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AN ISOPAVINE ALKALOID FROM *THALICTRUM MINUS*

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Key Word Index—*Thalictrum minus*; Ranunculaceae; isopavine alkaloid; isothalisopavine; isolation.

Abstract—A new alkaloid, isothalisopavine, was isolated from *Thalictrum minus* and shown to be 2,3,8-trimethoxy-7-hydroxyisopavine from spectral evidence. The known thalisopavine, from *T. dasycarpum*, is 2,3,8-trimethoxy-9-hydroxyisopavine. Isothalisopavine is the first of its class to be substituted unsymmetrically in the two aromatic rings. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Thalictrum* comprises ca 120 species of perennial herbaceous plants [1]. Over 200 alkaloids, almost all of the isoquinoline group, have been reported from this genus and a large number have biological activity (mainly hypotensive, antimicrobial and antitumor properties) [2–5]. *Thalictrum minus*, which is widely distributed in Bulgaria, has been studied for the past 30 years in relation to botanical, phytochemical [6–11] and alkaloid biosynthesis [12–15] aspects. Investigations of *T. minus* ssp. *minus* (known previously as *T. minus* ssp. *elatum*) and 26 other *T. minus* populations in Bulgaria led to well-defined chemosystematic correlations [1].

The aim of the present work was to continue studies on the alkaloid content of the above-ground parts of *T. minus* ssp. *minus*, with the aim of identifying minor alkaloids which might play an important role in biosynthetic processes or which might have some chemosystematic implications.

RESULTS AND DISCUSSION

From 1 kg of *T. minus* ssp. *minus*, 15 mg of a pure alkaloid was isolated, which was shown by spectroscopic methods to be 2,3,8-trimethoxy-7-hydroxyisopavine (**1**), herein named, isothalisopavine.

It was a non-crystalline solid, had $[\alpha]_D^{25}$ −169° (c 3.4 $CHCl_3$), a UV_{max} 286 (bathochromic base shift) and M_r 341 by $ESMSH^+$ and GC-mass spectrometry. The mass spectral data, along with the ^{13}C and ^1H NMR information (Table 1), suggested the molecular formula $C_{20}H_{23}NO_4$. This formula, the presence of three methoxyl groups by the NMR analysis and a base peak of *m/z* 204 (ion **2**) were consistent with data reported previously for thalisopavine (**3**) [16]. Although these data would also apply to the pavine alkaloid, platycerine (**4**) [17, 18], the mass spectrum of **1** showed a *m/z* 298 peak $[M-43]^+$. This peak can be used to distinguish between isopavines and pavines, which lack the loss of this unit ($CH_2{=}NCH_3$) [19]. The ^1H NMR doublets for **1** at δ 6.51 and 6.61 were typical of neighbouring protons on an aromatic ring and similar to those for platycerine (δ 6.4 and 6.8) [18]. A NOE enhancement was observed between the δ 6.61 aromatic proton and the δ 3.81 resonance for the methyl of a methoxyl group. Thus, structural possibilities **1** and **5** remained for the unknown alkaloid. It has been shown that irradiation of H-5 in the normally-substituted isopavines, such as **3**, causes enhancements in both of the *peri*-aromatic protons (H-4 and H-7) [20]. In the case of **1**, irradiation of the H-5 one proton doublet at δ 4.65 caused an enhancement in only one aromatic proton (δ 6.80). Similarly, irradiation of the H-12 one proton multiplet (δ 4.10) caused an enhancement in only one aromatic proton (δ 6.76). Thus, neither of the aliphatic methines was in proximity to the two aromatic protons and, hence, structure **5** could be ruled out. In corroboration, irradiation of H-10 caused enhancements only in H-9 and at δ 3.0, which was the resonance for one of the H-11 methylenes. Additionally, irradiation of the H-9 resonance (δ 6.61) caused enhancements at H-10 and at one OMe resonance (δ 3.8). The H-5 δ 4.65 resonance is anomalously deshielded but such an effect was also observed with **3** [18] and with the pavine, munitagine [21; R. Doskotch, pers. commun.], which is asymmetrically substituted similarly. This

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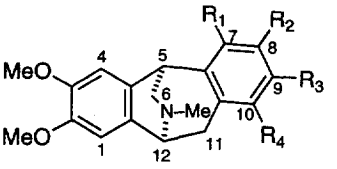
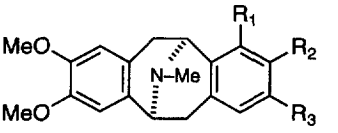
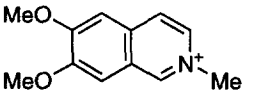


