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Alkaloids as taxonomic markers in some species of *Magnolia* L. and *Liriodendron* L.

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Abstract

Some *Magnoliaceae* cultivated in Poland were investigated: *Liriodendron tulipifera* L. and 6 species of magnolia: *Magnolia acuminata* L., *M. denudata* Desr., *M. kobus* DC., *M. obovata* Thunb., *M. salicifolia* Maxim. and *M. tripetala* L. For alkaloid detection in the leaves thin-layer chromatography was used. In *Liriodendron tulipifera* L. and in all the *Magnolia* species liriodenine which exhibits cytostatic activity was detected. The extract of *Liriodendron tulipifera* L. leaves showed, beside liriodenine other spots on the chromatogram than did the magnolias. Alkaloids can be utilized in the chemotaxonomy of *Magnoliaceae* as a diagnostic trait. The results of the investigations indicate a certain distinctiveness of *M. acuminata* L., in agreement with those obtained by way of numerical taxonomy. Most similar as regards alkaloid content in leaves are *M. obovata* Thunb. and *M. tripetala* L.

INTRODUCTION

Magnolia L. and *Liriodendron* L. are genera foreign to the Polish flora, they are bred only in botanical gardens and arboreta.

The species growing in Poland and other *Magnoliaceae* have been the object of numerous studies of both botanists and chemists on account of their significance in the phylogeny of plants, and also because they contain various chemical compounds with a pharmacological action.

Hegnauer (1969) mentions in "Chemotaxonomie der Pflanzen" the following compounds isolated from various *Magnolia* species: essential oils, alkaloids, polyphenols and compounds with a cyclic structure.

Particularly rich in chemical compounds in the *Magnoliaceae* family is *Liriodendron tulipifera* L. A full review of the literature dealing with the chemical composition of the examined species of the genus *Magnolia* L. and *Liriodendron* L. is given by Józefowicz (1977).

The present study was undertaken to find the relation between the ability of bio-synthesising alkaloids and the taxonomic position of several *Magnolia* species belonging to two subgenera and one of the *Liriodendron* species.

MATERIAL AND METHODS

As material served leaves of the following species:
Subgenus *Magnoliastrum* DC.
1. *Magnolia acuminata* L. (Kórnik Arboretum),
2. *Magnolia obovata* Thunb. (Rogów Arboretum),
3. *Magnolia tripetala* L. (Rogów Arboretum),
Subgenus *Gwillimia* Rot.
4. *Magnolia denudata* Desr. (Cracow University Botanical Garden),
5. *Magnolia kobus* DC. (Kórnik Arboretum),
6. *Magnolia salicifolia* Maxim. (Kórnik Arboretum).

The material was collected in the summer months — June and July — when the leaves were fully developed and green.

QUALITATIVE ANALYSIS OF THE ALKALOIDS

Preparation of extracts

The leaves were extracted in a Soxhlet apparatus: 15 g of powdered leaves of each of the 7 species studied was extracted with 96° ethanol so long as the extract gave a positive reaction with Mayer's reagent (ca. 10 h.). The extract was concentrated to a syrup consistency, 2 N HCl was added and the preparation was stored in the cold for 24 h. The precipitated resin-like contaminations were filtered off. The filtrate was alkalised with 10 per cent NH_4OH to pH 9 and was threefold shaken with chloroform. Combined chloroform extracts were evaporated on a water bath. The dry residue was dissolved in 1 ml 0.1 N HCl. The solution thus obtained which could contain tertiary bases was subjected to chromatographic analysis (solution A). After separation of the chloroform layer, the remaining aqueous solution which could contain quaternary bases was examined by the method described by Jerzmanowska (1967). To the alkaline water solution there were added 2 ml of freshly prepared 4 per cent Reinecke's ammonium salt solution and the whole was stored in a refrigerator for 24 h. The red crystalline sediment formed in the reaction with alkaloids was centrifuged, washed with water with ice, dried and dissolved in acetone. The acetone solution of the Reinecke fraction was used for further chromatographic analysis (solution B). Both solutions A and B precipitated with reagents for detection of plant alkaline compounds (Dragendorff's, Meyer's and Wagner's reagents, 5 per cent silico-tungstic acid solution).

PARTITION OF TERTIARY BASES BY THE METHOD OF THIN-LAYER CHROMATOGRAPHY

Chromatographic plates (20×20 cm) were coated with Merck silica gel. For this a gel suspension 1:3 was prepared in distilled water and the plates defatted in alcohol were coated with it. After coating with gel the plates were dried at room temperature and activated by heating in a drier at 105°C for 1 h. Solutions A from all the seven investigated species were placed on the plates. Simultaneously with solutions A, alkaloid standards in 0.1 per cent solution were placed on the same plates. These were tertiary alkaloids which, as results from the literature reports, were detected in the studied species of the genera *Magnolia* and *Liriodendron tulipifera*.

