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# The Polyketide Folding Mode in the Biogenesis of Isoshinanolone and Plumbagin from *Ancistrocladus heyneanus* (Ancistrocladaceae)[1]

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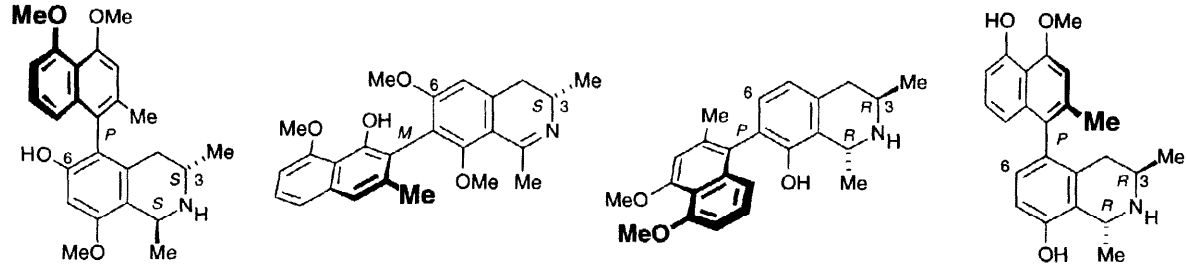
***Abstract***: The biosynthetic origins of the tetralone isoshinanolone and the related naphthoquinone plumbagin were investigated by feeding [$^{13}C_2$]-acetate to suspended callus cultures of *Ancistrocladus heyneanus*. The orientation of the acetate subunits was elucidated by a similar experiment using [2-$^{13}$C]-acetate. The polyketide folding mode found for isoshinanolone and plumbagin constitutes a further hint at the acetogenic nature of the naphthylisoquinoline alkaloids, which are typical of *A. heyneanus* and other species of Ancistrocladaceae and Dioncophyllaceae.


Ancistrocladaceae and the closely related Dioncophyllaceae are small families of palaeotropical lianas and the as yet only known sources of naphthylisoquinoline alkaloids, unique naturally occurring biaryls with marked biological activities (*i.a.* antimalarial and anti-HIV activities).[2,3] Thus, *Ancistrocladus heyneanus*, an Ancistrocladaceae species endemic to India, produces differently coupled naphthylisoquinolines such as ancistrocladine (**1**) and ancistrocladidine (**2**), which are oxygenated at C-6 and have an *S*-configuration at C-3,[4] whereas typical naphthylisoquinolines as produced by Dioncophyllaceae, are the molluscicidal dioncophylline A (**3**)[3,5] and the highly antimalarial dioncophylline C (**4**),[3,6] which are characterized by the lack of an oxygen function at C-6 and the *R*-configuration at C-3.[3]




**1** **2** **3** **4**

**Fig. 1.** Typical naphthylisoquinoline alkaloids from Ancistrocladaceae and Dioncophyllaceae.

These unusual structures hint at a likewise unprecedented biosynthetic origin of isoquinoline alkaloids from a joint open-chained hexaketide precursor **8**[2,3] (see Scheme 1), undergoing a stepwise

