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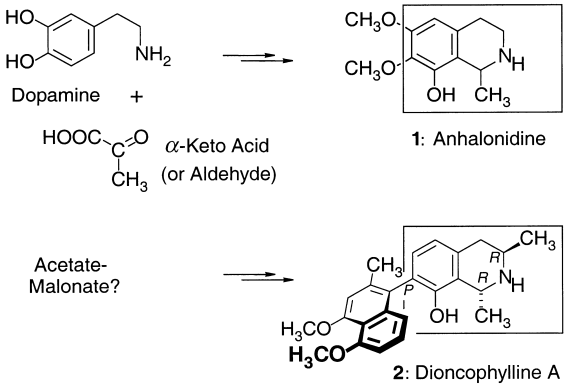
A New Biosynthetic Pathway to Alkaloids in Plants: Acetogenic Isoquinolines**

Gerhard Bringmann,* Michael Wohlfarth, Heiko Rischer, Matthias Grüne, and Jan Schlauer

Tetrahydroisoquinoline alkaloids constitute a large family of more than 2500 known,[1] mostly pharmacologically important, secondary plant metabolites—from simple 1-alkylisoquinolines like anhalonidine[2] (**1**, Scheme 1) to complex polycyclic benzylisoquinolines like the analgesic morphine.[3] Until now, it has been generally assumed that they are all derived from aromatic amino acids such as tyrosine through a common biosynthetic key step, the Pictet – Spengler condensation of 2-arylethylamines, like dopamine, with aldehydes (or α-keto acids). The ultimate structural diversity of isoquinolines found in nature[4] originates from the variation of the aldehyde precursor and subsequent transformations of the tetrahydroisoquinoline initially formed.



Scheme 1. Known biogenetic origin of tetrahydroisoquinolines such as anhalonidine (**1**) by Pictet – Spengler condensation[2] and the proposed novel pathway to dioncophylline A (**2**).

The structures of dioncophylline A (**2**, Scheme 1) and other naphthylisoquinoline alkaloids,[5] however, do not fit into this, hitherto, generally applicable biosynthetic scheme. In this paper, we report the discovery of a fundamentally different biosynthetic pathway to 1,2,3,4-tetrahydroisoquinoline alkaloids in higher plants; the entire carbon skeleton of dioncophylline A from *Triphyophyllum peltatum* (Dioncophyllaceae)[6] is unambiguously demonstrated to be synthesized by an acetate – polymalonate pathway.

Hints at the chemical plausibility of an acetogenic origin for naphthylisoquinoline alkaloids had already been obtained from early biomimetic polyketide cyclization reactions,[7] which had led to the efficient synthesis of both molecular parts from identical precursors and, thus, to first total syntheses of these compounds.[6]

Direct-feeding experiments with addition of [^{14}C]- and [^{13}C]-labelled acetate and malonate to cultivated or wild plants of the Ancistrocladaceae family did not yield significant incorporation rates. Cell cultures of *Ancistrocladus heyneanus* (Ancistrocladaceae) produced large amounts of naphthoquinones and tetralones[8] related to the naphthalene part of the alkaloids, but not the alkaloids themselves in sufficient quantities. More recently, we succeeded in establishing cell cultures of *T. peltatum* for the first time.[9] These proved to be capable of forming both naphthoquinones *and* naphthylisoquinolines, predominantly dioncophylline A (**2**).

The presumed precursor acetate was fed to these cultures in [$^{13}C_2$]-labelled form. After a previously optimized incubation time, **2** was isolated and investigated by high-field NMR spectroscopy.[10] The ^{1}H-decoupled ^{13}C NMR spectrum recorded on a 600 MHz system showed complex signal patterns. Due to overlapping signals and baseline noise obscuring the ^{13}C satellites, however, it was not possible to draw final conclusions. We addressed these problems by using the 2D INADEQUATE[11] experiment for the identification of direct C–C connectivities.[12] The signal-to-noise ratio was substantially improved by acquiring the data with a cryoprobe[13] on a 500 MHz NMR spectrometer.

