Labelled Precursors for Biosynthetic Studies on Naphthylisoquinoline Alkaloids†

Gerhard Bringmann*, Frank Pokorny, Matthias Wenzel,
Kathi Wurm, and Christoph Schneider

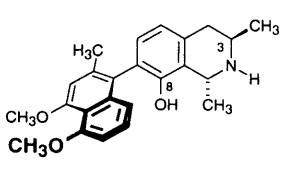
Institute of Organic Chemistry, University of Würzburg,
Am Hubland, 97074 Würzburg, Germany

Abstract: The isotope labelled monocyclic ketones **5** and **8**, postulated precursors to the presumably acetogenic naphthylisoquinoline alkaloids, have been synthesized for biogenetic experiments to Ancistrocladaceae and Dioncophyllaceae plants. Key step of the preparation of 1-(2'-[*carbonyl*-^{14}C]acetyl-3',5'-dibenzyloxyphenyl)-2-propanone ([^{14}C]-**13**) is the *C*-acetylation of the arylpropanone **10** with the mixed pivalic acetic anhydride ([^{14}C]-**11**). The resulting pyrylium salt [^{14}C]-**12**, which is stable and can be stored, is cleaved directly before the feeding experiment to give the diketone [^{14}C]-**13** and deprotected to give the free phenolic target molecule [^{14}C]-**5**. This synthetic route is applicable also to the prepation of 1-(2'-[$^{13}C_2$]acetyl-3'-hydroxyphenyl)-2-propanone ([$^{13}C_2$]-**5**) for biosynthetic experiments with NMR analysis. For the preparation of the oxygen-poorer ^{13}C-labelled diketone 1-(2'-[*methyl*-^{13}C]acetyl-3'-hydroxyphenyl)-2-propanone ([^{13}C]-**8**), an 'indanone-route' has been elaborated.

Key words: ^{13}C,^{14}C, naphthylisoquinolines, polyketides, acetogenic isoquinoline alkaloids

INTRODUCTION

Naphthylisoquinoline alkaloids like dioncophylline A (**1**) and ancistrocladine (**2**) constitute a rapidly growing novel class of natural products of increasing structural, pharmacological, chemotaxonomic, and biosynthetic interest.[2] The unusual substitution pattern of these alkaloids, in particular the methyl group at C-3 and the oxygen function at C-8, as well as the apparently acetogenic naphthalene substituent, hint at an unprecedented biosynthetic origin of tetrahydroisoquinoline alkaloids - not, as usually,[3] from aromatic amino acids, but from acetateunits, *via* β-pentaketones like **6** and monocyclic diketones like **5** and **8** (cf. Scheme 1).[4]



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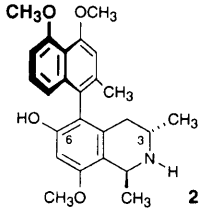

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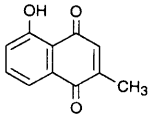

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† Part 79 in the series 'Acetogenic Isoquinoline Alkaloids'. For part 78, see lit.[1].

* Author to whom correspondence should be addressed.

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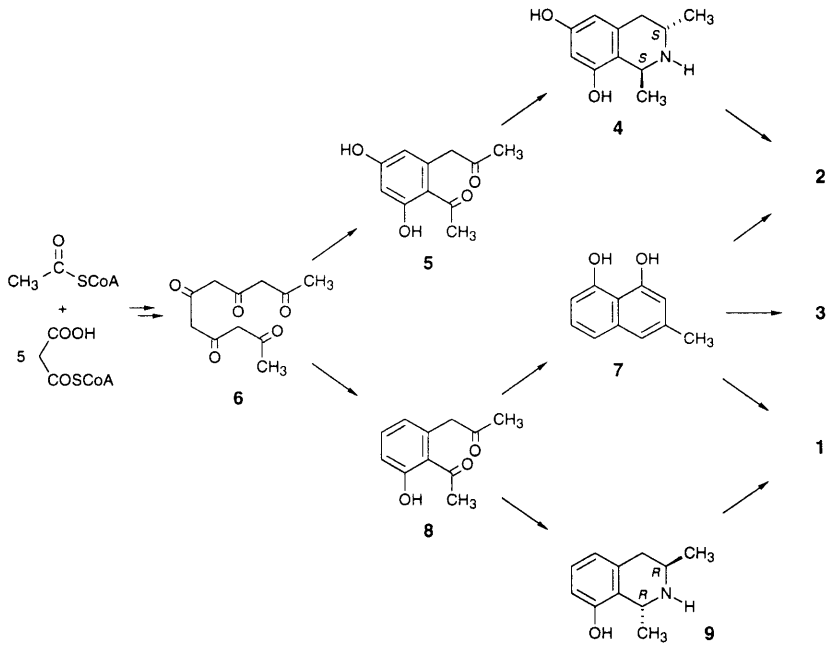


Scheme 1. Proposed[4] joint biosynthesis of acetogenic isoquinoline alkaloids and analogs like **3**.

