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Biosynthesis of naphthylisoquinoline alkaloids: synthesis and incorporation of an advanced $^{13}C_2$-labeled isoquinoline precursor

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Abstract—Biosynthetic studies on naphthylisoquinoline alkaloids involving a specifically [1,1′-$^{13}C_2$]-labeled dihydroisoquinoline **7** are described. The synthesized precursor **7** was fed to callus cultures of *Triphyophyllum peltatum* and the isolated secondary metabolites were characterized by spectroscopic methods (^{1}H, ^{13}C NMR, and INADEQUATE experiments). The unambiguous incorporation of the precursor into dioncophylline A and two minor naphthylisoquinolines, together with the formation of the labeled corresponding *trans*-configured tetrahydroisoquinoline, proves the implication of such advanced intermediates in the proposed biosynthetic pathway of naphthylisoquinoline alkaloids.

1. Introduction

Naphthylisoquinoline alkaloids,[1,2] like dioncophylline A (**1**),[3,4] isolated from various species of the tropical plant families Ancistrocladaceae and Dioncophyllaceae, constitute a rapidly growing class of structurally intriguing naturally occurring biaryl compounds.[5] Besides the presence of stereogenic centers, the compounds possess a rotationally hindered *C*,*C* or *C*,*N* biaryl axis, which forms the basis for axial chirality. Many of these remarkable natural products exhibit promising bioactivities. As an example, **1** displays antitrypanosomal[6] activities, while other members of this class of compounds show good antimalarial,[7–9] antileishmanial,[10] or anti-HIV[11] activities. The substitution patterns of these alkaloids do not fit into the—previously general—biosynthetic pathway to tetrahydroisoquinoline alkaloids from aromatic amino acids[12] but, rather, hint at an as yet unprecedented origin from acetate units,[1,2] which is also suggested by the co-occurrence of provenly[13,14] acetogenic naphthoquinones like plumbagin (**2a**) and droserone (**2b**), and tetralones like isoshinanolone (**3**, Fig. 1) in *Triphyophyllum peltatum* and the related plant species *Ancistrocladus heyneanus*.

Although early feeding experiments involving ^{14}C-labeled precursors encountered serious setbacks (low incorporation rates, insufficient quantities of isolated alkaloids), a major breakthrough succeeded by the establishment of tissue cultures of *T. peltatum*[14] that do produce appreciable amounts of dioncophylline A (**1**). With these cell cultures, feeding experiments with [1,2-$^{13}C_2$]-labeled acetate and INADEQUATE analysis of the isolated dioncophylline A (**1**), assisted by the cryoprobe technique, clearly revealed that the two molecular portions of **1**, the naphthalene and the isoquinoline part, are each formed from six acetate units, both apparently via the same β-pentaketo precursor **4** (Scheme 1).[15,16] Hence, these are the first acetogenic isoquinoline alkaloids in nature and—together with analogous results for the

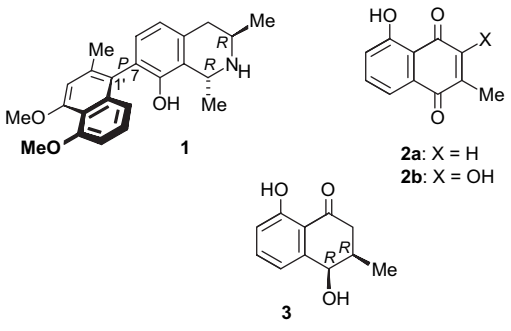

Figure 1. Dioncophylline A (**1**) and compounds related to its naphthalene portion, **2a/b** and **3**, from *Triphyophyllum peltatum* (Dioncophyllaceae).

