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Institute of Botany, University of Ferrara, Italy

Occurrence and Phylogenetical Implications of Quaternary Alkaloids in *Ranunculus serbicus (Ranunculaceae)*

By

Angelo BONORA, Barbara TOSI, Giuseppe DALL'OLIO and Alessandro BRUNI*)

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Summary

BONORA A., TOSI B., DALL'OLLIO G. & BRUNI A. 1990. Occurrence and phylogenetical implications of quaternary alkaloids in *Ranunculus serbicus (Ranunculaceae)*. – Phyton (Horn, Austria) 30 (2): 265–272. – English with German summary.

The occurrence of quaternary alkaloids in rhizomes of *Ranunculus serbicus* VIS. was studied by HPLC. The main components are the protoberberine-type alkaloids berberine, palmatine, columbamine and the aporphine-type alkaloid magnoflorine. The presence of quaternary alkaloids as well as of other compounds confirms the centrality of *Ranunculeae* in the family and indicates possible closer relationship between *Thalictreae* and *Ranunculeae.*

Zusammenfassung

BONORA A., TOSI B., DALL'OLIO G. & BRUNI A. 1990. Vorkommen und phylogenetische Bedeutung quaternärer Alkaloide in *Ranunculus serbicus (Ranunculaceae)*. – Phyton (Horn, Austria) 30 (2): 265–272. – Englisch mit deutscher Zusammenfassung.

Das Vorkommen quaternärer Alkaloide in den Rhizomen von *Ranunculus serbicus* VIS. wurde mittels HPLC untersucht. Die Hauptkomponenten sind Alkaloide vom Protoberberin-Typ, nämlich Berberin, Palmatin und Columbamin, sowie das Aporphin-Alkaloid Magnoflorin. Die quaternären Alkaloide, ebenso andere Verbindungen, bestätigen die zentrale Stellung der *Ranunculeae* innerhalb der Familie und weisen auf mögliche engere Beziehungen zwischen *Thalictreae* und *Ranunculeae.*

*) Prof. Dr. Alessandro BRUNI, Institute of Botany, University of Ferrara, Corso Porta Mare 2, I-44100 Ferrara, Italy.

1. Introduction

Comparisons with many diverse characters are commonly used to establish the natural relationships of the taxa within *Ranunculaceae.* In this respect, systematic research was carried out on karyotype (GREGORY 1941, GEOPFERT 1974), protein serology (JENSEN 1968), plastocyanin sequences (GRUND & al. 1981) and secondary substances, such as cyanogenic compounds, acrid principles and benzylisoquinoline alkaloids (TAYLOR & CAMPBELL 1969, DICKENMANN 1982, HEGNAUER 1986, BONORA & al. 1987). In the phylogenetic schemes concerning *Ranunculaceae,* a particular significance was given to the distribution of quaternary alkaloids (TAMURA 1968, DAHLGREN 1983, HEGNAUER 1986). In recent years there has been much interest in these compounds since they have been reported to have a taxonomic importance (see for references HEGNAUER 1988) and a variety of pharmacological properties, namely, antitumor, antibacterial and hypotensive activities (see for references SCHIFF 1987). Plants that contain these alkaloids have earned a special reputation in the folk medicine of different countries around the world (SCHIFF & DOSKOTCH 1970).

This investigation reports the quaternary alkaloid constituents of *Ranunculus serbicus,* and compares them with those stated to be present in *Thalictrum* species.

2. Materials and Methods

The plant material used consisted of rhizomes of *Ranunculus serbicus* Vis. *(Ranunculaceae)* which were collected in South Italy (Sila Mountains, Calabria) during summer 1988. Voucher specimens are deposited at the Herbarium of the Institute of Botany, University of Ferrara, Italy (FER).

Dried and ground rhizomes were defatted with petroleum ether and extracted with ethanol (95%). The ethanolic extract was then fractioned into non-phenolic and phenolic tertiary alkaloid fractions and a quaternary alkaloid fraction using established procedures (AL-KHALIL & SCHIFF 1986, BONORA & al. 1989). The quaternary alkaloid fractions were purified and combined appropriately after high performance thin-layer chromatography (HPTLC) monitoring.