This concept is in agreement with the co-occurrence of plumbagin (**3**), the oxidized form of the presumable precursor **7** to the naphthalene parts of all these alkaloids, in the same plants.[2] Due to its known[5] origin from acetate units, this naphthoquinone **3** can be considered as an 'acetogenic marker'. Other molecular alkaloid 'halves' of possible biogenetic relevance or their analogs that have been isolated from the same plants,[2] were similar naphthoquinones, but also tetrahydroisoquinolines closely related to the naphthalene-free compounds **4** and **9**.

Incorporation studies with higher - especially woody - plants are generally difficult,[6] because of the slow growth of the plants and problems in efficiently administering the precursors, and may result in low incorporation rates. Furthermore, general primary metabolites are often rapidly catabolized by the plants or by co-occurring microorganisms, or are incorporated into numerous other primary or secondary metabolites.

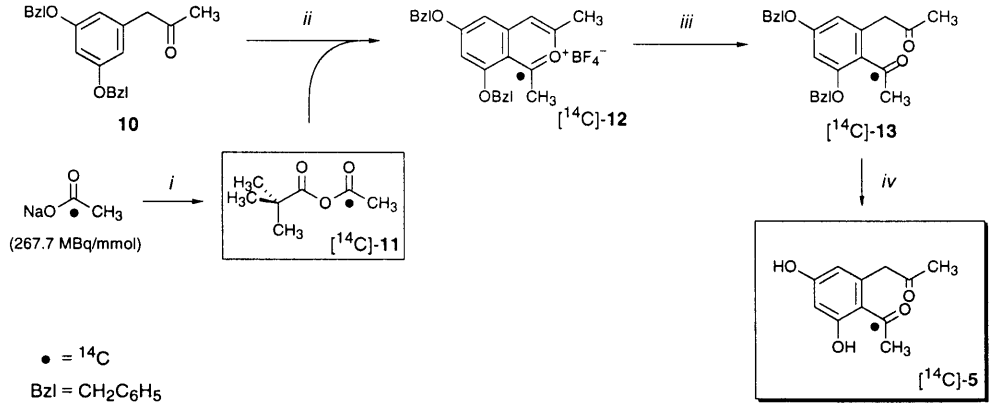
We have recently succeeded in optimizing the cultivation conditions[2,7-9] for several Ancistrocladaceae and Dioncophyllaceae species thus elaborating the experimental basis for systematic incorporation studies on naphthylisoquinoline alkaloids. First feeding experiments[4,10] with acetate and malonate using *Ancistrocladus* spp. showed small but significant incorporation rates into ancistrocladine (**2**) and plumbagin (**3**). Yet, in view of the mentioned problems with such

general precursors, better and more specific results should be obtained from feeding more 'advanced' - and thus more differentiated - precursors such as the monocylic diketones **5** and **8**, which constitute characteristic key compounds of our biosynthetic concept.

RESULTS AND DISCUSSION

Our approach to the preparation of the labelled diketone [^{14}C]-**5** is based on a synthetic access to this presumable precursor in a non-labelled form elaborated earlier.[11] Key step of that synthesis was the introduction of a C_2-unit by *C*-acetylation of the monoketone **10**[12,13] using acetic anhydride. In order to avoid the loss of half of the labelled material when analogously performing this crucial step with the symmetric anhydride of [1-^{14}C]acetic acid, we modified the procedure using the mixed pivalic acetic anhydride ([^{14}C]-**11**), which, due to the steric demand of the *t*Bu group, reacts on the acetyl part, exclusively. Furthermore, for security reasons, we now use HBF_4 as the activating acid instead of the explosive and water containing $HClO_4$ previously employed.[11] [^{14}C]-**11** is easily prepared according to a described non-labelling synthesis,[14] starting from the commercially available [1-^{14}C]-labelled sodium acetate and pivaloyl chloride.

