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CHEMOTAXONOMICAL SCREENING OF PHENOLIC GLYCOSIDES IN NORTHERN WILLOW TWIGS BY CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

The phenolic glycosides in the twig tissues of willows and common aspen (*Salicaceae*) have been extracted, purified and chromatographed using a SE-52 column. The glycosides screened were salicin, fragilin, picein, salidrosid, vimalin, triandrin, tremuloidin, populin, salicortin and grandidentatin. Each species exhibits a typical glycoside composition, which may change during the plant's life span. The glycosidic spectra may be used with certain reservations for the identification of unknown *Salix* and *Populus* species.

INTRODUCTION

Plants are able to produce a number of compounds containing one or more phenolic residues. Free phenolics are relatively rare in living plant tissues. They normally occur in conjugated forms, the most common of which are O-glycosides, where the sugars are linked through the oxygen atom of an alcohol or phenol[1].The phenolic glycosides form a signifcant part of the phenolic compounds in *Salicaceae* species. The first phenolic glycoside to be isolated was salicin, a glycoside of salicyl alcohol. Salicin and its acylated derivatives, fragilin, tremuloidin, populin and salicortin, appear to be restricted mainly to willows (*Salicaceae*), although salicin is also found in certain members of *Rosaceae*[1]. Other glycosides detected in *Salicaceae* are triandrin and vimalin, which are derivatives of cinnamic alcohols[2,3] and picein, which is the glycoside of hydroxyacetophenone. Among other glycosides, grandidentatin, salireposide and salidrosid have also been isolated from the bark or leaves of *Salicaceae*[4–9].

The complex but easy hybridization among *Salicaceae* species (especially in the group of *Diandrae*)[10] results in a number of morphological variations. Variable growth forms may also contribute to difficulties in classifying willow species taxonomically. Attempts have been made to use glycosidic diversity as a species marker, since there seem to be certain trends in the distribution of phenolic glycosides among species[3,11–13], but there have been no such attempts to investigate the willows occurring in northern Scandinavia, especially in Finland.

The aim of the present study was to determine the distribution of different phenolic glycosides in the twig tissues of several *Salicaceae* species and to detect any

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specific variations between species, as a tool for the chemotaxonomy of northern species. Capillary gas chromatography has been used throughout. It is a very sensitive and fast technique for screening phenolic glycosides, although preliminary derivatization of all the glycosides is required.

EXPERIMENTAL

Materials

The twigs used in this screening study were collected in February and May, 1982 and 1983 (except for *S. cinerea*, which was obtained in late October, 1983) near Joensuu, in Eastern Finland. Frozen twigs were immediately dried in an air circulating oven at 48°C overnight or until dry. For the 1983 twig samples, the bark and wood were separated from each other before drying. Homogenized samples were stored in air-tight containers at 4°C. The twig samples were taken from eight native species of *Salicaceae* (*S. caprea* L., *S. phylicifolia* L., *S. myrsinifolia* Salisb., *S. lapponum* L., *S. pentandra* L., *S. cinerea* L., *S. triandra* L., *Populus tremula* L.) and from two introduced, cultivated willow species (*S. viminalis* L. and *S. cv. aquatica*). For each composite sample, twigs of at least six clones were used. The lengths of the twigs used were 50 cm. Young twigs were 1–2 years old basal (juvenile) shoots, and old twigs were the top twigs of full grown plants.

Methods

The detailed methods for extraction and purification have been described previously[14]. The dried samples were extracted with acetone and ethyl acetate, eluted on a polyamide column, silylated with Tri-Sil Z (Pierce) and analysed by capillary gas chromatography. Two or three separate subsample analyses were used for each composite sample. The reference phenolic glycosides were salicin, fragilin, picein, salidrosid, vimalin, triandrin, tremuloidin, populin, salicortin and grandidentatin. The identification and quantification of the components was based on their retention times and the amounts of the reference glycosides.

RESULTS AND DISCUSSION

Willows (*Salix* sp.) and common aspen (*Populus tremula*) are very common, fast-growing woody species in Finland, varying from wet and moist riverbanks or lakesides to high arctic mountains. They reach heights of from 0.2 m (*S. myrsinites*) to as much as 15 m (*S. caprae*). *Populus tremula*, the only representative of its genus, can be as high as 30 m.

S. caprea, *S. phylicifolia* and *Populus tremula* are the most widespread *Salicaceae* species in Finland. *S. lapponum* is the most northerly of these species, occurring extensively in Northern and Central Finland. *S. triandra* is very rare, found only on flooded riverbanks in Northern Finland, and it may be sometimes a cultivation relic. *S. viminalis and S. cv. aquatica*, which are native to Central Europe, are found only in small cultivated fields in the study area. The other species investigated occur commonly in Southern and Central Finland[15].

