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Research Article

Synthesis of [*phenyl*-$^{13}C_6$]lachnanthocarpone and other ^{13}C-labelled phenylphenalenones

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Summary

[*phenyl*-$^{13}C_6$]Lachnanthocarpone ([*phenyl*-$^{13}C_6$]2,6-dihydroxy-9-phenylphenalen-1-one), a hypothetical intermediate in the biosynthesis of various natural phenylphenalenones, was prepared in four steps using [U-^{13}C]bromobenzene to introduce the label. Based on related methodologies further native phenylphenalenones such as [*phenyl*-$^{13}C_6$]anigorufone, [1-^{13}C]anigorufone and [4′-O$^{13}CH_3$]4′-methoxyanigorufone were synthesized in labelled form. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: anigorufone; ^{13}carbon; isotope labelling; Grignard reaction; lachnanthocarpone; phenylphenalenones

Introduction

Phenylphenalenones are secondary metabolites that have so far been found in the plant families Haemodoraceae,[1] Pontederiaceae,[2] Strelitziaceae,[3] and Musaceae.[4] In the latter family they are reported as phytoalexins. The biosynthesis of these compounds is a current research topic, and experimental evidence has been obtained for their formation from two phenylpropanoid units through condensation with malonyl-CoA and subsequent cyclization of the diarylheptanoid intermediate.[5,6] Recently, lachnanthocarpone[7] (**1**) has been hypothesized to be a candidate for the first cyclization product and a branching point for further biosynthetic conversion.[8,9] Two biosynthetic branches are suggested to start from compound **1**: one leading directly to 2-hydroxy-9-phenylphenalen-1-one (anigorufone, **2**) and the other to 1,2,5,6-

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tetraoxygenated 9-phenylphenalenones and subsequently to *oxa*-analogues of phenylphenalenones. In order to probe hypothetical biosynthetic relationships of phenylphenalenones by means of ^{13}C NMR spectroscopy, lachnanthocarpone (**1**) and further compounds of this type were required in labelled form.

The first synthesis of lachnanthocarpone (**1**) was accomplished in 10 steps starting from 4-(4,5-dimethoxy-2-methylphenyl)-butyric acid.[10] Bazan and Edwards[11] used an eight-step procedure to prepare compound **1** from diacetyldihydrocaffeic acid. However, since the yields are low, this biomimetic synthesis was only suitable for supporting biosynthetic theories rather than to provide a straightforward approach to this compound. A partial synthesis of lachnanthocarpone (**1**), albeit in low yields, was achieved by treating 6-methoxy-9-phenylphenalen-1-one with hydrogen peroxide and subsequent demethylation[12] but did not allow introduction of ^{13}C label. Addition of a Grignard reagent to the 9-position of perinaphthenone, followed by hydroxylation at C-2 through epoxidation of the 2,3-double bond, according to the Yang and Finnegan procedure,[13] was used by Cooke and Dagley[14] to synthesize 9-phenylphenalenones. This procedure is still the most convenient and generally useful method for synthesizing phenylphenalenones in very high yields. However, an attempt to synthesize a closely related compound, 2-hydroxy-6-methoxy-9-(4′-methoxyphenyl)-phenalenone resulted in a very low yield of 16% in the epoxidation step of the direct precursor, 6-methoxy-9-(4′-methoxyphenyl)-phenalenone. This low yield in a late step is unacceptable for the synthesis of lachnanthocarpone (**1**) in a labelled form. In this study, the Cooke and Dagley method[14] has been extended to 6-substituted phenylphenalenones, and used to prepare [*phenyl*-$^{13}C_6$]lachnanthocarpone ([*phenyl*-$^{13}C_6$]-**1**) in high yields.

Results and discussion

Retrosynthesis of phenylphenalenones

Due to the compact structure of most phenylphenalenones, especially the tricyclic nucleus, labelling with ^{13}C through a minimum of synthetic steps requires introducing the label with the lateral phenyl moiety at the *peri*-position of the perinaphthenone tricycle. The phenyl ring has to be attached to the phenalenone tricycle before introducing hydroxyl (at C-2) or deprotecting methoxyl substituents (at C-6 of lachnanthocarpone precursors). Grignard-type addition was considered the favored reaction for achieving high yields in this step (Figure 1). The α-hydroxy group would be established by opening the epoxide, which can be introduced via epoxidation of the 2,3-double bond of the 9-phenylphenalen-1-one intermediate by known procedures.[13–16] Phenylphenalenones bearing a methoxyl group can be readily labelled in the OCH_3 substituent. Removal of unlabelled *O*-methyl and methylation using a ^{13}C

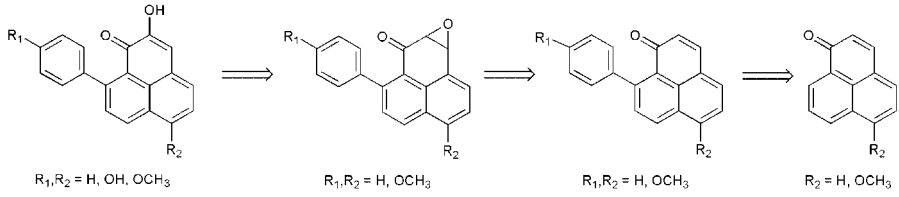

Figure 1. Retrosynthetic analysis of phenylphenalenones

labelled agent is required during synthesis, which in the other steps proceeds similarly to synthesis of the unmethylated phenylphenalenones.