Keywords: Naphthylisoquinoline alkaloids; Biosynthesis; Dioncophylline A; *Triphyophyllum peltatum*; $^{13}C_2$ labeling.
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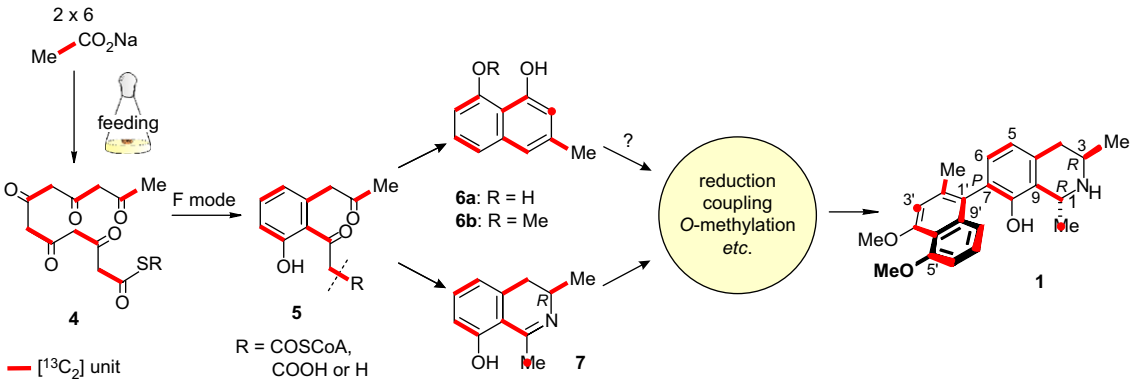
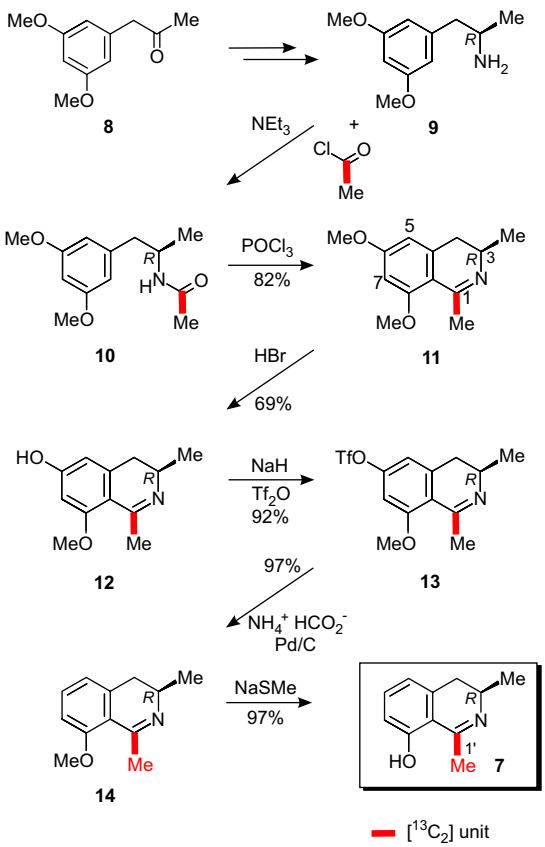


Scheme 1. Postulated biogenetic pathway to naphthylisoquinoline alkaloids; isotope labeling of $^{13}C_2$ units (in red) proven for **1**[15] and analogously assumed for the putative intermediates **4**–**7**.

related compounds **2a/b** and **3**[13,14]—the first experimentally proven examples of the so-called F folding mode[17] in higher plants (i.e., with two intact acetate derived C_2 units in the first ring).

According to our biosynthetic hypothesis (Scheme 1), the open-chain hexaketide molecule **4** should undergo aldol condensation and aromatization, to provide a monocyclic diketone of type **5**. Further aldol cyclization should give the corresponding naphthalene portion **6** or, by reductive amination of the (more reactive) acetonyl keto function of **5** (e.g., by transamination), deliver the respective primary amine (not shown), which would condense to form the dihydroisoquinoline **7**. These two molecular halves, **6** and **7**, hence divergently formed from identical precursors should then, convergently, be joined together according to the principle of phenol-oxidative biaryl coupling[5] thus, after some follow-up reactions, eventually yielding the complete alkaloid, here, e.g., dioncophylline A (**1**). In this paper, we report on the synthesis of a two-fold ^{13}C-labeled enantiomerically pure dihydroisoquinoline **7** (Scheme 2) and its successful incorporation into dioncophylline A (**1**) and other naphthylisoquinoline alkaloids, using our alkaloid producing tissue cultures of *T. peltatum*.



Scheme 2. Preparation of the [1,1′-$^{13}C_2$]-labeled assumed precursor **7**.

2. Results and discussion

Starting from the primary amine **9**, which is readily available from the arylacetone **8**,[18] the synthesis of the [1,1′-$^{13}C_2$]-labeled dihydroisoquinoline **7** was achieved based, in part, on a previously established[19] related synthetic procedure, which was further optimized in order to avoid loss of labeled material. The $^{13}C_2$ labeling was introduced by conversion of **9** into the amide **10** by using commercially available [1,2-$^{13}C_2$]-acetyl chloride, followed by Bischler–Napieralski cyclization to give the dihydroisoquinoline **11**. Deoxygenation at C-6 was performed by regioselective O-demethylation of **11**,[20] O-triflation of the resulting phenol **12**,[21] and subsequent Pd-catalyzed hydrogenation leading to the monooxygenated dihydroisoquinoline **14** in excellent yields. The hydrogenation step (with $NH_4^+HCO_2^-$, Pd/C) had to be monitored carefully by TLC to avoid over reaction leading to the *cis*-configured 8-*O*-methyltetrahydroisoquinoline. The synthesis of the target molecule **7** was completed by O-demethylation of **14** with sodium thiomethoxide in almost quantitative yield (Scheme 2).

With the labeled precursor **7** in hand, feeding experiments were performed using the alkaloid producing cell cultures of *T. peltatum* previously established by our group.[14] After the incubation, the callus cultures were harvested and the CH_2Cl_2/MeOH (1:1) extracts thereof analyzed by HPLC, which showed two significant peaks with the UV spectrum of naphthylisoquinoline alkaloids and a third peak with a UV trace of an isoquinoline molecular half.

