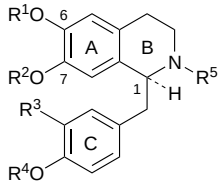
(*S*)-Norreticuline is the precursor for the biosynthesis of *Erythrina* alkaloids

Ulrich H. Maier and Meinhart H. Zenk*

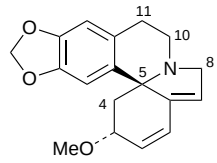
Lehrstuhl für Pharmazeutische Biologie, Universität München, Karlstrasse 29, D-80333 München, Germany

In contrast to previous assumptions, the isoquinoline alkaloid (*S*)-norreticuline is the biosynthetic precursor to *Erythrina* alkaloids and, furthermore, this precursor is asymmetrically incorporated into these spirocyclic alkaloids.

Erythrina-type alkaloids[1] suggest an unusual biosynthesis. Barton and co-workers[2,3] made the important observation that these spirocyclic alkaloids are formed by skeletal rearrangement from benzylisoquinoline alkaloids. Extensive application experiments of potential precursors to *Erythrina crista-galli* (Fabaceae) plants previously showed that (*S*)-norprotosinomenine **1** was the precursor for the *Erythrina* skeleton of erythraline **2**, although the incorporation was only in the range of 0.25%.[3–5] This finding was supported by the reported successful incorporation of labelled **1** into the *Erythrina* alkaloid cocculidine **3** (up to 0.66%) using plants of the taxonomically remote *Cocculus laurifolius* (Menispermaceae).[6,7] Norprotosinomenine **1** is a natural product and was isolated from *Erythrina variegata* (formerly *lithosperma*) fruits together with alkaloids possessing the *Erythrina*-type skeleton.[8]



1 R^1 = H, R^2 = Me, R^3 = OH, R^4 = Me, R^5 = H
4 R^1 = Me, R^2 = H, R^3 = H, R^4 = H, R^5 = H
5a R^1 = Me, R^2 = H, R^3 = OH, R^4 = Me, R^5 = H
5b R^1 = Me, R^2 = H, R^3 = OH, R^4 = Me, R^5 = Me
6 R^1 = Me, R^2 = H, R^3 = OMe, R^4 = H, R^5 = H
7 R^1 = H, R^2 = Me, R^3 = OMe, R^4 = H, R^5 = H
8 R^1 = H, R^2 = H, R^3 = H, R^4 = H, R^5 = H



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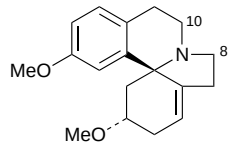

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In the past, only 6-methoxy-7-hydroxytetrahydrobenzylisoquinoline alkaloids of the coclaurine **4** and reticuline **5b** type were recognized as biosynthetic precursors of a multitude of isoquinoline alkaloids occurring in plants such as aporphine-, benzophenanthridine-, bisbenzylisoquinoline-, morphinane-, pavine- and protoberberine-type alkaloids.[9] Norprotosinomenine **1** with a differing 6-hydroxy-7-methoxy substitution pattern is up to now the only biosynthetic exception to a phenol-coupled group of alkaloids and would require evolutionarily the development of a completely different and specific set of enzymes for the early steps of *Erythrina*-type alkaloid biosynthesis, since the enzymes of the coclaurine **4**–reticuline **5b** pathway are highly specific and do not accept alkaloidal substrates with the 6-hydroxy-7-methoxy pattern. This fact and the unusual carbon skeleton of the spirocyclic alkaloids prompted us to reinvestigate the structure of the precursors of *Erythrina* alkaloids.

Feeding experiments (cotton wick method)[3] with all four isomeric nortetrahydrobenzylisoquinoline alkaloids **1**, **5a**, **6** (nororientaline) and **7** (norisoorientaline), each tritium-labelled in the 6- or 7-methoxy group of ring A, were conducted using 4–5 month old plants, the same age as were used previously.[3] Erythraline **2**, the major alkaloid which is distributed in all parts of *E. crista-galli*, was isolated and found to be unlabelled (< 0.0001%). Incubation of root, stem, leaf and flower tissue yielded the same result. Physiological transport experiments using ^{3}H-labelled **4**–**7** showed that these potential precursor alkaloids were not transported at all in these plants. As a consequence, tissue slices (3–4 mm thick) were allowed to float on the aqueous tracer solution (0.5 μCi; 25 nmol) under constant shaking (150 strokes min^{-1}) for 24 h. Again, all vegetative tissue yielded no incorporation into erythraline **2**. However, fruit tissue showed considerable incorporation into erythraline **2** (11.8%) but only with (*S*)-norreticuline **5a** as precursor and not with **1**, **6**, **7**. All of the (*R*)-configurated potential precursors **1**, **5a**, **6**, **7** as well as their N-Me congeners were not incorporated. (*S*)-[6-OC^3H_3]Coclaurine **4**, however, was under these conditions incorporated to 11%. This clearly suggests that a coclaurine **4**–norreticuline **5a** pathway is operative in *Erythrina* alkaloid formation while the hitherto assumed norprotosinomenine **1** is not at all metabolized (confirmed by autoradiography of the fruit and other organ extracts). In spite of the high rate of incorporation of **4** and **5a** into the *Erythrina* alkaloids it could not be excluded that de- and re-methylation of potential precursors occurred under these feeding conditions as previously observed in other systems.[10]