Synthesis of lachnanthocarpone

The synthesis of labelled lachnanthocarpone ([*phenyl*-$^{13}C_6$]-**1**) is shown in Figure 2. A method for the synthesis of 6-hydroxy-perinaphthenone (**3**) by condensing 2,7-dihydroxynaphthalene (**4**) with glycerol in the presence of phosphoric acid and an oxidizing agent was described earlier.[17] Using this method, compound **3** was obtained in low yields of approximately 8% after purification. No attempt to optimize this procedure was carried out, since a sufficient quantity was obtained to proceed and labelling was not yet involved in this step. Alternatively, compound **3** is accessible by cyclization of *β*-(4-methoxy-1-naphthyl)-propionic acid under dehydrogenation conditions.[17] Methylation using an ethanol-containing solution of diazomethane gave the desired methyl ether **5** in a quantitative yield. 6-Methoxy-perinaphthenone (**5**) was treated with [U-^{13}C]phenylmagnesium bromide and the crude product was dehydrogenated by the action of DDQ. [*phenyl*-$^{13}C_6$]6-Methoxy-9-phenylphenalen-1-one ([*phenyl*-$^{13}C_6$]-**6**) was obtained in 82% yield after purification by preparative TLC. The Yang and Finnegan[13] procedure for the epoxidation of a non-labelled sample of **6** was tested but no product was observed after 24 h. This problem has been overcome by employing the Jacobsen–Katsuki epoxidation,[15,16] which smoothly resulted in 2-hydroxy-6-methoxy-9-phenylphenalen-1-one (**7**) in 79% yield after purification by TLC. Manganese salen complexes are mainly used for the highly enantioselective epoxidation of alkenes, and a practical and economical method for the synthesis of this catalyst was available.[18] In our case, there was no need for an enantioselective epoxidation. However, the availability of this catalyst and the simplicity of the procedure made this type of epoxidation ideal for generating α-hydroxyketones from α,*β*-unsaturated carbonyl compounds such as **6**.

No attempt was made to isolate the epoxide because of its lability to acid treatment, which was concluded from the immediate color change from pale yellow to deep red that occurred during application to TLC plates of the dichloromethane fraction containing the crude product. Therefore, compound **7** was isolated directly and purified by preparative TLC. Demethylation of **7**

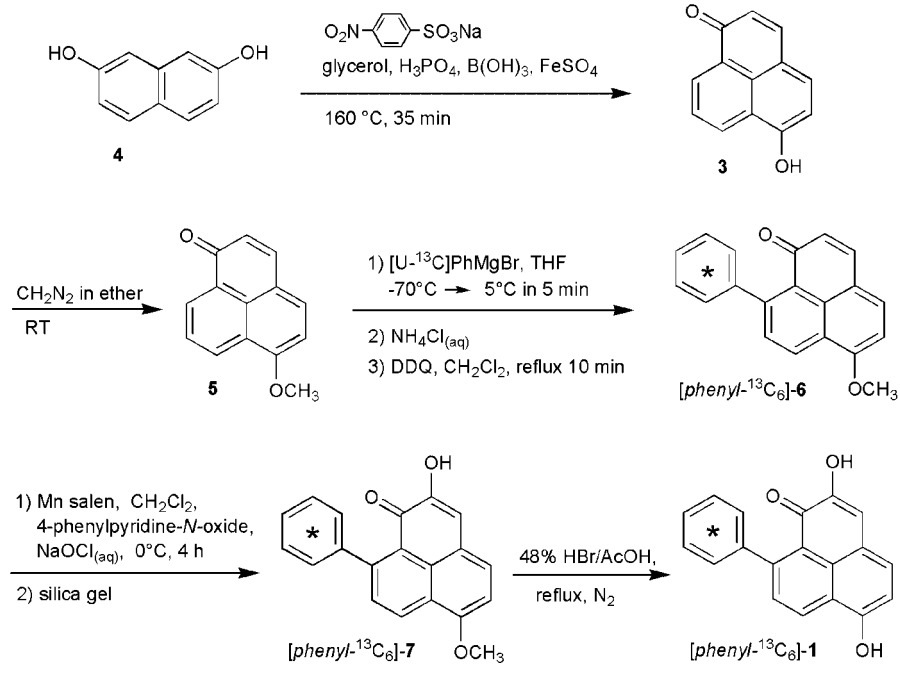

Figure 2. Synthesis of [*phenyl*-$^{13}C_6$]lachnanthocarpone ([*phenyl*-$^{13}C_6$]-1). * = [*phenyl*-$^{13}C_6$]

by HBr gave the target molecule, [*phenyl*-$^{13}C_6$]lachnanthocarpone ([*phenyl*-$^{13}C_6$]-**1**), in 84% yield. The total yield with respect to the labelled starting compound, [U-^{13}C]bromophenol, was 36%.

The ^{1}H and ^{13}C NMR signals of the uniformly labelled lateral phenyl ring of [*phenyl*-$^{13}C_6$]lachnanthocarpone ([*phenyl*-$^{13}C_6$]-**1**) and the synthetic intermediates [*phenyl*-$^{13}C_6$]-**6** and -**7** appear as multiplets. Interestingly, the resonance of H-8, due to $^3J_{H\text{-}8\text{–}H\text{-}7}$ = 8.5 Hz and $^3J_{H\text{-}8\text{–}C\text{-}1'}$ = 3.7 Hz, exhibits a doublet of doublets in the ^{1}H NMR spectrum (Figure 3). This characteristic coupling pattern represents a sensitive ^{1}H fingerprint signal, which can be used to detect metabolites derived from ([*phenyl*-$^{13}C_6$]-**1**) or analogously labelled compounds in plant crude extracts or semi-purified fractions of biosynthetic experiments.

Synthesis of phenylphenalenones without substitution at C-6

Labelled anigorufone[19] (^{13}C-**2**) and 4′-methoxyanigorufone[20] (^{13}C-**8**) were synthesized starting from perinaphthenone (**9**). [U-^{13}C]bromophenol and [1-^{13}C]bromophenol, respectively, were used to prepare the labelled Grignard reagents for the introduction of ^{13}C into the lateral phenyl substituent at C-9 of [*phenyl*-^{13}C]anigorufone ([*phenyl*-$^{13}C_6$]-**2**) and [1′-^{13}C]anigorufone ([1′-^{13}C]-**2**) (Figure 4).