Upon preparative HPLC isolation, the largest of these peaks provided dioncophylline A (**1**) as the main metabolite (Fig. 2),

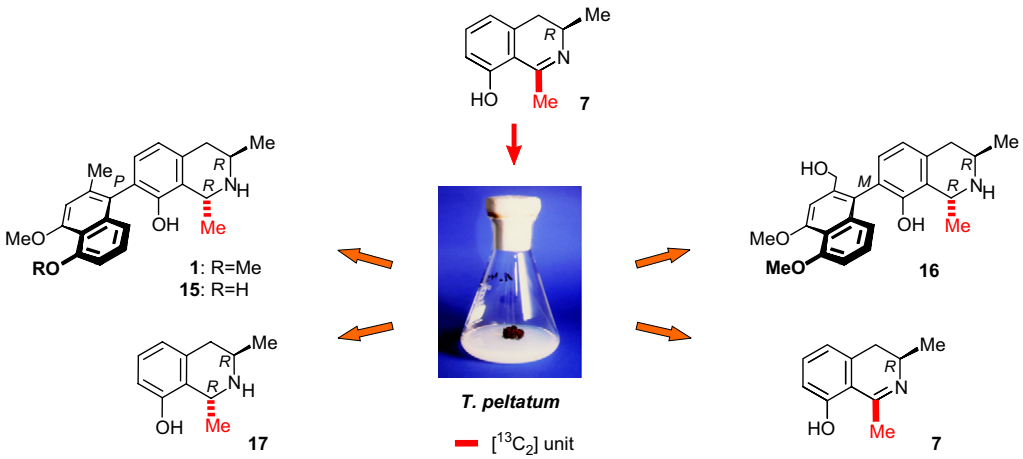


Figure 2. $^{13}C_2$-labeled metabolites isolated from callus cultures of *T. peltatum* after feeding [1,1′-$^{13}C_2$]-**7**.

initially contaminated by small quantities (ca. 11%) of its minor 5′-*O*-demethyl analog **15**,[22] but subsequently purified by HPLC (see Section 4.3). The second peak gave the side-chain oxygenated analog habropetaline A (**16**),[23] and the most polar one provided residual dihydroisoquinoline **7**, along with the *trans*-configured tetrahydroisoquinoline **17**—the latter two initially as a mixture, which was further resolved by HPLC (see Section 4.3).

The compound investigated first by ^{13}C NMR was the apparently newly formed *trans*-configured tetrahydroisoquinoline **17**, which had not been visible in the chromatogram of the untreated cell cultures and whose formation had thus been triggered by the application of the labeled compound **7**. As expected, it showed a very high labeling degree of ca. 94%. That this substrate was not an entirely unnatural metabolite of a likewise unnatural dihydroisoquinoline **7**, was made plausible by the exclusive formation of the respective *trans*-isomer; the corresponding *cis*-diastereomer was not detected. This hints at a specific, and thus an enzymatic process, since the chemical reduction of such dihydroisoquinolines, by catalytic hydrogenation or using common hydride transfer reagents like $NaBH_4$, normally produces *cis*,[19] while the directed chemical preparation of *trans* is synthetically more demanding and requires particular reaction conditions.[24] Moreover, the fact that the labeling degree is not complete (i.e., 100%), but 'only' 94% (determined by ^1H and ^{13}C NMR), reveals that the [1,1′-$^{13}C_2$]-labeled compound **17** is diluted by non-labeled—hence natural—material, so that **17** must be a normal, constitutional metabolite in the cell cultures of *T. peltatum*.

The recovered dihydroisoquinoline **7**, by contrast, was found to be fully labeled with no signs of the respective singlets for the non-labeled analog, which should just possess the natural ^{13}C abundance of ca. 1% (Fig. S2, Supplementary data). Nonetheless this as yet merely synthetic compound, which has never been found as such in nature, still might occur even in the non-treated cell cultures—in steady-state concentrations that can be very low, because it is usually immediately reduced to **17** and then coupled to dioncophylline A (**1**) or, vice versa, first coupled and then reduced. This may hint at a close organizational cooperation of the polyketide synthase (PKS) involved, with the respective reductase or the coupling enzyme, which may thus act as tailoring enzymes.[25] This in turn, may be the reason why the isoquinoline portion of naphthylisoquinoline alkaloids has (nearly) never been found free in nature,[26] never coupled to each other, but always to a naphthalene portion. From the fact that **17** is diluted by natural material, while **7** is fully labeled, one can further conclude that **7** and **17** are not in a (rapid) redox equilibrium with each other, because apparently **7** is reduced to **17**, but **17** is not oxidized back to **7** to a noticeable degree.

With all this information at hand—that **7** is at least accepted by a specific reductase, and is thus apparently accessible to the enzymes of the cells—the thrilling question was whether an incorporation had taken place into the isolated main alkaloid, the complete naphthylisoquinoline dioncophylline A (**1**). Thus the most important result of the present study was the unambiguous incorporation of **7** into **1** to the significant extent of ca. 5%, as clearly seen from the two distinct doublets for C-1 and Me-1 at 50.0 and 18.1 ppm, respectively (Fig. 3). These new peaks even exceeded the size of those resulting from 'non-labeled' dioncophylline A (**1**), i.e., with the natural ^{13}C abundance of ca. 1%. The results evidence the first successful incorporation of a precursor more advanced and differentiated than acetate, into a naphthylisoquinoline alkaloid.

