A comparative study of the alkaloid composition of *Isopyrum thalictroides* (*Ranunculaceae*)

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Abstract. A comparative study of the alkaloid composition of *Isopyrum thalictroides* (*Ranunculaceae*) from 15 natural localities in the Sofia floristic region was carried out for the first time. On the basis of the experimental data it has been proposed to divide the species into two chemotypes.

Key words: alkaloids, chemotypes, comparative phytochemical study, *Isopyrum thalictroides*, *Ranunculaceae*

Introduction

Genus *Isopyrum* (*Ranunculaceae*) is distributed worldwide with more than sixty species, but in Europe the only representative is *Isopyrum thalictroides* L. (Moulis & al. 1977). In earlier investigations we reported on the isolation and structural elucidation of ten new dimeric and six known monomeric isoquinoline alkaloids from the roots and rhizomes and the aerial parts of *I. thalictroides* from Mt Lyulin, Bonsovi Polyani locality (Philipov & Istatkova 1997, 1999; Istatkova & Philipov 2000, 2004) (Table 2). The present work describes a comparative phytochemical study of the species from 15 natural localities in the Sofia floristic region, while the above-mentioned habitat is assigned as standard.

Materials and methods

The plant material of *I. thalictroides* was collected in March-April, during flowering time, and identified by Dr A. Vitkova, Institute of Botany, Bulgarian Academy of Sciences. The voucher specimens were deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM) (Table 1).

Neutra alumina 90 (70–230 mesh, Merck) was used for column chromatography (CC), silica gel 60 PF_{254} (Merck) for preparative thin layer chromatography (PTLC) and aluminium sheets silica gel 60 F_{254} (Merck) for thin layer chromatography (TLC). Compounds were visualized by spraying with Dragendorff's reagent.

The plant material of *I. thalictroides* from each of the 15 localities was extracted in a Soxhlet apparatus and worked up in the same manner as described in Philipov & Istatkova (1997) and Istatkova & Philipov (2000) to obtain the total alkaloid mixtures (TAM). The TAM containing tertiary alkaloids were designated with "A" and these containing quaternary alkaloids with "B". The index "1" is for roots and rhizomes and "2" for the aerial parts. The amounts of the obtained TAM were presented in Table 1. Then, each TAM was divided into fractions with a smaller number of alkaloids. Finally, TLC comparison of the alkaloids in these fractions with the alkaloids isolated and identified from *I. thalictroides* of the standard locality (No 10, Table 1) was carried out.

Each TAM A_1 was worked up by CC with hexane-Me_2CO (increasing polarity 7:1, 7:2, 7:5, 1:1, 1:2, 1:4, 1:6, 1:10 and Me_2CO) and MeOH to obtain ten combined fractions. In them the following alkaloids were detected:

Table 1. Localities of the investigated plants and qualities of the total alkaloid mixtures

No	Localities	Time of collection	Voucher specimen (SOM)	Plant material, g		TAM, mg			
				Roots and rhizomes	Aerial parts	A_1	B_1	A_2	B_2
1.	German Monastery (Mt Lozenska)	1999	157012	29	18	173.87	24.52	18.53	15.07
2.	Urvich (Mt Lozenska)	1999	157005	22	13	185.23	26.35	22.83	6.67
3.	Eastwards under peak Polovrak (Mt Lozenska)	1999	157010	18	16	348.94	35.42	37.77	3.16
4.	Lovna Cheshma (Mt Lozenska)	2002	157009	18	6	185.24	3.49	9.67	1.95
5.	Lozen Monastery (Mt Lozenska)	2002	157008	12	3	110.00	3.88	10.95	1.62
6.	Westwards under peak Polovrak (Mt Lozenska)	2002	157011	19	4	212.53	2.85	14.50	1.91
7.	Beledie Han	2000	157156	15	14	145.87	16.70	16.39	2.77
8.	Beledie Han, Dedeva Glava	2001	157002	38	10	967.99	13.91	17.38	5.85
9.	Beledie Han, Ponor village	2001	157004	10	6	46.43	2.88	7.46	3.61
10.	Beledie Han, Tsarichina village	2001	157001	23	3	390.73	130.34	5.07	1.47
11.	Makotsevo village	2001	157006	59	16	364.71	18.04	28.02	2.42
12.	Knyazhevo	2001	157007	87	25	1523.18	27.06	61.27	17.36
13.	Gradoman village (Mt Lyulin)	2001	157157	42	8	946.00	19.84	9.07	2.30
14.	Cherniya Kos (Mt Lyulin)	2001	157000	68	15	649.54	12.88	21.56	6.46
15.	Bonsovi Polyani (Mt Lyulin) - standard locality	2001	156999	51	17	473.81	8.03	30.22	4.62