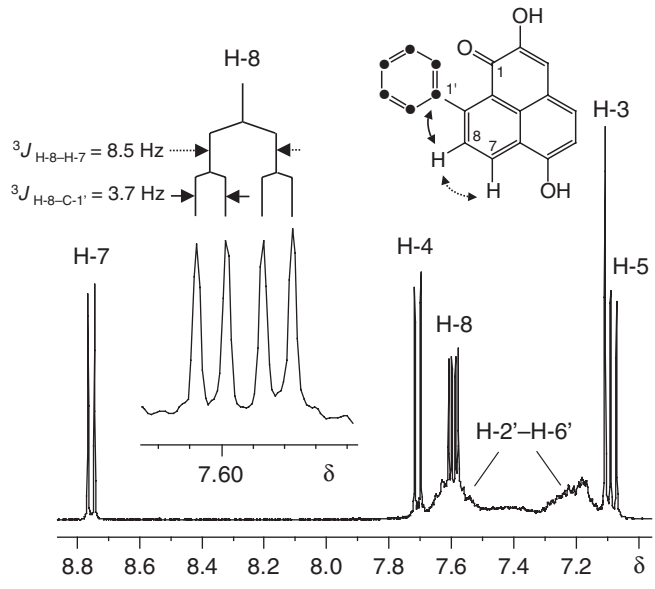

Figure 3. ^{1}H NMR spectrum of [*phenyl*-$^{13}C_6$]lachnanthocarpone ([*phenyl*-$^{13}C_6$]-1). The multiplicity of H-8 at δ 7.59 results from $^3J_{H-H}$ and $^3J_{H-C}$ couplings as indicated in the magnified detail. ● = ^{13}C

In the first step of the synthesis of [4′-O^{13}CH$_3$]4′-methoxyanigorufone[20] ([4′-O^{13}CH$_3$]-**8**), a Grignard reaction again led to the attachment of the lateral aryl moiety to C-9 of perinaphthenone (**9**). Ether cleavage of 9-(4′-methoxyphenyl)-phenalenone (**10**) made the 4′-*O*-position available for methylation, which was achieved by ^{13}CH$_3$I as a labelled precursor. The Yang and Finnegan[13] procedure for the epoxidation worked satisfactorily in the case of phenylphenalenones without substituent in position 6.

Conclusions

Phenylphenalenones were prepared in labelled form, bearing ^{13}C in the lateral substituent attached to C-9 of the phenalenone nucleus. Grignard addition of the aryl ring and hydroxylation at C-2 via epoxidation of the α,β-double bond were the key steps of this synthesis. While the Grignard reaction smoothly resulted in the desired products, two different procedures were needed in order to obtain appropriate yields in the epoxidation step. The Yang and Finnegan procedure worked well with phenylphenalenones lacking an *O*-substituent at C-6 but did not give satisfying results when 6-methoxy compounds were used. In contrast, the Jacobsen–Katsuki epoxidation proved superior in this case and was employed successfully in the synthesis of labelled lachnanthocarpone.

Different labelling patterns of phenylphenalenones are feasible for various purposes in biosynthetic experiments. For example, the fingerprint signal of

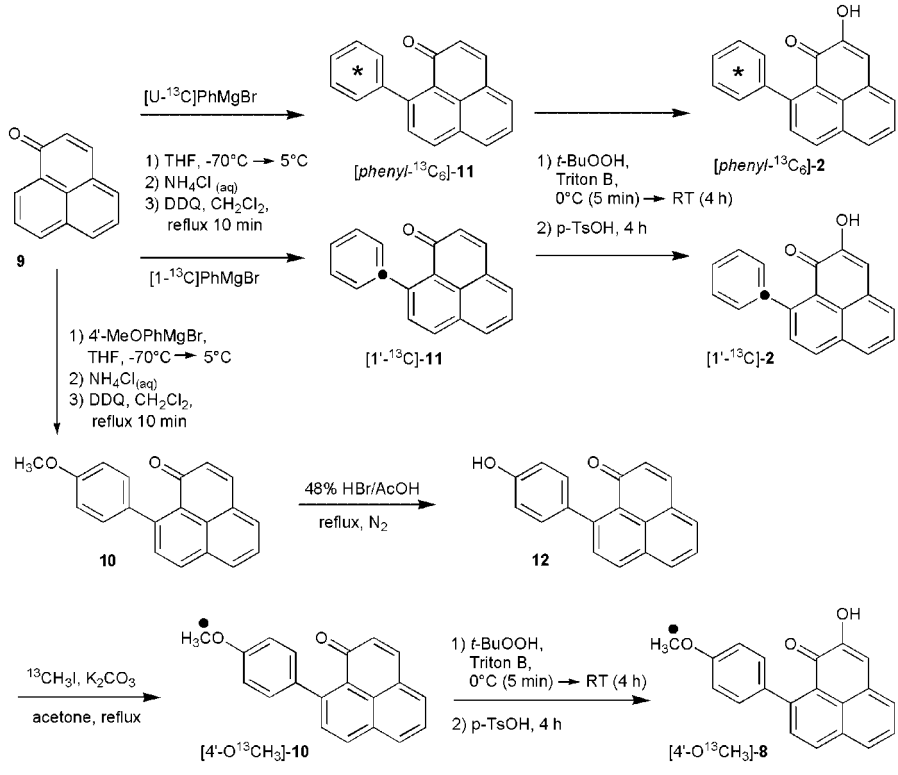

Figure 4. Synthesis of [*phenyl*-$^{13}C_6$]anigorufone ([*phenyl*-$^{13}C_6$]-2), [1-^{13}C]anigorufone [1-$^{13}C_6$]-2 and [4′-O^{13}CH$_3$]4′-methoxy-anigorufone ([4′-O^{13}CH$_3$]-8). * = [*phenyl*-$^{13}C_6$], ● = ^{13}C

H-8 in the ^{1}H NMR spectrum (Figure 3) allows sensitive detection of phenylphenalenones biosynthetically derived from ([*phenyl*-$^{13}C_6$]-labelled precursors. In contrast, in the ^{1}H spectra of singly 1′-^{13}C-labelled phenylphenalenones, such as [1′-^{13}C]anigorufone ([1′-^{13}C]-**2**), the doublet of H-8 is superimposed by the ^{1}H resonances of the phenyl moiety and therefore is less useful for biosynthetic studies. Instead, the ^{13}C signal of C-1′ of singly labelled compounds appears as a singlet and, in comparison with the multiplets of [*phenyl*-$^{13}C_6$]-labelled compounds, is detected more sensitively by ^{13}C NMR.