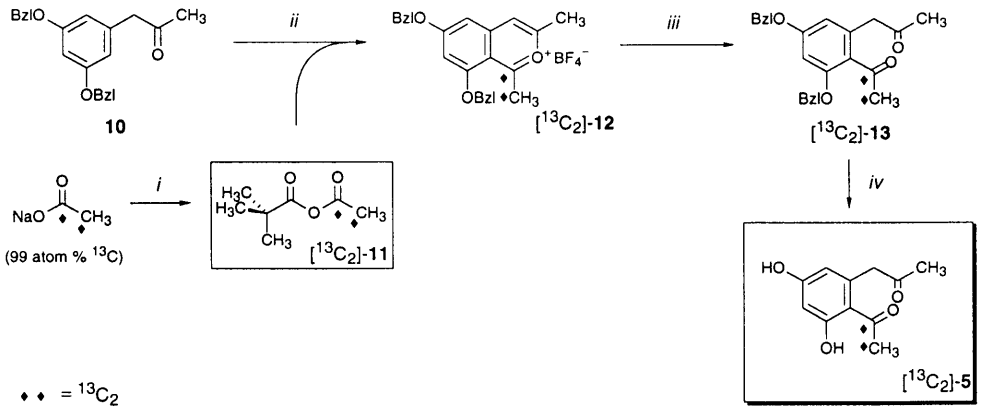
The acetylation step gives the expected labelled benzopyrylium salt [^{14}C]-**12**, which can be isolated and stored. Prior to use, it can be cleaved under mild conditions to give the diketone [^{14}C]-**13**, which is subsequently deprotected to give [^{14}C]-**5**. Due to the sensitivity of **5**, this cleavage



Scheme 2. Reagents and conditions: (*i*) $(CH_3)_3CCOCl$, Et_2O, reflux; 70%; 15.87 MBq, 70% radiochemical yield ('r.y.'). (*ii*) 54% HBF_4 in Et_2O, CH_2Cl_2; 70%; 11.1 MBq, 70% r.y. (*iii*) CH_2Cl_2, basic Al_2O_3; 92%; 9.86 MBq, 89% r.y. (*iv*) H_2/Pd-C (10%), MeOH; 70%; 7.7 MBq, 19.06 MBq/mmol, 78% r.y.

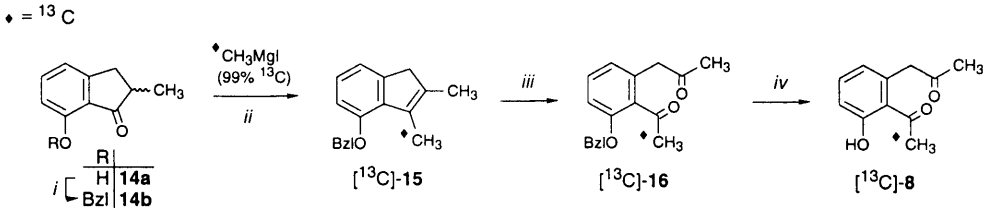
should be done directly before the feeding experiments. The radiochemical yield of this labelling synthesis based upon sodium [1-^{14}C]acetate as the radio precursor was 33.8%.

For the scheduled further incorporation experiments including a localization of the labelled position by NMR techniques rather than by tedious degradation procedures, we have likewise performed the synthesis of **5** in a ^{13}C-labelled form. In order to attain an optimum of sensitivity, we have labelled both of the two neighboring carbon atoms that are introduced *via* the anhydride methodology. Except for some minor experimental changes (due to practical and security reasons), this synthesis of [$^{13}C_2$]-**5**, as depicted in Scheme 3, is closely related to the corresponding radiolabelling synthesis shown above in Scheme 2.



Scheme 3. Reagents and conditions: (*i*) $(CH_3)_3CCOCl$, Et_2O, reflux; 86%. (*ii*) 54% HBF_4 in Et_2O, CH_2Cl_2; 80%. (*iii*) CH_2Cl_2, basic Al_2O_3; 87%; 98% isotopic yield ('i.y.'). (*iv*) H_2/Pd-C (10%), $CH_3CO_2C_2H_5$; 92%.