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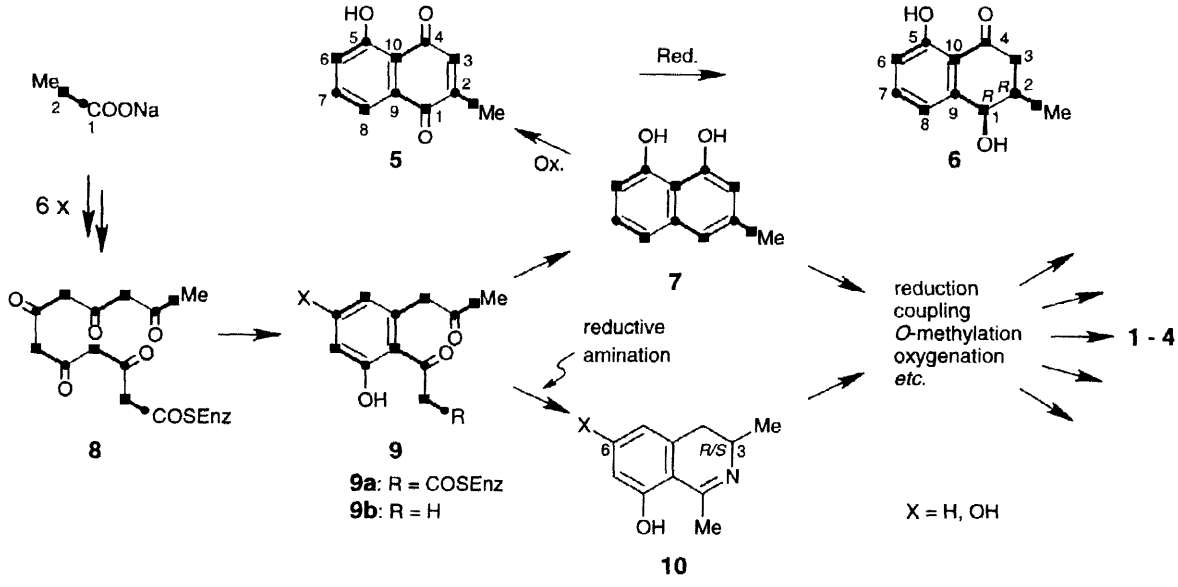
cyclization (*e.g. via* **9a** or **b**) both to the naphthalene and isoquinoline parts **7** and **10**, respectively, from which, by further modifications and the pivotal biaryl coupling step, in principle all of the known naphthylisoquinolines (including the anti-HIV active dimers, named michellamines[7,8]) should result. Previous biosynthetic feeding experiments on intact plants, as first cultivated by our group, showed only very low incorporation rates for both acetate and more specialized potential precursors like **9b** (X = H).[2,9-11] We have recently managed to grow cell cultures of *A. heyneanus*, which, regrettably, produce only very small quantities of naphthylisoquinoline alkaloids, but enhanced quantities of the naphthoquinone plumbagin (**5**) and the related tetralone isoshinanolone (**6**), instead. These two bicyclic compounds, which are well-known metabolites not only of Ancistrocladaceae and Dioncophyllaceae,[2,9,12-16] but also from closely related families (Plumbaginaceae, Droseraceae, Drosophyllaceae, Nepenthaceae),[17] should have a similar biosynthetic origin: **5** and **6** might be formed from the naphthalene moiety **7** of naphthylisoquinoline alkaloids, which, instead of being coupled to the isoquinoline part to give **1** - **4**, could be further oxidized to **5** and then reduced to **6**.[2,3] Biosynthetic results obtained on **5** and **6** should thus be of relevance for the biogenesis of naphthylisoquinoline alkaloids, too. Previous work on *Plumbago europaea* had revealed the biosynthetic origin of **5** from $^{14}$C-labelled acetate.[18] In this paper, we describe feeding experiments on the biosynthesis of both plumbagin and isoshinanolone with differently $^{13}$C-labelled acetate, establishing the folding mode of the polyketide chain.




**Scheme 1.** Proposed biosynthesis of **5** and **6** from acetate as deduced from labelling experiments, and presumable related origin of naphthylisoquinoline alkaloids.

Feeding experiments were performed on cell cultures grown from seeds germinated after surface sterilization, grown on modified liquid Linsmaier and Skoog media[19] with an addition of 5 mg/l 2,4-dichlorophenoxyacetic acid. 100 mg each of [2-$^{13}$C]-acetate or [$^{13}C_2$]-acetate were added to 100 ml liquid media with *ca.* 25 g of callus tissue, respectively, and incubated for 3 d. The calli (containing **5**) were subsequently separated from the media (containing **6**) by filtration.

Isoshinanolone (**6**) was isolated from the $CH_2Cl_2$ extracts of the media by column chromatography on deactivated silica gel ($CH_2Cl_2$), giving 1.1 mg of **6** (after feeding [2-$^{13}$C]-acetate) and 3.5 mg of **6** (after feeding [$^{13}C_2$]-acetate) as yellow oils. Plumbagin (**5**) was obtained by sublimation from the $CH_2Cl_2$ extracts of the calli (*ca.* 15 mg from each batch).