Experimental

Preparative TLC was performed on silica gel 60 F_{254} (1 mm layer thickness) (Merck, Darmstadt, Germany) and inspected under UV light (254 nm). Preparative column chromatography was conducted on silica gel 60 (particle size 0.040–0.063 mm) (Merck) using CH_2Cl_2 as an eluent. NMR spectroscopy: ^{1}H NMR, ^{1}H-^{1}H COSY, HMBC, and HMQC spectra were recorded on an

Avance DRX 500 NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5 mm TXI CryoProbe. ^{13}C NMR spectra were measured on a Bruker Avance 400 NMR spectrometer. The samples were measured in acetone-d_6 as a solvent. The spectra were referenced to TMS, which was used as an internal standard. Electron impact mass spectra (EI-MS) and high-resolution mass spectra (HREI-MS) were recorded on a MasSpec sector field mass spectrometer (Micromass Ltd., Manchester, UK) with a direct insertion probe. Labelled starting materials: ([1-^{13}C]bromobenzene (99% $^{13}C_1$) was from Deutero GmbH (Kastellaun, Germany), [U-^{13}C]bromobenzene (99% $^{13}C_6$) and $^{13}CH_3I$ (99% ^{13}C) were from Aldrich. Unlabelled chemicals were from Aldrich as well and used without purification.

*6-Hydroxy-perinaphthenone (**3**)*

A mixture of 2,7-dihydroxynaphthalene (**4**) (2 g), sodium nitrobenzenesulphonate (2.5 g), glycerol (5 g), H_3PO_4 (98%, 15 g), $FeSO_4$ (0.6 g) and $B(OH)_3$ (1 g) was heated at 160°C for 35 min. The mixture was poured into water and filtered. The residue was extracted with hot aqueous sodium carbonate and filtered, and the resulting solution was acidified with HCl to precipitate the product **3**. The solid was then washed with water and dried. The crude precipitate (0.5 g) was purified by column chromatography. TLC: $R_f = 0.3$ (Et_2O: *n*-hexane 7:1); EI-MS: *m/z* (rel. int.) 196 $[M]^+$ (100); HR-MS: found 196.051903 (calculated for $C_{13}H_8O_2$: 196.052430); ^1H NMR: δ 8.69 (H-7, d, $J = 8.0$ Hz), 8.57 (H-9, d, $J = 8.0$ Hz), 7.85 (H-8, dd, $J = 8.0$, 8.0 Hz), 7.83 (H-3, d, $J = 9.4$ Hz), 7.80 (H-4, d, $J = 8.0$ Hz), 7.08 (H-5, d, $J = 8.0$ Hz), 6.48 (H-2, d, $J = 9.4$ Hz); ^{13}C NMR: δ 185.4 (C-1), 159.6 (C-6), 143.0 (C-3), 134.8 (C-4), 131.1 (C-7), 130.8 (C-9a), 130.3 (C-9), 129.8 (C-9b), 126.8 (C-8), 126.7 (C-2), 125.6 (C-6a), 121.2 (C-3a), 110.4 (C-5).

*6-Methoxy-perinaphthenone (**5**)*

An ethanol-containing solution of diazomethane was prepared from Diazald® using the standard procedure. This solution was added dropwise to 6-hydroxy-perinaphthenone (**3**) (20 mg, 0.1 mmol) until gas evolution had ceased. The reaction mixture was dried under a stream of nitrogen gas and used directly in the next step. TLC: $R_f = 0.25$; EI-MS: *m/z* (rel. int.) 210 $[M]^+$ (100), 195 (26); HR-MS: found 210.067098 (calculated for $C_{14}H_{10}O_2$: 210.068080); ^1H NMR: δ 8.65 (H-7, dd, $J = 8.4$, 1.3 Hz), 8.56 (H-9, d, $J = 7.4$, 1.3 Hz), 7.91 (H-4, d, $J = 8.0$ Hz), 7.86 (H-3, d, $J = 9.7$ Hz), 7.86 (H-8, dd, $J = 8.4$, 7.4 Hz), 7.15 (H-5, d, $J = 8.0$ Hz), 6.51 (H-2, d, $J = 9.7$ Hz), 4.16 (6-O$\underline{CH_3}$, s); ^{13}C NMR: δ 185.2 (C-1), 160.5 (C-6), 142.9 (C-3), 134.9 (C-4), 131.0 (C-9), 130.4 (C-9a), 129.9 (C-7), 129.6 (C-9b), 127.5 (C-8), 127.4 (C-2), 126.2 (C-6a), 121.6 (C-3a), 106.5 (C-5), 57.0 (6-O$\underline{C}H_3$).