The obtained spectrum (Figure 1) only displays clear pairwise C–C correlations which originate from the acetate units incorporated intact, without bond cleavage, into dioncophylline A (**2**).[14] Closer examination led to the conclusion that the entire carbon skeletons of both molecular halves of **2** are derived from acetate and that they exhibit an identical polyketide folding pattern with respect to the corresponding isocyclic rings. Furthermore, it is obvious that in both molecular halves decarboxylations occur at homologous positions, leaving the ultimate 1-methyl carbon atom and 3′-C.[15] These findings unambiguously prove our biosynthetic postulate[6] of the acetogenic origin of these alkaloids.

[*] Prof. Dr. G. Bringmann, M. Wohlfarth, H. Rischer, Dr. M. Grüne, Dr. J. Schlauer
Institut für Organische Chemie
Universität Würzburg
Am Hubland, 97074 Würzburg (Germany)
Fax: (+49) 931-888-4755
E-mail: bringman@chemie.uni-wuerzburg.de

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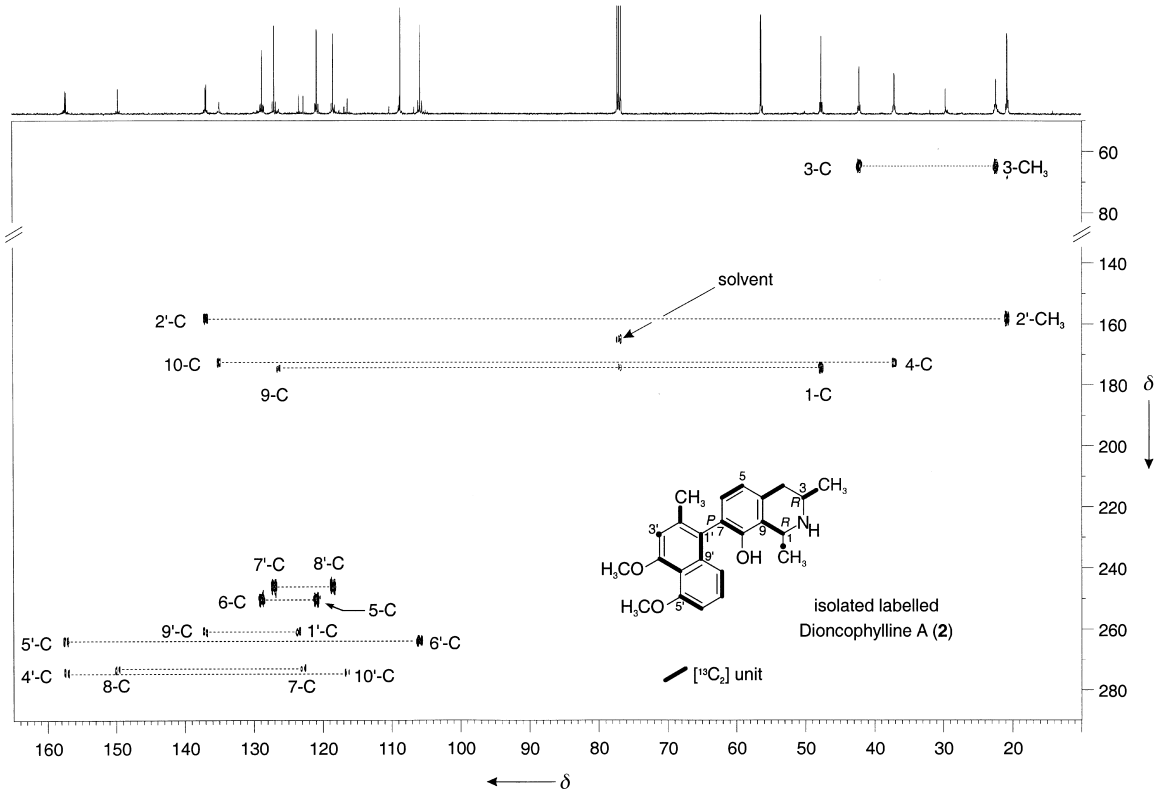
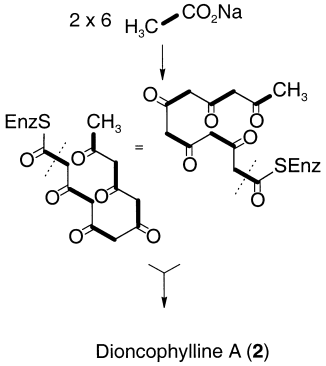


