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BIOSYNTHESIS OF THALICARPINE IN *THALICTRUM MINUS*

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Key Word Index—*Thalictrum minus*; Ranunculaceae; biosynthesis; alkaloids; thalicarpine; reticuline; isoboldine.

Abstract—Feeding experiments with ^{14}C-labelled reticuline, protosinomenine, orientaline, their *N*-nor-analogues and ^{3}H-labelled isoboldine have shown reticuline and isoboldine to be the most efficient precursors of thalicarpine in *Thalictrum minus*. A biosynthetic pathway for thalicarpine with reticuline and isoboldine at the benzylisoquinoline and aporphine stages respectively has been suggested. Support for this proposal has been provided by the demonstration by radioisotopic dilution that reticuline and isoboldine are minor constituents of the plant.

INTRODUCTION

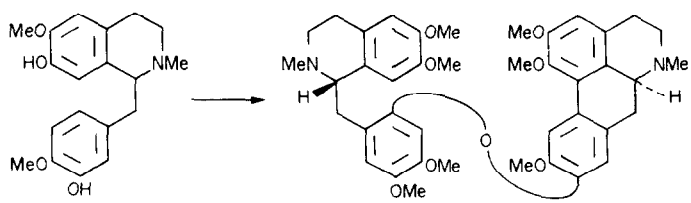
Recently [1] we demonstrated that radioactive reticuline (**1**) is incorporated into thalicarpine (**2**) (Scheme 1) by intact plants of *Thalictrum minus*. Chemical degradation located the label in both the aporphine and benzylisoquinoline moieties of **2**.

In the present paper, we report on the incorporation of the other possible benzylisoquinoline precursors and of an aporphine assumed to be an intermediate in their biological conversion to thalicarpine.

RESULTS AND DISCUSSION

The conversion of a benzylisoquinoline into thalicarpine is likely to be accomplished by phenolic oxidation by one or both of the two possible biosynthetic pathways represented by sequence A (Scheme 2): benzylisoquinolines (**3**) and (**4**)→bisbenzyl isoquinoline (**5**)→proaporphine–benzylisoquinoline (**6**)→aporphine–benzylisoquinoline (**2**); and sequence B (Scheme 3): benzylisoquinoline (**1**)→aporphine (**7**) and (**8**)→aporphine–benzylisoquinoline (**2**). The discovery of the proaporphine-benzylisoquinoline alkaloid pakistanamine and several of its aporphine-benzylisoquinoline analogues by Shamma *et al.* [2–4] in a number of *Berberis* spp., allowed him to suggest a biogenetic scheme, corresponding to sequence A for the biosynthesis of this alkaloid type in *Berberis* spp. Since (+)-reticuline (**1**) [5–7], laudanidine (**9**) [5, 8], (+)-isoboldine (**7**) [9] and (+)-*N*-methyllaurotetanine (**8**) [5, 10, 11] and none of the intermediates according to sequence A have been found to co-occur with thalicarpine in *Thalictrum* spp., we considered that sequence B was the more likely one to operate in *T. minus*. Taking into consideration the substitution pattern of thalicarpine (**2**), as well as the position of its oxygen bridge, orientaline (**10**) and protosinomenine (**11**) can also be regarded as possible precursors of its aporphine moiety. The substitution pattern of the base **12** is less suitable for conversion into an aporphine structure (Scheme 4).