Final separation, purification and quantitation of individual alkaloid components were achieved by analytical and micro-preparative IP-RP-HPLC (BONORA & al. 1989). The alkaloids were identified by a direct comparison [UV, IR, M. P. (if applicable)] of their chloride and/or iodide salts [prepared via passage of the column isolate through an anion exchange resin (chloride or iodide form)] with authentic samples and/or published data (see for refereces, CHATTOPADHYAY & al. 1983). HPLC instrumentation was a Varian Model 5020 equipped with a UV 100 detector used at 345 nm for protoberberine alkaloids and 310 nm for magnoflorine. A Gilson Model 201 was employed as fraction collector. HPTLC separations of eluates were performed on precoated plates, silica gel 60 F_{254} and NH_2 F_{254} S (Merck, Darmstadt, FRG), using the solvent systems most commonly employed for the quaternary alkaloids (CHATTOPADHYAY & al. 1983, BONORA & al. 1989). Qualitative chromatographic analyses were carried out on Camag TLC Scanner II spectrofluorodensitometer

equipped with K-400 filter and recording fluorescence excitation spectra, measured automatically „in situ", with a Hitachi D-2000 density computer. Full details of the isolation, identification and quantification are available on request.

3. Results and Discussion

Quaternary alkaloids are highly polar compounds and their HPLC analysis presents some problems due to their strong interaction with the absorption sites of the stationary phase. A procedure described in our previuos work was used to overcome these difficulties (BONORA & al. 1989). The alkaloids were identified to be aporphine-base magnoflorine (24.8 μg/g dry weight) and protoberberine-base berberine (10.9 μg/g dry weight), columbamine (2.8 μg/g dry weight) and palmatine (0.9 μg/g dry weight).

Magnoflorine is widely distributed in numerous genera of at least 13 families (HEGNAUER 1966, 1988, GUINAUDEAU & al. 1988). In *Ranunculaceae* it was demonstrated to be present in the subterranean organs of 16 genera and it was retained to be highly characteristic for infrafamiliar taxa (genera, subtribes, tribes) (WILLAMAN & LI 1970, GUINAUDEAU & al. 1984, 1988). To our knowledge the occurrence of magnoflorine in *R. serbicus* represents the first report concerning *Ranunculinae*. The alkaloid was known to be present in several members of the other two subtribes *Adonidinae* and *Trautvetteriinae* of the *Ranunculeae* tribe (JEFFS 1967, HEGNAUER 1988).

Berberine, columbamine and palmatine are rather common in *Angiospermae* (HEGNAUER 1966, 1988). Approximately 220 different protoberberine-type alkaloids were described (SCHIFF & DOSKOTCH 1970). Among these, berberine is doubtless the most important one for its biological properties (SCHIFF 1987). Columbamine, a jatrorrhizine isomer, is the central intermediate in the late stages of the biosynthesis of berberine and palmatine (RUEFFER & ZENK 1986). Berberine, columbamine and palmatine are protoberberine alkaloids playing important roles as precursors in the biosynthesis of a variety of related isoquinoline alkaloids and are widely distributed in *Ranunculaceae* (HANAOKA 1988).

Several literature reports demonstrate that individual quaternary alkaloids may be rather widespread in the *Polycarpicae* group, and in particular in *Annonaceae, Berberidaceae, Menispermaceae, Papaveraceae* and *Ranunculaceae.* Within the *Ranunculaceae,* the genera *Coptis* and *Thalictrum* remain the most prolific sources of quaternary alkaloids (Tab. 1). In *Thalictrum* the presence of all these quaternary alkaloids is well documented, while in *Coptis* the occurrence of magnoflorine, at first reported by BOIT 1961, was successively not confirmed (GUINAUDEAU & al. 1988). Among recent references, IKUTA & ITOKAWA 1983 report the presence of magnoflorine in extracts of commercial korean „*Coptis* rhizome" crude drugs by TLC. However, the taxonomic identification of this crude drug is not sure *(Jeffersonia dubia?)* and magnoflorine was not satisfactorily separated by TLC. In addition, many literature references where magnoflorine

Table 1.

Distribution of the quaternary alkaloids berberine (BER), palmatine (PAL), columbamine (COL) and magnoflorine (MAG) in subterranean organs of selected genera of *Ranunculaceae* (GUINAUDEAU & al. 1984, 1988; WILLAMAN & LI 1970, JEFFS 1967. *R. serbicus:* our data.)

