Stress-related polyketide metabolism of Dioncophyllaceae and Ancistrocladaceae

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Abstract

The discovery of a novel biosynthetic pathway to isoquinoline alkaloids is described. The naphthylisoquinoline alkaloid dioncophylline A, one of the most prominent representatives of a new class of structurally and pharmacologically intriguing secondary metabolites, is shown to originate from acetate units, both molecular halves, the isoquinoline part and the naphthalene portion, being formed from identical polyketide precursors. All other tetrahydroisoquinoline alkaloids previously investigated, ultimately originate from aromatic amino acids. The novel pathway to isoquinoline alkaloids (hence acetogenic) was proved by feeding experiments with ¹³C-labelled precursors administered to callus cultures of Triphyophyllum peltatum (Dioncophyllaceae), followed by NMR investigations using the potent cryoprobe methodology. The new pathway is largely stress-sensitive: upon exposure to chemical, biotic or physical stress, T. peltatum stops producing the isoquinoline part, so that the naphthalene moiety accumulates in the chemical form of naphthoquinones like plumbagin and droserone and the chiral tetralone isoshinanolone.

Key words: Isoquinoline alkaloids, biosynthesis, plant stress, Dioncophyllaceae, naphthylisoquinoline alkaloids, polyketide alkaloids, dioncophylline A.

Introduction

The very small palaeotropical families Dioncophyllaceae and Ancistrocladaceae including, for example, Triphyophyllum peltatum Airy Shaw from West Africa or Ancistrocladus heyneanus Wall. from India comprise most peculiar lianas, fascinating to botanists and natural products chemists. They produce a plethora of structurally unique naphthylisoquinoline alkaloids, among them dioncophylline A (I) (Bringmann and Pokorny, 1995; Bringmann et al., 1998a). These alkaloids are different from all the other c. 2500 isoquinolines isolated from plants (Kutchan et al., 1991; Bentley, 1998), not only

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Abbreviations: LC, liquid chromatography; NMR, nuclear magnetic resonance; MS, mass spectrometry; CD, circular dichroism; 2D INADEQUATE, two-dimensional incredible natural-abundance double-quantum experiment.

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due to their unusual structures (including a mostly rotationally hindered biaryl axis between the two molecular parts), but also because of their unprecedented biosynthetic origin: The structure of 1 does not fit into the generally accepted biosynthetic scheme for tetrahydroisoquinolines, which, as exemplified for morphine (3) in Fig. 1B, are known to originate from aldehydes (or α-keto acids), by a so-called Pictet–Spengler condensation with dopamine (2), thus ultimately arising from aromatic amino acids (Dalton, 1979; Bentley, 1998). The naphthylisoquinoline alkaloid dioncophylline A (1), by contrast, appears to be built up from acetate units, exclusively (Fig. 1A). In agreement with earlier hints from biomimetic polyketide cyclization reactions (Bringmann and Pokorny, 1995), it has been postulated that both molecular moieties, the naphthalene and isoquinoline parts, are synthesized from joint polyketide precursors (Bringmann and Pokorny, 1995).

This paper deals with the naphthylisoquinoline alkaloids, their structures, bioactivities, and ‘producers’ and with biosynthetic feeding experiments revealing, exemplarily for dioncophylline A (1), for the first time their acetogenic origin; furthermore, the stress-induced inhibition of this biosynthetic pathway and the production of acetogenic naphthoquinones and tetralones in Ancistrocladaceae and Dioncophyllaceae plants and in cell cultures after exposure to chemical, biotic, or physical stress is described.

Cultivation of the plants

For the biosynthetic experiments to be conducted, living, alkaloid-producing material was essential. Prior to the work described here, nothing was known about the cultivation of Dioncophyllaceae and Ancistrocladaceae plants in a greenhouse, so reliable growth parameters for these sensitive tropical lianas had first to be established (Bringmann et al., 1999c, f). More than ten naphthylisoquinoline-producing plants species are growing in the Botanical Garden of the University of Würzburg, including T. peltatum (Fig. 2), Ancistrocladus abbreviatus (Fig. 3A, B), and A. heyneanus (Fig. 3C, D) which were used to generate the cell cultures (Bringmann et al., 1998b, 2000a). With these alkaloid-producing systems available, it was possible to probe the biosynthetic postulate of an acetogenic origin of the alkaloids.

Naphthylisoquinolines, structurally unique alkaloids

The Dioncophyllaceae and Ancistrocladaceae are widely used in folk medicine, hinting at the presence of bioactive secondary metabolites. Indeed, phytochemical investigations have revealed that these plants produce a variety of structurally unique naphthylisoquinoline alkaloids. At the biaryl axis that connects the naphthalene moiety, most of these metabolites show the phenomenon of restricted rotation, leading
to stable atropisomers (rotational isomers). Some typical examples of the different coupling types by which the molecular halves can be joined together are shown in Fig. 4.

