Early Precursors in the Biosynthesis of Cularine-type Benzylisoquinoline Alkaloids

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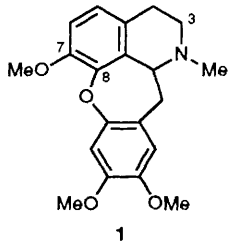
Cularine-type benzylisoquinoline alkaloids containing 7,8-substitution in the A-ring (crassifoline, cularine) are biosynthetically formed by an atypical and enzymatically catalysed Pictet–Spengler analogous (*ortho*) condensation of dopamine and an appropriate aldehyde.

The cularine-type alkaloids present a small group of benzylisoquinoline alkaloids which possess an intramolecular ether linkage between the A-ring of the isoquinoline nucleus and the 1-benzyl group.[1] The presence of this ether bridge is associated with the unusual 7,8-oxygenation pattern of the isoquinoline system. Cularine **1** and its congeners could be biosynthesized by at least one of two routes,[2] either by oxidative phenolic coupling of a preformed 1-benzylisoquinoline, containing already the 7,8-oxygenation pattern in the A-ring, or by phenolic coupling of a bis(2-phenylethyl)amine **2** followed by oxidative closure of the nitrogen-containing ring.[3] The former hypothesis proved to be correct in that it was shown that the 1-benzylisoquinoline crassifoline **3** is the specific precursor of the cularine-type alkaloids.[4,5] Crassifoline **3**, with its unusual 7,8-substitution pattern, could be formed either from the 6,7-substituted (*S*)-reticuline **4**, the principal precursor of isoquinoline alkaloids,[6] by hydroxylation at position 8 and subsequent dehydroxylation of position 6,[7] or by an unusual enzymatically catalysed Pictet–Spengler/



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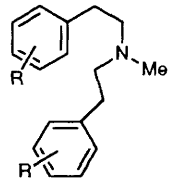

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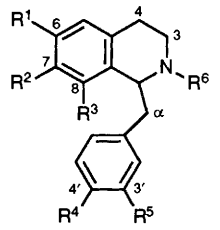
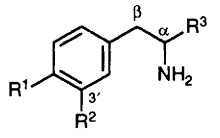
Bischler–Napieralsky-reaction with *ortho*-cyclisation possible yielding immediately the benzylisoquinoline precursor with 7,8-substitution. Furthermore, the biosynthetic origin of both the isoquinoline and the benzyl portion of the crassifoline **3** molecule should be explored. The question is whether or not this precursor molecule is also derived from two unequal C_6–C_2 units, as it has been known from 6,7-substituted isoquinolines.[8]



3; $R^1 = H$, $R^2 = R^4 = OMe$, $R^3 = R^5 = OH$, $R^6 = Me$
4; $R^1 = R^4 = OMe$, $R^2 = R^5 = OH$, $R^3 = H$, $R^6 = Me$
9; $R^1 = R^5 = R^6 = H$, $R^2 = R^3 = R^4 = OH$



5; $R^1 = OH$, $R^2 = H$, $R^3 = CO_2H$
6; $R^1 = R^2 = OH$, $R^3 = CO_2H$
7; $R^1 = OH$, $R^2 = R^3 = H$
8; $R^1 = R^2 = OH$, $R^3 = H$

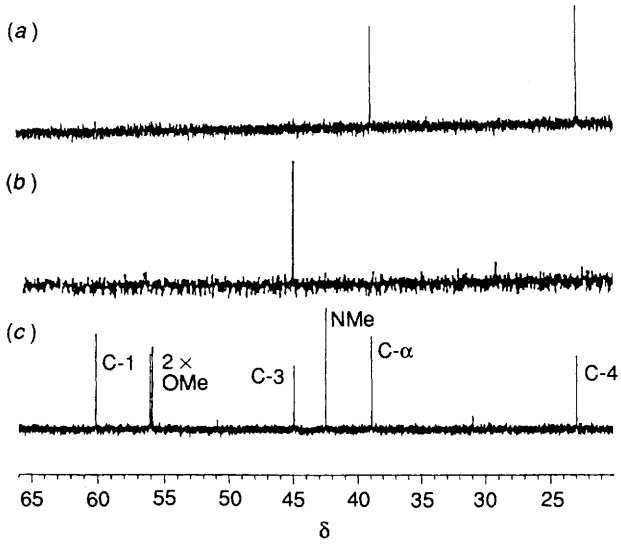


Fig. 1 Proton-decoupled ^{13}C NMR partial spectra of crassifoline **3**. (*a*) Biosynthesized from L-[β-^{13}C]tyrosine **5**; biosynthesized from [α-^{13}C]tyramine **7**; (*c*) unlabelled crassifoline **3**.

Feeding experiments of potential [^{13}C]-labelled precursors to small branches (10 g fresh weight) of the climbing *Corydalis claviculata* plants (feeding period of 72 h, at 21 °C and 80% humidity, normal day and night cycle) resulted in satisfactory incorporation of label into crassifoline **3**. Isolated by standard procedures[5] an average of 250 μg spectroscopically pure (UV, mass) **3** was obtained which was sufficient for ^{13}C NMR analysis of the enhanced signals (90 MHz, Bruker Aspect 3000) in $CDCl_3$.

