Pergamon

0031-9422(95)00959-0

Phytochemistry, Vol. 42, No. 2, pp. 535–537, 1996

0031-9422/96 $15.00 + 0.00

ISOQUINOLINE ALKALOID *N*-OXIDES FROM *THALICTRUM SIMPLEX*

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(*Received in revised form* 20 *November* 1995)

Key Word Index—*Thalictrum simplex*; Ranunculaceae; aporphine alkaloid *N*-oxides; phenanthrene alkaloid *N*-oxides; thalicsimidine *N*-oxide; thalihazine *N*-oxide; *N*-hydroxy-northalicthuberine.

Abstract—The new aporphine alkaloid, (+)-thalicsimidine *N*-oxide, was found together with the known aporphines, (+)-thalicsimidine, (+)-ocoteine, (+)-preocoteine and preocoteine *N*-oxide. Both novel phenanthrene alkaloid oxidation products, thalihazine *N*-oxide and *N*-hydroxy-northalicthuberine, were isolated together with the known thalihazine and northalicthuberine. The structures of the aporphine and phenanthrene alkaloids as well as their *N*-oxides were established from spectral analyses.

INTRODUCTION

Seven plant families, viz. Annonaceae, Berberidaceae, Magnoliaceae, Menispermaceae, Monimiaceae, Papaveraceae and Ranunculaceae, are known to produce aporphine *N*-oxides [1–5]. So far, three aporphine *N*-oxides have been isolated from the Ranunculaceae [1, 5]. Preocoteine *N*-oxide and thalicmidine *N*-oxide were found in the roots of *Thalictrum minus* [1], and (+)-leucoxylonine *N*-oxide in the aerial parts of *T. simplex* [5].

Nine phenanthrene *N*-oxides have been isolated from the Annonaceae, Fumariaceae, Menispermaceae and Ranunculaceae [4, 6, 7]. Six of the isolated alkaloid *N*-oxides were from members of the Annonaceae [4, 6]. The last three families are represented only by one *N*-oxide [4, 6, 7]. Thalicthuberine *N*-oxide is the first phenanthrene alkaloid *N*-oxide to be isolated from the Ranunculaceae [7].

Further examination of the alkaloid content of the aerial parts from *T. simplex* allowed the isolation and structural elucidation of eight isoquinoline alkaloids. Five of them are the known aporphine and phenanthrene alkaloids, (+)-ocoteine (**1**), (+)-preocoteine (**2**), (+)-thalicsimidine (**3**), preococteine *N*-oxide (**4**) and thalihazine (**6**). New representatives are the aporphine *N*-oxide, (+)-thalicsimidine *N*-oxide (**5**), and the new phenanthrene alkaloid oxidation products, thalihazine *N*-oxide (**8**) and *N*-hydroxy-northalicthuberine (**9**).

RESULTS AND DISCUSSION

Our ongoing investigation of the fractions from column chromatographic separation of the crude alkaloid mixture of *T. simplex* shows, on analytical TLC plates, that the less polar fraction A and the more polar fraction B are mixtures of alkaloids. These two fractions were subjected to gel filtration with acetone on a Sephadex LH-20 column [5]. Gel filtration of fraction A permitted the separation of the free bases **1**–**3** and **6** with M_rs ranging from 369 to 385. Fraction B yielded the alkaloid *N*-oxides **4**, **5** and **8**, and the *N*-hydroxy compound **9**. The structures of the known aporphine alkaloids (+)-ocoteine (**1**), (+)-thalicsimidine (**3**) and of the known phenanthrene alkaloid thalihazine (**6**) were confirmed by comparison of their spectroscopic data (^{1}H NMR and EI mass spectra) with those published in the literature [1, 6].

