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HPLC-NMR analysis of phenylphenalenones and a stilbene from *Anigozanthos flavidus*☆

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Abstract

Extracts from rhizomes and roots of *Anigozanthos flavidus* were analyzed by HPLC-NMR. Known phenylphenalenones and a stilbene dimer have been identified by means of reference spectra without isolation. New compounds of the phenylphenalenone type, including two dimers, were detected by HPLC-NMR and after isolation their structures were elucidated by conventional analytical methods.

Keywords: *Anigozanthos flavidus*; Haemodoraceae; HPLC-NMR; Phenylphenalenones; Stilbenes

1. Introduction

The direct coupling of HPLC and ^{1}H NMR spectroscopy is a straightforward analytical technique which is being used more and more to assign structures of natural products, avoiding time consuming isolation (Spring et al., 1995; Hötzel, Schlotterbeck, Albert, & Bayer, 1996; Wolfender, Rodriguez, Hostettmann, & Hiller, 1997; Schneider et al., 1998). *Anigozanthos flavidus*, which hitherto has not been phytochemically investigated, is used in this study to detect natural products by means of HPLC-NMR. This species is a member of the Haemodoraceae plant family, which is characterized by phenylphenalenones as the major chemotaxonomic markers (Cooke & Edwards, 1980). Phenylphenalenones from *Musa acuminata* and *Musa paradisiaca* (Musaceae) have been demonstrated to be active as phytoalexins (Luis et al., 1993, 1996) and nematicides (Binks, Greenham, Luis, & Gowen, 1997). Modified phenylphenalenones were found in the aquatic plant *Eichhornia crassipes* (Pontederiaceae) (Greca, Lanzetta, Molinaro, Monaco, & Previtera, 1992). In contrast to other members of the genus *Anigozanthos*, *A. flavidus* possesses large rhizomes, which are the main sites of phenylphenalenone accumulation in that species. In this paper we describe stopped-flow HPLC-NMR detection, isolation and structural elucidation of new phenylphenalenones, as well as the identification of known phenylphenalenones and a resveratrol dimer anigopreissin A, in extracts of the roots and the rhizomes of *A. flavidus*.

2. Results and discussion

The rhizomes and the roots of *A. flavidus* plants (10 g dw) were extracted with MeOH and the extract was partitioned between *n*-hexane–H_2O, chloroform–H_2O and ethyl acetate–H_2O. The organic phases were subjected to HPLC. The UV trace of the *n*-hexane extract exhibited two major peaks (**I**, R_t 29.04 min; **II**, R_t 20.59 min) which, along with three further peaks (**III**, R_t 15.57 min; **IV**, R_t 12.80 min, **V**, R_t 9.34 min), also occurred in the $CHCl_3$ extract (Fig. 1). The latter peak, **V**, was also found in the ethyl acetate extract. The five major components **I**–**V** were analyzed by stopped flow HPLC directly coupled with a NMR spectrometer, for ^{1}H NMR measurements.

The HPLC-^{1}H NMR spectra of **II** and **V** allowed direct identification, by means of reference spectra of

☆ Dedicated to Professor Günter Adam on the occasion of his sixty-fifth birthday.