The glycosidic spectra of willow species are shown in Fig. 1. The elution order for different glycosides on the SE-52 column is salicin, fragilin, picein, salidrosid,

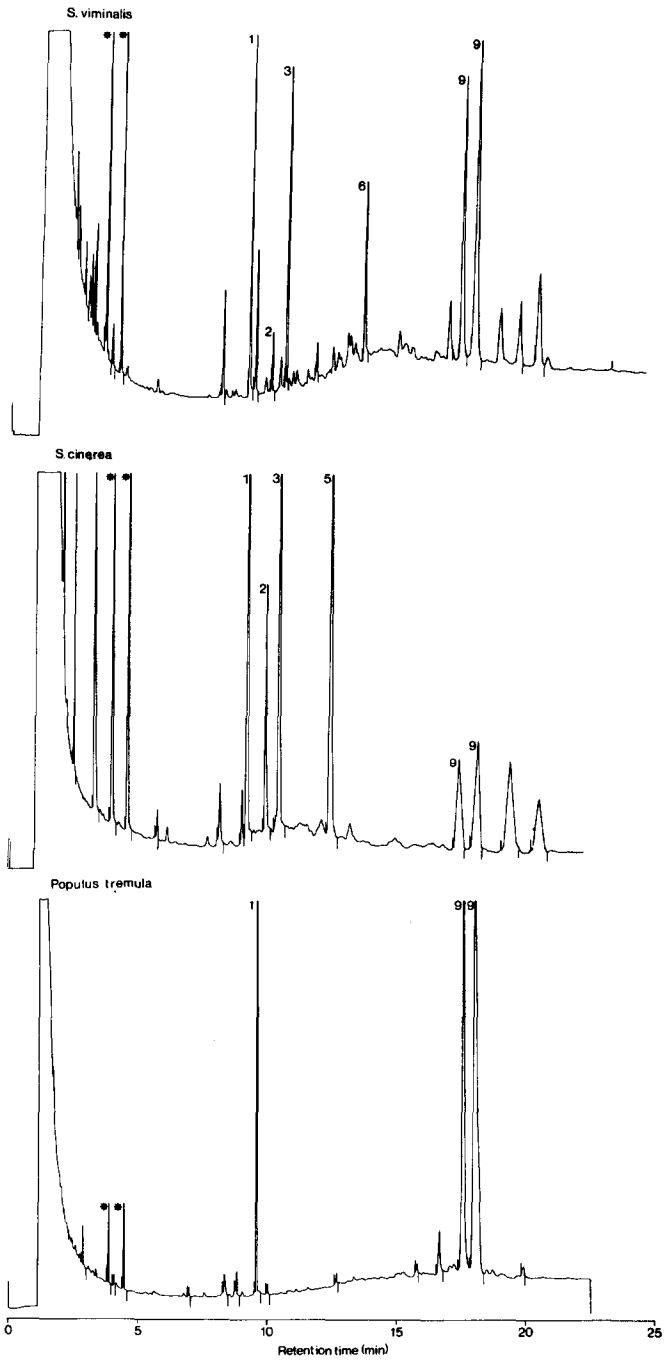

Fig. 1.

(Continued on p. 132)

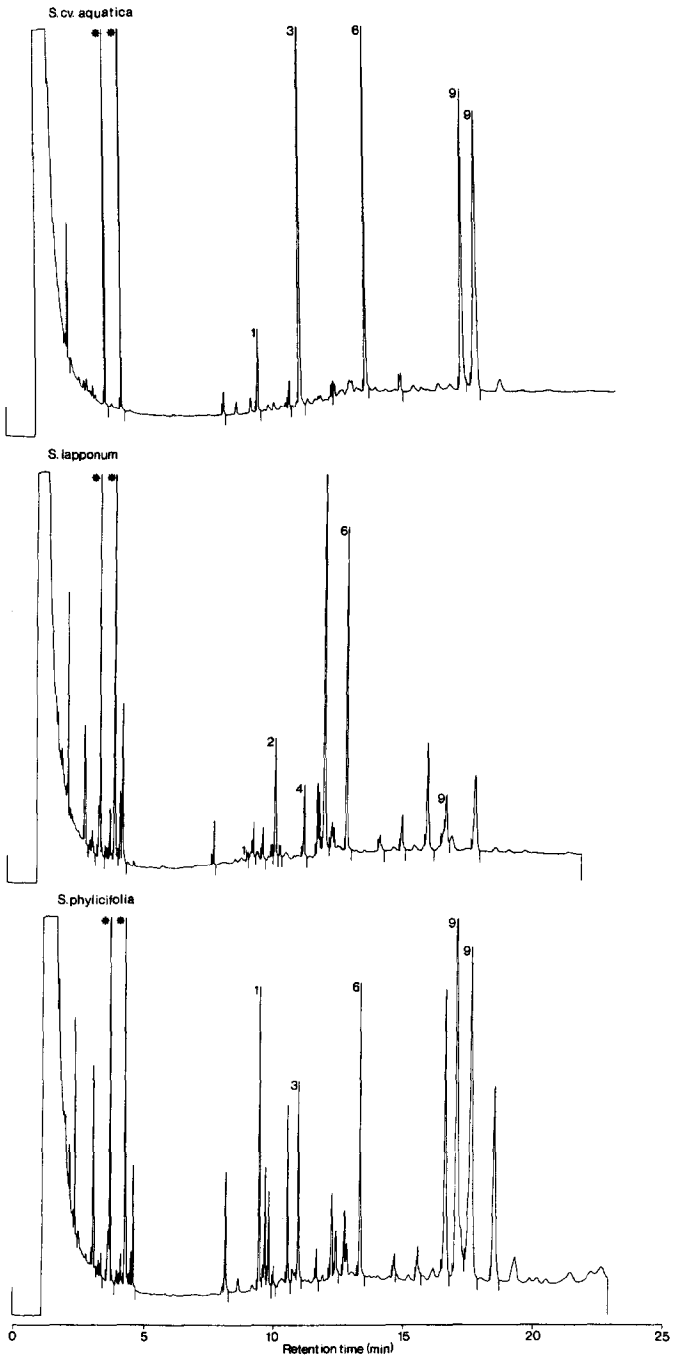
S.cv. aquatica
S. lapponum
S. phylicifolia
Retention time (min)
0
5
10
15
20
25

