Pergamon

PII: S0031-9422(96)00696-6

Phytochemistry, Vol. 44, No. 8, pp. 1591–1594, 1997

0031 9422/97 $17.00+0.00

ALKALOIDS FROM *ISOPYRUM THALICTROIDES*

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(*Received in revised form* 9 *September* 1996)

Key Word Index—*Isopyrum thalictroides*; Ranunculaceae; bisbenzylisoquinoline alkaloids; isopyruthaline; isopythaline.

Abstract—Two new bisbenzylisoquinoline alkaloids, isopyruthaline and isopythalines, were isolated from roots and rhizomes of *Isopyrum thalictroides*. The known alkaloid fangchinoline was isolated for the first time from a plant of the family Ranunculaceae. All structures were elucidated by physical and spectral studies. Copyright © 1997 Published by Elsevier Science Ltd

INTRODUCTION

The genus *Isopyrum* is distributed world-wide, with more than 60 species, but in Europe the only representative is *I. thalictroides*. To our knowledge chemical investigation of this species is quite limited and only 10 alkaloids of the bisbenzylisoquinoline, pseudoprotoberberine and aporphine type have been isolated from roots, rhizomes and aerial parts [1–4]. Recently, we have established anti-inflamatory, antimicrobial and immunological activity in extracts and alkaloid fractions of *I. thalictroides* of Bulgarian origin [5, 6]. Now, we have studied the chemical compositions of its roots and rhizomes, and 14 isoquinoline alkaloids have been isolated. In the present paper we report on the isolation and structural elucidation of the main alkaloids, namely the two new bisbenzylisoquinoline alkaloids, isopyruthaline (**1**) and isopythaline (**2**), and the known alkaloid, fangchinoline (**3**) [7–9], which has been found for the first time in a plant of the Ranunculaceae.

RESULTS AND DISCUSSION

Isopyruthaline (**1**) was isolated as an amorphous solid. The CI mass spectrum showed a $[M+NH_4]^+$ at *m/z* 760, which corresponds to the molecular formula $C_{42}H_{50}N_2O_{10}$, indicating a bisbenzylisoquinoline alkaloid. The $[M-1]^+$ at *m/z* 741 in the electron impact mass spectrum was in agreement with the same molecular formula. The base peak at *m/z* 220 and the next most intense peak at *m/z* 236 represent the two different dihydroisoquinoline cations obtained as a result of the cleavage of the central double benzylic bond.

The mass spectrum of **1** is typical of the spectra of bisbenzylisoquinoline alkaloids, containing a single tail-to-tail ether bridge [10, 11]. The ^1H NMR spectrum of **1** exhibits a six-proton singlet at δ 2.47 due to two NCH_3 groups and singlets at δ 3.53, 3.59, 3.72, 3.74, 3.79, 3.87 and 3.88 due to seven OCH_3 groups. The broad singlet at δ 5.94 corresponds to one methylenedioxy group. The aromatic protons H-8′, H-8 and H-10 are observed as singlets at δ 5.78, 6.38 and 6.02, respectively. The aromatic protons in the 1,4-disubstituted benzene ring C′ appear as two doublets, each of which represents two protons, with $J = 8.1$ Hz. The assignment of the chemical shifts of the protons and groups are in agreement with the two-dimensional NOESY experimental data. The two-dimensional NOESY spectrum shows significant correlations between H-8 (δ 6.38) and H-10 (δ 6.02), as well as between H-8 (δ 6.38) and OCH_3 groups at C-7 (δ 3.72) and C-6 (δ 3.74). The spatial relationships, which were not apparent from the planar diagram, are observed between H-8 (δ 6.38) and OCH_3 groups at C-13 (δ 3.87) and C-14 (δ 3.88). Effects between H-10 (δ 6.02) and H-11′, H-13′ (δ 6.50) and between H-10 (δ 6.02) and OCH_3 groups at C-7 (δ 3.72) and at C-12 (δ 3.79), C-13 (δ 3.87) and C-14 (δ 3.88) were visible. The NOE correlations observed between H-8, H-10 and OCH_3 group at C-7 of **1** closely resemble those reported for the same protons and groups in the alkaloid (+)-temuconine [12]. A significant correlation between H-8′ (δ 5.78) and the OCH_3 group at C-7′ (δ 3.59) was also visible. The presence of cross-peaks between the OCH_2O group (δ 5.94) and OCH_3 groups at C-5 (δ 3.53), C-6 (δ 3.74) and C-7 (δ 3.72) additionally confirmed the placement of these groups. The circular dichroism curve showed a negative Cotton effect at 209 nm and a positive Cotton effect at 294 nm, establishing the absolute configuration (1*S*,