The quantities of the likewise present minor alkaloid, 5′-*O*-demethyldioncophylline A (**15**), by contrast, were not sufficient to provide a good ^{13}C NMR spectrum after complete purification; still, the initial 11:89 mixture of **15** with the main alkaloid dioncophylline A (**1**) clearly showed an incorporation into **15**, too, exactly as that for **1** (Fig. S3, Supplementary data).

In the case of the other minor alkaloid, habropetaline A (**16**), by contrast, sufficient material was obtained after complete purification, which again, as already in the case of **1**, permitted to prove a significant incorporation of **7** to an extent of ca. 4% (for the respective spectra, see Fig. S19, Supplementary data).

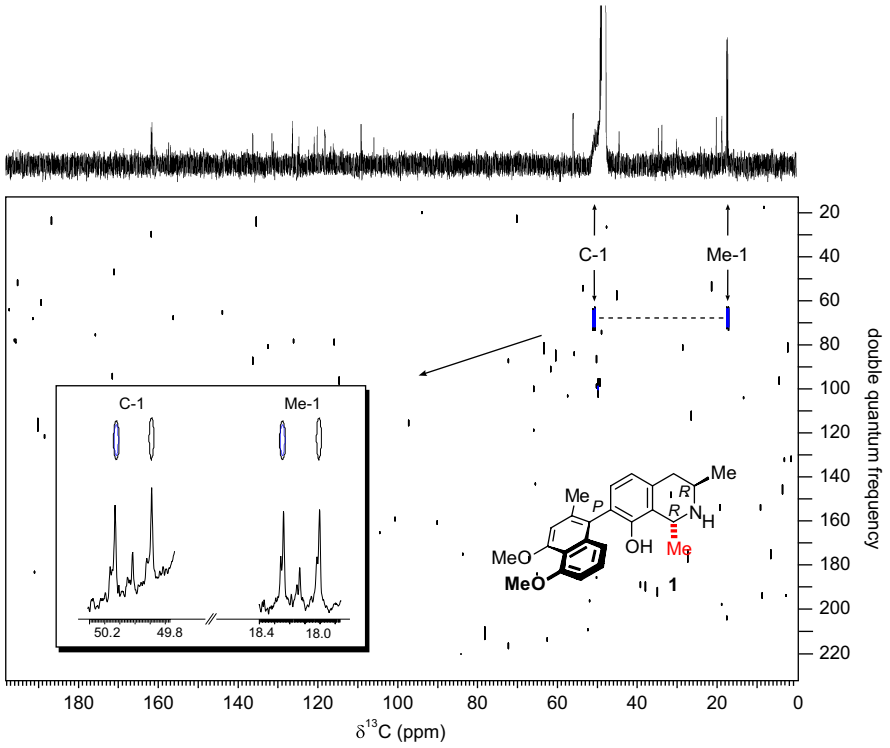


Figure 3. INADEQUATE spectrum (MeOH-d_4) of dioncophylline A (**1**) after feeding [1,1′-$^{13}C_2$]-dihydroisoquinoline **7** to callus cultures of *T. peltatum*. The expanded region (see included window left) indicates the two doublets of the $^{13}C_2$ unit of C-1 (50.0 ppm) and Me-1 (18.1 ppm) into which the $^{13}C_2$ label was incorporated.

3. Conclusions

This work constitutes the first proven incorporation of a more advanced biosynthetic precursor, viz. the dihydroisoquinoline **7**, into naphthylisoquinoline alkaloids, proving, for the first time, that the two molecular portions, the naphthalene and the isoquinoline, are formed separately, and are coupled to each other in a quite advanced form (i.e., not as an open-chain or monocyclic precursor). The smooth diastereoselective in vivo conversion of **7** to the respective *trans*-tetrahydroisoquinoline, **17** (and not to the *cis*-configured one, not shown), clearly indicates that **7** has access to the enzymes of the cell and is a substrate to its redox system and, in connection with its incorporation into the naphthylisoquinolines **1**, **15**, and **16**, possibly also to the respective coupling enzymes. The observed slight dilution of the labeling degree in **17**, but not in the reisolated **7**, does not necessarily mean that **17** is the immediate coupling precursor; it might also be that **7** is coupled and that normally very small portions are reduced to the tetrahydroisoquinoline **17**, while now, under the feeding conditions, larger quantities get reduced and thus remain uncoupled. Thus, it remains to be established, which of these two compounds, **7** or **17**, is the authentic coupling substrate, and also what exactly the naphthalene precursor is—whether it is a dihydroxy compound **6a** or its methyl ether **6b**. This work, as well as the search for the involved enzymes, is presently in progress.