According to our biosynthetic concept, the monocyclic precursor to the naphthalene part of **2** and to both molecular moieties of **1** is the analogous, yet oxygen-poorer, diketone **8**. Regrettably, the synthetic strategy that we had successfully used for the synthesis of **5**, was not applicable to **8** because of severe problems of regioselectivity in the *C*-acetylation step.[11] In the acylation of **10**, this problem had not arisen because of its symmetric substitution. For this reason, we have adopted a synthetic pathway previously elaborated for the methyl ether of **8**, starting from the indanone derivative **14b**, as preparated from the corresponding indanone **14a**.[11] Unfortunately, this procedure allows the introduction of only one carbon atom, leading to singly ^{13}C-labelled **8**, by transformation of **14b** into the indene derivative [^{13}C]-**15**, and subsequent oxidative cleavage to give the protected diketone [^{13}C]-**16**, which is finally debenzylated leading to [^{13}C]-**8** (cf. Scheme 4).



Scheme 4. Reagents and conditions: (*i*) $C_6H_5CH_2Br$, K_2CO_3, acetone; 99%. (*ii*) Et_2O, $\Delta \rightarrow H_2O/H^+$; 99%. (*iii*) $KMnO_4$, $NaIO_4$, DME/H_2O, pH 7.8; 44%, 98% i.y. (*iv*) H_2/Pd-Al_2O_3 (5%), MeOH; 32%, 96% i.y.

This first preparation of ^{14}C-, ^{13}C-, and $^{13}C_2$-precursors now opens the possibility of performing intensive biosynthetic studies with these presumable precursors to acetogenic isoquinoline alkaloids. Feeding experiments on *Ancistrocladus* and *Triphyophyllum* plants are in progress.

EXPERIMENTAL

Melting points were measured on a Reichert-Jung Thermovar hotplate and are uncorrected. Non-radioactive new intermediates were analyzed for the elements carbon and hydrogen at the Institute of Inorganic Chemistry, University of Würzburg. Labelled chemicals were purchased commercially and were used without further purification. The ^{14}C-labelled compounds were identified by TLC comparison (R_f, solvent) with the corresponding unlabelled authentic samples. The radiochemical yield is given in '% r.y.'. The ^{13}C content of the singly ^{13}C-labelled compounds was determined by measuring the intensity ratio of the central peak to the ^{13}C satellites in the ^1H NMR spectra at the labelled position. The ^{13}C content of the $^{13}C_2$-labelled compounds was measured by determining the signal area ratios of the doublets of the labelled and the singlets of the non-labelled compound in the ^{13}C inverse-gated decoupling experiment spectra. The isotopic yield is given in '% i.y.'. Before labelling all compounds were analogously synthesized in an unlabelled form; data of new unlabelled compounds are given. NMR spectra were recorded in $CDCl_3$ (unless otherwise mentioned), using a Bruker AC 200, a Bruker AC 250, and a Bruker DMX 600 spectrometer. The chemical shifts δ are given in parts per million (ppm) with the proton signals of the deuterated solvent as an internal reference for ^1H NMR. The coupling constants *J* are given in Hertz (Hz). Mass spectra were determined with a Finnigan MAT 8200 mass spectrometer. IR spectra were taken on a Perkin-Elmer 1420 infrared spectrophotometer. The wave numbers of the

vibrational absorptions are given in cm^{-1}, their intensities are denoted by: s (strong), m (medium), w (weak), and br (broad).

Preparation of [^{14}C]-5 and [$^{13}C_2$]-5:

Pivalic [2-^{14}C]acetic anhydride ([^{14}C]-**11**): To 88 mg (1.07 mmol) of sodium acetate, 0.84 mg (22.8 MBq, 2.22 GBq/mmol) sodium [1-^{14}C]acetate (Amersham, Braunschweig, Germany), 145 µl (1.17 mmol) of pivaloyl chloride, and 2 ml of dry diethyl ether were added with stirring. The suspension was refluxed for 5 h, filtered and the solvent was evaporated *in vacuo* to yield [^{14}C]-**11** (108 mg, 70%; 69.6% r.y., 15.9 MBq); unlabelled **11**: b.p. 45-70°C/27 Pa. IR (film): ν 2950 (s), 2850 (s), 1730-1750 (s), 1390 (w), 1360 (s). ^{1}H NMR (200 MHz): δ = 1.25 (d, *J* = 1.8 Hz, 9H, $C(CH_3)_3$), 2.22 (d, *J* = 1.8 Hz, 3H, CH_3). MS (70 eV): *m/z* (%) = 43 (100) [M^+ - $C_2H_9O_2$], 57 (53) [M^+ - $C_3H_3O_3$], 85 (21) [M^+ - $C_2H_3O_2$]. Anal. calcd. for $C_7H_{12}O_3$ (144.2): C, 58.3; H, 8.38. Found: C, 58.5; H, 8.58.