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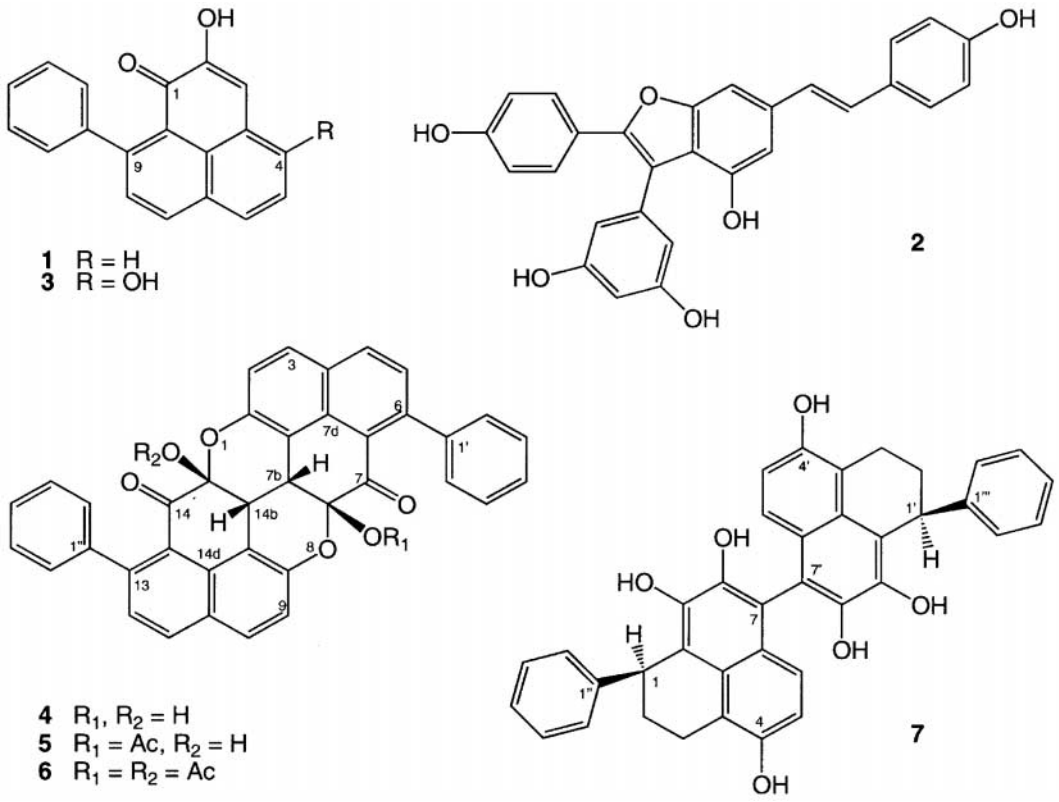

authentic compounds, previously isolated from *Anigozanthos preissii* (Hölscher & Schneider, 1997; Hölscher & Schneider, 1996). Since the spectra exhibited exclusively aromatic signals, there was no overlap with the residual solvent signals of HDO and MeCN. The ^{1}H NMR signals of **II** could be correlated with 2-hydroxy-9-phenylphenalen-1-one (anigorufone, **1**), which was first found in *Anigozanthos rufus* (Cooke &

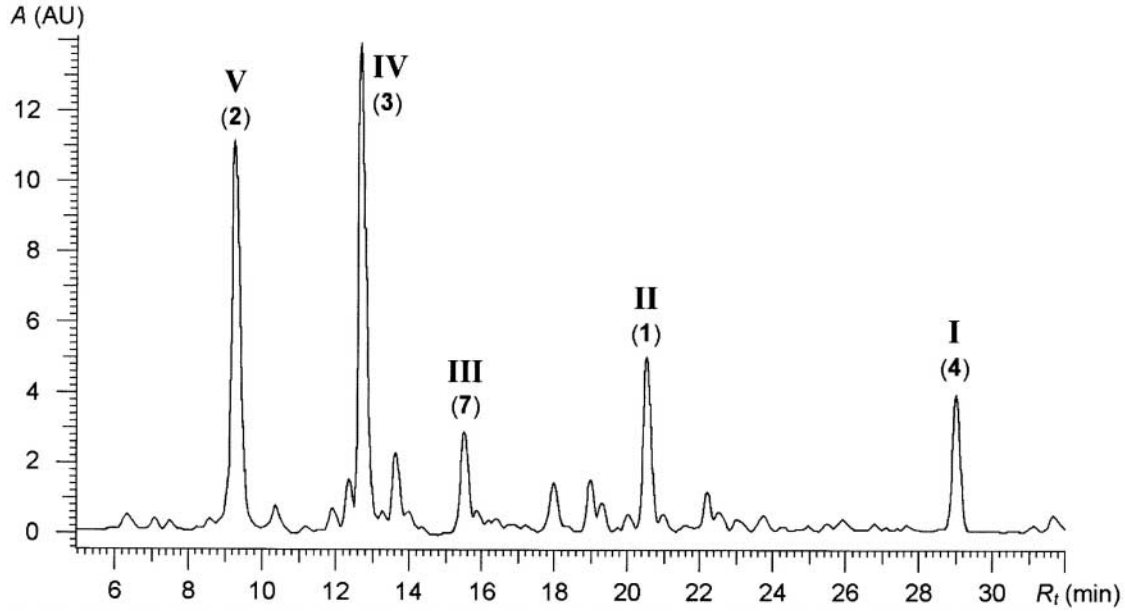

Fig. 1. HPLC chromatogram (UV 284 nm) of a chloroform soluble fraction from rhizomes and roots of *Anigozanthos flavidus*. Peaks **II** and **V** were directly identified by HPLC-NMR spectroscopy as compounds **1** and **2**, respectively. Peaks **I**, **III**, and **IV**, corresponding to compounds **4**, **7**, and **3**, were measured by HPLC-NMR prior to isolation and conventional structure confirmation.

