

Alkaloids from *Nigella arvensis* (*Ranunculaceae*)

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Abstract. The alkaloids glaucine, oxoglucine, predicentrine, bracteoline, isoboldine, N-methylglaucine, N-methylaurotetanine, and asimilobine were isolated for the first time from *Nigella arvensis* (*Ranunculaceae*).

Key words: aporphine alkaloids, *Nigella arvensis*, *Ranunculaceae*

Introduction

Ranunculaceae is one of the families widely distributed in Bulgaria and its species are rich in alkaloids (Assenov & Nikolov 1988). It is well known that some species belonging to family *Ranunculaceae*, as well as the alkaloids isolated from them are used in folk and official medicine. Thus, the species of the genera *Aconitum*, *Consolida* and *Delphinium* are used to treat epilepsy, migraine, radiculitis, and rheumatism (Stojanov & Kitanov 1960; Assenov & Nikolov 1988). A number of species of genus *Thalictrum* are applied in Bulgarian folk medicine as spasmolytic, hyper- and hypotensive remedies. There are data on the anticancer effect of alkaloids isolated from the species of this genus (Kupchan & al. 1963; Hahn & al. 1966; Suffness & Cordell 1985). *Nigella* seeds are used in Bulgarian folk medicine as tea with diuretic and antihelminthic effect (Jordanov & al. 1973; Petkov 1982).

Genus *Nigella* is represented by three species in Bulgaria, namely *Nigella sativa* L., *N. damascena* L. and *N. arvensis* L. (Tutin 1964; Markova 1970). Limited chemical studies of the alkaloid composition of *N. sativa* and *N. damascena* have been carried out

and several alkaloids of isoquinoline and indazole type have been isolated (Döpke & Fritsch 1970; Attar-Rahman & al. 1985a, b, 1992, 1995). To our knowledge, no phytochemical investigations of the alkaloid content of *N. arvensis* have been conducted.

This study describes the isolation and structural elucidation of eight aporphine alkaloids from *N. arvensis* growing in Bulgaria.

Materials and methods

Nigella arvensis was collected in July 1999, during the time of flowering, near Dolni Pasarel village (Sofia region). A voucher specimen was deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM 156997).

The IR spectra were taken on a Specord 75 IR. The mass spectra (MS) were obtained on a Hewlett Packard 6890 (direct inlet) at 70 eV. The ¹H NMR spectra were recorded on a Bruker DRX-250 in CDCl₃ and CD₃OD (for N-methylglaucine), with TMS as internal standard. Silica gel (70–230 mesh, Merck) was used for column chromatography (CC),

silica gel 60 PF₂₅₄ (Merck) for preparative thin layer chromatography (PTLC) and aluminium sheets silica gel 60 F₂₅₄ (Merck) for thin layer chromatography (TLC). Compounds were visualized by spraying with Dragendorff's reagent.

The whole plant (165 g), air-dried and ground, was extracted exhaustively in Soxhlet apparatus with 95 % EtOH. The ethanol extract was evaporated under reduced pressure, acidified with 3 % HCl and left overnight at room temperature. The insoluble non-alkaloid materials were removed by filtration and the filtrate was subjected to petroleum ether extraction to eliminate the rest of non-alkaloid substances. Thus purified, the acidic solution was made alkaline with 25 % NH₄OH (pH 9–10) and then extracted with CHCl₃. The combined CHCl₃ extracts were dried over anhydrous Na₂SO₄ and evaporated under reduced pressure to give a crude mixture of tertiary alkaloids (fraction A, 129.46 mg). To separate the quaternary alkaloids, the aqueous phase was acidified to pH 6–6.5 with conc. HCl, saturated solution of KI was added and then extracted with CHCl₃. The combined CHCl₃ extracts were dried over anhydrous Na₂SO₄ and evaporated under reduced pressure to give a crude mixture of quaternary alkaloids (fraction B, 93.08 mg).

By consecutive using of CC on silica gel with CHCl₃-Me₂OH (increasing polarity) and PTLC with petrol ether-CHCl₃-Me₂CO-MeOH (4:4:1:1), from fraction A were isolated: glaucine (11.10 mg), oxoglucine (8.71 mg), predicentrine (6.32 mg), bracteoline (5.63 mg), isoboldine (5.63 mg), N-methylaurotetanine (2.90 mg), and asimilobine (0.68 mg).

By PTLC with CHCl₃-MeOH-NH₄OH (30:8:0.4) from fraction B was isolated N-methylglaucine (4.27 mg).

Results and discussion

From the ethanol extract of a whole plant of *N. arvensis* the tertiary and quaternary alkaloid fractions were obtained. Eight known aporphine alkaloids were isolated from the alkaloid fractions by chromatographic procedures. The structures of the alkaloids were elucidated by direct comparison of their R_f values, IR, MS and ¹H NMR spectral data with those of the authentic samples.

The main alkaloids are glaucine (Kuzmanov & al. 1992) and oxoglucine (Philipov & al. 1998). The other alkaloids are predicentrine (Philipov & al.