From among tertiary alkaloids were used: assymilobine, liriodenine, nornuciferine, nuciferine, armepavine, norarmepavine and roemerine.

If the R_f value of one of the spots from the extract corresponded to the R_f of the standard substance cochromatography was applied, that is on the next chromatogram the standard and the substance isolated from the thin-layer chromatogram were placed at the same site. The 0.1 per cent alkaloid solution was used in the amount of 0.01 ml and the investigated solution in the amount of 0.02 ml.

For development of the chromatograms the following moving phases were used:

1. n-butanol—glacial acetic acid—water (4:1:5, v/v),
2. n-butanol—glacial acetic acid—water (100:30: water to saturation, v/v),
3. n-butanol—glacial acetic acid—water (100:4: water to saturation v/v),
4. n-butanol—water (to saturation, v/v),
5. chloroform—ethanol 95% (8:2, v/v),
6. chloroform—diethylamine (9:1, v/v),
7. 95 % ethanol—25 % ammonia (95:5, v/v).

The developed chromatograms were dried at room temperature and inspected in ultraviolet light. For chromatogram development Dragendorff's reagent was used after L i n s k e n s (1959).

The best partition of the spots from extracts of magnolia leaves was obtained in the least acid systems with n-butanol: n-butanol—water to saturation and n-butanol—glacial acetic acid—water (100:4:water to saturation, v/v). The above named two mobile phases were also used for two-dimensional chromatography.

Partition of quaternary bases by the method of thin-layer chromatography

Glass plates of 20×20 cm dimensions were used coated with silica gel Merck. On the prepared plate 0.02 ml of acetone solution of the Reinecke fraction (solution B) were placed. After drying the spots were

treated with two drops of 0.1 M silver nitrate for disrupting the complex combination of quaternary bases with Reinecke's salt. At the same time quaternary alkaloid standards of magnoflorine, magnocurarine, salicifoline, occurring in magnolias as well as choline chloride in 0.1 per cent solutions were placed on the same plates.

The following solvent systems were used for developing the chromatograms:

1. 96°-ethanol— 25% ammonia (95:5, v/v),
2. n-butanol—glacial acetic acid—water (100:4:water to saturation, v/v),
3. n-butanol—glacial acetic acid—water (4:1:5, v/v),
4. n-butanol—glacial acetic acid—water (100:30:water to saturation, v/v).

The partition of quaternary alkaloids was obtained when n-butanol—glacial acetic acid—water (100:30:water to saturation, v/v) was used in two-dimensional chromatography. All the chromatograms were inspected after drying in ultraviolet light.

RESULTS AND DISCUSSION

The chromatograms when seen in ultraviolet light after development with Dragendorff's reagent revealed the presence of alkaloids in the leaves of all the studied species. On the basis of the number of spots obtained in the chromatograms, a prevalence of tertiary over quaternary alkaloids was established. Identification of tertiary bases was facilitated by two-dimensional chromatography run for the extracts and a mixture of the corresponding standards.

Of the 6 examined magnolia species the richest in alkaloids proved to be *Magnolia acuminata*. In unidimensional and two-dimensional chromatography of leaf extracts of this species 8 spots were revealed 4 of which showed blue fluorescence in ultraviolet light, two showed orange and one yellow colour which became more intensive in UV light.

In the detection of alkaloids very helpful was their colour in ultraviolet light and after development with Dragendorff's reagent. The light yellow colour of liriodenine hardly visible before development became much more intensive in the UV. This made identification of this compound much easier. Assymilobine changed after a few days its colour from orange to brown and roemerine to grey-green. Armepavine and norarmepavine gave spots with blue fluorescence.

The same R_f values of standard alkaloids and the spots from the extracts on the chromatograms developed in various solvent systems and the characteristic colours and fluorescence in the UV indicate the presence in *Magnolia acuminata* leaves of liriodenine, assymilobine and roemerine not described to-date in this species as well as of armepavine and norarmepavine.

Among the unidentified alkaloids in *Magnolia acuminata* were two compounds fluorising blue and one orange and showing after development with Dragendorff's reagent orange colour.

