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BIOSYNTHESIS OF ADIANTIFOLINE AND THALMELATIDINE IN *THALICTRUM MINUS*

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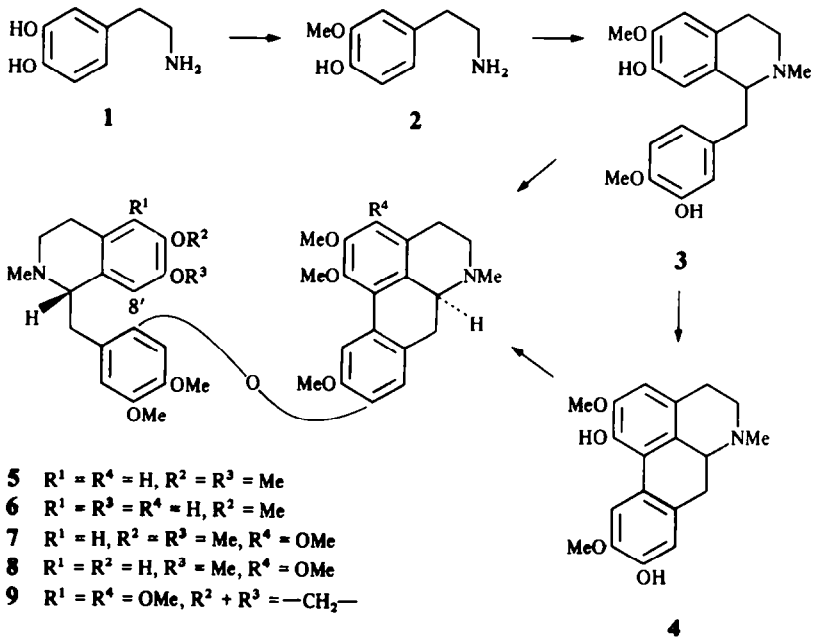
Key Word Index—*Thalictrum minus*; Ranunculaceae; biosynthesis; alkaloids; thalicarpine; thalmelatine; adiantifoline; thalmelatidine.

Abstract—Feeding experiments with *Thalictrum minus* using ^{14}C-labelled reticuline and ^{3}H-labelled thalicarpine and thalmelatine afforded radioactive adiantifoline and thalmelatidine. Our results led to the conclusion that the substitution pattern of these *Thalictrum* alkaloids can be formed at the stage of an aporphine-benzylisoquinoline structure.

INTRODUCTION

In a recent publication [1] we reported the results of our incorporation study leading to the conclusion that the substitution pattern of thalicarpine (**5**), the major aporphine-benzylisoquinoline alkaloid of *Thalictrum minus* subsp. *majus* (Ranunculaceae) is formed at the phenethylamine (**1–2**) stage. Thalicarpine (**5**) was proved to be formed through direct phenolic oxidative coupling of a fully formed 1,2,9,10-tetraoxygenated aporphine (isoboldine, **4**) with a tetrahydrobenzylisoquinoline (reticuline, **3**) [2–4] as shown in Scheme 1. The data obtained by Bhakuni *et al.* [5] were in accordance with ours.

Thalictrum minus is known to contain in its roots another group of aporphine-benzylisoquinoline alkaloids with adiantifoline (**7**) and thalmelatidine (**9**) as its representatives, whose substitution pattern is characterized by the presence of one or two additional oxygen-bearing substituents located at C-3 and C-5 [6–8]. Formation of an aporphine-benzylisoquinoline skeleton of this group of alkaloids is probably accomplished following the



5 $R^1 = R^4 = H$, $R^2 = R^3 = Me$
6 $R^1 = R^3 = R^4 = H$, $R^2 = Me$
7 $R^1 = H$, $R^2 = R^3 = Me$, $R^4 = OMe$
8 $R^1 = R^2 = H$, $R^3 = Me$, $R^4 = OMe$
9 $R^1 = R^4 = OMe$, $R^2 + R^3 = -CH_2-$

Scheme 1.

pathway established for thalicarpine (5). Here we report the results of our incorporation study concerning the biosynthetic stage at which these additional substituents are introduced.

RESULTS AND DISCUSSION

Introduction of these additional oxygenated substituents might be accomplished: (a) at the stage of an aporphine-benzylisoquinoline, such as thalicarpine (5) or thalmelatine (6), which is the second major alkaloid of this type found in *Thalictrum minus* [9] and whose hydroxyl and methoxyl groups are at the appropriate positions for formation of the methylenedioxy group of thalmelatidine (9). It would be interesting to know whether thalicarpine (5) can be converted into thalmelatine (6) by *O*-demethylation or *vice versa*. (b) At the benzylisoquinoline (reticuline, 3), and/or aporphine (isoboldine, 4) stage. (c) At as early a stage as phenethylamine (2).