6,8-Dibenzyloxy-1,3-[1-^{14}C]dimethyl-2-benzopyrylium tetrafluoroborate ([^{14}C]-**12**): A solution of 500 mg (1.45 mmol) 3',5'-dibenzyloxyphenyl-propanone **10** (14) in CH_2Cl_2 was treated with stirring with [^{14}C]-**11** for 5 min at room temperature and cooled down to 0°C. Portions of 0.1 ml of 54% HBF_4 in dry diethyl ether were cautiously added until completion of the reaction [TLC: petroleum ether / MTB (methyl *tert*-butylether) 1:2]. The solid was isolated by filtration and crystallization from the mother liquor to yield [^{14}C]-**12** (251.7 mg, 73%; 70% r.y., 11.1 MBq); unlabelled **12**: m.p. 187°C. IR (KBr): ν 1640 (m), 1600 (s), 1525 (m), 1230 (s), 1060 (s). ^{1}H NMR (250 MHz): δ = 2.69 (s, 3H, 3-CH_3), 3.14 (s, 3H, 1-CH_3), 5.29 (s, 2H, $OCH_2C_6H_5$), 5.42 (s, 2H, $OCH_2C_6H_5$), 6.88 (d, *J* = 2.0 Hz, 1H, 7-H), 7.30-7.50 (m, 11H, aromatic H), 7.80 (s, 1H, 4-H). MS (70 eV): *m/z* (%) = 49 (18) [BF_2^+], 91 (100) [$C_7H_7^+$]. Anal. calcd. for $C_{25}H_{23}O_3^+BF_4^-$ (458.3): C, 65.6; H, 5.1. Found: C, 65.2; H, 5.1.

*1-(2'-[*Carbonyl-^{14}*C]acetyl-3',5'-dibenzyloxyphenyl)-2-propanone* ([^{14}C]-**13**): A solution of the above generated [^{14}C]-**12** in 60 ml CH_2Cl_2 was filtered through 18 g basic Al_2O_3 (TLC quality, ICN), the column was washed with 150 ml of CH_2Cl_2 and the solvent was evaporated under reduced pressure to yield [^{14}C]-**13** (197.1 mg, 92.4%; 89% r.y., 9.86 MBq; TLC: R_f = 0.58, petroleum ether / MTB 1:2) as a colorless solid; unlabelled **13**: m.p. 81-82°C, (lit.[11] 82-83°C).

*1-(2'-[*Carbonyl-^{14}*C]acetyl-3',5'-dihydroxyphenyl)-2-propanone* ([^{14}C]-**5**): [^{14}C]-**13** (197.1 mg, 0.51 mmol) was dissolved in 10 ml of ethyl acetate and hydrogenated over 100 mg of Pd-C (10%) with ultrasonic assistance. After filtration and evaporation of the solvent *in vacuo*, 84 mg

(79%; 78% r.y., 7.7 MBq; TLC: R_f = 0.39, petroleum ether / MTB 1:2) of [^{14}C]-**5** were obtained as a colorless solid.

Preparation of [$^{13}C_2$]-5:

The synthesis was carried out analogously, starting with sodium [$^{13}C_2$]acetate (99 atom % ^{13}C, Sigma-Aldrich Chemie GmbH, D-89552 Steinheim, Germany). The following yields and data were obtained:

Pivalic [1,2-$^{13}C_2$]acetic anhydride ([$^{13}C_2$]-**11**): From 122 mg (1.45 mmol) sodium [$^{13}C_2$]acetate, 183 mg (86%) of the oily anhydride [$^{13}C_2$]-**11** were obtained.

6,8-Dibenzyloxy-1,3-[1,1'-$^{13}C_2$]dimethyl-2-benzopyrylium tetrafluoroborate ([$^{13}C_2$]-**12**): 183 mg (1.25 mmol) of [$^{13}C_2$]-**11** yielded 460 mg (80%) of [$^{13}C_2$]-**12**.

