



Alkaloids from *Isopyrum thalictroides*[☆]

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Abstract

Three new bisbenzylisoquinoline alkaloids, namely isothalictrine, isothalictine and isothalirine were isolated from roots and rhizomes of *Isopyrum thalictroides*. All structures were elucidated by the use of physical and spectral data. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Isopyrum thalictroides*; Ranunculaceae; Bisbenzylisoquinoline alkaloids; Isothalictrine; Isothalictine; Isothalirine

1. Introduction

In previous phytochemical study on *Isopyrum thalictroides* L. growing in Bulgaria we afforded two new and one known bisbenzylisoquinoline alkaloids (Philipov & Istatkova, 1997). Our further investigation of roots and rhizomes of *I. thalictroides* collected in the next year resulted in the isolation of the above mentioned alkaloids as well as of three new basic compounds. This paper deals with the isolation and structural elucidation of those three new bisbenzylisoquinoline alkaloids, named isothalictrine (1), isothalictine (2) and isothalirine (3). Their structures were established mainly by means of NMR spectroscopy, including NOE experiments and 2D shift correlations as well as by the data of MS, $[\alpha]_D$ and CD.

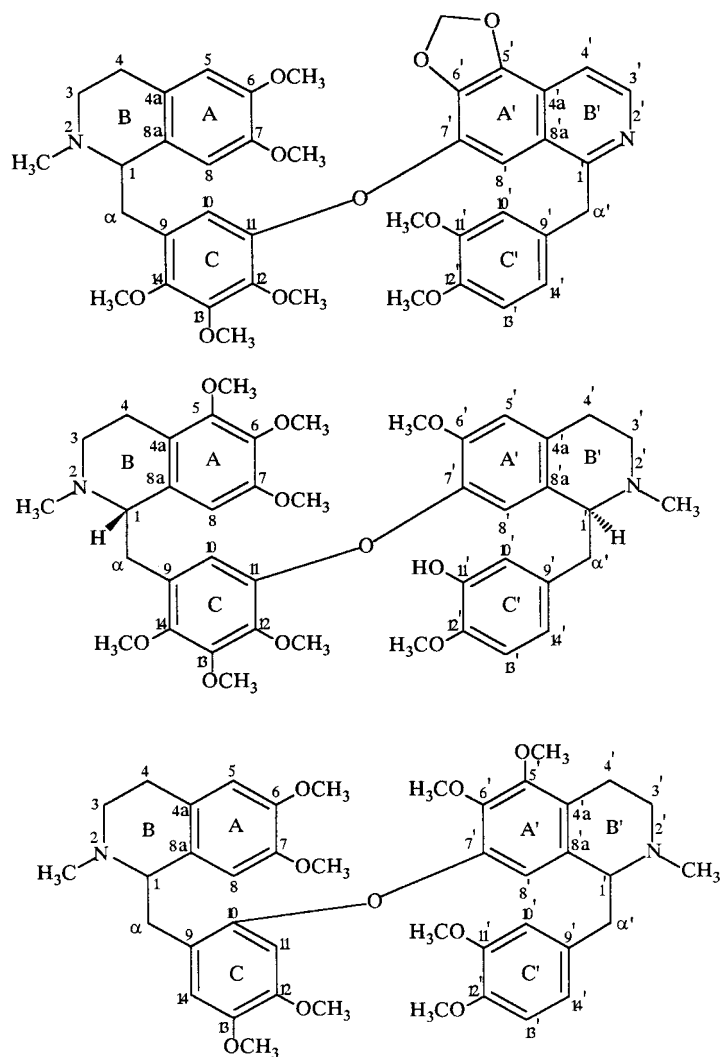
2. Results and discussion

Isothalictrine (1) was isolated as an amorphous solid. The CI mass spectrum showed a peak at m/z 725 $[M+H]^+$, which corresponded to the molecular formula $C_{41}H_{44}N_2O_{10}$. In agreement with this formula