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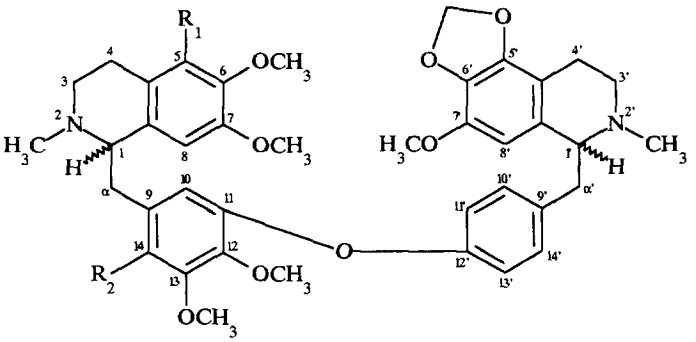

	H-1	H-1'	R_1	R_2
1	◢	·····	OCH_3	OCH_3
2	◢	◣	H	H

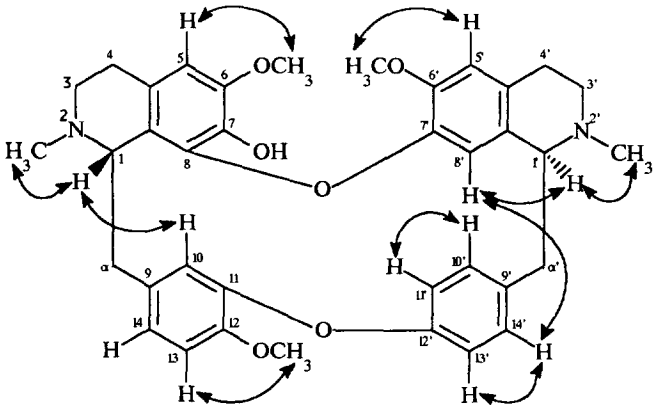

Fig. 1.Structures of alkaloids **1**–**3**. (←——→) NOESY experiment.

1′*S*) for **1** [13]. The positive specific rotation supported this configuration [14]. These spectral data suggested the structure and the stereochemistry of the new bisbenzylisoquinoline alkaloid as **1** (Fig. 1).

Isopythaline (**2**) was isolated as an amorphous solid. The $[M+NH_4]^+$ at *m/z* 700 in the CI mass spectrum corresponds to the molecular formula $C_{40}H_{44}N_2O_8$, and is characteristic of a bisbenzylisoquinoline alkaloid. The electron impact mass spectrum displays the base peak at *m/z* 206, representing the rings A and B. Another very strong peak at *m/z* 220 is due to rings A′ and B′. Weak peaks at *m/z* 476 $[M-206]^+$ and 462 $[M-220]^+$ are also observed. This cleavage pattern is typical of bisbenzylisoquinoline alkaloids, which have only tail-to-tail coupling [10, 11]. The ^1H NMR spectrum of **2** exhibits sharp singlets at δ 2.50 and 2.55 due to two NCH_3 groups and at δ 3.56, 3.60, 3.79, 3.82 and 3.83 due to five OCH_3 groups. The one-proton singlets at δ 5.99 and 6.05 correspond to one methylenedioxy group. The aromatic protons H-8′, H-8 and H-5 appear as singlets at δ 6.53, 6.55 and 6.86, respectively. The presence of one-proton doublets at δ 6.73 and 6.84 with $J = 1.8$ Hz is due to the *meta*-substituted protons H-10 and H-14. The aromatic protons in the 1,4-disubstituted benzene ring C′ are observed as an AA′XX′ spin system. The two-dimensional NOESY experiment shows the presence of NOE correlation contours between H-5 (δ 6.86) and the OCH_3 group at C-6 (δ 3.79), H-8′ (δ 6.53) and the OCH_3 group at C-7′ (δ 3.56), H-8 (δ 6.55) and the OCH_3 group at C-7 (δ 3.60). Like compound **1**, spatial relationships, which were not apparent from the planar diagram, are observed between H-8 (δ 6.55) and the OCH_3 group at C-13 (δ 3.83) and H-14 (δ 6.84). Cross-peaks between the OCH_2O group (δ 5.99, 6.05) and OCH_3 groups at C-7′ (δ 3.56), C-7 (δ 3.60) and C-6 (δ 3.79) are also observed. The absolute configuration (1*S*, 1′*R*) for **2** was assigned directly from the three negative Cotton effects at 208, 248 and 284 nm in the circular dichroism spectrum and by the negative specific rotation [13–16]. On the other hand, the absolute configuration (1*R*, 1′*S*) requires positive

Cotton efects at 200 and 280 nm and a positive specific rotation [13, 14]. According to these analyses the structure and the stereochemistry of the new bisbenzylisoquinoline alkaloid is **2**.

