

QUATERNARY ALKALOIDS FROM *Thalictrum minus* subsp. *elatum* (JACQ.) STOY. et STEFANOV

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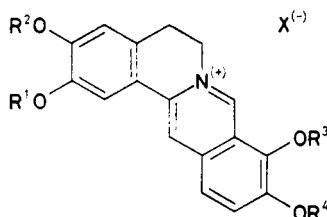
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Quaternary alkaloids berberine (chloride, 0.18%), and, after transformation into the iodides, magnoflorine (iodide, 1.06%), jatrorrhizine (iodide, 0.066%), thalphenine (iodide, 0.040%) and thalifendine (iodide, 0.021%) were isolated, after separation of the tertiary bases (0.38%), from the root of *Thalictrum minus* subsp. *elatum* (JACQ.) STOJ. et STEFANOV of Czechoslovak origin (1.8% alkaloid content). The aerial parts (0.013% alkaloids) in their quaternary fraction contain only negligible amounts of magnoflorine, berberine, and coptisine (which is the first time coptisine was found in the *Thalictrum* genus).

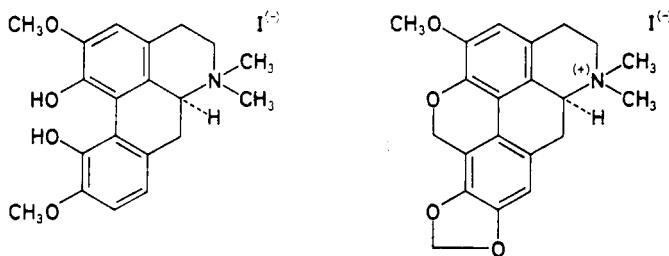
The *Thalictrum* L. genus of the *Ranunculaceae* family includes about 90 perennial species which are very rich in isoquinoline alkaloids. More than 200 alkaloids of various structural types obtained from this genus have been described so far, out of which the most current are the dimeric alkaloids of the bisbenzylisoquinoline and aporphinebenzylisoquinoline groups, monomeric alkaloids of aporphine and protoberberine groups, less current are alkaloids of the protopine, pavine, isopavine and other groups (cf. review^{1,2}). Widely distributed are quaternary alkaloids magnoflorine and berberine. The last alkaloid mentioned is — together with several related protoberberine alkaloids — responsible for the yellow colour of roots typical of the *Thalictrum* genus. The taxa often studied include the *T. minus* L. complex which is divided into many subspecies, varieties, and chemical races from which over 70 alkaloids have been isolated so far (cf. refs¹⁻³).

In the context of studies of quaternary isoquinoline alkaloids from plants growing in Czechoslovakia, in the present communication we pay attention to the quaternary alkaloids of *T. minus* subsp. *elatum* (JACQ.) STOJ. et STEFANOV which grows in the thermophyticum of this country. The only literature data about the occurrence of quaternary alkaloids in this subspecies can be found in the papers of authors^{4,5} who isolated from the roots of the plants cultivated in Poland magnoflorine and chromatographically proved the presence of berberine, jatrorrhizine, and thalifendine. The alkaloids of Czechoslovak population of *T. minus* subsp. *elatum* have not been studied so far. The plant material used in the present work was gathered in a natural locality in south-eastern Moravia. It is known⁶ that the plants from this region are hexaploid ($2n = 42$).

Rich in alkaloids was the root (about 1.8%) in which the quaternary alkaloids distinctly predominated. After separation of the tertiary bases (0.38%) we adopted the procedure usual in our papers (cf. e.g. refs^{7,8}) to obtain a fraction of quaternary non-phenolic protoberberines which on transformation into the chlorides gave berberine chloride (*Ia*; 0.18%). The slightly acidic (pH ca 6) aqueous phase was treated with potassium iodide and extracted with chloroform to obtain a fraction of iodides of strongly polar alkaloids wherefrom we separated magnoflorine iodide (*II*; 1.06%) which represents the major alkaloid of the plant. This alkaloid was found earlier (cf. refs^{1-5,9,10}) in several subspecies and races of *T. minus* L. from various geographical regions. No information is given about the occurrence of magnoflorine and/or other quaternary alkaloids in 26 Bulgarian populations of *T. minus* complex^{2,3} since the isolation method used in those studies was not oriented to isolation of strongly polar alkaloids.



