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PHENYLPHENALENONES FROM ROOT CULTURES OF *ANIGOZANTHOS PREISSII*

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Key Word Index—*Anigozanthos preissii*; Haemodoraceae; *in vitro* plants; phenylphenalenones; root cultures.

Abstract—Five new and four known compounds of the phenylphenalenone type were isolated from root cultures of *Anigozanthos preissii*. The structures were unambiguously established by spectrometric methods including 1D and 2D NMR analysis. The occurrence of these compounds in roots of greenhouse grown plants and aseptically grown *in vitro* plants has been also demonstrated.

INTRODUCTION

About 40 phenylphenalenones are known. The majority of these compounds are constitutive in the Haemodoraceae plant family, including *Anigozanthos* species [1]. They are also found in the aquatic plant *Eichhornia crassipes* (Pontederiaceae) [2] and in *Musa acuminata* and *M. paradisiaca* (Musaceae) where they function as phytoalexins [3, 4]. Due to the fact that phenylphenalenones accumulate within the rhizomes, root cultures of *A. preissii* were used to study their biosynthesis [5, 6]. These cultures are also a rich source of new phenylphenalenones. In this paper, the isolation and structural elucidation of five new and four known phenylphenalenones from cultured roots and roots of whole plants of *A. preissii* is described.

RESULTS AND DISCUSSION

Cultured roots of *A. preissii* were extracted with methanol and the extract was partitioned between *n*-hexane–H_2O, chloroform–H_2O and ethylacetate–H_2O. The organic extracts were further separated by medium pressure liquid chromatography (MPLC) on a reversed phase column followed by preparative TLC on silica gel, and finally purified by reversed phase HPLC.

The major compound of the *n*-hexane fraction was identified as 2-hydroxy-9-phenylphenalen-1-one (anigorufone, **1**), which is a known compound of *A. rufus* [7] and *M. acuminata* [8], and has recently been employed as the target molecule in biogenetic studies in *A. preissii* [5]. Three minor compounds, 2-methoxy-9-phenylphenalen-1-one (methoxyanigorufone, **2**), 2,3-dimethoxy-4-phenylphenalen-1-one (**8**) and 7,8-dimethoxy-6-phenylphenalen-1-one (**9**) were also isolated from the *n*-hexane fraction. Compound **2** was first found in *M. acuminata* [8]; compounds **8** and **9** are described here for the first time.

The chloroform extract contained one major compound, 2-hydroxy-9-(4-hydroxyphenyl)-phenalen-1-one (hydroxyanigorufone, **3**), and two new compounds in minor concentrations, 5-hydroxy-6-methoxy-7-phenylphenalen-1-one (**6**) and 5-hydroxy-2,6-dimethoxy-7-phenylphenalen-1-one (**7**). Compound **3** was first found in *A. rufus* [7] and has been detected also in *Conostylis setosa* [1] and in *M. acuminata* [9]. The occurrence of **3** as the major phenylphenalenone in root cultures of *A. preissii* gave rise to a study of the incorporation of putative biogenetic precursors [6]. Furthermore, a small amount of 2-hydroxy-9-(3,4-dihydroxyphenyl)-phenalen-1-one (dihydroxyanigorufone, **4**) was present in the chloroform fraction.

The major amount of **4**, a phenylphenalenone occurring in *A. rufus* [7], was isolated from the ethyl acetate extract. The novel phenylphenalenone dimer 3,3′-bis-[2-hydroxy-9-(4-hydroxyphenyl)-phenalen-1-one] (3,3′-bis-hydroxyanigorufone, **5**) also occurred in the ethyl acetate fraction in which recently a novel stilbene dimer was found [10].

Extraction with ethanol instead of methanol and HPLC analysis using an acetonitrile–H_2O gradient indicated the occurrence of the same metabolites. This confirmed that the methoxy compounds **2** and **6–9** are natural products and not artefacts originating from

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the workup procedure. Identification of all compounds was performed by EI mass spectrometry and NMR analysis. Assignment of ^1H and ^{13}C chemical shifts generally was based on ^1H NMR, ^{13}C NMR, ^1H–^1H COSY, HMQC, HMBC and NOESY experiments. The ^1H and ^{13}C NMR data for all identified compounds are given in Tables 1 and 2. For further analytical data of new compounds see Experimental.

Compound **1** was identified following the assignment strategy previously described [5]. The NMR data for **2** are very similar to those for **1**. An additional HMBC correlation between the singlet at δ 3.86 with C-2 (δ 154.6) indicated the position of the methoxyl group. The identification and assignment of the ^{13}C resonances of **3** recently was described [6]. The NMR data for **4** are different from those for **3** only in the phenyl ring signals.