Bioactivities of naphthylisoquinoline alkaloids

Naphthylisoquinoline alkaloids show promising bioactivities, exerting stress on a variety of human–pathogenic or crop-relevant micro-organisms, for example, against plant-pathogenic fungi (Bringmann et al., 1992) and anti-feeding and growth-retarding effects against herbivorous insects (Bringmann et al., 1997). Furthermore, some of the alkaloids are efficient agents of potential relevance for the treatment of severe tropical diseases such as African sleeping sickness, leishmaniasis and, in particular, malaria—in fact, using compounds like dioncophylline C (4, Fig. 4), malaria-infected mice can be fully healed (François et al., 1997; Bringmann and Feineis, 2000). Similarly dioncopeltine A (6, Fig. 4) and korupensamine A (5, Fig. 4) are also highly active.

Nitrogen-free metabolites in other Dioncophyllaceae: first hints at an acetogenic origin of the alkaloids?

By far the most active antimalarial naphthylisoquinoline alkaloids have been isolated from T. peltatum which constitutes a rich source of c. 20 such secondary metabolites, all of them structurally unprecedented (Bringmann et al., 1998a). It therefore seemed rewarding to investigate other phylogenetically related plants from the same family. However, besides T. peltatum, only two other Dioncophyllaceae species are known: Habropetalum dawei (Hutch. & Dalz.) Airy Shaw from Sierra Leone and Dioncophyllum thollonii Baillon from Gabon (Airy Shaw, 1951). Phytochemical investigations on these very rare plants yielded, unexpectedly, no sign of naphthylisoquinoline alkaloids, but abundant amounts of naphthalene-related, quite wide-spread, known natural products (Fig. 5) like isoshinanolone (10), plumbagin (11), and droserone (12) (Bringmann et al., 1999a, b, d).

However, by applying the efficient analytical ‘triad’ LC-MC/MS-NMR-CD (Bringmann et al., 1999a), naphthylisoquinoline alkaloids were detected in low quantities in these species, including known representatives like dioncophylline A (1), but also (mainly) new ones.

The bicyclic 10–12 proved to be valuable indicators of an acetogenic origin of the naphthylisoquinoline alkaloids, since one of them, plumbagin (11), is already...
Fig. 4. A selection of naphthylisoquinolines representing the biaryl coupling types detected in Dioncophyllaceae (red arrows) and Ancistrocladaceae (green arrows). In most cases (except for example 8, whose axis can freely rotate), the biaryl axis is configurationally stable and represents an additional element of chirality.

Fig. 5. Proposed biosynthesis of naphthylisoquinoline alkaloids like dioncophylline A (1), and the stress-induced (or constitutional) formation of the naphthalene-related compounds isoshinanolone (10), plumbagin (11) and droserone (12).
known to be formed from $^{14}$C-labelled acetate (Durand and Zenk, 1971).

**The concept of acetogenic isoquinoline alkaloids**

The hypothesis of an acetogenic origin of naphthylisoquinoline alkaloids as the first solely acetate-derived tetrahydroisoquinolines, is outlined in Fig. 5. Accordingly, both molecular halves of the alkaloids, the naphthalene part 15 and the isoquinoline portion 16, originate from six acetate units each, even via joint polyketide intermediates 13. The naphthoquinones and tetralones 10–12 abundantly occurring in *H. dawei* and *D. thollonii* are apparently derived from the free naphthalene part 15 of the alkaloids (Fig. 5), oxidized to give plumbagin (11), further oxygenated to droserone (12), or reduced to give isoshinanolone (10). Enhanced formation of 10–12 must relate to a block (or non-existence) of the reductive incorporation of nitrogen into the postulated joint diketo precursor 14 to give the dihydroisoquinoline 16. The capability to undertake this presumed key biosynthetic step, is restricted to the Dioncophyllaceae and Ancistrocladaceae, exclusively. Other taxonomically related plant families (Fig. 5, drawn in blue), including the closest phylogenetic neighbours to these plants, such as the Drosophyllaceae, the Droseraceae, the Nepenthaceae (all likewise carnivorous), and the Plumbaginaceae, cannot perform this remarkable biosynthetic step. They all produce nitrogen-free compounds like 10–12, but no naphthylisoquinoline alkaloids.