*[phenyl-$^{13}C_6$]6-Methoxy-9-phenylphenalen-1-one ([phenyl-$^{13}C_6$]-**6**)*

[U-^{13}C]Bromobenzene (0.17 ml, 1.6 mmol) was dissolved in dry THF (1 ml). This solution was added during a period of 20 min with a 1-ml syringe to small pieces of magnesium (39 mg, 1.6 mmol) in a 10-ml round-bottomed flask containing dry THF (0.5 ml) and a small crystal of iodine under N_2 atmosphere. After the addition, the solution was stirred for 20 min. An aliquot of this solution (0.15 ml, 0.15 mmol) was transferred to a 5-ml round-bottomed flask with a syringe purged with argon and cooled to -70°C in an acetone-dry ice bath. 6-Methoxy-perinaphthenone (**5**) (0.1 mmol, 21 mg) was dissolved in dry THF and added to the cold Grignard solution over a period of 10 min with stirring. The solution was warmed up to 5°C for 5 min and quenched with a saturated aqueous solution of NH_4Cl. After the crude mixture was partitioned with CH_2Cl_2 (50 ml) and water (50 ml), the organic phase was separated, dried with Na_2SO_4, and filtered. DDQ (23 mg, 0.1 mmol) was added and the solution refluxed for 15 min. Preparative TLC gave [*phenyl*-$^{13}C_6$]6-methoxy-9-phenylphenalen-1-one ([*phenyl*-$^{13}C_6$]-**6**) (24 mg, 82%). The rest of the Grignard reagent was used for the synthesis of [*phenyl*-$^{13}C_6$]anigorufone ([*phenyl*-$^{13}C_6$]-**2**) as described below. TLC: R_f = 0.70 (Et_2O: *n*-hexane 7:1). The labelled compound [*phenyl*-$^{13}C_6$]-**6** was completely used up in the next step and no analytical data were recorded. Analytical data of the unlabelled compound **6**, which was prepared under identical conditions: EI-MS: *m*/*z* (rel. int.) 285 $[M-1]^+$ (100), 279 (48); HR-MS: found 286.099107 (calculated for $C_{20}H_{14}O_2$: 286.099380); ^{1}H NMR: δ 8.61 (H-7, d, J = 8.3 Hz), 7.90 (H-4, d, J = 8.0 Hz), 7.77 (H-3, d, J = 9.7 Hz), 7.56 (H-8, d, J = 8.3 Hz), 7.16 (H-5, d, J = 8.0 Hz), 7.41-7.31 (H-2′–H-6′, m), 6.34 (H-2, *d*, J = 9.7 Hz), 3.73 (6-OC$\underline{H}_3$, s). ^{13}C NMR: δ 185.7 (C-1), 160.3 (C-6), 148.2 (C-9), 144.3 (C-1′), 141.0 (C-3), 134.2 (C-4), 131.4 (C-8), 128.9 (C-3′/5′), 128.1 (C-7), 127.9 (C-2), 127.7 (C-2′/6′), 127.3 (C-4′), 127.0 (C-9a), 125.7 (C-6a), 123.1 (C-9b), 122.5 (C-3a), 105.7 (C-5), 56.6 (6-O$\underline{C}H_3$).

*[phenyl-$^{13}C_6$]2-Hydroxy-6-methoxy-9-phenylphenalen-1-one ([phenyl-$^{13}C_6$]-**7**)*

[*phenyl*-$^{13}C_6$]6-Methoxy-9-phenylphenalen-1-one ([*phenyl*-$^{13}C_6$]-**6**) (24 mg, 82 μmol), (*S*,*S*)-(+)-*N*,*N*′-*bis*-(3,5-di-*tert*-butylsalicylidene)-1,2-diamino-cyclohexane manganese-(III)-chloride (Mn salen, 2.2 mg, 4 μmol) and 4-phenylpyridine-*N*-oxide (3.4 mg, 20 μmol) were dissolved in dichloromethane (5 ml) and the resulting solution cooled in an ice-water bath (solution A). Sodium hypochlorite solution (NaOCl, 5 ml, ca. 0.9M) was buffered with 2 ml of a sodium phosphate solution (0.05M) and the solution cooled in an ice-water bath (solution B). Solution B was promptly added to solution A under vigorous stirring and allowed to react for 3 h at 0°C. The mixture was separated, and the organic layer dried and applied directly to a preparative

TLC plate. Separation using the solvent system Et_2O: *n*-hexane 7:1 resulted in a red zone ($R_f = 0.9$), which gave 20 mg (79%) of ([*phenyl*-$^{13}C_6$]-**7**. EI-MS: *m*/*z* (rel. int.) 307 $[M-1]^+$ (100); HR-MS: found 308.112038 (calculated for $^{12}C_{14}$ $^{13}C_6H_{14}O_3$: 308.114424); ^{1}H NMR: δ 8.71 (H-7, d, J = 8.5 Hz), 7.80 (H-4, d, J = 7.8 Hz), 7.58 (H-8, dd, J = 8.4, 3.8 Hz), 7.67-7.51 and 7.30-7.13 (H-2′– H-6′, m), 7.14 (H-5, d, J = 7.8 Hz), 7.11 (H-3, s), 4.13 (6-OC$\underline{H}_3$, s); ^{13}C NMR: δ 180.7 (C-1), 158.0 (C-6), 150.1 (C-2), 149.8 (C-9), 144.2 (C-1', m), 133.2 (C-4), 131.5 (C-8), 129.9-127.0 (C-2' - C-6', C-7, C-9a, C-9b, m), 125.8 (C-6a), 123.0 (C-3a), 113.8 (C-3), 106.6 (C-5), 56.6 (6-O$\underline{C}H_3$). Analytical data of the unlabelled compound **7**, which was prepared under identical conditions: EI-MS: *m*/*z* (rel. int.) 301 $[M-1]^+$ (100); HR-MS: found 302.095491 (calculated for $C_{20}H_{14}O_3$: 302.094294); ^{1}H NMR: δ 8.71 (H-7, d, J = 8.5 Hz), 7.80 (H-4, d, J = 7.8 Hz), 7.58 (H-8, d, J = 8.5 Hz), 7.35-7.45 (H-2′– H-6′, brm), 7.14 (H-5, d, J = 7.8 Hz), 7.11 (H-3, s), 4.13 (6-OC$\underline{H}_3$, s); ^{13}C NMR: δ 180.7 (C-1), 158.0 (C-6), 150.1 (C-2), 149.8 (C-9), 144.2 (C-1′), 133.2 (C-4), 131.5 (C-8), 129.9 (C-7), 129.2 (C-3′/5′), 128.9 (C-2′/6′), 128.2 (C-4′), 127.2 (C-9a), 127.0 (C-9b), 125.8 (C-6a), 123.0 (C-3a), 113.8 (C-3), 106.6 (C-5), 56.6 (6-O$\underline{C}H_3$).