Compound **5** is a homodimer of **3**. It is the first homodimer of a phenylphenalenone to be isolated from the Haemodoraceae. The structure was established by means of the mass spectrum (*m/z* 574, $[M]^+$) and 1D and 2D NMR spectra. In contrast to **3**, the singlet resonance of H-3 in the ^1H NMR spectrum as well as the related cross peak with C-3 in the HMQC spectrum was lacking. Furthermore, the resonance of H-4 (δ 7.84) in the monomeric compound **3** was significantly shifted upfield in the dimer **5** to δ 7.67, coinciding now with the doublet of H-8.

The structure of **6** was also ascertained by EI mass spectrometry (*m/z* 302, $[M]^+$) and NMR analysis. The signal of the carbonyl atom (C-1, δ 185.3) showed ^1H–^{13}C three-bond long range correlations (HMBC) with two doublets due to H-3 (δ 7.86) and H-9 (δ 8.38). The signal of H-9 was assigned on the basis of the chemical environment, ^1H–^1H COSY correlation with H-8 (δ 7.57, $J = 7.6$ Hz) and HMBC connectivities with C-7 (δ 145.2). C-7 showed HMBC cross peaks with H-2′/6′ (δ 129.9), indicating the position of the phenyl ring. Furthermore, H-9, H-3 and H-4 (δ 7.71) exhibited HMBC cross signals with a common carbon atom (δ 125.0) which was, therefore, assigned to the central carbon C-9b. The *peri* position of H-3 with H-4 was confirmed by mutual HMBC connectivities and by direct ^1H–^{13}C correlations (HMQC). The HMBC cross signals of C-6 (δ 146.3) with 6-methoxyl (δ 3.15) and with H-4 indicated that the methoxyl group was attached to C-6 (δ 60.9). The chemical shift value of δ 3.15, which is typical for methoxyl hydrogen atoms in the *peri* position relative to a phenyl ring, was also consistent with the suggested structure [11]. Finally, the hydroxyl group had to be attached to the remaining carbon atom C-5 (δ 149.1).

The NMR signal assignment of **7** (*m/z* 332, $[M]^+$) followed the same strategy as for **6**, except that the signal of H-2 was absent and, consequently, that of H-3 (δ 7.16) collapsed to a singlet. The additional resonance in the ^1H NMR spectrum (δ 3.91) was due to a methoxyl group at C-2 as was deduced from the HMBC correlation with the adjacent C-2 (δ 55.7).

Compounds **6** and **7** represent a novel type of naturally occurring phenylphenalenone, bearing the phenyl group at C-7. In contrast, hitherto known phenylphenalenones of plant origin are usually 9-phenylphenalenones. Irenolone, a 4-phenylphenalenone from *M. acuminata* [3], is an exception. The phenylphenalenone nucleus obviously is of great structural variability. This might be due to keto–enol tautomerism during the biosynthesis and subsequent hydroxylations and methylations. Thus, a compound of the 4-phenylphenalenone type and the first phenylphenalenone bearing the phenyl group at C-6 were also found in root cultures of *A. preissii*.

Compound **8** (*m/z* 316, $[M]^+$) exhibited in its ^1H NMR spectrum three-spin systems and two methoxyl resonances. The proton H-9 (δ 8.58), which was part of a three spin system, exhibited HMBC correlations with C-1 (δ 181.3). Mutual HMBC cross signals between H-6/C-7 and H-7/C-6 were also due to the suggested structural feature. HMQC correlations completed the assignments of the protonated carbon atoms. The multiplet centred at δ 7.4 and integrating for five protons was readily assigned to the unsubstituted phenyl ring. The HMBC spectrum provided evidence for the position of the phenyl ring at C-4 by correlation of H-5 (δ 7.44) with C-4 (δ 144.7) and C-1′ (δ 145.2), respectively, and of the phenyl ring protons (H-2′/6′) and C-4. The above data implied that the methoxyl groups must be linked to C-2 and C-3. A weak four-bond HMBC correlation of H-5 with C-3 (δ 158.3) was used to discriminate between C-2 (δ 144.1) and C-3. Furthermore, the methoxyl resonance of 3-methoxyl (δ 3.49) was shielded with respect to 2-methoxyl (δ 3.89) by the *peri* phenyl ring, confirming the proposed assignment. HMBC connectivities between C-2 and 2-methoxyl and between C-3 and 3-methoxyl, respectively, were used to assign the methoxyl groups to the adjacent carbon atoms of the phenalenone nucleus.