Thomas, 1975) and served as a model compound in the study of phenylphenalenone biosynthesis in root cultures of *A. preissii* (Hölscher & Schneider, 1995). The HPLC-^{1}H NMR spectrum of **V** exactly matched the spectrum of anigopreissin A (**2**), a resveratrol dimer, previously reported from *A. preissii* and *Musa cavendish* (Hölscher & Schneider, 1996). The UV spectra and retention times of peaks **II** and **V** were also identical with those of the reference compounds **1** and **2**.

The HPLC-^{1}H NMR spectra of peaks **I**, **III**, and **IV** could not be assigned to any known phenylphenalenone and were designated as new compounds. The spectrum of **IV** exhibited signals of a non-substituted phenyl ring, two aromatic AB spin systems, and a single aromatic proton (Fig. 2A). This pattern indicated a phenylphenalenone bearing an additional substituent either at C-6, like 2,6-dihydroxyphenylphenalen-1-one (lachnanthocarpone) (Edwards & Weiss, 1970), at C-4, or a tautomer thereof. Isolation and structure assignment, mainly by heterocorrelated 2D NMR, confirmed this assumption and provided evidence for 4-hydroxyanigorufone (**3**) representing a novel compound. The signal of the carbonyl atom

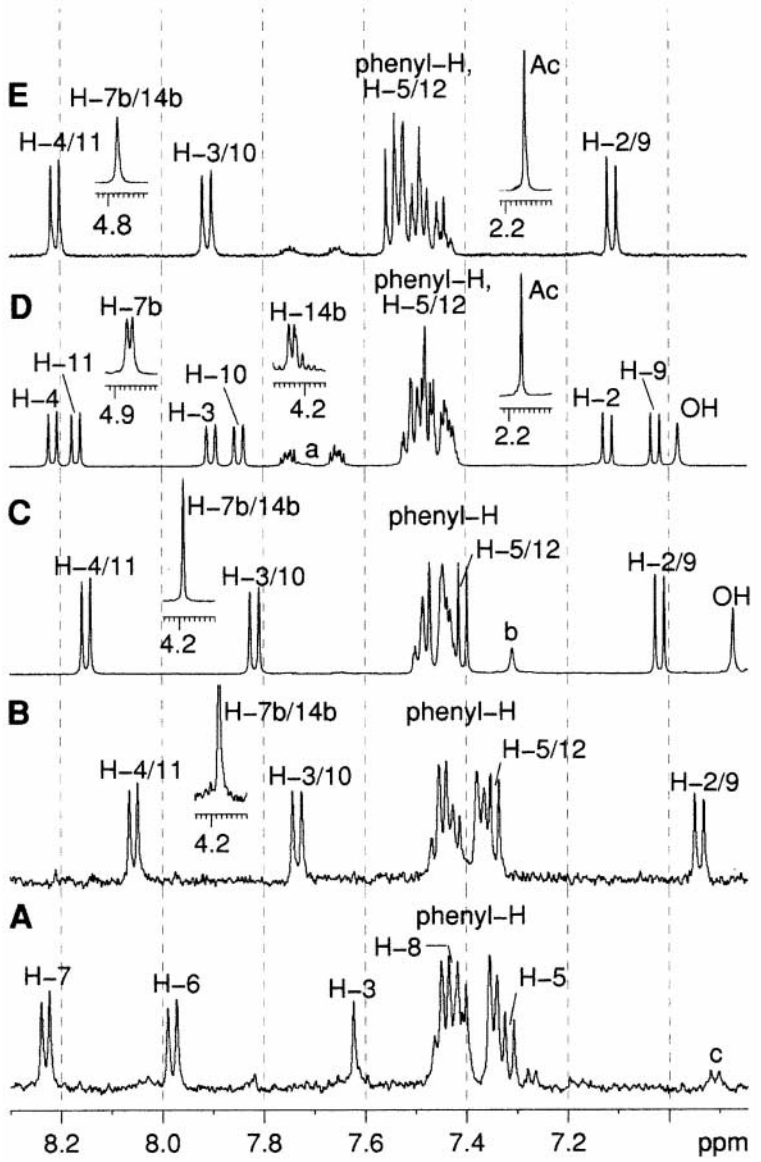