– **14** and **15** in the first;
– **1**, **4** and **9** in the second and third;
– **2** in the fourth and fifth;
– **3**, **5** and **6** in the sixth to ninth;
– **7** and **8** in the tenth.

For fractions in which the alkaloids **3** and **9** were not detected see Table 2.

Each TAM A_2 was worked up by PTLC with petroleum ether-$CHCl_3$-Me_2CO-MeOH (4:8:1:2) to obtain four combined fractions. In them the following alkaloids were detected:

– **10** in the first;
– **11** in the third.

Each TAM B_1 and B_2 were directly TLC compared because of their smaller quantities. The following alkaloids were detected:

– **16** in B_1;
– **12** and **13** in B_2.

The mobile phases petroleum ether-$CHCl_3$-Me_2CO-MeOH (4:4:1:1) for the alkaloids **1**, **2**, **4**, **5**, **6**, **9**, **10**, **14** and **15**, petroleum ethter-$CHCl_3$-Me_2CO-MeOH (4:8:1:2) for **3** and **11,** and $CHCl_3$-MeOH-NH_4OH (30:8:0.4) for **7**, **8**, **12**, **13** and **16** were used.

Results and discussion

A comparative phytochemical study of the species *I. thalictroides* from 15 natural localities in the Sofia floristic region was carried out. The obtained data showed differences in the alkaloid composition of the investigated plant material. It was found out that alkaloids **3** and **9** were not present in the plant material from the localities No 3, 13, 14 and 15 (Table 2). These two alkaloids are of the bisbenzylisoquinoline type and only they have two bridges in their molecules. Furthermore, **9** is a natural compound containing a nitrogen-formyl group, as well as C-3'–C-4' double bond in its structure. The presence of direct carbon-carbon "head-to-head" bridge determines it as the first representative of a new subgroup of bisbenzylisoquinoline alkaloids. These characteristics of **9** and the absence of alkaloids **3** and **9** in the plant material from the above- mentioned localities give us grounds to divide the species *I. thalictroides* into two chemotypes:

- The first one comprising the plant material from the localities in which all alkaloids were represented.

Table 2. Distribution of alkaloids in different localities

Alkaloid (references)	Localities														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Isopyruthaline (**1**) (Philipov & Istatkova 1997)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Isopythaline (**2**) (Philipov & Istatkova 1997)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fangchinoline (**3**) (Philipov & Istatkova 1997)	++	++	–	++	++	++	++	++	++	++	++	++	–	–	–
Isothalictrine (**4**) (Philipov & Istatkova 1999)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Isothalirine (**5**) (Philipov & Istatkova 1999)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Isothalicrine (**6**) (Philipov & Istatkova 1999)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Isopyruthaldine (**7**) (Istatkova & Philipov 2000)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Isopythaldine (**8**) (Istatkova & Philipov 2000)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Isothalmidine (**9**) (Istatkova & Philipov 2000)	++	++	–	++	++	++	++	++	++	++	++	++	–	–	–
Isocorydine (**10**) (Istatkova & Philipov 2000)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reticuline (**11**) (Istatkova & Philipov 2000)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Palmatine (**12**) (Istatkova & Philipov 2000)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Columbamine (**13**) (Istatkova & Philipov 2000)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Taliphine (**14**) (Istatkova & Philipov 2004)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Isothaliphine (**15**) (Istatkova & Philipov 2004)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
N-Methylglaucine (**16**) (Istatkova & Philipov 2004)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

a The localities are represented with their number in Table 1.

- The other chemotype comprising the plant material from the localities No 3, 13, 14 and 15 in which the alkaloids **3** and **9** were not found out.

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