In the ^{1}H NMR spectrum of **2**, four methoxyl groups at δ 3.95, 3.92, 3.91 and 3.86 were observed, together with three aromatic protons at δ 7.95, 6.78 and 6.25. The broad singlet at δ 6.25 disappeared upon adding D_2O to the solution. The EI mass spectrum of **2** showed the $[M]^+$ at m/z 371 and in the CI mass spectrum a low intensity ion at m/z 372 $[M + H]^+$. The three aporphine alkaloids, (+)-preocoteine, *O*-demethylpurpureine and (+)-thalibaicalidine, have the same M_r and give similar

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^{1}H NMR spectra because of the four substituents on the aromatic rings, one hydroxyl group and three methoxyl groups [1, 3]. It would be clear which of these three known alkaloids was isolated from *T. simplex* if the position of the hydroxyl group in **2** could be ascertained. A partial nOe experiment was performed. Intensive cross-peaks were observed between H-11 (δ 7.97) and 10-OMe (δ 3.92) and H-8 (δ 6.78) and 9-OMe (δ 3.91), but no cross-peaks were observed between H-11 and the other methoxyl groups at δ 3.95 and 3.86. Thus, it is clear that **2**, showing dextrarotatory optical activity, is the known alkaloid (+)-preocoteine.

Analogous nOe experiments were performed with **4**, which showed a ^{1}H NMR spectrum similar to that of **2**, except for the signal of the *N*Me group at δ 3.08, which was shifted from that of **2** [(**4**) − (**2**) = 0.52 ppm]. Partial nOe experiments showed that the hydroxyl group is situated on the first carbon atom. The alkaloid **4** was thus identified as the known, preocoteine *N*-oxide. The aporphine alkaloids, **1**, **2** and **4**, were isolated for the first time from *T. simplex*, while **3** has been previously found in this species [1].

The ^{1}H NMR spectrum of **5** was similar to that of **3** with the exception that the *N*Me signal was shifted downfield to δ 3.16 [(**5**) − (**3**) = 0.69 ppm]. The low intensity of the $[M]^+$ at *m/z* 401 present in the EI mass spectrum of **5**, as well as the fragment ion $[M-16]^+$ at *m/z* 385 are typical for *N*-oxides [8]. The $[M+H]^+$ ion in the CI mass spectrum of **5** was also of low intensity. Compound **5** showed dextrarotation like the base **3**, as H-6a of **5** appears in the ^{1}H NMR spectrum as a doublet of doublets at δ 4.41 ($J_1 = J_2 = 4.2$ Hz). A similar chemical shift of H-6a has been found for other aporphine *N*-oxides [9, 10]. Hence, **5** is the new *N*-oxide of the known base, (+)-thalicsimidine.

The phenanthrene nature of the new alkaloids, thalihazine *N*-oxide (**8**) and *N*-hydroxy-northalicthuberine (**9**), was also deduced from their ^{1}H NMR and mass spectral data. The ^{1}H NMR spectrum of **8** exhibited four aromatic protons, two singlets at δ 9.04 and 7.20 and two as AB system centred at δ 7.96 and 7.63 with a coupling constant of 9.1 Hz (H-9 and H-10 of the phenanthrene skeleton), three methoxyl groups at δ 4.05, 4.03 and 3.93 and one singlet for a methylenedioxy group at δ 6.10. The *N*Me group of **8** was shifted to δ 3.34 [(**8**) − (**6**) = 0.89 ppm] from that of **6** and the two 2H multiplets corresponding to the $CH_2CH_2NMe_2$ side-chain were shifted to δ 3.47 [(**8**) − (**6**) = 0.88 ppm] for the methylene protons and to δ 3.74 [(**8**) − (**6**) = 0.43 ppm] for the methylene protons. The *N*-oxide structure of **8** was supported by the presence of a $[M]^+$ at *m/z* 399, as well as the fragment ion $[M-16]^+$ at *m/z* 383 [8]. The CI mass spectrum of **8** showed a low intensity $[M+H]^+$ at *m/z* 400. Partial nOe experiments were performed and intensive cross-peaks were observed between H-5 (δ 9.04)/4-OMe (δ 3.93) and H-9 (δ 7.20)/H-7 (δ 7.63) and a low intensive cross-peak between 2-OMe (δ 4.05)/H-β (δ 3.74). Thus, it was ascertained that compound **8** is a new *N*-oxide, thalihazine *N*-oxide.