Fig. 2. ^{1}H NMR spectra of phenylphenalenones from *Anigozanthos flavidus* (500 MHz). The HPLC-NMR spectra (A and B) were obtained in the stopped flow mode in MeCN–D_2O; the signal of MeCN was set to δ 2.0. Conventional NMR spectra (C–D) were measured in Me_2CO-d_6 containing TMS as int. standard. A: HPLC-NMR spectrum of peak **IV** of the HPLC chromatogram, corresponding to 4-hydroxyanigorufone (**3**). B: HPLC-NMR spectrum of peak **I** of the HPLC chromatogram, corresponding to anigorootin (**4**). C: Conventional ^{1}H NMR spectrum of isolated anigorootin (**4**). D: ^{1}H NMR spectrum of the monoacetyl derivative (**5**) of anigorootin (**4**). E: ^{1}H NMR spectrum of the diacetyl derivative (**6**) of anigorootin (**4**). Impurity signals, (a): plastifier, (b) and (c): unknown.

(C-1, δ 179.8) showed ^{1}H–^{13}C long range correlations (HMBC) exclusively, with the singlet due to H-3 (δ 7.58). The signals of H-3 and H-6 (δ 7.99) exhibited HMBC connectivities with C-4 (δ 158.6), to which a hydroxyl group was attached. Mutual HMBC cross signals between H-6/C-7 (δ 135.9) and H-7 (δ 8.27)/C-6 (δ 133.0) were also in agreement with the suggested structure. Moreover, HMBC cross signals between the central carbon atom C-9b (δ 127.6) and the protons H-3, H-6 and H-7 were detected. C-9 (δ 149.6) showed HMBC cross peaks with phenyl ring protons, indicating the position of this phenyl substituent on the phenalenone skeleton. HMQC correlations completed the assignment of the protonated carbon atoms. Finally the second hydroxyl group had to be attached to the remaining carbon atom C-2 (δ 150.3). The structure of **3** was also confirmed by EI-MS (*m*/*z* 288, $[M]^+$).