We synthesized ^{14}C-labelled orientaline, protosinomenine, as well as their *N*-nor-analogues and fed them separately to intact plants of *T. minus* just prior to flowering. Similar experiments with labelled reticuline and nor-reticuline were performed simultaneously. The results obtained are summarized in Table 1. The nearly 10-fold higher rate of incorporation of reticuline compared to that of protosinomenine (**11**) can be only partially due to its incorporation into both parts of the thalicarpine molecule. Obviously, reticuline is a more efficient



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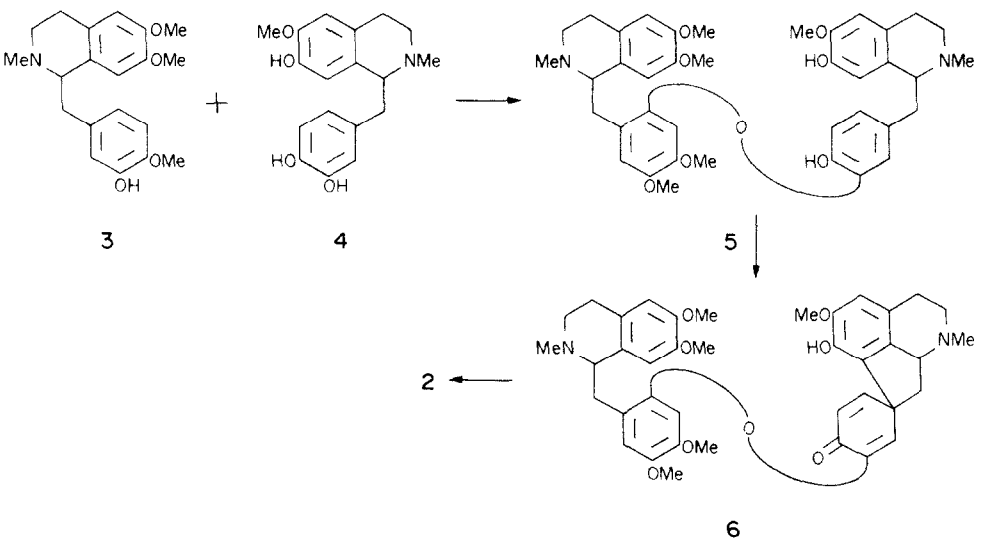
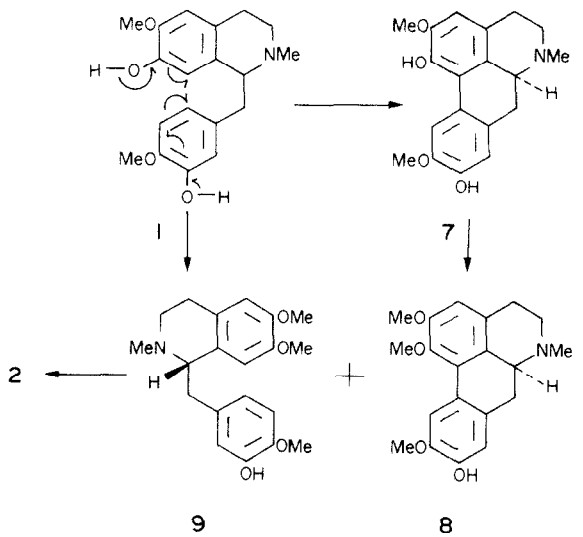
Scheme 1. Formation of thalicarpine from reticuline.



Scheme 2. Sequence A for the biosynthesis of thalicarpine (2).



