NMR COSY and HMBC NMR correlations are detailed in Table 1; ESIMS (positive mode) $m/z$ 333 $[M+H]^+$, 333 $[M+Na]^+$; ESIMS (negative mode) $m/z$ 331 $[M-H]^-$; HR-ESIMS displayed an $m/z$ at 333.1125 $[M+H]^+$ (calcd. for $C_{21}H_{17}O_4$: $m/z$ 333.1049).

5,6-Dimethoxy-7-phenyl-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1,3-dione (**12**): isolated as fine fluorescent yellow crystals: melting point (uncorr.) 256–258 °C; $C_{20}H_{14}O_5.0.5(H_2O)$, $M$ = 343.32$T$ = 130.0(2) K, $\lambda$ = 1.5418, Monoclinic, space group C2/c, $a$ = 18.455(2), $b$ = 8.077(1), $c$ = 21.455(2) Å, $\beta$ = 105.72(1)°, $V$ = 3078.7(6) Å$^3$, $Z$ = 8, $D_c$ = 1.481 mg M$^{-3}$ μ(Cu–Kα) 0.904 mm$^{-1}$, $F$(000) = 1432, crystal size 0.45 × 0.05 × 0.03 mm. 5526 reflections measured, 2765 independent reflections ($R_{int}$ = 0.071) the final $R$ was 0.0585 [$I > 2\sigma$ ($I$)] and $wR(F^2)$ was 0.1488 (all data). Crystallographic data for **12** has been deposited at the Cambridge Crystallographic Data Centre (CDDC 912701)[2] 12 Union Road, Cambridge, CB2 1EZ, UK (www.ccdc.cam.ac.uk/data_request/cif). NMR and mass spectrometric data for this compound was identical to that reported in the literature (Bazan and Edwards, 1976; Cooke and Segal, 1955; Dias et al., 2009b).

1-Phenyl-5,8,10-trioxa-cyclopenta[*a*]phenalene-4,6-dione [haemodordioxolane (**13**)]: isolated as a fluorescent yellow needles: melting point (uncorr.) 215–218 °C; UV $\lambda$ (log $\varepsilon$) 254 (3.7), 314 sh (3.0), 327 (3.1), 368 (3.0), 421 sh (2.4) nm; IR (film) $\nu$ cm$^{-1}$: 2956, 2926, 2854, 1770, 1728, 1679, 1463, 1379, 1265 cm$^{-1}$; $^1$H NMR (500 MHz, $d_6$-DMSO and $CDCl_3$) and $^{13}$C (125 MHz, $d_6$-DMSO and $CDCl_3$) details as well as the 2-D NMR COSY and HMBC NMR correlations are detailed in Table 2; ESIMS (positive mode): $m/z$ 319 $[M+H]^+$, HR-ESIMS displayed an $m/z$ at 319.0603 $[M+H]^+$ (calcd. for $C_{19}H_{11}O_5$: $m/z$ 319.0528).

11-*O*-β-D-glucopyranosyloxy-5-methoxy-6-oxabenzochrysen-1-one [haemodoroxychrysenose (**14**)]: isolated as a fluorescent pink powder; [α] +16.2° ($CH_3OH$, c 0.038); UV $\lambda$ (log $\varepsilon$) 236 sh (3.9), 266 sh (3.6), 288 sh (3.5), 371 sh (2.9), 402 (2.9), 537 (3.4) nm; IR (film) $\nu$ cm$^{-1}$: 3383, 2956, 2921, 2851, 1731, 1593, 1564, 1415, 1380, 1358, 1318 cm$^{-1}$; $^1$H NMR (500 MHz, $d_6$-DMSO) and $^{13}$C (125 MHz, $d_6$-DMSO) details as well as the 2-D NMR COSY and HMBC NMR correlations are detailed in Table 3; ESIMS (positive mode): $m/z$ 479 $[M+H]^+$, HR-ESIMS displayed an $m/z$ at 479.1337 $[M+H]^+$ (calcd. for $C_{26}H_{23}O_9$: $m/z$ 479.1264).

## 5. Conflict of interest statement

There are no financial/commercial conflicts of interest.

## Acknowledgments

The Marine And Terrestrial NAtural Product (MATNAP) research group thanks Mr. Allan Tinker for collection and identification of the plant specimens; Ms G. Ellis (University of Canterbury, Christchurch, New Zealand) for the biological testing; Ms Sally Duck (Monash University) for the high resolution mass spectrometric analysis and the award of an Australian Postgraduate Research Award (APA) scholarship.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2013.07.019.

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[2] CCDC 912701 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www/ccdc/cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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