Compound **9** showed ^{1}H NMR spectral data similar to those for the recently isolated phenanthrene base, northalicthuberine (**7**) [7]. The signal of the *N*Me group showed a noticeable shift to δ 3.54 [(**9**) − (**7**) = 1.1 ppm]. The two-proton multiplets of the side-chain were also shifted to lower field as followed for the methylene protons at δ 3.82 [(**9**) − (**7**) = 0.52 ppm] and for the methylene protons at δ 3.69 [(**9**) − (**7**) = 0.96 ppm]. Partial decoupling experiments were performed and coupling was observed between H-8 (δ 7.33) and H-9 (δ 7.52) but not between H-5 (δ 9.13) and other protons. In the EI mass spectrum of **9** $[M]^+$ at *m/z* 355 was observed, with the fragment ions $[M-16]^+$ at *m/z* 339 (83%) and $[M-17]^+$ at *m/z* 338. Other main fragment ions were observed at *m/z* 60 ($[CH_2=N(OH)Me]^+$) and apparently (from $[M-16]^+$) at *m/z* 44 ($[CH_2=MeNEt]^+$). Therefore, alkaloid **9** is *N*-hydroxy-northalicthuberine, isolated for the first time.

Authentic samples of thalihazine *N*-oxide and *N*-hydroxy-northalicthuberine were prepared by oxidation of the free bases thalihazine and northalicthuberine, respectively. The synthetic samples showed identical spectral characteristics to the natural ones.

EXPERIMENTAL

General. Specific rotation: in MeOH. IR as film. ^{1}H NMR: Bruker WM 250 and Bruker AC 300, in $CDCl_3$;

^{1}H NMR nOe experiments: Bruker ARX 300, in $CDCl_3$. EIMS and CIMS (NH_3, *iso*butane): direct inlet (70 eV).

Plant material. Aerial parts of *T. simplex* (1.5 kg, dry wt) were collected near Ulan Bator during the full flowering period at the end of July 1991. The species was identified by Prof. Ch. Sanchill and E. Ganbold (Institute of Botany, Mongolian Academy of Sciences). A voucher specimen (No 83) is deposited in the Herbarium of the Institute of Botany, Mongolian Academy of Sciences, Ulan Bator.

Extraction and isolation. Ground plant material was extracted successively with cold petrol and EtOH, and the residue after evap. of solvent was treated as described previously [5]. The less polar fr. A was eluted from a neutral alumina column with Et_2O–MeOH (97:3) and the more polar fr. B with Et_2O–MeOH (1:4). Gel filtration was performed according the procedures described in ref. [5].

Preparation of phenanthrene alkaloid N-*oxides.* Thalihazine (4 mg) and northalicthuberine (5 mg) were subjected separately to oxidation under the condition described previously [5]. Pure synthetic **8** (3 mg) and **9** (3.5 mg) were obtained by gel filtration on a Sephadex LH 20 column using Me_2 CO as eluent.

(+)-*Thalicsimidine* N-*oxide* (**5**). $C_{22}H_{27}NO_6$ (3 mg). $[\alpha]_D^{22}$ +46° ($c = 0.02$, MeOH). IR ν_{max}^{film} cm^{-1}: 3435, 2933, 2854, 1666, 1607, 1594, 1563, 1513, 1501, 1414, 1396, 1308, 1220, 1201, 1116, 1085, 1054, 754. ^{1}H NMR (300 MHz): 7.90 (1H, *s*, H-11), 6.85 (1H, *s*, H-8), 4.41 (1H, *dd*, $J_1 = 4.2$ Hz, $J_2 = 4.2$ Hz H-6a), 4.09 (2H, *d*, $J = 6.4$ Hz), 4.03 (2H, *d*, $J = 11.5$ Hz), 3.97 (3H, *s*, OMe), 3.95 (3H, *s*, OMe), 3.91 (6H, *s*, 2-OMe), 3.78 (3H, *s*, 1-OMe), 3.16 (3H, *s*, *N*Me), 3.07 (1H, *m*), 2.87 (1H, *d*, $J = 14$ Hz). EIMS *m/z* (rel. int.): 401 $[M]^+$ (8), 400 $[M-H]^+$ (10), 399 (25), 398 (25), 385 (100), 384 (75), 383 (29), 370 (58), 354 (46), 341 (54), 327 (17), 311 (21); CIMS (NH_3) *m/z* (rel. int.): 402 $[M+H]^+$ (8), 386 (100), 371 (2), 356 (6).