Ia, R¹+R²=CH₂; R³=R⁴=CH₃; X=Cl
Ib, R¹=R³=R⁴=CH₃; R²=H; X=I
Ic, R¹+R²=CH₂; R³=CH₃; R⁴=H; X=I
Id, R¹=H; R²=R³=R⁴=CH₃; X any anion
Ie, R¹+R²=R³+R⁴=CH₃; X any anion



II

III

After the separation of magnoflorine iodide the mother liquors gave as minor alkaloids jatrorrhizine iodide (*Ib*; 0.066%), thalifendine iodide (*Ic*; 0.021%) and the iodide of a nonphenolic alkaloid (0.040%) identical with thalphenine iodide¹¹ (*III*) according to its melting point, optical rotation, UV and mass spectra. The mass

spectrum exhibits characteristic peaks at m/z 351 ($C_{21}H_{21}NO_4$), 293 ($C_{18}H_{13}O_4$), 250 ($C_{16}H_{10}O_3$), 128 (HI), 127 (I) and by far predominant ions with m/z 58 (C_3H_8N). All these fragments are due to the methine formed under the measurement conditions by the Hofmann elimination of the quaternary salt (cf. ref.¹¹). Neither columbamine¹² (*Id*) nor corytuberine, which often accompanies magnoflorine in plants, was found in our plant material.

The aerial parts of *T. minus* subsp. *elatum* contained only small amount of almost exclusively tertiary alkaloids (0.013%) which was also found² in the Bulgarian hexaploid populations of the *T. minus* complex. In the quaternary fractions we detected only slight amounts of magnoflorine, berberine, coptisine (*Ie*) and another unidentified alkaloid. Not even traces of *trans*-N-methylcanadinium hydroxide, which was found¹³⁻¹⁵ as the chief alkaloid in aerial parts of subspecies and/or populations from Soviet Union, Bulgaria, and Turkey (*T. minus* subsp. *minus* L.), were detected in the subsp. *elatum* of the Czechoslovak population which was investigated by us.

From the results given it can be seen that the Czechoslovak population of *T. minus* subsp. *elatum* from south-eastern Moravia is — with its quaternary alkaloids — very close to the population studied by the Polish authors^{4,5}. The presence of thalphenine and coptisine was found in this taxon for the first time; at the same time this represents the first instance of finding coptisine in the *Thalictrum* genus. To some extent similar quaternary alkaloids are contained also in the taxonomically not closely specified "race B" of *T. minus*¹².

EXPERIMENTAL

The melting points were determined with a Mettler apparatus FP 51 and were not corrected. The mass spectra were obtained with a JEOL MS 100 spectrometer, the IR spectra were measured in Nujol on a Specord IR (Zeiss, Jena) apparatus, and the UV spectra in methanol on a Unicam SP 1800 spectrometer. The thin layer chromatography (TLC) was carried out on silica gel G (Merck) using the following solvent systems: S1 methanol-water-25% ammonia (15 : 3 : 1), S2 ethanol-water-25% ammonia (15 : 9 : 1), S3 1-propanol-water-85% formic acid (12 : 7 : 1), and on Silufol UV 254 plates (Kavalier, Czechoslovakia) using the following systems: S4 methanol-diethylamine (4 : 1) and S5 (1 : 1). The spots exhibiting fluorescence were detected in UV light (254 and 360 nm), the other spots were made visible by the reaction with potassium hexaiodoplatinate(IV).