1983), bracteoline (Mollov & Philipov 1979), isoboldine (Guinaudeau & al. 1975), N-methylglaucine (Guinaudeau & al. 1983), N-methylaurotetanine (Kande & al. 1994), and asimilobine (Philipov & al. 2000).

All alkaloids were isolated for the first time from genus *Nigella*.

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References

- Assenov, I. & Nikolov, S. 1988. Pharmacognosy. Meditsina & Fizkultura, Sofia (in Bulgarian).
- Atta-ur-Rahman, Malik, S., Cun-Heng, H. & Clardy, J. 1985a. Isolation and structural determination of nigelline, a novel alkaloid from the seeds of *Nigella sativa*. – *Tetrahedron Lett.*, **26**: 2759-2762.
- Atta-ur-Rahman, Malik, S., Ahmad, S., Choudhary, M. & Habib-ur-Rehman. 1985b. Nigellimine N-oxide: a new isoquinoline alkaloid from the seeds of *Nigella sativa*. – *Heterocycles*, **23**: 953-955.
- Atta-ur-Rahman, Malik, S., Hasan, S., Choudhary, M., Chao-Zhou, N. & Clardy, J. 1995. Nigellidine: a new indazole alkaloid from the seeds of *Nigella sativa*. – *Tetrahedron Lett.*, **36**: 1993-1996.
- Atta-ur-Rahman, Malik, S. & Zaman, K. 1992. Nigellimine: a new isoquinoline alkaloid from the seeds of *Nigella sativa*. – *J. Nat. Prod. (Lloydia)*, **55**: 676-678.
- Döpke, W. & Friltsch, G. 1970. Der Alkaloidgehalt von *Nigella damascena* L. – *Pharmazie*, **25**: 69-70.
- Guinaudeau, H., Leboeuf, M. & Cave, A. 1975. Aporphine alkaloids. – *Lloydia*, **38**: 275-338.
- Guinaudeau, H., Leboeuf, M. & Cave, A. 1983. Aporphinoid alkaloids, III. – *J. Nat. Prod. (Lloydia)*, **46**: 761-835.
- Hahn, R. A., Nelson, J. W., Tue, A. & Beal, J. L. 1966. Pharmacological activity of thalicarpine. – *J. Pharmac. Sci.*, **55**: 466-469.
- Jordanov, D., Nikolov, P. & Boichinov, A. 1973. Phytotherapy. Meditsina & Fizkultura, Sofia (in Bulgarian).
- Kande, K. M., Philipov, S. & Dutschewska, H. 1994. Alkaloids of *Stephania abyssinica*. – *Fitoterapia*, **55**: 90.
- Kupchan, M. S., Chakravati, K. K. & Yokayama, M. 1963. Thalictum alkaloids. Thalictarpine a new hypotensive alkaloid from *Thalictum dasycarpum*. – *J. Pharmac. Sci.*, **52**: 985-988.
- Kuzmanov, B., Philipov, S. & Deligiozova-Gegova, I. 1992. Comparative phytochemical and chemosystematic research of populations of *Glaucium flavum* Crantz in Bulgaria. – *Fitologiya*, **43**: 52-57.
- Markova, M. 1970. *Nigella* L. – In: Jordanov, D. (ed.), *Fl. Reipubl. Popularis Bulgaricae*. Vol. 4, pp. 50-54. In *Aedibus Acad. Sci. Bulgaricae, Serdicae* (in Bulgarian).

- Petkov, V.** 1982. Modern phytotherapy. Medtsina & Fizkultura, Sofia (in Bulgarian).
- Mollov, N. & Philipov, S.** 1979. Reaktionsfähigkeit von Aporphin-Alkaloiden gegenüber Formaldehyd. – Chem. Ber., **112**: 3737-3739.
- Philipov, S., Ivanovska, N., Istatkova, R., Velikova, M. & Tuleva, P.** 2000. Phytochemical study and cytotoxic activity of alkaloids from *Uvaria chamae* P. Beauv. – Pharmazie, **55**: 688-689.
- Philipov, S., Ivanovska, N. & Nikolova, P.** 1998. Glaucine analogues as inhibitors of mouse splenocyte activity. – Pharmazie, **53**: 694-698.
- Philipov, S., Petrov, O., Mollov, N.** 1983. Synthesis and properties of glaucine-guinol. – Tetrahedron, **39**: 1823-1827.
- Stojanov, N. & Kitanov, B.** 1960. Wilde Nutzpflanzen Bulgariens. Bulgarischen Acad. Wiss., Sofia (in Bulgarian).
- Suffness, M. & Cordell, G. A.** 1985. Antitumor Alkaloids. – In: **Brossi, A.** (ed.), The Alkaloids. Vol. **25**, pp. 171- 178. Acad. Press, INC., Orlando.
- Tutin, T. G.** 1964. *Nigella* L. – In: **Tutin, T. G. & al.** (eds), Flora Europaea. Vol. **1**, pp. 209-210. Cambridge Univ. Press, Cambridge.