Thalihazine N-*oxide* (**8**). $C_{22}H_{25}NO_6$ (3 mg). IR ν_{max}^{film} cm^{-1}: 3430, 2926, 2854, 1503, 1466, 1401, 1281, 1204, 1137, 1038, 757. ^{1}H NMR (300 HMz): 9.04 (1H, *s*, H-5), 7.96 (1H, *d*, $J = 9.1$ Hz, H-10), 7.63 (1H, *d*, $J = 9.1$ Hz, H-9), 7.20 (1H, *s*, H-8), 6.10 (2H, *s*, OCH_2O), 4.05 (3H, *s*, 2-OMe), 4.03 (3H, *s*, 3-OMe), 3.93 (3H, *s*, 4-OMe), 34.7 (2H, *m*, $ArCH_2$), 3.47 (2H, *m*, NCH_2), 3.34 [6H, *s*, $N(Me)_2$]. EIMS *m/z* (rel. int.): 399 $[M]^+$ (0.3), 383 $[M-16]^+$ (8), 369 (2), 354 (5), 338 (100), 323 (35), 307 (34), 291 (55), 280 (23), 265 (13), 237 (31), 58 (89); CIMS (NH_3) *m/z* (rel. int.): 400 $[M+H]^+$ (11), 384 (7), 370 (2), 356 (6), 340 (23), 339 (100), 338 (25), 323 (3).

N-*Hydroxy-northalicthuberine* (**9**). $C_{20}H_{21}NO_5$ (1.5 mg). IR ν_{max}^{film} cm^{-1} 3428, 2926, 1722, 1666, 1648, 1600, 1466, 1385, 1288, 1126, 1038, 757. ^{1}H NMR (250 MHz): 9.13 (1H, *s*, H-5), 7.75 (1H, *d*, $J = 9.2$ Hz, H-10), 7.52 (1H, *d*, $J = 9.2$ Hz, H-9), 7.33 (1H, *s*, H-8), 7.19 (1H, *s*, H-2), 6.10 (2H, *s*, OCH_2O), 4.03 (3H, *s*, 3-OMe), 3.90 (3H, *s*, 4-OMe), 3.82 (2H, *m*, $ArCh_2$), 3.69 (2H, *m*, NCH_2), 3.54 (3H, *s*, *N*Me). EIMS *m/z* (rel. int.): 355 $[M]^+$ (13), 339 $[M-16]^+$ (83), 338 $[M-17]^+$ (100), 324 (16), 308 (38), 295 (83), 281 (46), 265 (29), 237 (31), 60 (54), 44 (42); CIMS (isobutane) *m/z* (rel. int.): 356 $[M+H]^+$ (9), 340 (7), 339 (100), 338 (20).

Acknowledgements—The authors are grateful to the Swiss National Science Foundation and to the German Academic Exchange Service (DAAD) for financial support and for the fellowships awarded to M. P. V. We thank the members of the analytical departments of the Institute of Organic Chemistry, University Zurich, especially Dr Annalaura Lorenzi-Riatsch for CI and EIMS and Hans Frohofer for optical rotations, and Robert Tappe of the Institute of Organic Chemistry, University Köln, for performing partial ^{1}H NMR nOe experiments.

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