ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVIII, nr 2: 327—336
1979

Phenolic acids of native species of the *Rosa* L. genus in Poland

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(Received: May 2, 1978)

Abstract

Phenolic acids were identified in the leaves of 23 species of native roses and their quantitative proportions were determined by the method of two-dimensional paper chromatography. The common occurrence of ellagic and gallic acid in roses was confirmed and so was the regular presence of protocatechuic, caffeic, gentisic, p-hydroxybenzoic, p-hydroxyphenylacetic, p-cumaric, syringic, vanillic, ferulic and salicylic acids. A small amount of isoferulic acid was noted only in *Rosa gizellae* Borb. and *R. eglanteria* L. var. *comosa* (Ripart) Du Mortier. Homoprotocatechuic acid was revealed only in the species of the *Rosa rubiginosa* and *Rosa tomentosa* groups from the *Caninae* section. The taxonomically limited occurrence of some phenolic acids points to the systematic differentiating value of the latter within one genus.

INTRODUCTION

The species of the *Rosa* L. genus belong among the most highly variable ones and are, therefore, difficult to identify taxonomically, hence the difficulties in elaborating intrageneric systematics. Evidence of this is found in literature data sometimes widely discrepant (Schenk, 1957; Klášterský, 1966, 1969).

The more and more frequent in plant systematics application of chemotaxonomical methods allows sometimes to discover differences in chemical properties in species morphologically similar. This prompted us to use these methods in studies of the genus *Rosa* L.

Earlier investigations of the vitamin C content in rose hips (Krzaczek et al., 1970) indicated that its quantity is dependent above all on the systematic position of the plant, and only secondly on environmental conditions.

The aim of the present paper was the study of phenolic acids in the leaves of native rose species, collected during the blooming period. Marked differences in dependence on the species, and not on the vegetation period, in the content of these compounds were demonstrated in poplars (Klimczak, Kahl and Grodzińska-Zachwieja, 1972).

So far quercitine and kempherol glycosides, among them isoquercitrine, quercitrine, hyperine and the derivative of kempherol 3-glucoside p-cumaric acid as well as tannic compounds — galatonine and elagotanine (Hegnauer, 1974) have been discovered in the leaves of roses. Phenolic acids in the leaves of some rose species were investigated by Bate-Smith (1962) who detected in *Rosa bracteata* Wendl, *R. ace* and *R spinosissma* L. ellagic, caffeic and p-cumaric acidis and ferulic acid only in *R. bracteata.*

The subdivision of the genus and the nomenclature of the species were adopted after Klášterský (1968) and his later supplementations (1969, 1969a).

MATERIAL AND METHODS

Rose leaves for analysis were mostly taken from living specimens. They were collected from bushes growing in the rose collection of the Botanical Garden of the M. Curie-Skłodowska University in Lublin, where they had been transferred from natural habitats. The following species were examined *Rosa canina* L., *R. gallica* L., *R. glauca* Pourr., *R. jundzilli* Bess., *R. pimpinellifolia* L., *R. rubiginosa* L. var. *umbellata* (Leers) Du Mortier, *R. rugosa* Thunb., *R. subcanina* (Christ) Dalla, *R. villosa* L. Additionally leaves were collected from bushes growing on natural sites such as: *Rosa agrestis* Savi. var. *inodora* (Fr.) Borb. (locality Zarzecze near Nisko), *R.corymbifera* Borkh., *R. rubiginosa* L. var. *comosa* (Ripart) Du Mortier (Mięćmierz near Kazimierz on the Vistula), *R. caesia* Sm., *R. sherardii* Davies, *R. villosa* L. var. *ciliato-petala* (Bess.) Chrshan. and *R. vosagiaca* Desp. (Lublin — Rury), *R. tomentosa* Sm. (Kazimierz on the Vistula — Trzech Krzyży Mt.) For several taxons not easily available, material was taken from herbarium specimens: *R. elliptica* Tausch (Bolęcin Nowy near Leszno, 1965), *R. micrantha* Sm. (Gromadzice near Opatów, 1969), *R. mollis* Sm. (Hutki near Tomaszów Lub.. 1965), *R. obtusifolia* Desv. (Józefów near

Biłgoraj, 1969), *R. pendulina* L. (Duszniki, 1967) *R. gizellae* Borb. (Dwikozy near Sandomierz, 1969).