1: $R_1 = OH$, $R_2 = OMe$, $R_3 = R_4 = H$
3: $R_1 = R_4 = H$, $R_2 = OMe$, $R_3 = OH$
5: $R_1 = R_2 = H$, $R_3 = OMe$, $R_4 = OH$



4: $R_1 = OH$, $R_2 = OMe$, $R_3 = H$
6: $R_1 = H$, $R_2 = OMe$, $R_3 = OH$



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deshielding effect results when the C—H bond is neighboured by an eclipsing C—O bond. COSY and HMQC NMR spectra were used for the assignments given in Table 1 but complete assignments were frustrated by overlapping resonances for the H-6 and H-11 methylene protons, as well as those for the methoxyls. The large negative optical rotation is typical of all isopavines so far isolated and suggests the absolute configuration given.

None of the known isopavines possess the unsymmetrical aromatic substituent pattern of **1**. The structural relationship between **1** and **3** in the isopavine series is the same as that between **4** and isonorargemonine (**6**), in the pavine series. The structure thus fits with a biosynthetic route through reticuline, which has been shown to be applicable to other alkaloids from *T. minus* [12–15].

EXPERIMENTAL

Plant material

Above-ground parts of *T. minus* L. ssp. *minus* were collected in August 1996, from the south slopes of the Balkan Mountain, near Sliven, Bulgaria (voucher specimen No. 1169, Institute of Botany Herbarium, Bulgarian Academy of Science, Sofia).

Extraction and isolation

Air-dried, powdered material (1 kg) was exhaustively extracted with MeOH, the combined extracts evapd, the greenish mass obtained acidified (5% aq. HCl) and the mixt. filtered. The solution obtained (400 ml) was defatted with benzene (3 × 150 ml), basified to

Table 1. NMR spectral data for compound **1** ($CDCl_3$)

H1	6.76 *s*	C1	110.5
H4	6.80 *s*	C2	148.2*
H5	4.65 *d*, 4.8	C3	149.2*
H6	3.01 *dd*, 3.3, 18.0	C4	109.3
	3.8, under OCH_3's	C4a	128.5†
H9	6.61 *d* 8.1	C5	33.7
H10	6.51 *d*, 8.7	C5a	132.6†
H11	3.01 *dd*, 3.3, 18.0	C6	58.5
	3.8, under OCH_3's	C7	141.0*
H12	4.10 *m*	C8	144.3*
C2—OCH_3	3.84 *s*	C9	109.5
C3—OCH_3	3.84 *s*	C10	121.7
C8—OCH_3	3.81 *s*	C10a	127.0†
N—CH_3	2.59 *bs*	C11	36.1
		C12	63.2
		C12a	129.5†
		C2—OCH_3	56.2‡
		C3—OCH_3	56.1‡
		C8—OCH_3	56.0‡
		N—CH_3	45.2

*,†, ‡, Interchangeable.

pH 10 with aq. NH_3 and extracted with $CHCl_3$ (4 × 150 ml). The $CHCl_3$ soln was dried (Na_2SO_4) and evapd to yield 5.3 g of crude alkaloid mixt. Of this, 5 g was chromatographed (200 g silica gel 60, 0.063–0.200 mm), eluted with $CHCl_3$ followed by $CHCl_3$ containing gradually increasing proportions of MeOH. Isothalisopavine (**1**) was present as a minor component, along with thalmelatine, in frs eluted with 3% MeOH. Nearly pure **1** (27 mg) was obtained by prep. TLC (silica gel; $CHCl_3$–petrol–MeOH–Me_2CO, 4:4:1:1). Pure **1** (15 mg), a non-crystalline solid, was obtained by silica gel CC ($CHCl_3$–MeOH, 9:1).

Compound **1**. ^1H (300 MHz) and ^{13}C (75 MHz) NMR: Table 1. 2D and NOE NMR: 500 MHz. ESMSH$^+$ *m/z* 342 with a cone voltage 25 V. GC-MS: HP-1 12 m column, temp. prog. 100° (2 min) prog. at 25° min^{-1} to 290° (6 min). MS *m/z* (rel. intensity): 341 (48), 340 (50), 298 (17), 283 (17), 204 (100). Rt 9.6 min.

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