Extraction and Isolation of Alkaloids

The plants were gathered on July 23, 1986 in the phase of efflorescence and unripe fruits in a natural locality Hustopeče near Brno in south-eastern Moravia about in 330 m altitude above sea level and were dried at room temperature. The botanic determination of the plants was verified according to ref.⁶. The herbarium specimen is deposited in Biochemical Institute of Medical Faculty of Masaryk University, Brno. The roots (140 g) and aerial parts (620 g) were processed separately. The dry ground plant material was extracted with methanol in a Soxhlet

extractor, the solvent was distilled off, and the evaporation residue was extracted with 1% sulfuric acid, filtered, the insoluble portion was repeatedly washed with water, and the solution was filtered. The combined acidic filtrates were rid of lipid and other nonbasic constituents by three extractions with ether. The aqueous phase was alkalized with a solution of sodium carbonate and extracted four times with ether (portion A) whereafter a solution of sodium hydroxide was added to increase the pH value above 13, and the extractions with ether were repeated four times (portion B). Then the alkaline aqueous phase was acidified to pH about 6 with 20% sulfuric acid, saturated KI solution was added in excess, and the mixture was extracted twenty times with chloroform or with a mixture of chloroform and ethanol (4 : 1 by vol.) until negative reaction with the Mayer reagent (portion I).

Roots

After usual acid-base purification procedure, the portion A gave 0.53 g amorphous bases (0.38% of dry root) which were not further investigated yet. The ethereal solution of portion B was immediately treated with solid citric acid to give a rich lemon-yellow precipitate of the citrates. The ether was distilled off, and the intensively yellow crystalline evaporation residue was dissolved in hot water, the solution was filtered and, while hot, treated with hydrochloric acid. On cooling, 0.25 g crystalline berberine chloride was obtained. The chloroform solution of fraction I was filtered with charcoal and the solvent was distilled off. The crystalline evaporation residue was recrystallized from methanol to give 1.45 g magnoflorine iodide, and the mother liquor gave 82.0 mg jatrorrhizine iodide on recrystallizations from methanol. The non-crystallizing residue was divided (cf. ref.¹⁶) into a nonphenolic (I₁) and a phenolic (I₂) portion. The portion I₁ was repeatedly recrystallized from methanol to give 55.5 mg thalphenine iodide, and the portion I₂ similarly gave 31.4 mg magnoflorine iodide, 29.4 mg thalifendine iodide, and 10.6 mg jatrorrhizine iodide. The main component in the mother liquor was represented by an unidentified alkaloid with R_F 0.12 in S1. TLC also proved the absence of columbamine (S1, S3, S4, S5) and corytuberine (S1, S2, S3).

Aerial Parts

The procedure was the same as above for roots and gave 0.08 g of the purified bases of portion A (0.013% of the dry aerial parts). In portion B (1.5 mg yellow bases: 0.00024%), TLC (S1, S2, S4, S5) detected berberine and coptisine which were identified by co-chromatography with the authentic samples. The amorphous portion I contains, according to TLC (S1, S2, S3), only slight amounts of magnoflorine and of two unidentified alkaloids with R_F 0.04 and 0.18 (in S1).

Characterization of Isolated Alkaloids

The alkaloids isolated were identified by their melting points and mixed melting points, UV and IR spectra, and — in some cases — by mass spectra, optical rotation, and TLC comparison with the authentic samples (except for thalphenine whose sample was not available). The yields in % of dry root are given in parenthesis.

Magnoflorine iodide (II; 1.06): from methanol colourless prisms in druses, m.p. 264—265°C, no depression with the reference sample. The optical rotation, UV and IR spectra, and R_F values (S1, S2, S3) were identical with those of the authentic sample¹⁷.

Berberine chloride (Ia; 0.18): from dilute hydrochloric acid lemon-yellow needles, m.p. 208 to 210°C; identification by means of mixed melting point, UV and IR spectra, and TLC (S1, S4, S5).

Jatrorrhizine iodide (Ib; 0.066) crystallizes from methanol in two interconvertible forms; the lower-melting one forms orange needles, m.p. 208–209°C (refs^{12,18} give m.p. 207–209°C and 208–210°C, respectively), the higher-melting form has not yet been described in literature – it forms orange prisms, m.p. 227–228°C. Both the forms were also observed in a sample from *Berberis vulgaris*, and the samples from *T. minus* showed no m.p. depression with them. The mixtures of both forms melted between the m.p. of the lower- and higher-melting forms. The identity with the authentic sample was confirmed by UV and IR spectra and TLC (S1, S3, S4, S5).