In the case of **9** (*m/z* 316, $[M]^+$) the major information for the structural assignment was again provided by NMR analysis. A series of NOE interactions proved the spatial neighbourhood of the phenyl ring protons (centred at δ 7.40) with 7-methoxyl (δ 3.34), between 7- and 8-methoxyl (δ 4.06), and between 8-methoxyl and H-9 (δ 8.35). Furthermore, HMBC connectivities of the doublet of H-4 (δ 7.82) with C-3 (δ 142.4) and C-6 (δ 143.8) as well as of both the doublet of H-3 (δ 7.90) and the singlet of H-9 with C-1 (δ 184.4) were also in agreement with the suggested structure.

EXPERIMENTAL

Plant material. Root cultures of *A. preissii* L. were initiated from surface sterilized seeds on solid MS medium [12] at 22° in the dark. After germination, roots (*ca* 2 cm in length) were excised and placed on fresh medium. After *ca* 12 weeks, a root aggregation had developed and was cultivated for several passages. Root aggregations of *ca* 4 g (fr. wt) were transferred to MS liquid medium (140 ml in 300-ml Erlenmeyer

Table 1. ^{1}H NMR spectral data (500 MHz, acetone-d_6, TMS int. standard) for phenylphenalenones **1–9** from root cultures of *A. preissii*

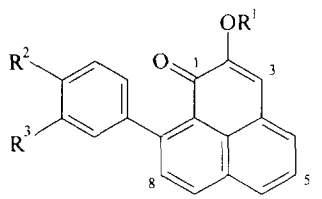
H	**1** δ *J* (Hz)	**2** δ *J* (Hz)	**3** δ *J* (Hz)	**4** δ *J* (Hz)	**5*** δ *J* (Hz)	**6** δ *J* (Hz)	**7** δ *J* (Hz)	**8** δ *J* (Hz)	**9** δ *J* (Hz)
2						6.58 *d* (9.7)			6.63 *d* (9.8)
3	7.18 *s*	7.15 *s*	7.15 *s*	7.14 *s*		7.86 *d* (9.7)	7.16 *s*		7.90 *d* (9.8)
4	7.88 *d* (7.1)	7.86 *d* (7.0)	7.84 *d* (7.0)	7.81 *d* (7.1)	7.67 *d* (8.0)	7.71 *s*	7.62 *s*		7.82 *d* (7.3)
5	7.70 *dd* (7.1, 8.2)	7.66 *dd* (7.0, 8.1)	7.66 *dd* (7.0, 8.0)	7.65 *dd* (7.1, 8.2)	7.54 *t* (8.0)			7.44 *d* (8.4)	7.35 *d* (7.3)
6	8.09 *d* (8.2)	8.05 *d* (8.1)	8.05 *d* (8.0)	8.04 *d* (8.2)	8.06 *d* (8.0)			8.13 *d* (8.4)	
7	8.41 *d* (8.2)	8.34 *d* (8.3)	8.36 *d* (8.2)	8.32 *d* (8.3)	8.41 *d* (8.4)			8.36 *d* (7.7)	
8	7.63 *d* (8.2)	7.58 *d* (8.3)	7.62 *d* (8.2)	7.62 *d* (8.3)	7.67 *d* (8.4)	7.57 *d* (7.6)	7.58 *d* (7.6)	7.85 *t* (7.7)	
9						8.38 *d* (7.6)	8.44 *d* (7.6)	8.58 *d* (7.7)	8.35 *s*
2′			7.27 *d* (8.4)	6.89 *s*	7.35 *d* (8.5)				
3′			6.92 *d* (8.4)		6.95 *d* (8.5)				
4′	7.38 7.47 *m*	7.34 7.43 *m*				7.40 7.46 *m*	7.42–7.47 *m*	7.34–7.45 *m*	7.36–7.44 *m*
5′			6.92 *d* (8.4)	6.82 *d* (8.0)	6.95 *d* (8.5)				
6′			7.27 *d* (8.4)	5.74 *d* (8.0)	7.35 *d* (8.5)				
2-OMe		3.86 *s*					3.91 *s*	3.89 *s*	
3-OMe								3.49 *s*	
6-OMe						3.15 *s*	3.13 *s*		
7-OMe									3.34 *s*
8-OMe									4.06 *s*

* Resonances of identical monomers.