The HPLC-^{1}H NMR spectrum of peak **I**, representing compound **4**, exhibited the same set of signals as compound **3** (Fig. 2, A and B). However, one of the doublets appeared at relatively high field (δ 6.84) and the singlet was significantly shifted upfield to δ 4.18, indicating an aliphatic methine proton. For complete structure elucidation compound **4** was isolated. The molecular weight, determined by EI-MS (*m*/*z* 574 $[M]^+$), suggested a dimeric molecule. The signals of the aromatic phenylnaphthaline moieties were easily assigned by the strategy used for compound **3**, and other phenylphenalenones (Hölscher & Schneider, 1997). The ^{1}H–^{1}H COSY correlations between H-5/12 (δ 7.41)/H-4/11 (δ 8.15) and H-2/9 (δ 7.02)/H-3/10 (δ 7.82), the HMQC correlations of the protonated carbon atoms C-2/9 (δ 119.8), C-3/10 (δ 129.9), C-4/11 (δ 134.8) and C-5/12 (δ 129.2), and the mutual HMBC cross signals between H-3/10/C-4/11 and H-4/11/C-3/10 were in agreement with the suggested structure of **4**. The signals of oxygenated C-1a/8a (δ 153.0) and resonances of the remaining quaternary carbon atoms of the phenylnaphthalene moieties C-7c/14c (δ 109.4), C-3a/10a (δ 129.1), C-6/13 (δ 145.8), C-6a/13a (δ 124.5), and C-7d/14d (δ 133.7) as well as the positions of the phenyl rings at C-6 and C-13 were also assigned by HMBC. The HMBC connectivities of the H-7b/H-14b signal (δ 4.19) were most important in establishing the link between both monomers. Within one monomeric unit, H-7b/H-14b exhibited cross signals via three bonds with the carbonyl atoms (C-7/14, δ 191.0), with C-1a/8a, and with the central carbon atoms of the phenalene nuclei C-7d/14d. The cross signal between H-7b/H-14b and C-7a/14a (δ 94.3) was due to both a three bond connectivity between both units and a correlation within one unit via two bonds. A HMBC spectrum optimized to detect correlations via two bonds exhibited a strong cross signal between H-7b/14b and C-7b/14b of the other monomer. The tall signal of the hydroxyl protons (δ 6.78, Fig. 2C) showed couplings with C-7a/14a, C-7b/14b, and C-7/14. The absence of a proton on C-7a/14a and the chemical shift of δ 94.3, which is consistent with the deshielding effect of two oxygen substituents at that carbon, indicated that hemiacetalic groups are present in compound **4**. A similar structural feature was recently found in phenylphenalenone dimers isolated from rhizomes of a *Musa* hybrid (Luis, Lahlou, Andrés, Echeverri, & Fletcher, 1997). Compound **4** contained four stereochemical centers C-7a, C-7b, C-14a and C-14b, theoretically accounting for eight diastereomers and eight enantiomers. Only the enantiomers have to be considered here because compound **4** is definitely a homodimer as indicated by the single set of signals in the HPLC-^{1}H NMR spectrum (Fig. 2B) and the ^{1}H-NMR spectrum of the isolated compound **4** (Fig. 2C). Acetylation of **4** afforded the monoacetyl derivative **5** and the diacetyl derivative **6**. Both acetyl derivatives were suitable to diminish the number of questionable stereoisomers. The NOESY spectrum of **6** exhibited a spatial proximity between one or both acetyl groups with H-7b and/or H-14b. Consequently, structures with 7a*S*,7b*R*,14a*S*,14b*R* and 7a*R*,7b*S*,14a*R*,14b*S* configuration, which carry acetyl groups on the opposite face of the molecule to H-7b and H-14b, could be ruled out. To establish the relative configuration of **4**, the conformation of the central carbon–carbon bond between C-7b and C-14b was estimated by measuring $^{3}J_{\text{H-7b–H-14b}}$. This coupling constant of 5.70 Hz, indicating a *gauche* type conformation, was obtained from satellite doublets of the H-7b/14b signal in the ^{1}H NMR spectrum of **4**. A similar value of 5.53 Hz for $^{3}J_{\text{H-7b–H-14b}}$ was directly taken from the signals of H-7b (δ 4.87) and H-14b (δ 4.23) in the monoacetyl derivative **5**, which by acetylation of one hydroxyl appeared as two doublets (Fig. 2D). In a similar manner, the other signals of both monomers are no longer equivalent and, therefore, appear twice in the spectrum of **5**. As expected, the ^{1}H NMR spectrum of the diacetyl derivative **6** exhibited only a single set of signals (Fig. 2E). The *gauche* conformation of the bond between C-7b and C-14b of **4** is only consistent with 7b*S*,14b*S* and 7b*R*,14b*R* configuration, while all stereoisomers with 7b*S*,14b*R* or 7b*R*,14b*S* show antiperiplanar configuration. Thus, the relative configuration of the four stereocenters of **4** must be all-*R* or all-*S*, and the relative configuration is arbitrarily reported as *rel*-(7a*R*,7b*R*,14a*R*,14b*R*)-7b,14b-dihydro-7a,14a-dihydroxy-6,13-diphenyl-(7H,14H)-diphenalen[2,3,3a,4-b,c,d:2,3,3a,4-g,h,i]pyrano[4,3-c]pyran-7,14-dione, given the trivial name anigorootin (**4**). Compound **4** can be considered as a dimer of 4-hydroxy-anigorufone (**3**).

The aromatic part of the HPLC-^{1}H NMR spectrum of peak **III** exhibited resonances of an unsubstituted phenyl ring (δ 7.03, d, 8.0 Hz, 2H; δ 7.12, t, 7.3 Hz,