is the peak at m/z 723 $[M-1]^+$ in the EI mass spectrum. The base peak at m/z 573, due to the loss of ring C' from the molecular ion, as well as the other important peaks at m/z 206 and 151 directly proved a bisbenzylisoquinoline alkaloid containing a single head-to-tail ether bridge (Bruneton, Shamma, Minard, Freyer, & Guinaudeau, 1983; Chalandre, Guinaudeau, & Bruneton, 1985; Wang, Zhao, Lai, Shizuri, & Yamamura, 1993). The 1H NMR spectrum of 1 exhibits a three-proton singlet at δ 2.47 due to one NCH_3 group. The lack of second signal for NCH_3 group and the presence of two doublets at δ 7.43 and 8.29 with $J=5.8$ Hz for the two aromatic protons H-4' and H-3', respectively, suggested a fully aromatic ring B'. The downfield doublets at δ 4.35 and 4.43 with $J=14.6$ Hz for the both H- α' supported this assertion (Patra et al., 1986; Abouchacra, Leboeuf, Guinaudeau, & Cave, 1987). Also in agreement were the ^{13}C NMR spectrum and the DEPT experiment, in which only four upfield signals for secondary carbons were observed. The downfield signal at δ 158.8 in the ^{13}C NMR spectrum is for C-1' (Table 1). The 1H NMR spectrum of 1 also showed sharp singlets for seven OCH_3 groups and one broad singlet for two protons due to one methylenedioxy group. In the aromatic region of the spectrum singlets for four protons as well as signals for an ABX spin system corresponding to the protons in ring C' (Table 2) were observed. The carried out NOE experiments are in agreement with the proposed structure of

[☆] Part 2 in the series "Alkaloids from *Isopyrum thalictroides*". For Part 1 see Philipov and Istatkova, 1997.

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1. It is of importance that irradiation of H-8' (δ 7.37) gave a 12.4% NOE of OCH₃ group at C-12 (δ 3.98) and irradiation of OCH₃ group at C-12 led to a 4.6% NOE of H-8', so that ring A' is located close to ring C. On the other hand, the observed NOE between H-8 (δ 6.46) and H-10 (δ 6.01) as well as the presence of three OCH₃ groups in ring C suggested a C-11,C-7' ether bridge. The rest of the important NOE data are given in Fig. 1. The assignments of the chemical shifts of all carbons and hydrogens were made by the use of HMQC and HMBC experiments. The data of HMBC spectrum are summarized in Fig. 1. The compound **1** is racemate, since the specific rotation is 0°. All these data suggested the structure of the new bisbenzylisoquinoline alkaloid *racem.* isothalictrine as **1**.

Isothalictrine (**2**) was isolated as an amorphous solid. The CI mass spectrum of **2** displayed a $[M+H]^+$ peak at m/z 745, corresponding to the molecular formula C₄₂H₅₂N₂O₁₀. A weak $[M-1]^+$ peak at m/z 743 in the EI mass spectrum confirmed this molecular composition. The base peak in the spectrum is at m/z 236 and the next most intensive peak, due to the loss of

ring C' from the molecular ion, is at m/z 607. The small peak at m/z 137 was also observed and it suggested the presence of one OH group and one

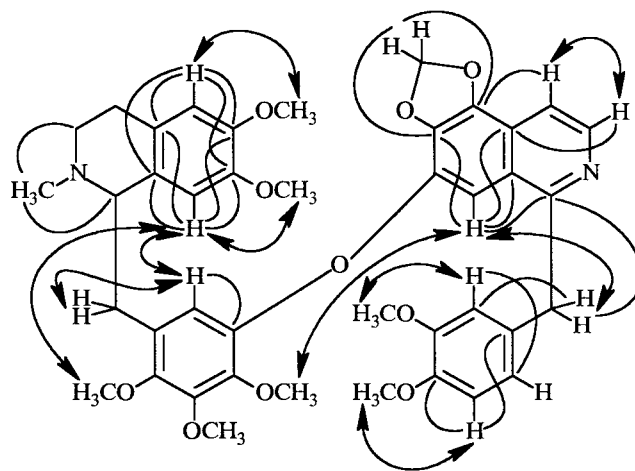


Fig. 1. Main NOE and HMBC experimental data of compound **1** (the arrows are for NOE and — is for HMBC).