Liriodenine was found in the leaves of all the examined *Magnolia* species. According to the colour intensity, it may be supposed that *Magnolia obovata* contains this compound in largest quantity. The presence of this alkaloid in magnolias growing in Poland is valuable since, as known from the literature, liriodenine inhibits *in vitro* development of tumours of the human nosopharynx (Neville, Cooke, 1969).

Less numerous were spots on chromatograms from *Magnolia kobus*, *M. obovata* and *M. tripetala*. From among the tertiary alkaloids, beside liriodenine, chromatography indicated the presence in these three species of assymilobine. One spot with lowest R_f value (0.34) could not be identified. Chromatograms of the tertiary bases from the leaves of these three species differ only in the intensity of colour of the spots. No qualitative differences were found.

The tertiary bases from *Magnolia denudata* gave on chromatograms also 3 spots: one corresponding to liriodenine, the second to assimilobine and the third not identified with lowest R_f value (0.17).

Poorest in alkaloids was *Magnolia salicifolia* in which only traces of liriodenine were found.

The chromatograms of quaternary bases revealed also differences in the number of spots according to species. Most spots (4) gave the leaves of *M. acuminata*. The R_f value and characteristic colour indicated the presence of magnoflorine and magnocurarine. Two spots were not identified, both with higher R_f values, one of them showed in UV light yellow colour.

The extract from *M. kobus* gave 2 spots, probably due to magnoflorine and magnocurarine. One spot was obtained from *M. denudata*, *M. obovata* and *M. tripetala*, probably representing magnoflorine. Trace amounts of alkaloid with similar R_f were obtained from *M. salicifolia*.

The results of chromatographic analysis and R_f values of the alkaloids are shown in Tables 1 and 2.

Most quaternary bases were found in *M. acuminata*, less in *M. kobus* and trace amounts in the remaining species.

The alkaloids identified in the magnolias not only confirm, but supplement the phytochemical studies described in the literature.

Noteworthy is the finding of liriodenine in the leaves of all the examined *Magnolia* species. From literature reports to date it is known that in six of the *Magnolia* species compared in the present study this alkaloid has been detected in the leaves, bark, wood and roots of *M. obovata* (Ito, Yoshida, 1966; Ito, Asai, 1974). So far assimilobine was also only found in this species (Ito, Asai, 1974). Magnoflorine was detected by other authors only in the leaves of *M. acuminata*

Table 1

Distribution of alkaloids in some *Magnoliaceae*

No	Species	Occurrence of alkaloids (R_f values)																				
		tertiary bases																quaternary bases				
		0.62	0.57	0.54	0.49	0.45	0.45	0.43	0.41	0.38	0.36	0.34	0.34	0.26	0.19	0.17	0.11	0.50	0.44	0.43	0.40	0.36
		—	asm	—	lir	nor arm	—	roem	nor nuc	—	—	arm	—	—	—	—	—	—	—	—	mcr	mfl
	MAGNOLIA L. subgen. *Magnoliastrum* DC																					
1	*M. acuminata* L.	—	++	—	++	++	—	+	—	+	—	++	—	—	+	—	+	+	++	—	+	+++
2	*M. obovata* L.	—	++	—	+++	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	+
3	*M. tripetala* L.	—	+++	—	++	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	+
	subgen. *Gwillimia* Rottl.																					
4	*M. denudata* Desr.	—	+	—	+	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+
5	*M. kobus* DC	—	+	—	++	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	++	+++
6	*M. salicifolia* Maxim.	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr.
	LIRIODENDRON																					
7	*L. tulipifera* L.	+	—	+++	+	—	+	—	++	—	+	—	—	tr.	—	—	—	—	—	tr.	—	—

Notations: asm — assimilobine, lir — liri odenine, norarm — norarmepavine, roem — roemerine, nornuc — nornuciferine, arm — armepavine, mcr — magnocurarine, mfl — magnoflorine, no name under R_f means the alkaloid was not identified. Spot intensity determinations on chromatograms: — none, tr. — trace of alkaloid, + weak colour, ++ distinct colour, +++ intensive colour