Fig. 1.

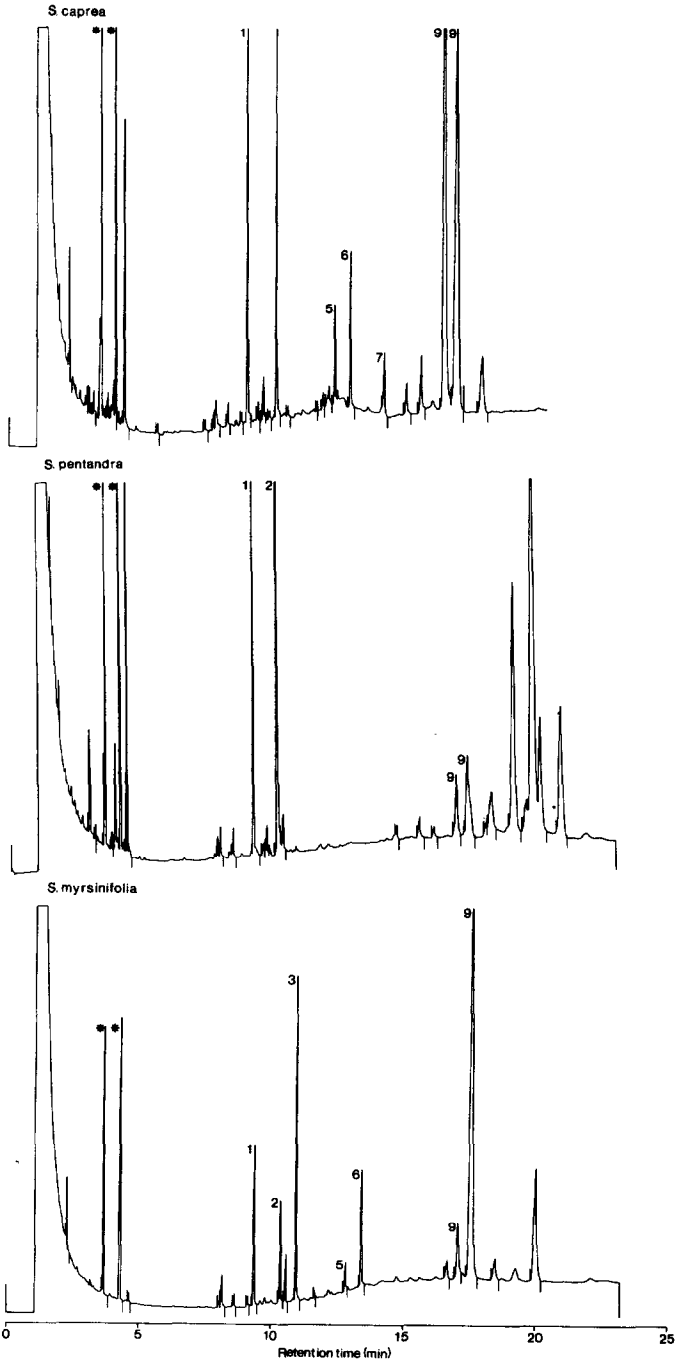

Fig. 1. Gas chromatograms of trimethylsilyl derivatives of different phenolic glycosides in the bark of willows and common aspen (*Salix* sp. and *Populus tremula*). For *S. cinerea* the whole twig samples were used. The samples were analysed on a silica SE-52 capillary column, 25 m × 0.32 mm I.D., liquid phase of 0.25 μm. The temperature program was started at 190°C, then increased at 8°C/min to 295°C. Nitrogen was used as a carrier gas (flow-rate 1.2 ml/min). The splitting ratio was 1:20 and the injected volume was 1 μl (except for *S. myrsinifolia*, 0.5 μl). Peaks: 1 = salicin; 2 = fragilin; 3 = picein; 4 = salidrosid; 5 = vimalin; 6 = triandrin; 7 = tremuloidin; 8 = populin; 9 = salicortin; * = glucose.

TABLE I

DISTRIBUTION OF PHENOLIC GLYCOSIDES IN THE BARK OF *SALIX* sp. AND *POPULUS TREMULA*

Twig samples were taken in February 1983. Results are expressed as means of three subsample analyses (mg/g on the dry weight basis).

Species	*Salicin*	*Fragilin*	*Picein*	*Salidrosid*	*Vimalin*	*Triandrin*	*Tremuloidin*	*Salicortin*	*Total ± S.E.*
S. caprea									
young	3.300	0.091	0.081	0	0.432	1.703	0	19.190	24.690 ± 1.100
old	0.588	0	0	0	0.207	0.163	0	2.727	3.684 ± 0.079
S. pentandra									
young	4.941	13.0.30	0	0	0	0	0	2.799	20.750 ± 1.168
old	0.973	11.497	0	0	0	0	0	0.927	13.400 ± 1.134
S. myrsinifolia									
young	2.932	1.772	5.477	0.097	0.536	2.900	0	21.500	35.220 ± 0.544
old	1.950	8.948	4.589	0	1.120	0.217	0	12.360	29.180 ± 1.500
S. phylicifolia									
young	1.235	0	1.220	0	0	6.598	0.359	13.548	22.960 ± 3.050
old	0.439	0	3.074	0	0	3.352	0.746	13.684	21.310 ± 1.241
S. lapponum									
old	0.047	0.102	0	0.660	0.156	2.406	0	0.887	4.258 ± 0.504
S. viminalis									
young	0.572	0.120	0.371	0	0	0.647	0	3.463	5.533 ± 0.312
S. cv. aquatica									
young	0.745	0	7.014	0	0	5.735	0.332	8.580	22.410 ± 1.677
P. tremula									
old	1.979	0	0	0	0	0	0	14.590	16.569 ± 3.640

vimalin, triandrin, tremuloidin, populin, salicortin and grandidentatin[14]. All the authentic compounds give single and symmetrical peaks, except salicortin, which yields two separate peaks, which are close to each other. Each species exhibits the species--specific composition of different glycosides, especially the main ones. In addition to one or more main peaks, several minor known components are also found. In the region of known glycosides some willows yield quite high unknown peaks, which do not correspond with any of the reference compounds screened.