4. Experimental

4.1. General

All reactions were carried out under a nitrogen atmosphere with magnetic stirring. Analytical TLC was performed on silica gel precoated glass plates (60 F_{254}, Merck). Visualization was accomplished by irradiation with UV light at 254 nm and 365 nm. Flash chromatography was performed using silica gel (0.063 mm, Merck). Melting points were obtained on a Stuart Scientific SMP10 instrument and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz). Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were taken on a JASCO FT-IR-410 spectrometer. HRMS were obtained on a micrOTOF-focus mass spectrometer (Bruker Daltonik GmbH). The following chemicals were purchased and used as received: NaH (Fluka), Tf_2O (Sigma Aldrich), ammonium formate (Riedel-de Häen), Pd/C (Fluka), and NaSMe (ACROS). CH_3CN, CH_2Cl_2, DMF, and toluene were dried over CaH_2; MeOH was dried over sodium, and all solvents were freshly distilled prior to use. [1,2-$^{13}C_2$]-Labeled acetyl chloride (99% labeling degree) was purchased from Aldrich and used without any further purification. Deactivated silica gel was prepared by mixing silica gel with 7.5 vol % NH_3. All labeled synthetic compounds showed a $^{13}C_2$ degree of ≥98.5 according to ^{1}H and ^{13}C NMR. The IR data of compounds **10**, **11**, and **12** are in agreement with those of the non-labeled analogs[19] and are given in the Supplementary data.

4.2. Synthesis of [1,1′-$^{13}C_2$]-8-hydroxy-3,4-dihydro-1,3-dimethylisoquinoline

4.2.1. *N*-[(*R*)-1-(3,5-Dimethoxyphenyl)propan-2-yl]-[1,2-$^{13}C_2$]-acetamide (10). Amine **9**·HCl (2.74 g, 11.8 mmol, 1.0 equiv) was placed under nitrogen in a flame-dried flask. Dichloromethane (80 mL) and freshly distilled NEt_3 (2.63 g, 26.0 mmol, 2.2 equiv) were added and the mixture was cooled to 0 °C. After addition of

[1,2-$^{13}C_2$]-acetyl chloride (1.00 g, 12.4 mmol, 1.05 equiv), the reaction mixture was stirred for 30 min at room temperature and then quenched with 2 N HCl. The organic layer was washed twice with 2 N HCl, then with water, dried over $MgSO_4$, and the solvent was removed under reduced pressure to give **10** in quantitative yield as white crystals; mp 83–84 °C (CH_2Cl_2); lit.[19,27] mp 85 °C (CH_2Cl_2/cyclohexane); $[\alpha]_D^{20}$ +23.5 (*c* 0.08, CH_2Cl_2); lit.[19,27] $[\alpha]_D^{20}$ +10.1 (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.33 (s, 3H, H-2′, H-4′, and H-6′), 5.30 (br s, 1H, NH), 4.22–4.28 (m, 1H, H-2), 3.78 (s, 6H, OCH_3), 2.79 (dd, $^2J_{HH}$=13.4 Hz, $^3J_{HH}$=5.8 Hz, 1H, H-1), 2.64 (dd, $^2J_{HH}$=13.4 Hz, $^3J_{HH}$=7.2 Hz, 1H, H-1), 1.94 (dd, $^1J_{HC}$=128.0 Hz, $^2J_{HC}$=6.0 Hz, 3H, $COCH_3$), 1.12 (d, $^3J_{HH}$=6.7 Hz, 3H, CH_3-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2 (d, J_{CC}=51.0 Hz, $COCH_3$), 163.6 (C-3′, C-5′), 143.0 (C-1′), 110.3 (C-2′ and C-4′), 101.3 (C-6′), 58.1 (OCH_3), 48.8 (C-2), 45.5 (C-1), 26.3 (d, J_{CC}=51.0 Hz, $COCH_3$), 22.8 (CH_3-3); EIMS (70 eV) *m/z* (rel int.) 239.0 (17) $[M]^+$, 178.0 (100) $[M^+-NHCOCH_3]$; HRESIMS 262.1327 ([M+Na]$^+$, 262.1330 calcd for [$^{13}C_2$]-$C_{13}H_{20}NO_3Na$).