Table 1

¹H NMR spectral data (δ) of alkaloids 1–3

H	1	2	3
1	3.77–3.81 (1H, m)	3.54–3.62 (1H, m)	3.88–3.92 (1H, m)
2	2.47 (3H, s, NCH ₃)	2.50 (3H, s, NCH ₃)	2.47 (3H, s, NCH ₃)
5	6.73 (1H, s)	3.85 (3H, s, OCH ₃)	6.55 (1H, s)
6	3.71 (3H, s, OCH ₃)	3.82 (3H, s, OCH ₃)	3.83 (3H, s, OCH ₃)
7	3.73 (3H, s, OCH ₃)	3.49 (3H, s, OCH ₃)	3.56 (3H, s, OCH ₃)
8	6.46 (1H, s)	5.81 (1H, s)	6.14 (1H, s)
10	6.01 (1H, s)	5.98 (1H, s)	—
11	—	—	6.38 (1H, s)
12	3.98 (3H, s, OCH ₃)	3.74 (3H, s, OCH ₃)	3.71 (3H, s, OCH ₃)
13	3.87 (3H, s, OCH ₃)	3.85 (3H, s, OCH ₃)	3.77 (3H, s, OCH ₃)
14	3.88 (3H, s, OCH ₃)	3.84 (3H, s, OCH ₃)	6.57 (1H, s)
1'	—	3.88–3.92 (1H, m)	3.60–3.62 (1H, m)
2'	—	2.50 (3H, s, NCH ₃)	2.47 (3H, s, NCH ₃)
3'	8.29 (1H, d, J = 5.8 Hz)	—	—
4'	7.43 (1H, d, J = 5.8 Hz)	—	—
5'	6.19 (2H, s, OCH ₂ O)	6.53 (1H, s)	3.87 (3H, s, OCH ₃)
6'	6.19 (2H, s, OCH ₂ O)	3.78 (3H, s, OCH ₃)	3.86 (3H, s, OCH ₃)
8'	7.37 (1H, s)	6.42 (1H, s)	6.02 (1H, s)
10'	6.57 (1H, d, J = 1.8 Hz)	6.38 (1H, d, J = 1.8 Hz)	6.59 (1H, d, J = 1.8 Hz)
11'	3.76 (3H, s, OCH ₃)	—	3.73 (3H, s, OCH ₃)
12'	3.83 (3H, s, OCH ₃)	3.75 (3H, s, OCH ₃)	3.78 (3H, s, OCH ₃)
13'	6.67 (1H, d, J = 8.1 Hz)	6.59 (1H, d, J = 8.2 Hz)	6.60 (1H, d, J = 8.2 Hz)
14'	6.52 (1H, dd, J = 8.1 and 1.8 Hz)	6.54 (1H, dd, J = 8.2 and 1.8 Hz)	6.49 (1H, dd, J = 8.2 and 1.8 Hz)
CH ₂	2.52–3.14 (4H, m)	2.54–2.60 (1H, m)	2.50–2.66 (2H, m)
	3.48–3.60 (2H, m, 2H- α)	2.62–2.98 (7H, m)	2.70–2.90 (5H, m)
	4.35 (1H, d, J = 14.6 Hz, H- α')	3.00–3.30 (4H, m)	2.92–3.36 (5H, m)
	4.43 (1H, d, J = 14.6 Hz, H- α')		

OCH₃ group in ring C'. A confirmation for the hydroxyl group being in this moiety is the absorption at 3537 cm⁻¹ in the IR spectrum. The position of the hydroxyl substituent at C-11' was suggested by the NOE and HMBC experimental data (Fig. 2). The above discussed mass spectral fragmentation of **2** proved a single bridged bisbenzylisoquinoline alkaloid involving head-to-tail ether coupling (Bruneton et al., 1983; Chalandre et al., 1985; Wang et al., 1993). The ¹H

NMR spectrum of **2** revealed the presence of six-proton singlet at δ 2.50 due to two NCH₃ groups and sharp singlets for eight OCH₃ groups. The aromatic region of the spectrum displayed singlets for four protons as well as signals for an ABX spin system, corresponding to the protons in ring C' Table 2. The proposed structure of **2** was confirmed by the NOE experiments. As in **1**, irradiation of H-8' (δ 6.42) created a 11.6% NOE of OCH₃ group at C-12 (δ 3.74) and irradiation of OCH₃ group at C-12 resulted in a 4.1% NOE of H-8'. On the other hand, the observed NOE between H-10 (δ 5.98) and H-1 (δ 3.54–3.62) as well as the presence of three OCH₃ groups in ring C suggested a C-11, C-7' ether bridge. The other important NOE are represented in Fig. 2. The data of HMQC and HMBC Fig. 2 experiments enable the location of all carbons and hydrogens. The absolute configuration at C-1 and C-1' of **2** was established by the data of circular dichroism curve and specific rotation, which are very similar to those of the alkaloids malekulatine and *O,O*-dimethylmalekulatine (Cassels & Shamma, 1980; Bruneton et al., 1983). The positive specific rotation and the three positive Cotton effects at 240, 273 and 293 nm directly assigned the absolute configuration (1*S*,1'*S*). According to these analyses the structure and the stereochemistry of the new bisbenzylisoquinoline alkaloid (1*S*,1'*S*) isothalicrine is **2**.