1-(2'-[$^{13}C_2$]acetyl-3',5'-dibenzyloxyphenyl)-2-propanone ([$^{13}C_2$]-**13**): Cleavage of the above produced [$^{13}C_2$]-**12** (460 mg, 1.01 mmol) as described for the preparation of [^{14}C]-**13** gave 339 mg (87%) colorless needles of [$^{13}C_2$]-**13**, m.p. 81-82°C, (lit.[11] 82-83°C). The peak areas of the ^{13}C signals at δ = 205.6 and 32.56 showed a $^{13}C_2$ content of >98% (measured by inverse-gated decoupling experiments). ^{1}H NMR (200 MHz): δ = 2.17 (s, 3H, 3-CH_3), 2.48 [dd, $^1J(^{13}CH)$ = 128 Hz, $^2J(^{13}CH)$ = 6.13 Hz, 3H, 2''-$^{13}CH_3$], 3.79 (s, 2H, 1-CH_2), 5.04 (s, 2H, O$CH_2C_6H_5$), 5.06 (s, 2H, O$CH_2C_6H_5$), 6.37 [d, ^4J(H,H) = 2.0 Hz, 1H, 6'-H], 6.56 [dd, ^4J(H,H) = 1.98 Hz, $^4J(^{13}CH)$ = 1.51 Hz, 1H, 4'-H], 7.27-7.44 (m, 10H, aromatic H). ^{13}C NMR (150.03 MHz, [D_4]methanol): δ = 29.69 (3-C), 32.63 (d, 2''-C), 43.70 (1-C), 71.18, 71.97 (3'-O$CH_2C_6H_5$, 5'-O$CH_2C_6H_5$), 100.32 (4'-C), 111.48 (6'-C), 124.72 (2'-C), 128.68, 128.93, 129.07, 129.25, 129.59, 129.68 (aromatic C), 137.40 (1'-C), 160.26, 162.52, (3'-C, 5'-C), 205,60 (d, 1''-C), 208.46 (2-C). MS (70 eV) *m/z* (%): 391 (0.1) [M^+ + 1], 390 (1.1) [M^+], 299 (0.8) [M^+ - 91], 91 (100) [$C_7H_7^+$].

1-(2'-[$^{13}C_2$]acetyl-3',5'-dihydroxyphenyl)-2-propanone ([$^{13}C_2$]-**5**): Hydrogenation of 339 mg (0.87 mmol) of [$^{13}C_2$]-**13** gave 167 mg (92%) as colorless needles; m.p. 132-133°C, (lit.[11] 130-131°C). ^{1}H NMR (200 MHz): δ = 2.15 (s, 3H, 3-CH_3), 2.47 [dd, $^1J(^{13}CH)$ = 128 Hz, $^2J(^{13}CH)$ = 5.98 Hz, 3H, 2''-$^{13}CH_3$], 3.72 (s, 2H, 1-CH_2), 6.12 [dd, ^4J(H,H) = 2.3 Hz, $^4J(^{13}CH)$ = 0.31 Hz, 1H, 6'-H], 6.28 [dd, ^4J(H,H) = 2.3 Hz, $^4J(^{13}CH)$ = 1.23 Hz, 1H, 4'-H].

Preparation of [^{13}C]-8:

7-Benzyloxy-2-methyl-1-indanone (**14b**): A suspension of 1.80 g (11.1 mmol) of the indanone **14a**,[11] 6.60 g (28.4 mmol) of benzylbromide, and 1.40 g (0.10 mmol) of dry K_2CO_3 in 160 ml of

dry acetone was stirred under Ar for 24 h at room temperature. After filtration the solvent was removed under reduced pressure and the residue was chromatographed on silica gel with CH_2Cl_2 to yield 2.71 g (99%) **14b** as an oil, b.p. 181°C/6.67 Pa. IR (KBr): ν = 1690 (s), 1590, 1490 (s), 1265, 1145 (w). ^{1}H NMR (200 MHz): δ = 1.23 (d, *J* = 7.2 Hz, 3H, 2-CH_3), 2.59 (m, 2H, 3-CH_2), 3.25 (m, 1H, 2-H), 5.17, (s, 2H, O*C*H_2C$_6$H$_5$) 6.72, (d, *J* = 8.2 Hz, 1H, 6-H), 6.90 (d, *J* = 7.5 Hz, 1H, 4-H), 7.15-7.50 (m, 6H, aromatic H). MS (70 eV) *m/z* (%): 252 (15) [M$^+$], 91 (100) [$C_7H_7^+$], 77 (4) [$C_6H_5^+$]. Anal. calcd. for $C_{17}H_{16}O_2$ (252.3): C, 80.93; H, 6.39. Found: C, 81.23; H, 6.40.