Table 2

Chromatographic characterisation of alkaloids occurring in some *Magnoliaceae*

Substance identified	R_1	Colour appearance day light	UV (356 nm)
Tertiary bases			
Unknown (from *L. tulipifera*)	0.62	orange	none
Assimilobine	0.57	orange→brown	none
Unknown (from *L. tulipifera*)	0.54	red	none
Liriodenine	0.49	orange	yellow
Norarmepavine	0.45	orange	blue
Unknown (from *L. tulipifera*)	0.45	orange	none
Roemerine	0.43	red→grey-green	orange
Nornuciferine	0.41	red→brown	orange
Unknown (from *M. acuminata*)	0.38	orange	orange
Unknown (from *L. tulipifera*)	0.36	orange	none
Armepavine	0.34	orange	blue
Unknown (from *M. kobus*, *M. obovata*, *M. tripetala*)	0.34	orange	none
Unknown (from *L. tulipifera*)	0.26	orange	none
Unknown (from *M. acuminata*)	0.19	orange	blue
Unknown (from *M. denudata*)	0.17	orange	none
Unknown (from *M. acuminata*)	0.11	orange	blue
Quaternary bases			
Unknown (from *M. acuminata*)	0.50	orange	none
Unknown (from *M. acuminata*)	0.44	orange	yellow
Unknown (from *L. tulipifera*)	0.43	orange	none
Magnocurarine	0.40	orange	none
Magnoflorine	0.36	red	none

(Kapadia, Shah, 1964). It was isolated also from the roots of *M. denudata* (Nakano, 1956), the branches of *M. kobus* (Nakano, Uchijama, 1956) and the wood and bark of *M. obovata* (Ito, Yoshida, 1966).

The present results indicate that alkaloids can be useful as diagnostic traits in comparative phytochemistry of *Magnolia* species. On the basis of the obtained tertiary base chromatograms it is easy to distinguish *M. acuminata* from the remaining species on account of the largest number of alkaloid spots.

Worth noting is the easy distinction on the basis of chromatograms between *M. kobus* and *M. salicifolia*. To the morphological similarity of these two species does not correspond a similar chemical composition as regards alkaloids in the leaves. In *M. kobus* are probably present assimilobine, liriodenine, magnoflorine and magnocurarine, while *M. salicifolia* revealed only the presence of liriodenine.

As compared with other species *M. denudata* contains but few alkaloids. Its chromatographic characteristic indicates the presence of

liriodenine and magnoflorine. The chromatogram of tertiary alkaloids from the leaves of this species differs distinctly from others.

As far as the yield of alkaloids in the studied magnolias is concerned, they may be ordered as follows: *M. acuminata, M. kobus, M. obovata, M. tripetala, M. denudata, M. salicifolia.*

Liriodendron tulipifera requires a separate discussion. As compared with magnolias this species is rich in alkaloids. The spots obtained on the chromatograms showed intensive colour and large sizes. Tertiary bases gave 7 spots, among which, on the basis of R_f values and the characteristic colour, liriodenine and nornuciferine could be identified. The remaining spots were not very compact. Good partition of the alkaloids of this species probably requires different methods. The quaternary bases gave only one unidentified spot with weak colour after development with Dragendorff's reagent.

By the chromatographic methods applied the chromatogram of *Liriodendron tulipifera* could be distinguished from those of the 6 *Magnolia* species examined.

It results from chromatographic analysis that for all the species subjected to analysis, both *L. tulipifera* and *Magnolia* L. the presence of liriodenine — the alkaloid specific for the family *Magnoliaceae* — is characteristic.

For further phytochemical and pharmacological studies the leaves of 4 *Magnolia* species can be recommended: *M. acuminata, M. kobus, M. obovata* and *M. tripetala* as well as *Liriodendron tulipifera* as containing alkaloids particularly rich in cytostatically active liriodenine.

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Alkaloidy jako wskaźnik taksonomiczny w niektórych gatunkach magnolii i tulipanowca

Streszczenie

Przedmiotem badań były hodowane w Polsce magnoliowate: *Liriodendron tulipifera* L. i sześć gatunków magnolii: *Magnolia acuminata* L., *M. denudata* Desr., *M. kobus* DC., *M. obovata* Thunb., *M. salicifolia* Maxim. i *M. tripetala* L. W ich liściach poszukiwano alkaloidów, posługując się metodą chromatografii cienkowarstwowej. W *Liriodendron tulipifera* L. i we wszystkich gatunkach magnolii wykryto liriodeninę, która odznacza się aktywnością cytostatyczną. Wyciąg z liści *Liriodendron tulipifera* L. poza liriodeniną dał inne plamy na chromatogramie niż badane magnolie. Alkaloidy mogą być wykorzystywane jako cecha diagnostyczna w chemotaksonomii magnoliowatych. Z przeprowadzonych badań wynika pewna odrębność *M. acuminata* L. od pozostałych gatunków, co jest zgodne z wynikami uzyskanymi drogą taksonomii numerycznej. Najbardziej podobne pod względem składu jakościowego alkaloidów zawartych w liściach są: *M. obovata* Thunb. i *M. tripetala* L.