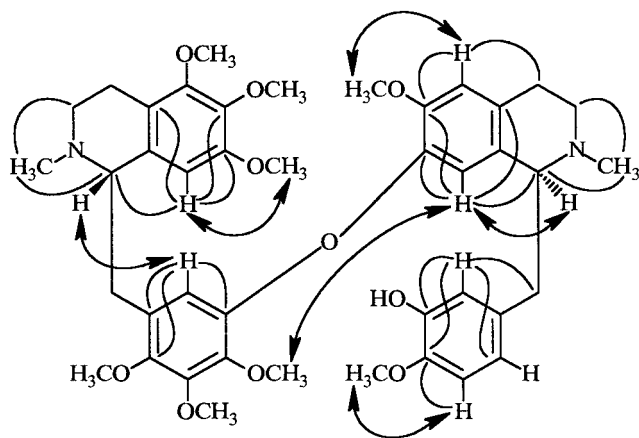


Fig. 2. Main NOE and HMBC experimental data of compound **2** (the arrows are for NOE and — is for HMBC).

Table 2

 ^{13}C NMR spectral data (δ) of alkaloids **1–3**

C	1	2	3
1	64.3	63.1	63.3
2	42.4 (NCH ₃)	41.7 or 42.7 (NCH ₃)	42.4 (NCH ₃)
3	46.7	19.8 or 47.2	46.6
4	40.3	20.8 or 45.5	25.1
4a	122.5	119.0	125.2
5	112.5	150.8 or 151.1	111.1
6	146.1	140.5	147.5
7	148.5	150.8	146.7
8	104.2	107.0	111.1
8a	122.5	131.6 or 133.4	128.8
α	20.0	45.5 or 20.8	35.3
9	133.7	122.6	122.4
10	111.3	111.1	147.9
11	141.0	149.3	103.6
12	145.4	148.3	148.1
13	145.9	151.1 or 150.8	145.0
14	151.2	141.1	114.7
1'	158.1	64.7	64.4
2'	—	42.7 or 41.7 (NCH ₃)	42.4 (NCH ₃)
3'	139.9	47.2 or 19.8	46.6
4'	111.8	33.5	20.0
4'a	118.8	122.0	122.5
5'	141.9	114.7	151.1
6'	137.3	145.1	141.5
7'	149.9	147.4	149.1
8'	100.7	104.0	112.1
8'a	123.6	133.4 or 131.6	122.6
α'	35.7	39.2	40.2
9'	131.8	131.8	131.8
10'	112.3	116.4	112.3
11'	148.4	145.3	148.5
12'	147.3	145.5	147.3
13'	110.7	110.6	110.8
14'	121.3	120.7	121.4
OCH ₂ O	102.9	—	—
5-OCH ₃	—	60.4 or 60.5	—
6-OCH ₃	56.0	60.9	55.7
7-OCH ₃	56.1	55.5	55.5
12-OCH ₃	56.3	56.2	56.1
13-OCH ₃	60.9	60.4 or 60.5	56.2
14-OCH ₃	60.4	60.7	—
5'-OCH ₃	—	—	60.4
6'-OCH ₃	—	56.2	61.0
11'-OCH ₃	55.7	—	55.7
12'-OCH ₃	55.7	55.7	55.8

Isothalirine (**3**) was isolated as an amorphous solid. The $[\text{M} + \text{H}]^+$ peak at m/z 729 in the CI mass spectrum corresponded to the molecular formula $\text{C}_{42}\text{H}_{52}\text{N}_2\text{O}_9$. In accordance with this molecular composition was the presence of weak molecular ion at m/z 728 in the EI mass spectrum. The observed fragmentation of **3** is the same as in **1** and **2**, namely the presence of a base peak at m/z 206 and a peak at m/z 577 due to the loss of ring C' from the molecular ion. These data proved a bisbenzylisoquinoline alkaloid with only one head-to-tail ether link (Bruneton et al., 1983; Chalandre et al., 1985; Wang et al., 1993). The

^1H NMR spectrum of **3** displayed a six-proton singlet at δ 2.47 due to two NCH₃ groups and eight sharp singlets for corresponding OCH₃ groups. Alike **1** and **2**, the protons in ring C' appeared again as an ABX spin system. The rest of the aromatic protons were observed as five singlets in the aromatic region Table 2. The proposed structure of **3** was confirmed by the NOE experiments. Irradiation of H-8' (δ 6.02) gave a 3.5% NOE of H-11 (δ 6.38) and a 5.9% NOE of OCH₃ group at C-12 (δ 3.71). Irradiation of H-11 and of OCH₃ group at C-12 led to a 3.7% NOE and a 1% NOE of H-8', respectively. Irradiation of H-11 created