*[phenyl-$^{13}C_6$]2,6-Dihydroxy-9-phenylphenalen-1-one ([phenyl-$^{13}C_6$]lachnanthocarpone, [phenyl-$^{13}C_6$]-***1***)*

Compound ([*phenyl*-$^{13}C_6$]-**7** (20 mg, 65 μmol) was refluxed in a N_2 atmosphere with AcOH (4.4 ml) and 48% HBr (0.56 ml) for 10 h. After cooling to room temperature, the crude product was subjected to liquid–liquid distribution between H_2O (10 ml) and CH_2Cl_2 (10 ml). Preparative TLC of the organic phase resulted in [*phenyl*-$^{13}C_6$]lachnanthocarpone ([*phenyl*-$^{13}C_6$]-**1**) (16 mg, 84%). TLC: $R_f = 0.8$ (Et_2O: *n*-hexane 7:1); EI-MS: *m*/*z* (rel. int.) 293 $[M-1]^+$ (100); HR-MS: found 294.100182 (calculated for $^{12}C_{13}$ $^{13}C_6H_{12}O_3$: 294.098773); ^{1}H NMR: δ 8.75 (H-7, d, J = 8.5 Hz), 7.67-7.51 and 7.30-7.13 (H-2′–H-6′, brm), 7.70 (H-4, d, J = 8.0 Hz), 7.59 (H-8, dd, $^3J_{H\text{-}8\text{–}C\text{-}1'} = 3.7$ Hz, $^3J_{H\text{-}8\text{–}H\text{-}7} = 8.5$ Hz), 7.11 (H-3, s), 7.08 (H-5, d, J = 8.0 Hz); ^{13}C NMR: δ 180.3 (C-1), 157.1 (C-6), 149.6 (C-9, d, $^1J_{C\text{-}9\text{–}C\text{-}1'}$ = 55 Hz), 149.5 (C-2), 144.1 (C-1′, m), 133.4 (C-4), 130.9 (C-8), 130.3 (C-7, d, $^3J_{C\text{-}7\text{–}C\text{-}1'} = 3.7$ Hz), 129.8-126.6 (C2′–C6′, C-9a, C-9b, m), 124.9 (C-6a), 122.0 (C-3a), 113.8 (C-3), 110.5 (C-5). Analytical data of the unlabelled compound **1**, which was prepared under identical conditions: EI-MS: *m*/*z* (rel. int.) 287 $[M-1]^+$ (100); HR-MS: found 288.078178 (calculated for $C_{19}H_{12}O_3$: 288.078644); ^{1}H NMR: δ 8.75 (H-7, d, J = 8.5 Hz), 7.70 (H-4, d, J = 8.0 Hz), 7.59 (H-8, d, 8.5 Hz,), 7.35-7.45 (H-2′–H-6′, m), 7.11 (H-3, s), 7.08 (H-5, d, J = 8.0 Hz); ^{13}C NMR: δ 180.3 (C-1), 157.1 (C-6), 149.6 (C-9), 149.5 (C-2), 144.1 (C-1′), 133.4 (C-4), 130.9 (C-8), 130.3 (C-7), 129.2 (C-3′/5′), 128.6 (C-2′/6′), 127.8 (C-4′), 128.6 (C-9a), 126.9 (C-9b), 124.9 (C-6a), 122.0 (C-3a), 113.8 (C-3), 110.5 (C-5).

*[phenyl-$^{13}C_6$]9-phenylphenalen-1-one ([phenyl-$^{13}C_6$]-**11**)*

The Grignard reagent (1.35 ml, 1.35 mmol) prepared from [U-^{13}C]bromophenol as described above was also used for the synthesis of [*phenyl*-$^{13}C_6$]9-phenylphenalen-1-one ([*phenyl*-$^{13}C_6$]-**11**) which was obtained in ca. 80% yield (168 mg). TLC: R_f = 0.8 (Et_2O: *n*-hexane 7:1); ^{1}H NMR: δ 8.37 (H-7, d, J = 8.3 Hz), 8.22 (H-6, dd, J = 8.2, 1.1 Hz), 7.98 (H-4, dd, J = 8.2, 1.1 Hz), 7.89 (H-3, d, J = 9.7 Hz), 7.73 (H-5, dd, J = 8.2, 8.2 Hz), 7.60 (H-8, dd, $^3J_{H\text{-}8\text{–}C\text{-}1'}$ = 3.8 Hz, $^3J_{H\text{-}8\text{–}H\text{-}7}$ = 8.3 Hz), 7.41-7.36 (H-2′–H-6′, m), 6.51 (H-2, d, J = 9.7 Hz); ^{13}C NMR: δ 185.2 (C-1), 147.7 (C-9), 143.7 (C-1′, m), 140.7 (C-3), 134.2 (C-7, d, $^3J_{C\text{-}7\text{–}C\text{-}1'}$ = 3.7 Hz), 132.4 (C-6a), 132.2 (C-6), 132.0 (C-4), 131.9 (C-8), 130.5 (C-2), 129.8-126.8 (C-2′–C-6′, C-3a, C-5, C-9a, C-9b). Analytical data of the unlabelled compound **11**, which was prepared under identical conditions: EI-MS: *m*/*z* (rel. int.) 256 $[M]^+$ (100); HR-MS: found 256.087554 (calculated for $C_{19}H_{12}O$: 256.088815); ^{1}H NMR: δ 8.37 (H-7, d, J = 8.3 Hz), 8.22 (H-6, dd, J = 8.2, 1.1 Hz), 7.98 (H-4, dd, J = 8.2, 1.1 Hz), 7.89 (H-3, d, J = 9.7 Hz), 7.73 (H-5, dd, J = 8.2, 8.2 Hz), 7.60 (H-8, d, J = 8.3 Hz), 7.41-7.36 (H-2′–H-6′, m), 6.51 (H-2, d, J = 9.7 Hz); ^{13}C NMR: δ 185.2 (C-1), 147.7 (C-9), 143.7 (C-1′), 140.7 (C-3), 134.2 (C-7), 132.4 (C-6a), 132.2 (C-6), 132.0 (C-4), 131.9 (C-8), 130.5 (C-2), 129.0 (C-3a), 128.7 (C-9b), 128.5 (C-2′/C-6′), 128.3 (C-3′/C-5′), 127.2 (C-4′), 127.1 (C-5), 126.2 (C-9a).