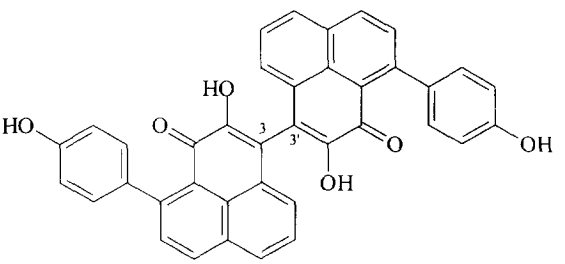
Table 2. ^{13}C NMR spectral data (125 MHz, acetone-d_6, TMS int. standard) for phenylphenalenones **1**–**9** from root cultures of *A. preissii*

C	1	2	3	4	5*	6	7	8	9
1	180.7	179.7	180.7	180.6	180.1	185.3	180.6†	181.3	184.4
2	151.3	154.6	151.4	151.4	148.9	128.3	152.7†	144.1	129.0
3	113.1	112.2	112.8	112.8	117.9	142.7	113.8	158.3	142.4
3a	130.0	129.9	129.9	129.9	129.3	125.7	n.d.‡	123.9	128.1
4	131.0	130.2	130.9	130.9	130.2	125.3	123.1	144.7	130.7
5	127.9	127.8	127.7	127.7	127.7	149.1	149.4†	132.4	130.9
6	130.2	129.6	130.1	130.1	130.5	146.3	143.8	131.2	143.8
6a	132.5	132.6	132.3	132.3	132.5	127.1	126.8†	132.3	126.7
7	136.2	135.0	136.1	136.0	136.5	145.2	145.5	135.8	152.3[a]
8	132.0	132.2	132.5	132.5	132.5	131.7	131.5	127.5	152.2[a]
9	149.1	148.1	149.7	149.7	149.9	127.4	127.4	130.7	117.9
9a	124.7	126.7	124.6	124.7	124.4	129.8	130.0	130.3	126.7
9b	125.7	126.2	126.0	125.9	126.3	125.0	121.9†	126.0	125.1
1′	143.6	144.2	134.5	135.1	134.5	143.8	143.8†	145.2	144.7
2′	128.9	128.9	130.7	116.7	130.8	129.9	130.0	128.7	127.9
3′	128.8	128.8	115.7	145.6[a]	115.8	127.9	127.8	128.2	129.2
4′	127.8	127.6	157.9	145.7[a]	158.0	127.7	127.7	127.3	127.3
5′	128.8	128.8	115.7	115.9	115.8	127.9	127.8	128.2	129.2
6′	128.9	128.9	130.7	120.8	130.8	129.9	130.0	128.7	127.9
2-OMe		55.7					55.7	60.4	
3-OMe								60.9	
6-OMe						60.9	60.9		
7-OMe									56.9
8-OMe									60.9

* Resonances of identical monomers.
† Chemical shifts were obtained from the ^1H detected HMQC and HMBC spectra (500 MHz).
‡ Not detected because of poor signal-to-noise ratio and/or overlapping with other signals.
[a] May be reversed in the same column.



1 $R^1, R^2, R^3 = H$
2 $R^1 = CH_3$; $R^2, R^3 = H$
3 $R^1, R^3 = H$; $R^2 = OH$
4 $R^1 = H$; $R^2, R^3 = OH$



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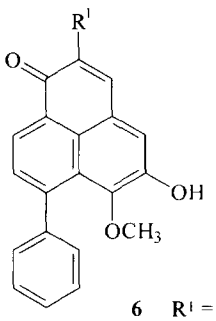
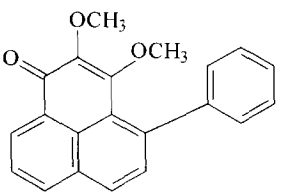


6 $R^1 = H$
7 $R^1 = OCH_3$



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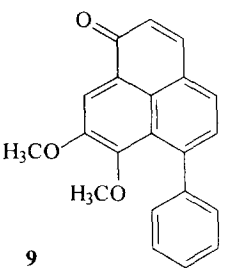

9

flasks) and maintained at 22° on a gyratory shaker (100 rev min^{-1}) under permanent light (600 lux). The aseptically grown plants were regenerated from root cultures and maintained in hydroponic growth vessels. *In vitro* plants were adapted to soil culture and grown under greenhouse conditions (22°).