Figure 1. 2D-INADEQUATE NMR spectrum ([^{2}H]$CHCl_3$, 30 °C) of dioncophylline A (**2**) from bond-labeling experiments with sodium [$^{13}C_2$]acetate; the pairwise ^{13}C-^{13}C correlations of incorporated [$^{13}C_2$] units are visible.

Accordingly, starting from six acetate units each, through identical intermediate β-polycarbonyl precursors, both the naphthalene and the isoquinoline halves of dioncophylline A are formed divergently by cyclization and, in the case of the isoquinoline moiety, nitrogen incorporation. The resulting two molecular halves are then joined together convergently to give the full alkaloid, dioncophylline A (Scheme 2). Further



Scheme 2. The new biosynthetic pathway to isoquinolines involving purely acetogenic precursors.

metabolic steps such as reductions, oxidations, or methylations result in the known multitude of structures,[6] all based on common precursors.

The now successfully established biogenetic origin of the naphthylisoquinoline alkaloid dioncophylline A (**2**) by the acetate–polymalonate pathway paves the way for further studies on key steps of this interesting biosynthesis and the corresponding enzymes. It furthermore emphasizes the great importance of polyketide metabolism,[16] which is well-known in microorganisms,[17] but has so far been underestimated in higher plants.

Experimental Section

[1,2-$^{13}C_2$]NaOAc (99% isotopic enrichment) was purchased from Promochem (Wesel, Germany). A sterile-filtered solution of 0.05 M labelled NaOAc in distilled water, adjusted to pH 5.8, was used for the feeding experiments.

Callus cultures of *T. peltatum* were established as previously reported.[9] For feeding experiments, they were cultivated on the same solid medium as used for general maintenance. During a period of two months, 250 mg of labelled NaOAc were administered in total to 832 mg (dry weight) of callus.

The lyophilized callus material was extracted with MeOH and 1% 0.1N HCl (v/v). The methanolic extract was diluted with an equal amount of water, neutralized with NH_3, and reextracted with CH_2Cl_2. After evaporation of the solvent the organic phase was purified by reverse-phase MPLC (Lobar C-18, MeOH/H_2O (70/30) and 0.1‰ TFA (v/v)), normal-phase MPLC (Lobar Si-60, CH_2Cl_2/MeOH (9/1) and 1% NEt_3 (v/v)), and preparative HPLC on a chiral-phase column (Chiralcel OD, hexane/*i*PrOH (8/2) and 1‰ NEt_3 (v/v)) to yield 1.7 mg dioncophylline A (**2**). Unlabeled **2**, (2.5 mg) isolated from root material of *T. peltatum*,[18] was added to this sample. The final purification step by column chromatography (deactivated silica gel, CH_2Cl_2/MeOH (80/1)) gave 4.0 mg of **2**.

For the assignment of the ^1H and ^{13}C signals, NMR spectra of an unlabelled sample in [^{2}H]$CHCl_3$ were recorded on a Bruker DMX600 spectrometer, using standard 1D as well as 2D HMQC, HMBC, COSY, TOCSY, and ROESY experiments. To establish C–C connectivities, a 2D INADEQUATE[19] spectrum (optimized for $^1J(^{13}C,^{13}C) = 50$ Hz) of the labelled dioncophylline A was acquired at 125.7 MHz on a Bruker DRX500 spectrometer within a measurement time of 39 h. For this experiment, a prototype of a 5 mm ^{13}C cryoprobe with a ^1H decoupling coil was used with the coils and the preamplifier was cooled to 20 K.

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