4-Benzyloxy-3-[^{13}C]methyl-2-methyl-2-indene ([^{13}C]-**15**): 1.00 g (3.94 mmol) indanone [^{13}C]-**14b** in 10 ml dry diethyl ether was treated at 0°C with a solution of [^{13}C]methyl magnesium iodide [500 mg magnesium and 119 mg, 8.28 mmol [^{13}C]methyl iodide (Promochem, Wesel, Germany)] in 40 ml of diethyl ether. The mixture was stirred at room temp. for 40 min, then refluxed for 1 h, cooled down to 0°C, and finally hydrolyzed with 5 ml of 2 N HCl under vigorous stirring. The organic layer was separated, the aqueous phase was extracted 5 times with MTB, and the combined organic phases were dried over Na_2SO_4. After removal of the solvent, filtration through silica gel with MTB / petroleum ether (1:4) yielded 1.92 g (97%) of [^{13}C]-**15**, which after crystallization from the same solvent by slow evaporation at 5°C gave 985 mg (99%) colorless needles; m.p. 53-55°C. IR (KBr): ν = 1620, 1490, 1565 (s), 1260, 1155 (s). ^{1}H NMR (250 MHz): δ = 2.00 (s, 3H, 2-CH_3), 2.24 [d, ^1J(^{13}CH) = 125.3 Hz, 3H, 3-$^{13}CH_3$], 3.23 (m_c, 2H, 1-CH_2), 5.09 (s, 2H, O*C*H_2C$_6$H$_5$), 6.81 (dd, *J* = 6.7, 2.2 Hz, 1H, 5-H), 6.96-7.07 (m, 2H, 6- and 7-H), 7.23-7.50 (m, 5H, aromatic H) - a quantitative calculation of the intensity ratio of the ^{1}H signals at δ = 2.24 was hampered by the overlap of the signal at δ = 2.00. MS (70 eV) *m/z* (%): 252 (7) [M$^+$ + 1], 251 (32) [M$^+$], 160 (59) [M$^+$ - 91], 91 (100) [$C_7H_7^+$]. Anal. calcd. for unlabelled $C_{18}H_{18}O$ (250.3): C, 86.36; H, 7.25. Found: C, 85.99; H, 7.51.

*1-(2'-[*Methyl-*^{13}C]acetyl-3'-benzyloxyphenyl)-2-propanone* ([^{13}C]-**16**): To a solution of 985 mg (3.93 mmol) [^{13}C]-**15**, 13.7 ml dimethoxyethane and 5.5 ml water at pH 7.8 (adjusted with 0.05 N NaOH and 0.05 N HCl) 163 mg (1.03 mmol) $KMnO_4$ and then 12.8 mg (59.8 mmol) $NaIO_4$ were added in portions with stirring at 0°C in an Ar atmosphere. After further stirring at room temp. for 84 h, the mixture was extracted with MTB. The resulting organic layer was dried over $MgSO_4$ and chromatographed on silica gel with MTB / petroleum ether (1:1). Reisolated starting material [^{13}C]-**15** (320 mg) was reacted again using the same reaction conditions, to give 605 mg (460 mg from the first preparation) of [^{13}C]-**16**, which was crystallized from MTB / petroleum ether (1:1) to

give 448 mg (41%) colorless needles; m.p. 66-68°C. IR (KBr): ν 1705 (s), 1680 (s), 1590, 1570 (s), 1240, 1160 (s). ^{1}H NMR (250 MHz): δ = 2.18 (s, 3H, CH_2COCH_3), 2.50 [d, $^1J(^{13}CH)$ = 128.3 Hz, 3H, $CO^{13}CH_3$], 3.78 (s, 2H, CH_2COCH_3), 5.09 (s, 2H, $OCH_2C_6H_5$), 6.74 (d, *J* = 7.5 Hz, 1H, 4'-H), 6.92 (d, *J* = 8.2 Hz, 1H, 6'-H), 7.22-7.40 (m, 6H, aromatic H). The ^{1}H NMR signals of the methyl group at δ = 2.50 showed a central peak to ^{13}C satellite intensity ratio of 1.5:98.5. MS (70 eV) *m/z* (%): 284 (0.3) [M^+ + 1], 283 (1.7) [M^+], 91 (100) [$C_7H_7^+$]. Anal. calcd. for $C_{17}{}^{13}C_1H_{18}O_3$ (283.3): C, 76.66; H, 6.40. Found: C, 76.24; H, 6.61. Anal. calcd. for unlabelled $C_{18}H_{18}O_3$ (282.3): C, 76.57; H, 6.43. Found: C, 76.95; H, 6.65.