**Stress-induced naphthoquinone formation**

This assumed transamination (plus cyclization) step 14$\rightarrow$16 in Ancistrocladaceae and Dioncophyllaceae can, however, be easily blocked (Fig. 5, pointed out in blue), by all sorts of chemical (e.g. by elicitors like methyl jasmonate), physical (e.g. in plants wounded mechanically), or biotic stress (see below), thus leading to the formation of the isocyclic compounds 10–12, apparently needed as ‘chemical weapons’.

An illustration of this, hinting at a possible chemoecological role for all these acetogenic products in the interaction between the plants and their herbivores, is, for example, the East African species *A. robertsoniorum*. When wounded by insects, this species produces large quantities of pure crystalline droserone (12) of such a high quality that one can perform an X-ray structure analysis (Fig. 6), directly on these ‘biogenic crystals’ (Peters et al., 1995).

Another example is *A. heyneanus*: when attacked by the holoparasite *Cuscuta reflexa*, it forms large amounts of plumbagin (11) around the haustoria (Fig. 7), thus ultimately killing the parasite and also some of its own cells, so that a brown scar remains, but the parasite is repelled and *Ancistrocladus* can survive (Bringmann et al., 1999e).

**Biogenesis of acetogenic naphthoquinones and tetralones**

The stress-induced naphthalene formation is also predominant in cell cultures of *A. heyneanus* and can,
in turn, be used for biosynthetic feeding experiments with doubly $^{13}$C-labelled acetate. These investigations confirm the postulated acetogenic origin of the three bicyclic compounds 10–12 (Fig. 8), with the full folding mode of the polyketide chain, including the site where one C-atom gets lost by decarboxylation (Bringmann et al., 1998b).

This result was, however, only partially satisfying. It did give concise information on the polyketide origin of the nitrogen-free compounds 10–12; an acetogenic origin of the alkaloids, however, although more plausible, still remained unproved, just because the cell cultures of A. heyneanus did not produce enough dioncophylline A (1).

Finally proved: the acetogenic polyketide origin of dioncophylline A—a new biosynthetic pathway to isoquinoline alkaloids!

The breakthrough finally came from cell cultures of T. peltatum, which have recently been established for the first time, starting from fresh seeds (Fig. 2E). Besides again forming naphthoquinones (Bringmann et al., 2000a), these tissue cultures produced sufficient quantities of dioncophylline A (1), so that feeding experiments with $^{13}$C$_2$-labelled acetate became possible. Subsequently a small quantity (c. 1 mg) of 1 was isolated and investigated by NMR, and this time, a clear incorporation of $^{13}$C$_2$-units was found over the naphthalene part. This was to be expected from the structural relationship to the naphthalene analogues 10–12, but has now been verified for the first time for a naphthylisoquinoline alkaloid.

In the isoquinoline part, one clear single $^{13}$C$_2$-unit (C5–C6) was identified, in perfect agreement with the biosynthetic hypothesis, but it was impossible to assign any further acetate units because of an unfavourable signal-to-noise ratio. After renewed extensive purification of the alkaloid and elimination of the last minor paramagnetic impurities and, in particular, by applying the 2D-INADEQUATE technique (Budrus and Bauer, 1987; Podkorytov, 1999), in combination with the novel NMR cryoprobe methodology (Styles et al., 1984, 1989), the full polyketide folding pattern was clearly and unambiguously identified. The spectrum (Fig. 9) unequivocally shows the entire carbon skeletons of both halves of dioncophylline A (1) to be derived from acetate units, with identical incorporation patterns for the two molecular portions (Bringmann et al., 2000b). Dioncophylline A is thus the first unambiguously proven acetogenic tetrahydroisoquinoline alkaloid: a novel, though highly stress-sensitive, pathway to plant isoquinoline alkaloids has been discovered.

Summary and outlook

With the acetogenic nature of naphthylisoquinoline alkaloids established, further work on these interesting secondary metabolites will now focus on other remarkable (mostly unique) details of the biosynthesis, such as

1. the identification of the polyketide synthase, which (unless influenced by stress) always seems to produce a 1:1 ratio of the naphthalene and isoquinoline building blocks,

2. the unique incorporation of nitrogen into a ketone precursor (not into an $\alpha$-keto acid or an aldehyde, as usual), and

3. the remarkable coupling enzymes, which, for most of the alkaloids, join the two molecular halves together with high regio- and stereoselectivity.

Of similar interest is the correlation of chemo-taxonomical results with those of molecular phylogeny of African and Asian naphthylisoquinoline-producing plants (Meimberg et al., 2000), a rewarding task for the future.
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References


Fig. 9. 2D INADEQUATE NMR spectrum of dioncophylline A (1) from bond-labelling experiments with sodium $^{13}$C$_2$-acetate-visualizing intact incorporation of C$_2$-units by the pairwise $^{13}$C,$^{13}$C-correlations.