*[phenyl-$^{13}C_6$]2-Hydroxy-9-phenylphenalen-1-one ([phenyl-$^{13}C_6$]anigorufone, ([phenyl-$^{13}C_6$]-**2**)*

[*phenyl*-$^{13}C_6$]9-Phenylphenalen-1-one ([*phenyl*-$^{13}C_6$]-**11**) (168 mg, 0.6 mmol) was subjected to Yang and Finnegan peroxidation[13] (for a protocol see Cooke and Dagley),[14] which after separation by preparative TLC resulted in [*phenyl*-$^{13}C_6$]anigorufone ([*phenyl*-$^{13}C_6$]-**2**, 110 mg, 66%). TLC: R_f = 0.86 (Et_2O: *n*-hexane 7:1); EI-MS: *m*/*z* (rel. int.) 277 $[M\text{-}1]^+$ (100); HR-MS: found 278.103359 (calculated for $^{12}C_{13}\,^{13}C_6H_{12}O_2$: 278.103859); ^{1}H NMR: δ 8.41 (H-7, d, J = 8.2 Hz), 8.09 (H-6, d, J = 8.2 Hz), 7.88 (H-4, d, J = 7.1 Hz), 7.70 (H-5, dd, J = 8.2, 7.1 Hz), 7.63 (H-8, dd, $^3J_{H\text{-}8\text{–}C\text{-}1'}$ = 3.8 Hz, $^3J_{H\text{-}8\text{–}H\text{-}7}$ = 8.2 Hz), 7.61-7.49 and 7.33-7.21 (H-2′–H-6′, brm), 7.18 (H-3, s); ^{13}C NMR: δ 180.7 (C-1), 151.3 (C-2), 149.1 (C-9, brd, $^1J_{C\text{-}9\text{–}C\text{-}1'}$ = 55 Hz), 143.6 (C-1′, m), 136.2 (C-7, d, $^3J_{C\text{-}7\text{–}C\text{-}1'}$ = 3.7 Hz), 132.5 (C-6a), 132.0 (C-8), 131.0 (C-4), 130.2 (C-6), 130.0 (C-3a), 129.6-127.1 (m, C-2′–C-6′,C-5), 125.8 (C-9b, d, $^3J_{C\text{-}9b\text{–}C\text{-}1'}$ = 3.7 Hz), 124.7 (C-9a), 113.1 (C-3). Analytical data of the unlabelled compound **2**, which was prepared under identical conditions, were identical with those of the natural product.[21]

*[1'-^{13}C]9-phenylphenalen-1-one ([1'-^{13}C]-**11**)*

The procedure used for the synthesis of [*phenyl*-$^{13}C_6$]9-phenylphenalen-1-one ([*phenyl*-$^{13}C_6$]-**11**) was used analogously, except that the Grignard reagent

(1.5 ml, 1.5 mmol) was prepared from [1-^{13}C]bromophenol. [1′-^{13}C]9-Phenylphenalen-1-one ([1′-^{13}C]-**11**) was obtained in 82% yield (316 mg). TLC: $R_f = 0.8$ (Et_2O: *n*-hexane 7:1); EI-MS: *m/z* (rel. int.) 256 $[M-1]^+$ (100); HR-MS: found 257.090149 (calculated for $^{12}C_{18}{}^{13}CH_{12}O$: 257.092170); ^{1}H and ^{13}C NMR data matched those of unlabelled compound **11** except the enhanced ^{13}C signal of C-1′ at δ 143.7.

[1′-^{13}C]2-Hydroxy-9-phenylphenalen-1-one ([1′-^{13}C]anigorufone, [1′-^{13}C]-2)

[1′-^{13}C]Anigorufone ([1′-^{13}C]-**2**) (80 mg, 60%) was synthesized from [1′-^{13}C]9-phenylphenalen-1-one ([1′-^{13}C]-**11**) using the procedure described above. EI-MS: *m/z* (rel. int.) 273 $[M]^+$ (100), 272 $[M-1]^+$ (64); HR-MS: found 273.087662 (calculated for $^{12}C_{18}{}^{13}CH_{12}O_2$: 273.087085); ^{1}H and ^{13}C NMR data matched those of unlabelled anigorufone[21] (**2**) except the enhanced signal of C-1′ at δ 143.6