(±)-[1-^{14}C]Reticuline, [8′-^{3}H]thalicarpine and [8′-^{3}H]thalmelatine were separately fed to intact plants of *Thalictrum minus*. The data obtained (Table 1) indicate that both thalicarpine (5) and thalmelatine (6) are incorporated into adiantifoline (7) and thalmelatidine (9). Reticuline (3), already proved to be a precursor of thalicarpine, is also incorporated into adiantifoline presumably via thalicarpine and is diluted by the large pool of the latter, which accounts for its small incorporation. The large incorporation of thalmelatine into thalicarpine and the lack of incorporation of thalicarpine into thalmelatine (Table 1), indicate that the latter is not produced by *O*-demethylation of thalicarpine, while the reverse conversion is operating in the plant.

The incorporation of thalmelatine (6) and thalicarpine (5) into adiantifoline (7) and thalmelatidine (9) observed in our experiments supports the suggestion that the substitution pattern of adiantifoline and thalmelatidine is formed at the aporphine-benzylisoquinoline stage. The smaller incorporation of thalmelatine (6) into thalmelatidine (9) than that of thalicarpine (5) is an indication that the *ortho* hydroxyl–methoxyl substitution of the former, apparently favourable for the formation of the methylenedioxy group of thalmelatidine, does not give it any priority over thalicarpine (5). The formation of the methylenedioxy group of thalmelatidine (9) possibly involves another hydroxy–methoxyl intermediate, such as *O*-desmethyladiantifoline (8), an alkaloid also found to occur in *Thalictrum minus* [6, 8].

On the basis of these results it can be concluded that the following biogenetic pathway is operating in *Thalictrum minus*: reticuline → thalmelatine → thalicarpine → adiantifoline, thalmelatidine.

It is noteworthy that our investigation with the precursors reticuline, thalmelatine and thalicarpine revealed a comparatively small incorporation. Elucidation of the reasons for this observation, as well as the contribution of a pathway starting from an appropriately three-substituted phenethylamine, is the subject of our work now in progress.

Table 1. Tracer experiments on *Thalictrum minus*

Precursor	Isolated product	Specific incorporation (%)
[8′-^{3}H]Thalicarpine	Thalmelatine	0
	Adiantifoline	0.48
	Thalmelatidine	0.14
[8′-^{3}H]Thalmelatine	Thalicarpine	2.62
	Adiantifoline	0.13
	Thalmelatidine	0.06
(±)-[1-^{14}C]Reticuline	Adiantifoline	0.10

EXPERIMENTAL

Counting methods. The radioactivities were determined by liquid scintillation counting with a Packard (Model 3320) Tri-Carb liquid scintillation spectrometer. The counts obtained were not corrected for self-absorption.

Synthesis of the precursors. (±)-[1-^{14}C]Reticuline was prepared by a standard method [10] with a sp. act. of 2.57×10^{7} dpm/mmol. Tritium was introduced specifically at C-8′ of thalmelatine by the method of ref. [11]. Thalmelatine (45 mg) in tritiated H_2O (0.6 ml; activity 25 mCi) and DMF (0.3 ml) was heated under N_2 (sealed tube) for 100 hr at 100°. The solvent and H_2O were removed from the resulting mixture and the residue was purified by prep. TLC [plates: silica gel G (type 60) Merck, $CHCl_3$–petrol–MeOH–Me_2CO, 4:4:1:1] to give pure [8′-^{3}H]thalmelatine (28 mg) with a sp. act. of 4.87×10^{8} dpm/mmol. To a soln of [8′-^{3}H]thalmelatine (18 mg) in MeOH (1 ml) was added a soln of an excess of CH_2N_2 in Et_2O and the mixture was allowed to stand for 4 days at 5°. Evaporation of the excess reagent and the solvent left a residue, which was purified by prep. TLC [plates: silica gel G (type 60) Merck, $CHCl_3$–petrol–MeOH–Me_2CO, 4:4:1:1] to give pure [8′-^{3}H]thalicarpine (10 mg) with a sp. act. of 4.86×10^{8} dpm/mmol.

Feeding experiments. Labelled reticuline, thalicarpine and thalmelatine, as aq. solns of their hydrochlorides, were separately fed by the cotton wick method to intact two-year-old *Thalictrum minus* plants originating from the south slopes of the Balkan Mountain near the town of Sliven, Bulgaria, in the stage of blossoming. (The voucher specimen No. 1169 is deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Science, Sofia). The plants were allowed to grow for 14 days in the field and the above-ground parts were then worked up for thalicarpine and thalmelatine and the roots for adiantifoline and thalmelatidine.

Isolation and purification of the alkaloids. The isolation of the crude alkaloid mixture was carried out as previously described [2]. The crude alkaloid mixture from the above-ground parts were chromatographed on a column of neutral alumina (activity II) using an Et_2O–NH_3 mixture. The isolated thalicarpine and thalmelatine were further purified by prep. TLC [plates: silica gel G (type 60) Merck, $CHCl_3$–petrol–MeOH–Me_2CO, 4:4:1:1]. The same procedure was used for adiantifoline and thalmelatidine. Finally the isolated alkaloids were recrystallized from Et_2O several times to constant radioactivity.

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