In order to exclude the influence of genetic factors on the chemical differences, the material for phenolic acid analysis in the particular species was taken in each sample from one bush, analyses of 2—3 samples from various bushes being made for most taxons.

Phenolic acids for chromatography were isolated by the modified method of Bate-Smith (1962). Ten grammes of fresh or 5 g of dried leaves were homogenized with 100 cm^3 of a 2 N HCl aqueous solution and then hydrolyzed for 20 min under reflux in a boiling water bath. The hydrolyzate was filtered through filter paper. The filtrate was shaken after cooling four times with 30 cm^3 of ethyl ether. The ether layer was washed out with a 5 per cent aqueous solution of $NaHCO_3$. The carbonate layer was acidified with 10 per cent HCl and washed once more with ethyl ether. The ether fraction was dried (anhydrous Na_2SO_4) and the ether was evaporated. A semi-crystalline residue was obtained which after dissolution in 1 cm^3 of ethanol was used for chromatography.

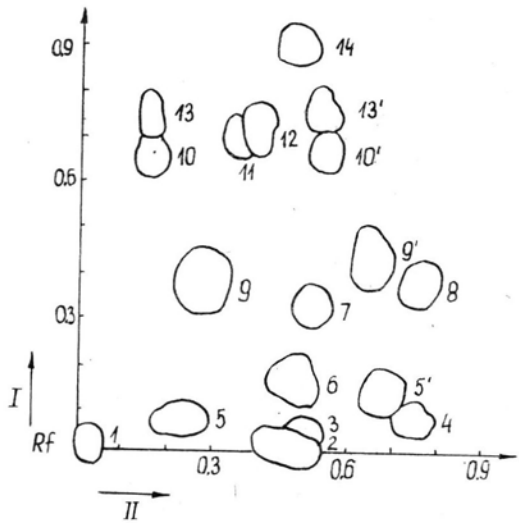

Fig. 1. A two-dimensional paper chromatogram of phenolic acids from the leaves of species of the *Rosa* L. genus. Number and staining of spots are given in table 1. The cis isomers are marked with an apostroph.

Two-dimensional paper chromatography was run on Whatman No. 1 paper (29 × 29 cm) in the following developing systems (Griffiths, 1957; Smith, 1958): direction I: benzene-acetic acid-water (6 : 7 : 3) direction II: sodium formate-formic acid-water (10 : 1 : 200). The chromatograms were inspected under UV light (ca. 366 nm) before and

Table 1

Phenolic acids spots after a two-dimensional chromatography (fig. 1) in UV light and after spraying with various reagents

Spots numbers	Identified acids	UV	UV+NH_3	2% $FeCl_3$	dSa	dNa
1	ellagic	—	—	brown	light brown	red-brown
2	gallic	—	absorbing	grey	light brown	green-red
3	gentisic	blue	yellow	navy blue	grey	grey-green
4	homoprotocatechuic	—	—	blue	pink	red-brown
5	caffeic	blue	blue	brown-green	brown	brown
6	protocatechuic	—	absorbing	brown-blue	brown	red-brown
7	p-hydroxybenzoic	—	—	—	yellow	red
8	p-hydroxyphenylacetic	—	—	—	pink	violet
9	p-cumaric	—	blue	—	red	brown-blue
10	isoferulic	violet-blue	yellow	—	light red	violet-red
11	syringic	—	—	—	red	blue
12	vanillic	—	—	brown	orange	violet
13	ferulic	blue	blue	—	violet	blue
14	salicylic	blue	blue	violet	yellow	orange

Developing reagents (Bary et al., 1950, Randerath, 1962): dSa—diazotized sulfanilic acid in 10% Na_2CO_3, dNa—diazotized p-nitroaniline.

after treatment with NH_3 and in daylight after development with the following reagents: diazotized sulphanilic acid in 10 per cent Na_2CO_3 (Bary et al., 1950), diazotized p-nitroaniline (Randrath, 1962) and 2 per cent aqueous $FeCl_3$ solution. The spots were identified on the basis of simultaneous two-dimensional chromatograms run for a mixture of the corresponding standards and on the basis of R_f, fluorescence and colour reactions with the developers, identical as those of these standards (Fig. 1, Table 1). It should be mentioned that during migration in the second direction cinammic acid derivatives separate into *trans* and *cis* isomers.