Ranunculaceae genera	BER	PAL	COL	MAG
Aconitum				×
Adonis				×
Anemonella				×
Aquilegia	×	×		×
Caltha				×
Clematis				×
Consolida				×
Coptis	×	×	×	(×)
Delphinium				×
Helleborus				×
Hydrastis	×			
Isopyrum				×
Nigella				×
Ranunculus serbicus	×	×	×	×
Thalictrum	×	×	×	×
Trautvetteria				×
Trollius				×
Xanthorrhiza	×			×

( ): identification not surely documented

was supposedly identified may be in error because of its similarity in properties with *N, N*-dimethyllindcarpine (STERMITZ & al. 1980). Consequently a re-examination of the occurrence of magnoflorine in the *Ranunculaceae* family is desirable. For these reasons, we have preferred to compare our results of *Ranunculus serbicus* with the recent literature data on the distribution of berberine, palmatine, columbamine and magnoflorine only in the subterranean organs of selected *Thalictrum* species (Tab. 2). In *R. serbicus* the quaternary alkaloid content is very small, in particular when it is compared with the one of *T. foliolosum*. However, magnoflorine and palmatine reach quantities that are comparable with those of *T. lucidum*. Other alkaloids of *R. serbicus* are substantially present in smaller amounts than in *Thalictrum* species.

This is the first reported isolation and quantitation of four quaternary alkaloids from the subterranean organs of a *Ranunculus species*. Some

Table 2.

Distribution of berberine (BER), palmatine (PAL), columbamine (COL) and magnoflorine (MAG) in *Ranunculus serbicus* and species of *Thalictrum*. Amounts are expressed in ‰ DW.

		BER	PAL	COL	MAG	Refereces
T. lucidum	roots	0.06	0.001	0.006	0.03	Wu & al. 1976
T. glandulosissimum	roots rhizomes	0.08	0.07	0.03	0.1	Lou & al. 1987
T. foliolosum	roots	2.0	0.08	0.01	×	Chattopadhyay & al. 1983
T. minus	callus	6.7	×	×	×	Ikuta & Itokawa 1982
T. cultratum	roots	1.0	0.03	0.03	0.5	Gao & al. 1987
T. longistylum	roots	×	×	×	×	Wu & al. 1977 a
T. javanicum	whole plant	×	×	×	×	Bahadur & Shukla 1983
T. alpinum	roots	0.7	0.006	0.04	×	Wu & al. 1980
T. podocarpum	roots	×	×	×	×	Wu & al. 1977 b
R. serbicus	rhizomes	0.01	0.001	0.003	0.025	our data

× = compounds identified but not quantified.

Table 3.

Distribution of quaternary alkaloids and protoanemonin in the four main chemically characterized groups of *Ranunculaceae*. The groups are combined on the basis of HEGNAUER'S suggestions (1986) adding new data on the occurrence of quaternary alkaloids and of protoanemonin.

Groups	Selected genera	Chemical characters
I	*Hydrastis, Coptis, Xanthorrhiza, Aquilegia, Isopyrum, Thalictrum*	large amounts of quaternary alkaloids, small amounts of protoanemonin in Aquilegia and Thalictrum
II	*Caltha, Trollius, Nigella, Delphinium, Aconitum, Adonis*	small amounts of quaternary alkaloids, small amounts of protoanemonin in Caltha
III	*Anemone, Clematis, Ranunculus, Helleborus*	small amounts of quaternary alkaloids in Ranunculus, large amounts of protoanemonin
IV	*Eranthis, Actaea, Cimicifuga*	no protoanemonin, no quaternary alkaloids

authors considered the absence of quaternary alkaloids in *Ranunculeae* phylogenetically significant (see Tab. 3). For this reason, together with other parameters, the *Thalictreae-Isopyreae-Coptideae* group and the *Ranunculeae* could not be closely related (TAMURA 1968, TAYLOR & CAMPBELL 1969, DECAMPS & BONNET 1976 a, b, HEGNAUER 1986). Our results however, confirm the centrality of *Ranunculeae* in the family and indicate possible closer relationships between the tribes of *Thalictreae* and *Ranunculeae*. This fact is in contrast with other systematic parameters such as the morphological ones, but it is in accordance with data concerning the distribution of protoanemonin and with those obtained by cumulative multifactorial analysis (DECAMPS & BONNET 1976 a, b, HEGNAUER 1986). In a recent review, HEGNAUER 1986 has divided *Ranunculaceae* in four main groups which probably represent evolutionary lines. Tab. 3 shows the distribution of quaternary alkaloids and protoanemonin in selected genera of *Ranunculaceae* on the basis of HEGNAUER'S scheme (1986) and of literature reports (for protoanemonin see BONORA & al. 1987). The study on the distribution of quaternary alkaloids and protoanemonin in the *Ranunculaceae* could lead to a new insight into generic and tribal relationships and therefore may contribute to the elucidation of the taxonomic distances existing among the taxa of this polymorphic family. Further investigations are in progress to evaluate the distribution of quaternary alkaloids and protoanemonin in *Ranunculaceae* species.

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