1H; δ 7.19, dd, 8.0 Hz, 7.3 Hz, 2H) and two doublets of an AB spin system (δ 6.71, d, 9.5 Hz; δ 6.64, d, 9.5 Hz). Further signals at (δ 4.77 brs, 1H), a doublet at δ 2.96 (16.9 Hz, 1H) and a triplet at δ 2.24 (16.9 Hz, 1H) indicated a non-aromatic part of this unknown compound. Two additional methylene group signals, which were superimposed by the large solvent signal of MeCN in the HPLC-NMR spectrum, were detected in the ^{1}H NMR spectrum of the isolated component **III**. The dimeric structure of compound **7** was indicated by mass spectral data (*m*/*z* 582, M^+) and finally elucidated based on HMBC, HMQC, and 2D NOESY experiments. Attachment of the phenyl ring to the carbon atom resonating at δ 39.0 (C-1/1′) was proven by HMBC connectivities with H-2″/6″/2‴/6‴ (δ 7.13) and between its attached protons H-1/1′ (δ 4.92) with C-2″/6″/2‴/6‴ (δ 129.0) and C-1″/1‴ (δ 146.3) as well. A NOESY correlation between H-1/1′ and H-2″/6″/2‴/6‴ confirmed this structural feature. Further HMBC cross peaks of H-1/1′ with C-2/2′ (δ 30.3), C-3/3′ (δ 19.0), C-9b/9b′ (δ 128.6) and C-9/9′ (δ 143.7) were in agreement with the suggested structure. Occurrence of a carbonyl group in *peri* position to the phenyl ring, which is usual for many phenylphenalenones (Cooke & Edwards, 1980), could be ruled out by the chemical shift of C-9/9′ which was due to a hydroxyl function. The broad singlet of H-1/1′ ($\Delta_{1/2}$ = 9.7 Hz) indicated an equatorial position of this proton and an axial phenyl ring. Moreover, the large *trans*-axial coupling constant between H-2a/2a′ and H-3a/3a′ (13.5 Hz) was indicative of a rigid semichair conformation of this ring. HMBC cross peaks of H-3a/3a′/H-3e/3-e′ (δ 2.46/3.15) and H-6/6′ (δ 6.83) with C-4/4′ (δ 150.2) via three bonds and a two-bond correlation between H-5/5′ (δ 6.78) and C-4/4′ proved the positions of hydroxyl groups at C-4 and C-4′. The AB protons H-5/5′ and H-6/6′ were attached to the carbon atoms resonating at δ 115.6 (C-5/5′) and δ 124.4 (C-6/6′), as demonstrated by HMQC. The quaternary carbon atoms C-3a/3a′ (δ 116.9), C-6a/6a′ (129.2), C-9a/9a′ (δ 118.4), and C-9b/b′ were assigned by further HMBC connectivities. Moreover, a three-bond HMBC correlation of H-6/6′ with the quaternary carbons C-7/7′ resonating at δ 114.8 indicated the position of the linkage between both monomeric units. Due to the lack of any CH-correlation and the chemical shift of δ 146.2 the remaining signal was assigned to the hydroxylated carbon atoms C-8/8′ of (1,1′*S**)-2,2′,3,3′-tetrahydro-4,4′,8,8′,9,9′-hexahydroxy-1,1′-diphenyl-7,7′-di-[(1H)-phenalen] (**7**).

Dihydrophenalenes, structurally related to compound **7**, are known from *E. crassipes* (Pontederiaceae) (Greca et al., 1992) and *Musa acuminata* cultivars (Luis et al., 1995; Kamo et al., 1998) but to date have not been reported as constituents of the Haemodoraceae. It is not known whether dihydrophenalenes are intermediates of the phenylphenalenone biosynthesis. However, the occurrence of the dihydrophenalene dimer **7** in a species of the Haemodoraceae described here is more likely to be a transformation product than a phenylphenalenone precursor.

These investigations have demonstrated that HPLC-NMR is a convenient method for distinguishing between known and unknown compounds in plant extracts, it provides rapid information about unknown structures without isolation, and avoids laborious isolation of known compounds.

3. Experimental

3.1. Plant material

The plants of *A. flavidus* DC. were obtained from the Botanical Garden of the University of Halle grown under greenhouse conditions at a minimum temperature of 20°C.

3.2. Isolation and purification

Roots of the whole plants (1 kg fr. wt) were frozen with liquid N_2, ground, and exhaustively extracted with MeOH at room temp. The MeOH extract was evapd (<40°C) and partitioned between *n*-hexane–H_2O, $CHCl_3$–H_2O and EtOAc–H_2O. The organic phases were subjected to MPLC on RP-18, initial eluent MeOH–H_2O (1:1), stepwise increase of the MeOH–H_2O ratio to 1:0. MPLC fractions were separated by TLC on silica gel $60F_{254}$, toluene–Me_2CO 4:1 and 2:1, respectively. Final purification was performed by prep. HPLC on Nucleosil 7 C 18 (250 × 20 mm), MeCN–H_2O 17:3, UV 284 nm. Analyt. HPLC on LiChrospher 100 RP-18 (250 × 4 mm), 5 μm; 0.8 ml min^{-1}, linear gradient MeCN–H_2O (0.1% TFA) from 1:1 to 4:1 in 20 min and 9:1 in 25 min, followed by isocratic elution for another 10 min at a ratio of 9:1; diode array detection at 200–450 nm.