RESULTS AND DISCUSSION

The present investigations are the first attempt at revealing the qualitative composition of phenolic acids in the leaves of native rose species for the purpose of ascertaining their eventual taxonomic differentiating value. The results of analyses confirmed the presence in the plant material of phenolic acids previously found in roses (Bate-Smith, 1962; Hegnauer, 1974) such as: ellagic, gallic, caffeic, p-cumaric and ferulic acid. The following acids were found: protocatechuic, gentisic, p-hydroxybenzoic, vanillic and salicylic, new for roses, but occurring in species of other genera belonging to *Rosaceae* (Karrer, 1958; Hegnauer, 1974). Moreover, homoprotocatechuic, p-hydroxyphenylacetic, isoferulic and syringic acids were detected, so far not known to be present in the genus *Rosa* — or in other plants of the *Rosaceae* family. Thus, as the result of the phytochemical investigations performed, the phenolic acid composition characteristic for the particular rose species native for the flora of Poland (with the exception of *R. rugosa* Thunb.) could be established. The taxonomic distribution of these compounds within the plants supplies new information on their biochemical structure and on the kinship between the studied taxonomical units. The new chemical data give grounds for intrageneric classification based mainly on morphological traits.

If we consider the composition of phenolic acids in all the studied taxons (Table 2), a biochemical marked distinction may be noted between the goup of nonglandular species belonging to the sections: *Pimpinellifoliae, Cassiorhodon, Rosa* and, among the *Caninae*, only the *R. canina* group, and the remaining species of the section *Caninae* classified to the *R. tomentosa* and *R. rubiginosa* groups. For this group of roses the regular occurrence of resin glands on the leaves is characteristic. The distinguished groups were designated as chemical complexes I and II, respectively.

Table 2

The occurrence of phenolic acids in the taxons of the *Rosa* L. genus

Taxons	Phenolic acids													
I chemical complex	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sect. *Pimpinellifoliae* DC.														
R. pimpinellifolia L.	+++	+++	++		+++	++	++	++	+++		+	++	++	+
Sect. *Cassiorhodon* Dumort.														
R. majalis J. Herrm.	++	+++	+++		++	+++	+++	++	+++		+	+++	++	++
R. glauca Pourr.	++	+++	++		+++	++	++	+	+++		+	++	+	+++
R. rugosa Thunb.	++	++	++		++	++	++	+	++		+	++	++	++
R. pendulina L.	++	++	++		++	++	+++	+	++		+	+++	+	++
Sect. *Rosa* (*Gallicanae* DC.)														
R. gallica L.	++	++			++	+	+	+	++			++	++	++
Sect. *Caninae* DC.														
R. jundzillii Bess.	++	++	++		++	+	++	+	+++		+	++	++	++
R. canina group														
R. canina L.	++	++	+++		++	+	+	+	+++		+	++	+++	++
R. vosagiaca Desp.	++	+++	++		+++	++	++	++	++		+	++	++	+++