1-(2'-[Methyl-*^{13}C]acetyl-3'-hydroxyphenyl)-2-propanone* ([^{13}C]-**8**): Catalytic hydrogenation of 60 mg (40.4 μmol) [^{13}C]-**16** with Pd-Al_2O_3 (5%) in 10 ml methanol, followed by chromatography on silica gel with MTB / petroleum ether (1:1) as the eluent and subsequent crystallization from acetone / petroleum ether (1:1) gave 17 mg (32%) of the free diketone [^{13}C]-**8**; m.p. 114-116°C (lit.[16] 125°C). IR (KBr): ν 3250 (br), 1680, 1660 (s), 1590, 1570, 1460 (w), 1260, 1170 (s). ^{1}H NMR (250 MHz, [D_4]methanol): δ = 2.14 (s, 3H, CH_2COCH_3), 2.51 [d, $^1J(^{13}CH)$ = 128.2 Hz, 3H, $CO^{13}CH_3$], 3.78 (s, 2H, CH_2COCH_3), 6.65 (d, *J* = 7.5 Hz, 1H, 4'-H), 6.80 (d, *J* = 8.3 Hz, 1H, 6'-H), 7.19 (dd, *J* = 7.7, 8.1 Hz, 1H, 5'-H). The ^{1}H signals of the methyl group at δ = 2.51 showed a central peak to ^{13}C satellite intensity ratio of 1.6:98.4. MS (70 eV) *m/z* (%): 194 (2) [M^+ + 1], 193 (8) [M^+], 175 (18) [M^+ - H_2O], 151 (22) [M^+ - CH_2CO], 135 (100) [M^+ - CH_2COCH_3], 44 (15) [$^{13}CH_3CO^+$], 43 (75) [CH_3CO^+].

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REFERENCES AND NOTES

1. Bringmann, G., Holenz J., Aké Assi, L., Zhao, C., and Hostettmann, K. - Planta Med., 1996 (in press).

2. Bringmann, G. and Pokorny, F. - The Alkaloids (Ed.: Cordell, G.A.), *vol. 46*, Academic Press, New York, 1995, pp. 127-271.

3. The Chemistry and Biology of Isoquinoline Alkaloids (Eds.: Phillipson, J.D., Roberts, M.F., and Zenk, M.H.), Springer, Berlin, 1985.

4. Bringmann, G. - The Alkaloids (Ed.: Brossi, A.) *29*, Academic Press, New York, 1986, pp. 141-184.

5. Durand, R. and Zenk, M.H. - Tetrahedron Lett.: 3009-3012 (1971).

6. Brown, S.A. and Wetter, L.R. - Prog. Phytochem. 3: 1-45 (1972).

7. Bringmann, G., Pokorny, F., and Zinsmeister, H.-D. - Palmengarten (Frankfurt/M., Ger.) 55/3: 13-18 (1991).

8. Bringmann, G., Schneider, C., Pokorny, F., Lorenz, H., Fleischmann, H., Sankaranarayanan, A.S., Almeida, M.R., Govindachari, T.R., and Aké Assi, L. - Planta Med. 59 *(Suppl.)*: 623-624 (1993).

9. Bringmann, G., Haller, R.D., Bär, S., Isahakia, M.A., and Robertson, S.A. - Palmengarten (Frankfurt/M., Ger.) 58: 148-153 (1994).

10. Bringmann, G., Pokorny, F., Stäblein, M., Govindachari, T.R., Almeida, M.R., and Ketkar, S.M. - Planta Med. 57 *(Suppl. 2)*: 98 (1991).

11. Bringmann, G. and Jansen, J.R. - Liebigs Ann. Chem.: 2116-2125 (1985).

12. Hill, R.A., Carter, R.H., and Staunton, J. - J. Chem. Soc., Perkin Trans. 1: 2570-2576 (1981).

13. Prepared according to lit.[15] 35%; m.p. 53°C (lit.[12] oil).

14. Krimen, L.I. - Organic Synthesis 50 (Ed.: Breslow, R.), Wiley & Sons, New York, 1970, pp. 1-3.

15. Hass, H.B., Susie, A.G. and Heider, R.L. - J. Org. Chem. 15: 8-14 (1950).

16. Bringmann, G. - Liebigs Ann. Chem.: 2105-2115 (1985).