Thalphenine iodide (III; 0.040): colourless prisms, m.p. 205–206°C (methanol), $[\alpha]_D^{21} + 57^\circ \pm 3^\circ$ (*c* 0.13, methanol); ref.¹¹ gives m.p. 198–199°C (water-acetone) and for the chloride $[\alpha]_D + 69^\circ$ (ethanol); when recalculated for the optical rotation of the quaternary cation, the values of the chloride and the iodide are practically identical, viz. $[M]_D = 291$ and 293°, respectively. UV spectrum, λ_{\max} , nm (log ε): 223 (4.36), 236 sh (4.20), 282 sh (3.62), 290 (3.73), 319 (3.91), 330 (3.85); λ_{\min} 270 (3.51) and 300 (3.67) is consistent with the spectrum in ref.¹¹, the same being true of the mass spectrum with the characteristic peaks *m/z* 351, 293 and 250 (cf. ref.¹¹), 128, 127, and 58.

Thalifendine iodide (Ic; 0.021): from methanol yellow needles incompletely melting at 220 to 225°C, at higher temperatures it darkens and carbonizes; the same behaviour in the mixture with the substance prepared by oxidation of (±)-tetrahydrothalifendine (vide infra); refs^{9,12} give m.p. 207 and 210°C, respectively. UV spectrum, λ_{\max} , nm (log ε): 229 (4.56), 278 (4.37), 353 (4.33); λ_{\min} 351 (4.23) and 313 (3.97) in good accordance with both literature data¹² and spectrum of the authentic sample; UV spectrum (methanol with 0.02M NaOH): λ_{\max} 212 (4.54), 225 (4.52), 291 (4.47), 377 (4.36); λ_{\min} 218 (4.51), 261 (4.13), and 328 (3.90). Also the R_F values (S1, S4, S5) were identical with those of the authentic sample.

Oxidation of (±)-Tetrahydrothalifendine to Thalifendine

(±)-Tetrahydrothalifendine¹⁹ (2.0 mg) was dissolved in 4 ml 10% acetic acid, treated with a solution of 16 mg mercuric acetate in 4 ml 10% acetic acid, and heated on a water bath 45 min. After addition of sodium formate the heating was continued for another 10 min, and the mercury produced by reduction was removed by filtration. The filtrate was treated with saturated solution of potassium iodide and extracted five times with chloroform. The solvent was evaporated and the residue was recrystallized from methanol to give 2.2 mg thalifendine iodide as yellow needles with incomplete melting at 220–225°C (gradual carbonization at higher temperatures). The UV spectrum and R_F values (S1, S4, S5) were identical with those of the substance isolated from the root of *T. minus* subsp. *elatum*.

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Quaternary Alkaloids from *Thalictrum minus* subsp. *elatum* (JACQ.) STOY. et STEFANOV

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Collect. Czech. Chem. Commun. 57, 573 (1992)

p. 573, in Papername:

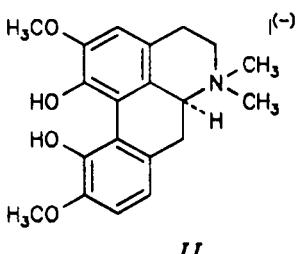
For STOY. read STOJ.

p. 574, in formula Ie:

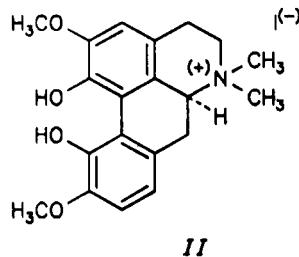
For $R^4 = \text{CH}_3$ read $R^4 = \text{CH}_2$

p. 574, in formula II:

For



read



Study of Spatial Distribution of Kinetic Energy of Turbulence in a Cylindrical System with Turbine Impeller and Baffles

Ivan Fořt, Petr Ettler, František Kolín, Juris Vanags and Maris A. Rikmanis

Collect. Czech. Chem. Commun. 57, 1053 (1992)

p. 1057, Eq. (II):

For $E = \bar{e}/[\rho_1 (\pi d n)^2]$ read $E = 2 \bar{e}/[\rho_1 (\pi d n)^2]$