NMR data were measured on a DMX 600 spectrometer (Bruker) operating at 600 MHz $^1$H and 150 MHz $^{13}$C frequencies. The relative labelling intensities ($I_S$) were determined by comparison of the proton-decoupled $^{13}$C NMR spectra of labelled *vs.* unlabelled **5** and **6** after feeding [$^{13}C_1$]-acetate (relative peak areas standardized by the area of the peak at $\delta$ = 136.0 ppm and 136.9 ppm for **5** and **6**, corresponding to C-7[20] of the respective molecule, see Tables 1 and 2). $^1J$ ($^{13}$C,$^{13}$C) coupling constants and patterns in **5** and **6** obtained from cultures fed with [$^{13}C_2$]-acetate were determined by 1D-INADEQUATE and 2D-INADEQUATE experiments.

The $^{13}$C NMR spectrum of isoshinanolone (**6**) obtained from cultures of *Ancistrocladus heyneanus* fed with [2-$^{13}$C]-acetate showed a uniform incorporation rate of *ca.* 6% with enhanced signals at C atoms 2-$CH_3$, 1, 8, 6, 10, and 3 (Table 1). The data prove the biosynthesis of **6** from acetate subunits, the methylene positions alternating with carbonyl-derived ones in the polyketide. The coupling constants and patterns obtained from the 1D-INADEQUATE and 2D-INADEQUATE experiments with **6** obtained from cultures fed with [$^{13}C_2$]-acetate showed pairwise coupling of the C atoms 2-$CH_3$/2, 1/9, 8/7, 6/5, and 10/4. C-3 remained isolated, confirming the folding of the polyketide as in Scheme 1 and decarboxylation at C-3.

**Table 1.** $^{13}$C NMR data for labelled **6** after application of differently $^{13}$C-labelled acetate.

| | Isoshinanolone (**6**) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C atom | 2-$CH_3$ | 2 | 1 | 9 | 8 | 7 | 6 | 5 | 10 | 4 | 3 |
| $\delta$ [ppm] | 16.15 | 34.43 | 71.18 | 145.0 | 118.6 | 136.9 | 118.2 | 162.7 | 115.4 | 204.7 | 40.70 |
| $I_S$[a] [%] | 5.7 | 0.2[c] | 4.8 | 0 | 5.7 | 0 | 6.5 | 0 | 6.2 | 0 | 6.4 |
| $^1J$[b] [Hz] | 36.0 | 36.0 | 45.4 | 45.0 | 55.1 | 54.8 | 66.4 | 66.6 | 52.2 | 52.2 | (32.1)[d] |

[a] After feeding [2-$^{13}$C]-acetate. - [b] After feeding [$^{13}C_2$]-acetate. - [c] The enhancement is below the limit of significance (0.5 %), C-3 can be assumed unlabelled. - [d] Coupling disappeared when labelled acetate diluted with unlabelled acetate was applied.

Plumbagin (**5**) obtained from the feeding experiments showed an incorporation rate of *ca.* 2-3% (Table 2). The methylene positions at C atoms 2-$CH_3$, 1, 8, 6, 10, and 3, and coupling of C-atoms 2-$CH_3$/2, 1/9, 8/7, 6/5, and 10/4, while C-3 remained isolated. The labelling pattern of plumbagin (**5**) thus fully corresponds to that of isoshinanolone (**6**), suggesting a similar biogenesis of the two metabolites.

**Table 2.** $^{13}$C NMR data for labelled **5** after application of differently $^{13}$C-labelled acetate.

| | Plumbagin (**5**) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C atom | 2-$CH_3$ | 2 | 1 | 9 | 8 | 7 | 6 | 5 | 10 | 4 | 3 |
| $\delta$ [ppm] | 16.50 | 149.6 | 184.7 | 132.0 | 119.2 | 136.0 | 124.1 | 161.1 | 115.1 | 190.2 | 135.4 |
| $I_S$[a] [%] | 2.7 | 0 | 2.2 | 0 | 2.4 | 0 | 2.2 | 0 | 4.5 | 0 | 2.1 |
| $^1J$[b] [Hz] | 43.9 | 43.9 | 51.3 | 51.6 | 55.2 | 55.2 | 66.1 | 66.5 | 53.9 | 54.1 | (50.2)[d] |

[a,b,d] See Table 1.

These feeding experiments establish the folding mode for the intermediate polyketide chain **8** in the biogenesis of **5** and **6**. Analogous investigations on the biosynthetic origin of the naphthylisoquinoline alkaloids are in progress.

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20. Contrary to the IUPAC rules, but for simpler comparability of the incorporation sites, **5** and **6** are given analogous atom numbers in this paper.