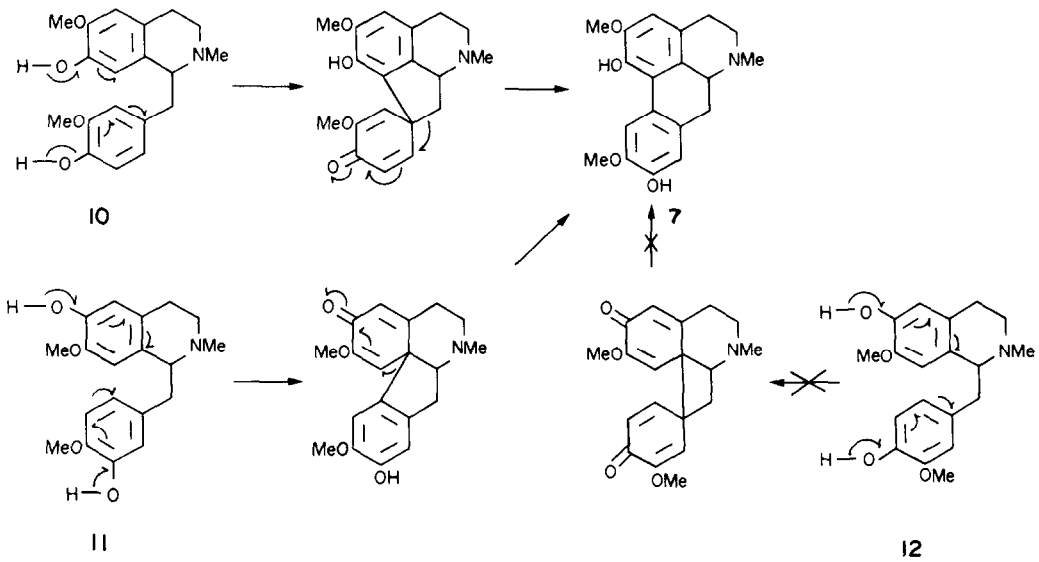
Scheme 3. Sequence B for the biosynthesis of thalicarpine (2).

precursor of the aporphine moiety than **11**, i.e. an *o,p*-rather than a *p,p*-oxidative cyclization is the preferred one for the formation of this moiety.

The aporphine alkaloid isoboldine (**7**) is an intermediate in the transformation of reticuline (**1**) into thalicarpine (**2**) according to sequence B. This substance or its 1-*O*-methyl derivative (**8**) has a phenolic group in the position required for formation of the oxygen bridge of thalicarpine by intermolecular oxidative coupling with a suitably substituted phenolic benzylisoquinoline. (+)-[8-^{3}H]Isoboldine was prepared by selective demethylation of (+)-glaucine [12] followed by proton exchange in $^{3}H_2O$. When administered out of season to an intact plant of *T. minus*, it gave rise to radioactive thalicarpine with an incorporation (0.50%) high enough to suggest that this aporphine is an efficient precursor in thalicarpine biosynthesis and to support sequence B as the one which operates in *Thalictrum* spp. Additional support for the biosynthetic route given by sequence B was sought by a search for reticuline and isoboldine in *T. minus*. Though reticuline and isoboldine have not

Table 1. Incorporation of ^{14}C-labelled precursors into thalicarpine

Precursor	Total activity of precursor (10^{-6} × dpm)	Total activity of isolated thalicarpine (10^{-2} × dmp)	Incorporation (%)
(±)-[1-^{14}C]Reticuline	4.8	8500	1.76
(±)-[1-^{14}C]Norreticuline	8.9	9900	1.12
(±)-[1-^{14}C]Protosinomenine	3.5	630	0.16
(±)-[1-^{14}C]Norprotosinomenine	6.4	6.4	0.01
(±)-[1-^{14}C]Orientaline	9.1	180	0.02
(±)-[1-^{14}C]Nororientaline	5.9	5.9	0.01
(+)-[8-^{3}H]Isoboldine	2.6	1300	0.50



Scheme 4. Formation of isoboldine (**7**) from either orientaline (**10**) or protoinomenine (**11**).

been found in *T. minus* so far, its detection in other *Thalictrum* spp. is in accordance with the results of our incorporation experiments. Our attempts to detect them in the alkaloidal fraction of *T. minus* by means of conventional phytochemical methods failed. However, positive results were obtained by the isotopic dilution method. The constant specific radioactivity exhibited after recrystalization of reticuline and isoboldine (as an oxalate and picrate respectively) isolated from a plant incubated in a $^{14}CO_2$ atmosphere provided evidence for their presence in *Thalictrum* spp.

Summarizing the results obtained, it can be concluded that the biosynthesis of thalicarpine, the major aporphine-benzylisoquinoline aklaloid in *T. minus*, operates by the sequence: reticuline → isoboldine → thalicarpine, presumably via *N*-methyllaurotetanine and laudanidine.