4.2.2. [1,1′-$^{13}C_2$]-(3*R*)-3,4-Dihydro-6,8-dimethoxy-1,3-dimethylisoquinoline (11). Under nitrogen, a solution of **10** (2.83 g, 11.8 mmol, 1.0 equiv) and freshly distilled $POCl_3$ (2.13 g, 13.8 mmol, 1.15 equiv) in CH_3CN (40 mL) was stirred at 70 °C for 45 min. After removal of the solvent under reduced pressure, the residue was carefully quenched by addition of an aqueous ammonia solution at 0 °C and the aqueous layer was extracted with CH_2Cl_2. Drying of the organic layer over $MgSO_4$ and removal of the solvent under reduced pressure yielded the dihydroisoquinoline **11** as a brown oil (2.30 g, 10.4 mmol, 82%, 2 steps); $[\alpha]_D^{20}$+69 (*c* 0.10, MeOH); lit.[19,27] $[\alpha]_D^{20}$+139 (*c* 0.6, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.35 (s, 1H, H-7), 6.31 (s, 1H, H-5), 3.82 (s, 6H, OCH_3), 3.24–3.36 (m, 1H, H-3), 2.59 (dd, $^2J_{HH}$=15.6 Hz, $^3J_{HH}$=4.2 Hz, 1H, H_{eq}-4), 2.42 (ddd, $^1J_{HC}$=128.0 Hz, $^2J_{HC}$=6.7 Hz, J_{HH}=1.8 Hz, 3H, CH_3-1), 2.34 (dd, $^2J_{HH}$=15.5 Hz, $^3J_{HH}$=13.1 Hz, 1H, H_{ax}-4), 1.35 (d, $^3J_{HH}$=6.7 Hz, 3H, CH_3-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0 (d, J_{CC}=48.8 Hz, C-1), 164.7 (C-6), 161.8 (C-8), 145.1 (C-10), 107.0 (C-5), 99.9 (C-7 and C-9), 58.1 (OCH_3), 54.0 (C-3), 38.0 (C-4), 30.2 (d, J_{CC}=48.8 Hz, CH_3-1), 24.5 (CH_3-3); EIMS (70 eV) *m/z* (rel int.) 221.2 (100) $[M]^+$, 206.1 (44) $[M^+-CH_3]$, 191.1 (17) $[M^+-2CH_3]$; HRESIMS 222.1397 ([M+H]$^+$, 222.1399 calcd for [$^{13}C_2$]-$C_{13}H_{18}NO_2$).

4.2.3. [1,1′-$^{13}C_2$]-(3*R*)-3,4-Dihydro-6-hydroxy-8-methoxy-1,3-dimethylisoquinoline (12). Dimethoxydihydroisoquinoline **11** (575 mg, 2.59 mmol, 1.0 equiv) was stirred in 12 mL of 62% hydrobromic acid for 45 min at 110 °C. Excess HBr was removed under reduced pressure, the residue was treated with water and all volatiles were removed in vacuo. After column chromatography on deactivated silica gel, using an increasing gradient of CH_2Cl_2/MeOH (100:3 up to 100:7) (v/v) as an eluent, the monohydroxy compound **13** (376 mg, 1.78 mmol, 69%) was obtained as a yellow-brownish solid; mp 213 °C (CH_2Cl_2/MeOH);[19,28] $[\alpha]_D^{20}$+284 (*c* 0.1, MeOH);[19,28] ^{1}H NMR (250 MHz, CD_3OD) δ 6.00 (s, 1H, H-7), 5.98 (s, 1H, H-5), 3.82 (s, 3H, OCH_3), 3.55–3.75 (m, 1H, H-3), 2.82 (dd, $^2J_{HH}$=15.6 Hz, $^3J_{HH}$=4.9 Hz, 1H, H_{eq}-4), 2.59 (dd, $^2J_{HH}$=15.6 Hz, $^3J_{HH}$=11.8 Hz, 1H, H_{ax}-4), 2.51 (ddd, $^1J_{HC}$=131.0 Hz, $^2J_{HC}$=6.1 Hz, J_{HH}=0.9 Hz, 3H, CH_3-1), 1.33 (d, $^3J_{HH}$=6.4 Hz, 3H, CH_3-3); ^{13}C NMR (100 MHz, CD_3OD) δ 169.2 (d, J_{CC}=41.2 Hz, C-1), 167.5 (C-6 and C-8), 142.7 (C-10), 115.6 (C-5), 101.8 (C-7), 55.8 (OCH_3), 49.1 (C-3), 36.7 (C-4), 23.9 (d, J_{CC}=41.2 Hz, CH_3-1), 18.9 (CH_3-3); EIMS (70 eV) *m/z* (rel int.) 207.2 (100) $[M]^+$, 192.2 (37) $[M^+-CH_3]$; HRESIMS 208.1242 ([M+H]$^+$, 208.1242 calcd for [$^{13}C_2$]-$C_{10}H_{16}NO_2$).