Isolation and purification. Cultured roots (440 g fr. wt), roots of *in vitro* plants (175 g fr. wt) and roots of greenhouse plants (140 g fr. wt), respectively, were frozen with liquid N_2, ground, and exhaustively extracted with MeOH at room temp. The MeOH extract was evapd (< 40°) and partitioned between *n*-hexane–H_2O, $CHCl_3$–H_2O and EtOAc–H_2O. MPLC: RP-18; initial eluent MeOH–H_2O (1:1), stepwise increase of the MeOH–H_2O ratio to 1:0. TLC: silica gel 60 F_{254}; toluene–Me_2CO (4:1) and (2:1), respectively. Prep. HPLC: Nucleosil 7 C18 (250 × 20 mm); MeCN–H_2O (17:3); UV 284 nm. Analyt. HPLC: LiChrospher 100 RP-18 (250 × 4 mm), 5 μm; MeOH–H_2O (13:7); 0.6 ml min^{-1}; diode-array detection at 200–450 nm. The amounts of isolated compounds were as follows: **1**: 17.6 mg from cultured roots, 7.0 mg from roots of *in vitro* plants, 5.6 mg from roots of greenhouse plants; **2**: 0.9, 0.4, 0.3; **3**: 13.2, 5.3, 4.2; **4**: 0.4, 1.1, 0.8; **5**: 0.9, 0.4, 0.3; **6**: 1.3, 0.5, 0.4; **7**: 0.5, 0.2, 0.2; **8**: 0.3, 1.2, 1.0; **9**: 0.4, 1.4, 1.1.

NMR spectrometry. NMR (Bruker DRX 500): 500.13 MHz (^1H), 125.75 MHz (^{13}C), Me_2CO-d_6, TMS as int. standard. ^1H NMR, ^1H–^1H COSY, HMBC, HMQC and NOESY experiments were recorded in a 2.5 mm inverse detection microprobe head; broadband decoupled ^{13}C and DEPT spectra were determined using a 2.5 mm broadband microprobe head.

3,3′-*bis*-[2-*Hydroxy*-9-(4-*hydroxyphenyl*)-*phenalen*-1-*one*] (3,3′-*bis*-*hydroxyanigorufone* (**5**). Orange–red crystals, mp 205–210° (EtOH). EIMS (70 eV): *m/z* (rel. int.): 574 $[M]^+$ (46), 546 (100), 516 (26), 488 (29), 287 (88); UV λ_{max}^{MeOH} nm: 215, 244, 274, 310, 369, 429, IR ν_{max}^{KBr} cm^{-1}: 3396, 3285, 1646, 1610, 1588, 1552, 1520, 1498, 1444, 1399, 1381, 1347, 1276, 1252, 1214, 1182, 1174, 1057, 840, 819: ^1H NMR Table 1; ^{13}C NMR Table 2.

5-*Hydroxy*-6-*methoxy*-7-*phenylphenalen*-1-*one* (**6**). Yellow crystals, mp 185–187° (EtOH). EIMS (70 eV): *m/z* (rel. int.): 302 $[M]^+$ (100), 287 (64); UV λ_{max}^{MeOH} nm: 274, 369, 463; IR ν_{max}^{Kbr} cm^{-1}: 3427, 2960, 2927, 1635, 1565, 1398, 1355, 1155, 823, 764, 702; ^1H NMR Table 1; ^{13}C NMR Table 2.

5-*Hydroxy*-2,6-*dimethoxy*-7-*phenylphenalen*-1-*one* (**7**). EIMS (70 eV): *m/z* (rel. int.): 332 $[M]^+$ (100); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

2,3-*Dimethoxy*-4-*phenylphenalen*-1-*one* (**8**). Yellow crystals, mp 184–186° (CH_2Cl_2). EIMS (70 eV): *m/z* (rel. int): 316 $[M]^+$ (72), 301 (100), 286 (16), 271 (21); UV λ_{max}^{MeOH} nm: 243, 342, 362; IR ν_{max}^{KBr} cm^{-1}: 3370, 1634, 1615, 1573, 1554, 1385, 1243, 1065, 954, 857; ^1H NMR: Table 1; ^{13}C NMR Table 2.

7,8-*Dimethoxy*-6-*phenylphenalen*-1-*one* (**9**). Yellow crystals, mp 126–128° (Me_2CO). EIMS (70 eV): *m/z* (rel. int.): 316 $[M]^+$ (100), 301 (38), 286 (28); UV λ_{max}^{MeOH} nm: 212, 268, 429; IR ν_{max}^{KBr} cm^{-1}: 3410, 2927, 1638, 1579, 1564, 1455, 1395, 1292, 1218, 1079, 1017, 851, 764, 705; ^1H NMR Table 1; ^{13}C NMR Table 2.

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