3.3. LC-NMR

LiChrospher 100 RP-18 (250 × 4 mm), 5 μm; linear gradient MeCN–D_2O (0.1% TFA) from 1:1 to 4:1 in 20 min and 9:1 in 25 min, followed by isocratic elution for another 10 min at a ratio of 9:1; UV 284 nm; 0.8 ml min^{-1}; stopped-flow mode. A Merck-Hitachi LiChrograph L-6200A gradient pump was fitted with a Bruker DRX 500 NMR spectrometer (4 mm inverse detection LC probehead, detection volume 120 μl). ^{1}H Spectra (500.13 MHz) were measured with a spectral width of 12,000 Hz, and data were acquired into 32,000 data points. An acquisition time of 1.36 s and a relaxation delay of 1.80 s were used. Double solvent

suppression of MeCN and the residual water in the MeCN–D_2O gradients were performed by presaturation, applying standard Bruker pulse sequences. For calibration, the suppressed signal of MeCN was set to δ 2.0.

For HPLC-NMR measurements the evaporated extract (300 μg dry wt) was dissolved in 100 μl MeOH-d_4.

3.4. NMR of isolated compounds

Bruker DRX 500 NMR spectrometer: 500.13 MHz (^{1}H), 125.75 MHz (^{13}C), Me_2CO-d_6, TMS as int. standard. ^{1}H NMR, ^{1}H–^{1}H 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 run using a 2.5 mm broadband microprobe head.

3.5. 4-Hydroxy-9-phenylphenalen-1-one (3)

Red crystals, 3.9 mg, mp 115°C (CH_2Cl_2). EIMS (70 eV): *m/z* (rel int.) 289 (11), 288 (54), 287 (100), 271 (4); UV λ_{max}^{MeOH} nm (ϵ): 224 (3.7), 272 (3.6), 330 (1.0), 448 (1.5); IR ν_{max}^{KBr} cm^{-1}: 2371, 1605, 1547, 1490, 1432, 1357, 1213, 1057, 915, 847, 825, 758, 702, 638, 582; R_t 12.8 min. ^{1}H NMR: δ 7.04–7.44 (5H, m, phenyl protons, 7.40 (1H, d, J = 8.7 Hz, H-5), 7.42 (1H, d, J = 8.2 Hz, H-8), 7.58 (1H, s, H-3), 7.99 (1H, *d*, J = 8.7 Hz, H-6), 8.27 (1H, d, J = 8.2 Hz, H-7). ^{13}C NMR: δ 107.4 (C-3), 113.1 (C-3a), 118.9 (C-5), 124.2 (C-9a), 127.6 (C-9b), 127.7 (C-4′), 128.2 (C-6a), 128.7 (C-3′ and C-5′), 128.9 (C-2′ and C-6′), 129.5 (C-8), 133.0 (C-6), 135.9 (C-7), 144.3 (C-1′), 149.6 (C-9), 150.3 (C-2), 158.6 (C-4), 179.8 (C-1).

3.6. rel-(7aR,7bR,14aR,14bR)-7b,14b-Dihydro-7a,14a-dihydroxy-6,13-diphenyl-(7H,14H)-diphenalen[2,3,3a,4-b,c,d:2,3,3a,4-g,h,i]pyrano[4,3-c]pyran-7,14-dione (Anigorootin, 4)