4.2.4. [1,1′-$^{13}C_2$]-(3*R*)-3,4-Dihydro-8-methoxy-1,3-dimethylisoquinolin-6-yl trifluoromethanesulfonate (13). 6-Hydroxydihydroisoquinoline **12** (103 mg, 0.49 mmol, 1.0 equiv) was placed under nitrogen in a flame-dried Schlenk flask and CH_2Cl_2 (8 mL) was added. After cooling the flask to 0 °C, NaH (44.0 mg of a 60% dispersion in oil, 1.09 mmol, 2.2 equiv) was added and the solution was stirred for 30 min at 0 °C. A solution of Tf_2O (115 mg, 0.54 mmol, 1.1 equiv) in CH_2Cl_2 (15 mL) was added dropwise at 0 °C over a period of 15 min and after stirring for 5 min, the reaction mixture was directly filtered through a short pad of silica gel and then washed with CH_2Cl_2 giving **13** as a yellow oil (155 mg, 0.45 mmol, 92%); $[\alpha]_D^{20}$ −65.4 (*c* 0.1, CH_2Cl_2); IR (KBr) ν_{max} 2967, 1606, 1466, 1606, 1305, 1244, 1217, 1141, 1120, 979, 834 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.71 (s, 2H, H-5, H-7), 3.88 (s, 3H, OCH_3), 3.25–3.35 (m, 1H, H-3), 2.65 (dd, $^2J_{HH}$=15.8 Hz, $^3J_{HH}$=4.6 Hz, 1H, H_{eq}-4), 2.43 (ddd, $^1J_{HC}$=128.0 Hz, $^2J_{HC}$=7.0 Hz, J_{HH}=0.9 Hz, 3H, CH_3-1), 2.36 (dd, $^2J_{HH}$=15.8 Hz, $^3J_{HH}$=13.7 Hz, 1H, H_{ax}-4), 1.38 (d, $^3J_{HH}$=6.7 Hz, 3H, CH_3-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5 (d, J_{CC}=50.2 Hz, C-1), 158.9 (C-8), 150.9 (C-6), 143.4 (C-10), 118.7 (q, $^3J_{CF}$=320.9 Hz), 112.6 (C-5 and C-9), 104.2 (C-7), 56.3 (OCH_3), 51.5 (C-3), 34.8 (C-4), 27.7 (d, J_{CC}=50.2 Hz, CH_3-1), 22.0 (CH_3-3); EIMS (70 eV) *m/z* (rel int.) 339.0 (13) $[M]^+$, 206.1 (100) $[M^+-Tf]$; HRESIMS 340.0730 ([M+H]$^+$, 340.0736 calcd for [$^{13}C_2$]-$C_{11}H_{15}F_3NO_4S$).

4.2.5. [1,1′-$^{13}C_2$]-(3*R*)-3,4-Dihydro-8-methoxy-1,3-dimethylisoquinoline (14). A suspension of **13** (101 mg, 0.29 mmol, 1.0 equiv), ammonium formate (78.0 mg, 1.23 mmol, 4.0 equiv), and 5 mg Pd/C in MeOH (7 mL) was stirred under nitrogen at 80 °C (using a preheated oil bath) for 5 min. The reaction mixture was filtered through a short pad of Celite and washed with MeOH. After removal of the eluent under reduced pressure, the obtained residue was purified by column chromatography on deactivated silica gel using an increasing gradient of CH_2Cl_2/MeOH (100:3 up to 100:7) giving **14** (55.0 mg, 0.28 mmol, 97%) as a yellow oil; $[\alpha]_D^{20}$ −96.4 (*c* 0.1, CH_2Cl_2); IR (KBr) ν_{max} 2963, 2940, 1592, 1473, 1326, 1275, 1159, 1091, 1033 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (dd, ^3J=7.3 Hz, ^3J=8.2 Hz, 1H, H-6), 6.84 (d, J=8.2 Hz, 1H, H-5 or H-7), 6.77 (d, J=7.3 Hz, 1H, H-5 or H-7), 3.86 (s, 3H, OCH_3), 3.25–3.35 (m, 1H, H-3), 2.63 (dd, $^2J_{HH}$=15.6 Hz, $^3J_{HH}$=4.6 Hz, 1H, H_{eq}-4), 2.46 (ddd, $^1J_{HC}$=128.0 Hz, $^2J_{HC}$=7.0 Hz, J_{HH}=2.1 Hz, 3H, CH_3-1), 2.35 (dd, $^2J_{HH}$=15.8 Hz, $^3J_{HH}$=14.7 Hz, 1H, H_{ax}-4), 1.38 (d, $^3J_{HH}$=6.7 Hz, 3H, CH_3-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9 (d, J_{CC}=48.7 Hz, C-1), 158.2 (C-8), 140.4 (C-10), 132.0 (C-6), 119.9 (C-5 and C-9), 110.3 (C-7), 55.4 (OCH_3), 50.9 (C-3), 34.3 (C-4), 27.0 (d, J_{CC}=48.7 Hz, CH_3-1), 21.1 (CH_3-3); EIMS (70 eV) *m/z* (rel int.) 191.1 (100) $[M]^+$,

176.1 (33) [M^+-CH_3]; HRESIMS 192.1294 ([M+H]$^+$, 192.1294 calcd for [$^{13}C_2$]-$C_{10}H_{16}NO$).