*9-(4′-Methoxyphenyl)-phenalen-1-one (**10**)*

A Grignard solution (15 ml, 5.2 mmol) was prepared from 4-bromoanisol (0.7 ml, 5.2 mmol) and used for the synthesis of 9-(4′-methoxyphenyl)-phenalen-1-one[22] (**10**), which was obtained from perinaphthenone (**9**) in 65% yield (950 mg). TLC: R_f = 0.53 (Et_2O: *n*-hexane 7:1); EI-MS: *m/z* (rel. int.) 285 $[M-1]^+$ (100), 271 (27); HR-MS: found 286.101036 (calculated for $C_{20}H_{14}O_2$: 286.099380); ^{1}H NMR: δ 8.32 (H-7, d, J = 8.3 Hz), 8.19 (H-6, dd, J = 8.2, 1.1 Hz), 7.94 (H-4, dd, J = 7.1, 1.1 Hz), 7.85 (H-3, d, J = 9.7 Hz), 7.70 (H-5, dd, J = 8.2, 7.1 Hz), 7.61 (H-8, d, J = 8.3 Hz), 7.32 (H-2′/6′, d, J = 8.7 Hz), 6.94 (H-3′/5′, d, J = 8.7 Hz), 6.50 (H-2, d, J = 9.7 Hz), 3.87 (O$\underline{C}H_3$, s); ^{13}C NMR: δ 185.6 (C-1), 160.1 (C-4′), 147.9 (C-9), 141.0 (C-3), 136.0 (C-1′), 134.6 (C-7), 132.8 (C-8), 132.7 (C-6a), 132.6 (C-6), 132.4 (C-4), 131.1 (C-2), 130.6 (C-2′/6′), 129.4 (C-3a), 129.4 (C-9b), 127.4 (C-5), 126.7 (C-9a), 114.3 (C-3′/5′), 55.5 (O$\underline{C}H_3$).

*9-(4′-Hydroxyphenyl)-phenalen-1-one (**12**)*

9-(4′-Methoxyphenyl)-phenalen-1-one (**10**) (900 mg, 3.1 mmol) was treated with 48% HBr (7 ml) in acetic acid (55 ml) and refluxed for 6 h. The resulting 9-(4′-hydroxyphenyl)-phenalen-1-one (**12**) was purified by column chromatography (760 mg, 90%). TLC: R_f = 0.37 (Et_2O: *n*-hexane 7:1); EI-MS: *m/z* (rel. int.) 271 $[M-1]^+$ (100); HR-MS: found 272.084846 (calculated for $C_{19}H_{12}O_2$: 272.083730); ^{1}H NMR: δ 8.31 (H-7, d, J = 8.3 Hz), 8.18 (H-6, dd, J = 8.2, 1.1 Hz), 7.94 (H-4, dd, J = 7.1, 1.1 Hz), 7.85 (H-3, d, J = 9.7 Hz), 7.69 (H-5, dd, J = 8.2, 7.1 Hz), 7.61 (H-8, d, J = 8.3 Hz), 7.35 (H-2′/6′, d, J = 8.7 Hz), 6.89 (H-3′/5′, d, J = 8.7 Hz), 6.50 (H-2, d, J = 9.7 Hz); ^{13}C NMR: δ 185.6 (C-1), 157.7 (C-4′), 148.3 (C-9), 140.9 (C-3), 134.9 (C-1′), 134.6 (C-7), 133.0

(C-8), 132.64 (C-6a), 132.61 (C-6), 132.3 (C-4), 131.2 (C-9b), 130.7 (C-2′/6′), 129.4 (C-3a), 130.8 (C-2), 127.3 (C-5), 126.7 (C-9a), 115.8 (C-3′/5′).

*[4′-O^{13}CH$_3$]9-(4′-Methoxyphenyl)-phenalen-1-one ([4′-O^{13}CH$_3$]-**10**)*

9-(4′-Hydroxyphenyl)-phenalen-1-one (**12**) (170 mg, 0.7 mmol) was dissolved in acetone (20 ml). ^{13}CH$_3$I (0.46 ml, 0.8 mmol) and K_2CO_3 (482 mg, 0.7 mmol) were added and the mixture refluxed for 4 h. The resulting [4′-O^{13}CH$_3$]9-(4′-methoxyphenyl)-phenalen-1-one ([4′-O^{13}CH$_3$]-**10**) was purified by column chromatography (110 mg, 55%). TLC: R_f = 0.53 (Et_2O: *n*-hexane 7:1); EI-MS: *m*/*z* (rel. int.) 286 [M-1]$^+$ (100); HR-MS: found 287.099998 (calculated for $^{12}C_{19}{}^{13}CH_{14}O_2$: 287.098264); ^{1}H and ^{13}C NMR data matched those of the unlabelled compound **10** except that the methoxyl signal (δ 3.87) appeared as a doublet ($^1J_{C-H}$ = 143.8 Hz) in the ^{1}H NMR spectrum and the signal of 4′-OCH$_3$ at δ 55.5 was enhanced in the ^{13}C NMR spectrum.

*[4′-O^{13}CH$_3$]2-Hydroxy-9-(4′-methoxyphenyl)-phenalen-1-one ([4′-O^{13}CH$_3$]-**8**)*

The introduction of the hydroxyl group at C-2 of [4′-O^{13}CH$_3$]9-(4′-methoxyphenyl)-phenalen-1-one ([4′-O^{13}CH$_3$]-**10**) (50 mg, 0.17 mmol) was achieved using the Yang and Finnegan[13] procedure. The resulting [4′-O^{13}CH$_3$]2-hydroxy-9-(4′-methoxyphenyl)-phenalen-1-one ([4′-O^{13}CH$_3$]-**8**) was obtained in 75% yield (40 mg) after purification by TLC: R_f = 0.58 (Et_2O: *n*-hexane 7:1); EI-MS: *m*/*z* (rel. int.) 302 [M-1]$^+$ (100), 287 (34), 272 (58); HR-MS: found 303.097991 (calculated for $^{12}C_{19}{}^{13}CH_{14}O_3$: 303.097649); ^{1}H NMR: δ 8.38 (H-7, d, *J* = 8.3 Hz), 8.07 (H-6, dd, *J* = 8.2, 1.0 Hz), 7.86 (H-4, dd, *J* = 7.2, 1.0 Hz), 7.68 (H-5, dd, *J* = 8.2, 7.2 Hz), 7.64 (H-8, d, *J* = 8.3 Hz), 7.36 (H-2′/6′, d, *J* = 8.8 Hz), 7.02 (H-3′/5′, d, *J* = 8.8 Hz), 3.89 (OC$\underline{H}_3$, d, $^1J_{C-H}$ = 143.8 Hz); ^{13}C NMR data matched those of the natural product[20] except the enhanced signal of 4′-OCH$_3$ at δ 55.6

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