The third bisbenzylisoquinoline alkaloid from this species was identified by physical and spectral data ($[\alpha]_D$, UV, IR, ^{1}H NMR, and mass spectrometry) as fangchinoline [7–9]. The circular dichroism spectrum and a two-dimensional NOESY experiment supported this structure. Alkaloid **3** was isolated for the first time from a species of the Ranunculaceae.

EXPERIMENTAL

General. UV: EtOH. IR: KBr discs. ^{1}H NMR and 2D NOESY: 250 MHz in $CDCl_3$ 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). Prep.TLC: silica gel GF_{254}. Spray reagent for TLC: Dragendorff's reagent.

Plant material. Isopyrum thalictroides L. was collected in May 1994 during the time of flowering from Ljulin mountain near Sofia. A voucher specimen, (SOM-319) is deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences.

Extraction and isolation. Air-dried and ground roots and rhizomes (500 g) were extracted exhaustively with 95% EtOH at room temp. The combined EtOH extracts were evapd under red. pres., acidified with 5% HCl (50 ml) and left overnight at room temp. Insol. non-alkaloid material was removed by filtration and the filtrate subjected to Et_2O extraction (4 × 50 ml) to eliminate the rest of the non-alkaloid substances. Thus purified, the acidic soln was made alkaline with 25% NH_4OH (pH 9–10) and then extracted with $CHCl_3$ (6 × 100 ml). The $CHCl_3$ extracts were combined, dried (Na_2SO_4) and evapd under red. pres. to give 1.9 g of a crude mixt. of tertiary alkaloids. By consecutive using of CC on silica gel with $CHCl_3$–MeOH (increasing polarity), then on neutral alumina with hexane–Me_2CO (increasing polarity) and, finally, by prep.TLC with petrol–$CHCl_3$–Me_2CO–MeOH (4:4:1:1) from the tertiary alkaloid mixt. the alkaloids **1** (9.0 mg), **2** (7.0 mg) and **3** (6.8 mg) were isolated.

Isopyruthaline (**1**). UV λ_{max} nm (log ε): 208 (6.02), 232 sh (5.65), 283 (5.14). IR ν_{max} cm^{-1}: 2850, 1600, 1510, 1460, 1270, 1220, 1020, 850, 750, 660. ^{1}H NMR ($CDCl_3$): δ 2.47 (6H, *s*, $2NCH_3$), 2.55–2.85 (6H, *m*), 2.99–3.11 (4H, *m*), 3.53 (3H, *s*, 5-OCH_3), 3.59 (3H, *s*, 7′-OCH_3), 3.72 (3H, *s*, 7-OCH_3), 3.74 (3H, *s*, 6-OCH_3), 3.78–3.84 (4H, *m*), 3.79 (3H, *s*, 12-OCH_3), 3.87 (3H, *s*, 13-OCH_3), 3.88 (3H, *s*, 14-OCH_3), 5.78 (1H, *s*, H-8′), 5.94 (2H, *br s*, OCH_2O), 6.02 (1H, *s*, H-10), 6.38 (1H, *s*, H-8), 6.50 (2H, *d*, J = 8.1 Hz, H-11′, H-13′), 6.61 (2H, *d*, J = 8.1 Hz, H = 10′, H-14′). CIMS (NH_3) *m/z* (rel. int.): 760 $[M+NH_4]^+$ (30), 759 $[M+NH_4-H]^+$ (63), 743 $[M+H]^+$ (100), 729 $[M+NH_4-OCH_3]^+$ (5), 712 $[M+NH_4-H-OCH_2O]^+$ (4). EIMS *m/z* (rel. int.): 741 (12), 635 (5), 607 (18), 591 (26), 522 (2), 506 (2), 356 (7), 340 (27), 236 (87), 220 (100), 206 (7), 191 (5). CD $\Delta\varepsilon$ (nm): −13.3 (209), 5.1 (240), −0.1 (277), 0.7 (294). $[\alpha]_D$ +21° ($CHCl_3$; *c* 0.45).