4.2.6. [1,1′-$^{13}C_2$]-(3*R*)-3,4-Dihydro-8-hydroxy-1,3-dimethylisoquinoline (7). To a solution of **14** (90.0 mg, 0.47 mmol, 1.0 equiv) in DMF (4 mL), NaSMe (148 mg, 2.11 mmol, 4.5 equiv) was added under nitrogen and the mixture was stirred at 160 °C for 45 min. Repeatedly, water was added and all volatiles were removed in vacuo. The resulting residue was purified by column chromatography on deactivated silica gel, using CH_2Cl_2/MeOH (100:8) as an eluent, to afford **7** (81.0 mg, 0.47 mmol, 97%) as yellow crystals; mp 195–197 °C (dec., CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ +67.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 2924, 1685, 1606, 1465, 1205, 1135, 800 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (dd, ^3J=8.9 Hz, ^3J=7.0 Hz, 1H, H-6), 6.93 (d, J=8.9 Hz, 1H, H-5 or H-7), 6.86 (d, J=7.0 Hz, 1H, H-5 or H-7), 3.93–4.05 (m, 1H, H-3), 2.85 (dd, $^1J_{HC}$=131.0 Hz, $^2J_{HC}$=6.2 Hz, 3H, CH_3-1), 3.11 (dd, $^2J_{HH}$=16.4 Hz, $^3J_{HH}$=5.2 Hz, 1H, H_{eq}-4), 2.87 (dd, $^2J_{HH}$=16.4 Hz, $^3J_{HH}$=11.5 Hz, 1H, H_{ax}-4), 1.45 (d, $^3J_{HH}$=6.7 Hz, 3H, CH_3-3); ^{13}C NMR (100 MHz, CD_3OD) δ 178.3 (d, J_{CC}=40.4 Hz, C-1), 163.0 (C-8), 140.3 (C-10), 139.8 (C-6), 120.6 (C-5), 117.6 (C-7 and C-9), 50.2 (C-3), 34.6 (C-4), 24.8 (d, J_{CC}=40.1 Hz, CH_3-1), 18.0 (CH_3-3); EIMS (70 eV) *m/z* (rel int.) 177.2 (100) [M]$^+$, 162.2 (44) [M^+-CH_3], 148.2 (43); HRESIMS 178.1138 ([M+H]$^+$, 178.1143 calcd for [$^{13}C_2$]-$C_{11}H_{14}NO$).

4.3. Feeding experiments

Callus cultures of *T. peltatum* were cultivated as previously described.[14] The [1,1′-$^{13}C_2$]-labeled precursor **7** (77.6 mg) was administered aseptically, in triplicate, to 5.84 g (dry weight) callus for a period of nine weeks. The harvested, dried callus material was extracted with CH_2Cl_2/MeOH (1:1) and after evaporation of the solvent in vacuo, the crude organic extract was purified by preparative HPLC on a Symmetry RP_{18} column (Waters, 19×300 mm, 7 μm) employing the following gradient: H_2O (A)/CH_3CN (B) and 0.05% TFA (v/v); flow rate 12 mL min^{-1}; 0 min 5% B, 30 min 70% B, 35 min 100% B, 40 min 100% B, 41 min 5% B, 46 min 5% B. This yielded a mixture of dioncophylline A (**1**) along with its 5′-*O*-demethylated analog (**15**, t_R=20.6 min, 14 mg), habropetaline A (**16**, t_R=16.4 min, 4 mg, 4% $^{13}C_2$ labeling degree), and a mixture of *trans*-configured tetrahydroisoquinoline **17** together with reisolated **7** (t_R=11.3 min, 14 mg). Further purification on a Chromolith Semi Preparative RP_{18} column (100×10 mm) using H_2O (A)/CH_3CN (B) and 0.05% TFA (v/v); flow rate 10 mL min^{-1}; 0 min 0% B, 2 min 0% B, 8 min 15% B, 23 min 35% B, 25 min 50% B, 27 min 50% B, 27.5 min 0% B, 30 min 0% B, gave dioncophylline A (**1**, 4.9 mg, 5% $^{13}C_2$ labeling degree), while the tetrahydroisoquinoline **17** (7 mg, 94% $^{13}C_2$ labeling degree) and its dihydro analog **7** (7 mg, 98.5% $^{13}C_2$ labeling degree) were separated by the following gradient: flow rate 12 mL min^{-1}; 0 min 0% B, 4 min 0% B, 4.5 min 2% B, 10 min 2% B, 10.5 min 5% B, 16 min 5% B, 16.5 min 25% B, 20 min 0% B, 30 min 0% B.

Structural elucidation of the isolated compounds was achieved by 1D- (for **1**, **7**, **15**, **16**, and **17**) and 2D-NMR (HMQC, HMBC, COSY, and NOESY, for **1**, **15**, and **16**) experiments and, in the case of **1**, **7**, and **17**, by co-elution with available authentic **1** and synthetically prepared **7** and **17**. The isolated natural products **1**, **15**, and **16** were spectroscopically identical with the materials isolated earlier.[3,22,23] In order to establish the C–C connectivities for labeled dioncophylline A (**1**) and its 5′-*O*-demethylated analog, **15**, a 2D-INADEQUATE spectrum was measured on a Bruker DMX600 spectrometer. The labeling degree of all isolated compounds was determined by ^{13}C NMR.

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Supplementary data

Spectroscopic details for compounds **17** and **18**. Copy of the INADEQUATE spectrum of the mixture of **1** and **15**. Copies of the ^{1}H and ^{13}C NMR spectra for **1**, **7**, **10**–**14** and **16**–**18**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.12.022.

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27. Lit.[19] value is for the non-labeled analog.
28. Lit.[19] value is only given for the hydrobromide.