To confirm specific incorporation and also to reinvestigate whether the later biosynthetic pathway to *e.g.* erythraline involves a symmetrical intermediate,[4,5] (*S*)-[1-^{13}C]-**1**,-**4**,-**5a**,-**6**,-**7** were synthesized by standard methods,[11] the correct position of the label was verified in each case by NMR analysis and the compounds applied at 10^{-4} M concentration to fruit slices (dry weight 18.2 g) as above. By isolation an average of 6 mg spectroscopically pure (UV, mass) erythraline **2** was obtained which was used for ^{13}C NMR analysis of the enhanced signals (90.6 MHz, Bruker Aspect 3000) in $CDCl_3$. (*S*)-[1-^{13}C]Norprotosinomenine **1** yielded erythraline **2** that was unlabelled [Fig. 1(*a*)]. In contrast, (*S*)-[1-^{13}C]coclaurine **4** yielded **2** that was labelled at C-10 [Fig. 1(*b*), enrichment of 5.5% ^{13}C, ^{13}C atom excess calculated from mass spectral data]. (*S*)-[1-^{13}C]Norreticuline **5a** also labelled only C-10 of erythraline **2** [Fig. 1(*c*), enrichment of 9.3%]. If a symmetrical intermediate is occurring *en route* to the later steps to erythraline as postulated before,[4,5] ^{13}C enrichment in the case of the **4** and **5a** feeding experiment would have occurred both at C-10 and C-8. Since at C-8 no enrichment is seen [Fig. 1(*b*), (*c*)], we have to conclude that *Erythrina* alkaloid biosynthesis does *not* involve a symmetrical intermediate of the diphenoquinone type. The previously observed labelling both of C-8 and C-10 of an *Erythrina*-type alkaloid after feeding [2-^{14}C]tyrosine[12] is easily explained. Norcoclaurine **8**, the precursor of coclaurine **4** and norreticuline **5a**, is formed from two different

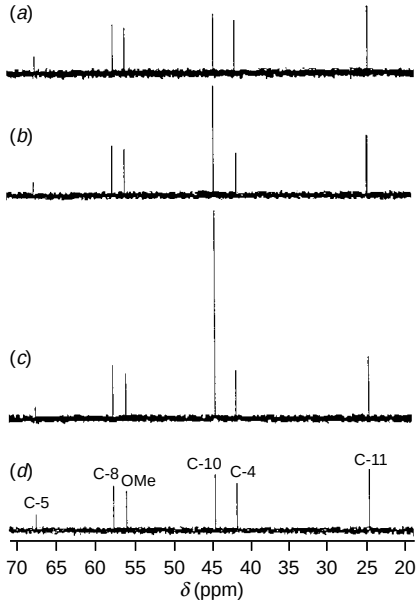
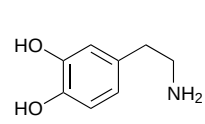


Fig. 1 Proton-decoupled ^{13}C NMR partial spectrum of erythraline **2** (*a*) isolated after a feeding experiment with (*S*)-[1-^{13}C]norprotosinomenine **1**; (*b*) biosynthesized from (*S*)-[1-^{13}C]coclaurine **4**; (*c*) biosynthesized from (*S*)-[1-^{13}C]norreticuline **5a**; (*d*) unlabelled erythraline **2**

C_6–C_2 precursors analogous to all other 6,7-substituted isoquinoline alkaloids found in nature.[9,13,14] Tyrosine supplies both halves of the *Erythrina* alkaloids, while dopamine **9** is incorporated into the isoquinoline half of **4**, **5a**, **8** only. The benzyl part of these precursors is derived from 4-hydroxyphenylacetaldehyde **10** which in turn is also derived from tyrosine *via p*-hydroxyphenylpyruvate and subsequent decarboxylation to yield the aldehyde.[9] Thus the symmetrical labelling of an *Erythrina* alkaloid by [2-^{14}C]tyrosine in the reported experiment[12] is explained.

Finally, to probe the experiments reported on the incorporation of norprotosinomenine **1** into the *Erythrina*-type alkaloids in *Cocculus laurifolius*[6,7] we repeated these application experiments with the same plant. Again no incorporation of [1-^{13}C]-**1** into any of the *Cocculus* alkaloids was observed. **1** is, therefore, not a precursor to *Erythrina* alkaloids.



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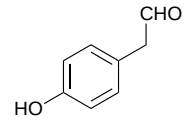

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Future work on the biosynthesis of *Erythrina* alkaloids has to concentrate on the enzymes involved in (*S*)-norreticuline **5a** formation and on the later steps beyond precursor **5a** involving a non-symmetrical intermediate.

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Footnote and References

* E-mail: zenk@lrz.uni-muenchen.de

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