Isopythaline (**2**). UV λ_{max} nm (log ε): 207 (6.43), 236 (6.10), 260 (5.79), 283 (5.74). IR ν_{max} cm^{-1}: 2840, 1600, 1510, 1460, 1260, 1220, 1020, 850, 750, 660. ^{1}H NMR ($CDCl_3$): δ 2.50 (3H, *s*, 2-NCH_3), 2.55 (3H, *s*, 2′-NCH_3), 2.72–2.84 (6H, *m*), 3.17–3.23 (4H, *m*), 3.56 (3H, *s*, 7′-OCH_3), 3.60 (3H, *s*, 7-OCH_3), 3.72–3.78 (4H, *m*), 3.79 (3H, *s*, 6-OCH_3), 3.82 (3H, *s*, 12-OCH_3), 3.83 (3H, *s*, 13-OCH_3), 5.99, 6.05 (each 1H, *s*, OCH_2O), 6.53 (1H, *s*, H-8′), 6.55 (1H, *s*, H-8), 6.73 (1H, *d*, J = 1.8 Hz, H = 10), 6.78 (2H, *d*, J = 8.6 Hz, H-11′, H-13′), 6.84 (1H, *d*, J = 1.8 Hz, H-14), 6.86 (1H, *s*, H-5), 7.00 (2H, *d*, J = 8.6 Hz, H-10′, H-14′). CIMS (NH_3) *m/z* (rel. int.): 700 $[M+NH_4]^+$ (25), 222 $[C_{12}H_{14}NO_3+2H]^+$ (100), 220 $[C_{12}H_{14}NO_3]^+$ (35), 208 $[C_{12}H_{16}NO_2+2H]^+$ (60), 206 $[C_{12}H_{16}NO_2]^+$ (30). EIMS *m/z* (rel. int.): 681 (2), 356 (3), 340 (3), 476 (2), 462 (2), 206 (100), 220 (80), 191 (10). CD $\Delta\varepsilon$ (nm): −6.7 (208), −1.2 (248), −0.6 (284). $[\alpha]_D$ −6° ($CHCl_3$; *c* 0.35).

Fangchinoline (**3**). UV λ_{max} nm (log ε): 208 (6.10), 240 sh (5.53), 283 (4.99). IR ν_{max} cm^{-1}: 3530, 2800, 1610, 1590, 1500, 1440, 1270, 1210, 1120, 1060, 1020, 960, 840, 750. ^{1}H NMR ($CDCl_3$): δ 2.33 (3H, *s*, 2-NCH_3), 2.37–2.45 (1H, *m*), 2.53–2.57 (1H, *m*), 2.63 (3H, *s*, 2′-NCH_3), 2.66–3.02 (7H, *m*), 3.23–3.31 (1H, *m*), 3.36 (3H, *s*, 6′-OCH_3), 3.39–3.65 (2H, *m*), 3.74–3.78 (1H, *m*, H-1), 3.78 (3H, *s*, 6-OCH_3), 3.88–3.94 (1H, *m*, H-1′), 3.93 (3H, *s*, 12-OCH_3), 6.05 (1H, *s*, H-8′), 6.29 (1H, *s*, H-5), 6.32 (1H, *dd*, J = 8.3, 2.3 Hz, H-10′), 6.53 (1H, *s*, H-5′), 6.56 (1H, *s*, H-10), 6.80 (1H, *dd*, J = 8.3, 2.3 Hz, H-11′), 6.86 (2H, *br s*, H-13, H-14), 7.14 (1H, *dd*, J = 8.3, 2.3 Hz, H-13′), 7.34 (1H, *dd*, J = 8.3, 2.3, H-14′). EIMS *m/z* (rel. int.): 608 (87), 607 (34), 594 (10), 578 (5), 471 (4), 417 (12), 416 (8), 381 (100), 367 (40), 350 (5), 335 (5), 321 (5), 192 (60), 191 (90), 174 (30), 168 (20). CD $\Delta\varepsilon$ (nm): −51.9 (198), 51.9 (219), −4.5 (248), 5.5 (284). $[\alpha]_D$ +132° ($CHCl_3$; *c* 0.34).

Acknowledgements—This work was supported by Grant No. X-513 from the National Fund for Scientific Research, Bulgaria. We thank Ms I. Kleiber and Mr U. Walter, Hohenheim University, Stuttgart, for the mass spectra and Dr P. Denkova for fruitful discussion. Thanks are also due to Ms M. Slavcheva for help in collecting plant material.

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