[^{13}C]Precursors were synthesized by standard techniques and the correct position of the label verified by NMR analysis. L-[β-^{13}C]Tyrosine **5** yielded crassifoline **3** which was labelled at C-3 and C-α [Fig. 1(*a*), enrichment of 10% ^{13}C and 8% ^{13}C atom excess respectively, calculated from mass spectral data]. In contrast, crassifoline **3** was labelled only in the isoquinoline portion by L-[3′-^{18}O]DOPA (DOPA = 3,4-dihydroxyphenylalanine) **6** (yielding 26% enrichment at O-8), by [α-^{13}C]tyramine **7** (12.5% enrichment at C-3) and by [α-^{13}C]dopamine **8** (19% enrichment at C-3), without scrambling of the label to other positions. This labelling pattern corresponds exactly to the situation found in the 6,7-substituted benzylisoquinoline alkaloids[8,9] and clearly demonstrates also for the 7,8-substituted cularine-type alkaloids and their precursors that the isoquinoline and benzyl portion have

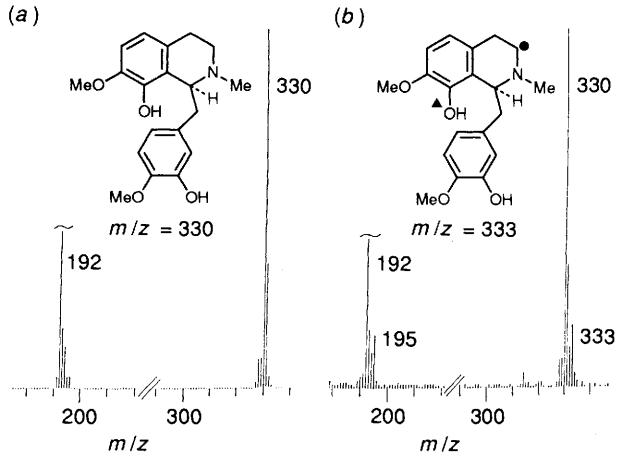
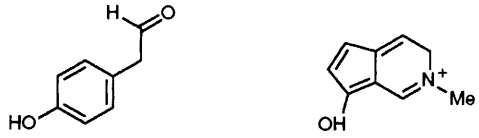


Fig. 2 Partial mass spectra (chemical ionization; isobutane) of crassifoline **3**. (*a*) Unlabelled crassifoline; (*b*) crassifoline isolated after a feeding experiment with [3′-^{18}O, α-^{13}C]dopamine. ●, ^{13}C-label; ▲, ^{18}O-label.



11 **10**

different biogenetic origins. By analogy to the situation of the reticuline pathway[9–12] it is to be expected that not a 7,8,3′,4′-tetraoxygenated but rather a 7,8,4′-trioxygenated intermediate **9** is the first alkaloidal intermediate in the crassifoline–cularine alkaloid pathway.

In order to prove whether an unusual Pictet–Spengler/Bischler–Napieralsky-type reaction is operative in this plant yielding the 7,8-oxygenated isoquinoline precursor by cyclisation *ortho* to the oxygen function in dopamine **8**, doubly labelled [3′-^{18}O, α-^{13}C]dopamine with 90% ^{18}O and 99% ^{13}C atom excess respectively was synthesized. For the introduction of the ^{18}O-label into 3-acetyl-4-hydroxyphenyl-[1-^{13}C]acetonitrile,[13] the Dakin oxidation using $H_2{}^{18}O_2$ was used. If crassifoline **3** was biosynthesized *via* 8-hydroxylation and 7-dehydroxylation, the oxygen label should be lost in the transition of dopamine into **3**. If both labels are retained, however, dopamine is directly incorporated into a 7,8-oxygenated cyclisation product **9**. Feeding experiments of the doubly labelled dopamine to *C. claviculata* plants yielded a 19% incorporation into **3**. NMR analysis of crassifoline **3** showed clearly that only C-3 of this alkaloid contained ^{13}C-enrichment (14% incorporation by MS). No scrambling of the label to other carbon atoms had occurred. Mass spectral analysis (chemical ionization) of **3** (which was separated from any isomer by GC and its purity confirmed) showed a protonated molecular ion peak $(M + H)^+$ with $m/z = 330$ in an unlabelled control, while the sample from the labelling experiment showed clearly an additional isotope peak at $m/z = 333$ (Fig. 2).

High-resolution measurement MS yielded the required peak of $m/z = 333.1745$ demonstrating clearly the incorporation of both the [^{13}C]- and the [^{18}O]-label. The fragmentation pattern of crassifoline **3** yielded a fragment ion with $m/z = 148$ **10**, which contained only the single oxygen originally present at C-3′ of dopamine. Beyond any doubt, [3′-^{18}O, α-^{13}C]dopamine is incorporated intact into crassifoline **3**. Isolation of cularine **1** from the same feeding experiment showed an incorporation of dopamine **8** of 6%. C-3 of this alkaloid **1** was specifically ^{13}C-labelled and confirmed the role of **3** as precursor[4,5] to the cularine alkaloid family.

Based on the results presented here, the cularine-type alkaloids with the 7,8-dioxygenation pattern are formed from two different C_6–C_2 precursors analogous to the 6,7-substi-

tuted isoquinoline alkaloids.[8] Tyrosine supplies both halves of the cularine alkaloids, while DOPA, dopamine and tyramine are incorporated only into the isoquinoline half of this alkaloid class. The unequal distribution of label between the two halves of the benzylisoquinolines is not compatible with symmetrical intermediates of type **2**. It is postulated that a 7,8,4′-trioxygenated intermediate **9** is the first biosynthetic precursor to the cularine alkaloids. A 6,7-substituted precursors for the cularine alkaloids could be excluded by feeding experiments using [3′-^{18}O, α-^{13}C]dopamine which clearly demonstrated the incorporation of ^{18}O into **1** and **3**. The key entry reaction for dopamine might be an enzymatic condensation of dopamine **8** with 4-hydroxyphenylacetaldehyde **11** to yield **9** in an unusual *ortho* cyclisation.

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