Yellow solid, 2.3 mg. EIMS (70 eV): *m/z* (rel. int.) 574 [M]$^+$ (6), 288 (46), 287 (61); UV λ_{max}^{MeOH} nm (ϵ): 249 (3.9), 284 (3.4), 358 (1.5), 448 (1.5); IR ν_{max}^{KBr} cm^{-1}: 3446, 2924, 2852, 1700, 1617, 1559, 1491, 1448, 1386, 1348, 1223, 1141, 885, 847, 758, 702; R_t 29.0 min. ^{1}H NMR: δ 4.19 (2H, *s*, $^3J_{H\text{-}7b–H\text{-}14b}$ = 5.7 Hz, H-7b and H-14b), 6.78 (2H, s, OH-7a and OH-14a), 7.02 (2H, d, J = 8.9, H-2 and H-9), 7.41 (2H, d, J = 8.3, H-5 and H-12), 7.43–7.48 (10H, m, phenyl protons), 7.82 (2H, d, J = 8.9, H-3 and H-10), 8.15 (2H, d, J = 8.3, H-4 and H-11). ^{13}C NMR: δ 33.7 (C-7b and C-14b), 94.3 (C-7a and C-14a), 109.4 (C-7c and C-14c), 119.8 (C-2 and C-9), 124.5 (C-6a and C-13a), 128.1 (C-4′ and C-4″), 128.8 (C-3′, C-3″, C-5′ and C-5″), 129.1 (C-3a and C-10a), 129.2 (C-5 and C-12), 129.5 (C-2′, C-2″, C-6′ and C-6″), 129.9 (C-3 and C-10), 133.7 (C-7d and C-14d), 134.8 (C-4 and C-11), 142.7 (C-1′ and C-1″), 145.8 (C-6 and C-13), 153.0 (C-1a and C-8a), 191.0 (C-7 and C-14). Acetylation of **4** with Ac_2O–pyridine (1:1) for 2 h at 20°C afforded the monoacetyl derivative (**5**) and the diacetyl derivative (**6**) in a ratio of about 1:1. Separation by TLC on silica gel $60F_{254}$, toluene–Me_2CO 4:1. ^{1}H NMR of **5**: δ 2.18 (3H, s, acetyl protons), 4.23 (1H, d, $^3J_{H\text{-}14b–H\text{-}7b}$ = 5.5 Hz, H-14b), 4.87 (1H, d, $^3J_{H\text{-}7b–H\text{-}14b}$ = 5.5 Hz, H-7b), 6.97 (1H, s, OH-14a), 7.03 (1H, d, J = 8.9 Hz, H-9), 7.12 (1H, d, J = 8.9 Hz, H-2), 7.42–7.53 (10H, m, phenyl protons), 7.85 (1H, d, J = 8.9 Hz, H-10), 7.90 (1H, d, J = 8.9 Hz, H-3), 7.47 (1H, d, J = 8.3 Hz, H-5), 7.48 (1H, d, J = 8.4 Hz, H-12), 8.17 (1H, d, J = 8.4 Hz, H-11), 8.22 (1H, d, J = 8.3 Hz, H-4). Selected ^{13}C NMR data of **5**: δ 21.1 (acetyl–CH_3), 32.5 (C-7b), 33.1 (C-14b), 169.3 (acetyl–CO). ^{1}H NMR of **6**: δ 2.16 (6H, s, acetyl protons), 4.78 (2H, s, H-7b and H-14b), 7.11 (2H, d, J = 8.8 Hz, H-2 and H-9), 7.45–7.49 (10H, m, phenyl protons), 7.55 (2H, d, J = 8.2 Hz, H-5 and H-12), 7.91 (2H, d, J = 8.8 Hz, H-3 and H-10), 8.28 (2H, d, J = 8.2 Hz, H-4 and H-11). Selected ^{13}C NMR data of **6**: δ 21.9 (acetyl–CH_3), 32.9 (C-7b and C-14b), 169.0 (acetyl–CO).

3.7. (1R*)-4,4′,8,8′,9,9′-hexahydroxy-1,1′-diphenyl-7,7′-di-[2,2′,3,3′-tetrahydro-(1H)-phenalen] (7)

Yellow solid, 1.1 mg. EIMS (70 eV): *m/z* (rel. int.) 582 [M]$^+$ (4), 292 (45); R_t 15.6 min. UV (obtained from diode array detected HPLC run) λ_{max} (nm): 216, 240 (sh), 272 (sh), 341; ^{1}H NMR: δ 2.19 (1H, dddd, J = 16.5, 13.5, 4.6, 4.6, H-2a), 2.33 (1H, dddd, J = 16.5, 4.5, 2.4, 2.3, H-2e), 3.15 (1H, brd, $\Delta_{1/2}$ = 15.9, H-3e), 2.46 (1H, ddd, J = 16.5, 13.5, 4.5, H-3a), 4.92 (1H, brs, $\Delta_{1/2}$ = 9.7, H-1), 6.78 (1H, d, J = 9.1, H-5), 6.83 (1H, d, J = 9.1, H-6), 7.13 (2H, dd, J = 7.5, 1.2, H-2′ and H-6′), 7.16 (1H, dd, J = 7.5, 1.2, H-4′), 7.25 (2H, dd, J = 7.5, 7.5, H-3′ and H-5′), ^{13}C NMR: δ 19.0 (C-3), 30.3 (C-2), 39.0 (C-1), 115.6 (C-5), 116.9 (C-3a), 118.4 (C-9a), 124.4 (C-6), 126.5 (C-4′), 128.6 (C-9b), 128.8 (C-3′ and C-5′), 129.0 (C-2′ and C-6′), 129.2 (C-6a), 143.7 (C-9), 146.2 (C-8), 146.3 (C-1′), 150.2 (C-4).

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