R. subcanina (Christ.) D. T. et Sarnth.	++	++	++		+++	+	+	+	+++		+	++	+	++
R. caesia Sm.	++	++	++		++	+	++	+	+++		+	++	++	+
R. obtusifolia Desv.	+++	+	+++		++	+	++	+	+++		+	+	+	+
R. corymbifera Borkh.	++	+	++		++	+	+	+	+++		+	++	++	++
II. chemical complex														
R. tomentosa group														
R. tomentosa Sm.	++	++	++	++	++	+	+++	+	+++		+	++	+	++
R. sherardii Davies	++	++	+	++	++	+	+		++		+	++	+	+
R. villosa L. var. *ciliato-petala* (Bess.) Chrshan.	++	+++	+	+	+++++++++				+++		+	++	++	+
R. mollis Sm.	++	++	++	+	+++	++	+	+	+++		+	++	++	++
R. rubiginosa group														
R. rubiginosa L. var. *umbellata* (Leers) Du Mortier	++	+++++	++	+++	+	++	+	+++			+	++	+	++
var. *comosa* (Ripart) Du Mortier	++	+++	++	++	++	+	++	++	+++	+	+	++	+	++
R. elliptica Tausch	+++++	++	+	++	++	+	+	++			++	+++	++	++
R. agrestis Savi var. *inodora* (Fr.) Borbas	+++++	++	+	++	+	++	++	+++			+	+	+	++
R. gizellae Borb.	++	++	+++	++	++	+++	++	+	+++	+	+	++	++	++
R. micrantha Borrer ex Sm.	++	+++	++	+	++	+	++	+	+++		+	++	+	++
R. caryophyllacea Bess.	+++++++++		+	+++	+	++	++	+++			+	+	++	+++

Explanation: name of phenolic acids given in Tab. 1. + — small amount, ++ — average, +++ — large amount.

Chemical complex I is characterized by the absence in all species of homoprotocatechuic acid, whereas in the other complex it is present in all species. In view of the systematically strictly conditioned presence of homoprotocatechuic acid in morphologically differing rose taxons, this compound seems to be a taxonomic element within the *Rosa* genus. This feature suggests different development lines of both these groups.

Among the species of complex I, only *Rosa gallica* L. shows a difference in the phenolic acid composition — gentisic and syringic acids being absent in this species. This fact confirms the correctness of classifying *R. gallica* L., to a separate monotypic section on the basis of morphological traits. The remaining species of complex 1 (Table 2) show an uniform qualitative composition of their phenolic acids. The lack of differences in the content of these compounds in *Rosa jundzilli* Bess. should be stressed here. This feature supports the view that this species should be classified to the section *Caninae after* Christ (1873), notwithstanding the morphological similarity to *R. gallica* L. Keller (1931) and Khshanovski (1958) for instance were of a different opinion.

Chemical complex II characterized by the presence of homoprotocatechuic acid includes the species belonging to the *R. rubiginosa* and *R. tomentosa* groups (section *Caninae*) which morphologically differ markedly. This feature may be a microtrait of taxonomic value since it seems to imply common links in the origin of the species of chemical complex II and points to their close kinship.

Thus, the present investigations demonstrated the nonuniform physiological character of the species classified to the *Caninae* section in the taxonomy of the genus included in Flora Europaea.

Among the species of the *R. rubiginosa* group, *R. gizellae* Borb. and *R. rubiginosa* L. var. *comosa* (Ripart) Du Mortier are characterized by the ability of synthesising isoferulic acid. Interpretation of this trait seems premature at present and further studies are required on the species of the *R. rubiginosa* group. The presence of this trait might for example be an indice of a righer stage of differentiation of the taxons, and we may be dealing here with a parallelism of isoferulic acid formation. This hypothesis supports the view of Klášterský (1969a), who raised the taxon *R. agrestis* Savi var. *gizellae* (Borb.) Schlimpert to the rank of species. The consistent composition of phenolic acids in *R. caryophyllacea* Bess. and other species of the *R. rubiginosa* group confirms its appartenance to the latter (Besser, 1816), in spite of morphological differences between the specimens found on the territory of Poland (in the first place fewer glands on the leaves than in the typical forms).

Within the species of the *R. tomentosa* group, an interesting division into two subgroups is noticeable. *R. sherardii* Dav. and *R. villosa* L.,

namely, do not contain p-hydroxyphenylacetic acid, in contrast to *R. mollis* Sm. and *R. tomentosa* Sm. This feature may contribute to the elucidation of the still controversial taxonomic rank of these species. According to the present authors this trait confirms the view that these units should be considered as species (Klášterský, 1968), and, above all, it supports the opinion that *R. mollis* Sm. should be separated from *R. villosa* L. in its broad sense (Keller, 1931).