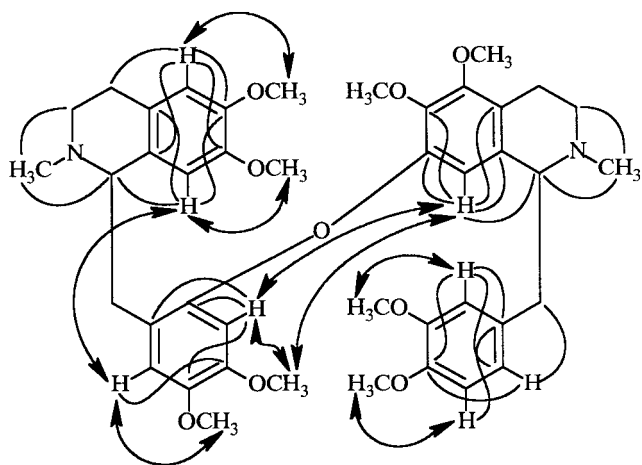


Fig. 3. Main NOE and HMBC experimental data of compound **3** (the arrows are for NOE and \sim is for HMBC).

a 12.3% NOE of OCH₃ group at C-12 and irradiation of OCH₃ group at C-12 resulted in a 6.6% NOE of H-11. These data give a reason to suppose that the ether link is at C-10, C-7'. The other important NOE are represented in Fig. 3. The final structure was established by the means of HMQC and HMBC Fig. 3 spectra. The alkaloid **3** is racemate, since $[\alpha]_D$ is 0°. All these data proved that the structure of the new bis-benzylisoquinoline alkaloid *racem.* isothalirine is **3**.

3. Experimental

3.1. General

UV: EtOH. IR: CHCl₃. ¹H, ¹³C NMR spectra and 2D experiments: 250 and 400 MHz in CDCl₃ with TMS as int. standard. MS: 70 eV. CD: MeOH. CC: silica gel (Merck, Kieselgel 60, 70–230 mesh) and neutra alumina (Merck, Aluminiumoxid 90, act. II–III Brockmann, 70–230 mesh). PTLC: silica gel GF₂₅₄. Spray reagent for TLC: Dragendorff's reagent.

3.2. Plant material

Isopyrum thalictroides L. was collected in May 1995 during the time of flowering from Ljulin mountain near Sofia. A voucher specimen (SOM-320) is deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences.

3.3. Extraction and isolation

Air-dried and ground roots and rhizomes (1500 g) were worked up to obtain 6.0 g crude mixture of tertiary alkaloids. By the same manner as described in

(Philipov & Istatkova, 1997) the alkaloids **1** (21.0 mg), **2** (6.3 mg) and **3** (5.0 mg) were isolated.

3.4. Isothalicrine (**1**)

UV λ_{max} nm (log ϵ): 208 (6.61), 236 sh (6.32), 259 (6.29), 285 (5.84). IR ν_{max} cm⁻¹: 3020, 2920, 2840, 1600, 1510, 1400, 1260, 1210, 1020, 860, 750, 650. ¹H NMR: see Table 2. ¹³C NMR: see Table 1. CIMS (NH₃) m/z (rel. int.): 725 [M+H]⁺ (100). EIMS m/z (rel. int.): 723 (10), 573 (100), 352 (25), 236 (8), 206 (21), 151 (21). $[\alpha]_D$ 0° (CHCl₃; c 0.15).

3.5. Isothalicrine (**2**)

UV λ_{max} nm (log ϵ): 208 (6.62), 236 sh (6.06), 285 (5.74). IR ν_{max} cm⁻¹: 3537, 3020, 2930, 2850, 1600, 1500, 1450, 1210, 1195, 860, 750, 650. ¹H NMR: see Table 2. ¹³C NMR: see Table 1. CIMS (NH₃) m/z (rel. int.): 745 [M+H]⁺ (100). EIMS m/z (rel. int.): 743 (2), 607 (55), 372 (10), 236 (100), 206 (11), 137 (12). CD $\Delta\epsilon$ (nm): 5.8 (240), 0.7 (273), 2.6 (293). $[\alpha]_D$ +33° (CHCl₃; c 0.31).

3.6. Isothalirine (**3**)

UV λ_{max} nm (log ϵ): 208 (7.20), 236 sh (6.82), 285 (6.39). IR ν_{max} cm⁻¹: 3020, 2930, 2840, 1600, 1510, 1410, 1250, 1210, 1020, 850, 750, 660. ¹H NMR: see Table 2. ¹³C NMR: see Table 2. CIMS (NH₃) m/z (rel. int.): 729 [M+H]⁺ (100). EIMS m/z (rel. int.): 728 (4), 591 (5), 577 (75), 371 (6), 340 (10), 206 (100). $[\alpha]_D$ 0° (CHCl₃; c 0.25).

Acknowledgements

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