EXPERIMENTAL

Counting methods. ^3H and ^{14}C were measured by liquid scintillation counting. Samples were counted in 5 ml scintillation fluid (1 l. dioxan, 0.3 g POPOP, 7 g PPO and 100 g naphthalene). The counts obtained were not corr. for self-absorption. The incorporations of activity were calc. as:

$$\frac{100 \times \text{Total activity of isolated thalicarpine}}{\text{Total activity of precursor fed}}.$$

For this purpose the final constant sp. act. (cpm/mg) of the thalicarpine was used and this was multiplied by the quantity (mg) of thalicarpine isolated which was of good chemical purity.

Synthesis of benzylisoquinoline precursors. (±)-[1-^{14}C]Reticuline, (±)-[1-^{14}C]orientaline, (±)-[1-^{14}C]protosinomenine and their (±)-[1-^{14}C]-*N*-nor-analogues were prepared by standard methods [13–15].

(+)-Isoboldine. (+)-Glaucine (3 g), dry $AlCl_3$ (4.2 g) and nitrobenzene (18 ml) were heated for 20 min at 120°. After cooling Si gel (10 g) was added and the black powder obtained was eluted from a short column with petrol. After removing the first fraction containing nitrobenzene, alkaloids were eluted with an Et_2O–NH_3 mixture. The crude alkaloidal mixture was chromatographed on a column of Al_2O_3 (neutral) using Et_2O followed by Et_2O–MeOH. 430 mg (+)-isoboldine was obtained.

Tritiation of (+)-isoboldine. (+)-Isoboldine (60 mg) in ^3H_2O (0.6 ml; activity 25 mCi) containing potassium-*t*-butoxide (75 mg) was heated under N_2 (sealed tube) for 100 hr at 100°. The (+)-[8-^{3}H]isoboldine was purified through its hydrochloride after crystallization from MeOH to constant sp. act. The position of the label was determined by NMR spectroscopy of deuteriated (+)-isoboldine obtained under the same conditions with D_2O.

Feeding experiments. Labelled compounds, as aq. solns of the hydrochlorides, were introduced through cotton wicks passed through the stems of young vigorously growing *T. minus* plants. The plants were left 14 days and then worked up for thalicarpine.

Isolation and purification of thalicarpine. Stems and leaves of the plants were extracted with MeOH. The combined alcoholic extracts were concd under red. pres. to give a greenish viscous mass which was extracted with 5% HCl (5 × 20 ml). The aq. acidic soln was defatted with C_6H_6 (4 × 20 ml), basified (pH 10) with NH_3 and then extracted with $CHCl_3$ (3 × 25 ml). The combined $CHCl_3$ extracts were washed with H_2O, dried (Na_2SO_4) and evaporated to afford a crude alkaloid mixture, which was then subjected to prep. TLC [Si gel-G (Merk), $CHCl_3$–petrol–MeOH–Me_2CO, 4 : 4 : 1 : 1 : 1). The isolated thalicarpine was further converted into its picrate (mp 151–152°) and crystallized from EtOH to constant sp. act.

Isotopic dilution. Three *T. minus* plants were incubated for 7 hr in an atmosphere of $^{14}CO_2$ (3 mCi). After 36 hr the plants were harvested and the material percolated in MeOH (300 ml) containing (±)-reticuline (50 mg) and (+)-isoboldine (50 mg). The combined MeOH extracts were worked up to afford a crude alkaloidal mixture. After prep. TLC (as above) the reticuline and isoboldine were isolated, converted into their oxalate (mp 156–157°) and picrate (mp 163–165°) respectively and further crystallized from EtOH to constant sp. act.: reticuline, 1.4×10^5 dpm/mmol; isoboldine, 6.9×10^4 dpm/mmol.

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