In the analysis of phenolic acids composition within the groups *R. tomentosa* and *R. rubiginosa* the similarity in their biochemical structure indicates a link between these species. If we confront these traits with the known morphological data, it seems doubtful whether they can be classified to *Caninae*. The present results rather suggest that these two groups of species should be placed in a separate section with appropriate subsections.

The other phenolic acids revealed, cannot be of chemotaxonomic value within the genus *Rosa* L. in view of their common occurrence in the studied roses.

Acknowledgments:

The authors would like to thank Prof. Dr. hab. Stanisław Kohlmünzer (Medical Academy, Cracow) and Doc. Dr. hab. Lucjan Świątek (Medical Academy, Łódź) for making the standards of some acid available to them.

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Fenolokwasy krajowych gatunków rodzaju Rosa L.

Streszczenie

Metodą dwukierunkowej chromatografii bibułowej zbadano występowanie fenolokwasów w liściach 23 krajowych gatunków róż. Wyniki analizy chromatograficznej zestawiono w tab. 2. W gatunkach rodzaju *Rosa* L. potwierdzono występowanie następujących fenolokwasów: elagowego, galusowego, kawowego, p-kumarowego i ferulowego. Stwierdzono także kwasy: protokatechowy, homoprotokatechowy, gentyzowy, p-hydroksybenzoesowy, p-hydroksyfenylooctowy, izoferulowy, syryngowy, wanilinowy i salicylowy, wykryte u tych roślin po raz pierwszy. Niektóre z podanych fenolokwasów charakteryzują się ograniczonym występowaniem w obrębie rodzaju *Rosa* L., co pozwala wnioskować o wartości taksonomicznej tych związków, a także wskazuje na pokrewieństwa filogenetyczne badanych gatunków. Na tej podstawie wyróżniono dwa kompleksy chemiczne gatunków.

Rozmieszczenie taksonomiczne w obrębie rodzaju wykazuje kwas homoprotokatechowy (Tab. 2). Związek ten nie jest syntetyzowany przez I kompleks chemiczny gatunków należących do sekcji: *Pimpinellifoliae, Cassiorhodon, Rosa* i grupy *Rosa canina* z sekcji *Caninae,* podczas gdy występuje on u gatunków z grupy *Rosa tomentosa* i grupy *R. rubiginosa* sekcji *Caninae* (II kompleks chemiczny).

Wśród gatunków I kompleksu chemicznego wyróżnia się *Rosa gallica* L., u której brak jest kwasów gentyzowego oraz syryngowego i w konsekwencji gatunek ten posiada jakościowo najmniej fenolokwasów.

W II kompleksie chemicznym róż, syntetyzujących kwas homoprotokatechowy, różnice w obrębie grupy *R. rubiginosa* wykazuje *R. gizellae* Borb. i *R. rubiginosa* L. var. *comosa* (Ripart) Du Mortier. Tylko u tych taksonów wykryto obecność kwasu izoferulowego. W grupie *R. tomentosa* ściśle ograniczone występowanie wykazuje kwas p-hydroksyfenylooctowy. Występuje on u *R. mollis* Sm. i *R. Tomentosa* Sm., natomiast *R. sherardii* Davies i *R. villosa* L. odróżniają się jego brakiem.

W przybliżeniu określono też proporcje ilościowe wykrytych fenolokwasów. Do głównych składników frakcji fenolowej należy zaliczyć kwasy: elagowy, galusowy, gentyzowy, protokatechowy, p-hydroksybenzoesowy, p-kumarowy, wanilinowy i salicylowy.

Uzyskane wyniki badań rzucają nowe światło na fenolokwasy badanej grupy roślin i dostarczają nowych danych taksonomicznych dla rodzaju *Rosa* L., pozwalających uzupełnić dotychczasową klasyfikację wewnątrzrodzajową.