It would seem to be possible to identify the willow species on the basis of their phenolic glycosides if the whole chromatogram is examined closely, comparing the relative proportions of all peaks.

All the species investigated are able to synthesize phenolic glycosides commonly found in *Salicaceae*, although the total yield differed considerably among species. The twigs used in this study were collected in midwinter (except for those of *S. cinerea*), so the level of glycosides should be at a maximum[16].

Salicin and salicortin were the most widespread chemical indicators in all the species screened, although the content of salicin in some species was very low (Tables I–III). The very labile glycoside, salicortin[4] was the main bark glycoside, except in *S. pentandra* and *S. lapponum*. It was absent from the twigs of *S. triandra*, which appears to be unique in that it contained high amounts of a rare glycoside, salidrosid (Table III). The only other species to contain salidrosid were *S. myrsinifolia* and *S. lapponum*. The salidrosid content in the latter was substantial, when compared with the total glycoside amount. *S. pentandra* produced only salicin and its two acylated derivatives (fragilin and salicortin), of which fragilin was detected in very high quantities. Populin and grandidentatin were not detected in any of the bark, wood or twig extracts screened. However, Thieme[4] has shown the presence of grandidentatin in the Central European twigs of *S. caprea* and *S. pentandra*. Vimalin was also a quite rare and trace glycoside except in old *S. myrsinifolia* winter bark and in old *S. cinerea* autumn twigs.

The synthesis of phenolics by a plant is known to change with the plant's age and even during the day[16–18]. The ability to produce phenolic glycosides (*e.g.*, salicortin) is reported to decrease more than two-fold during the growth period[16]. In my study the bark from juvenile twigs always contained higher amounts of total glycosides than the corresponding bark of old twigs (Table I). Especially noteworthy is the young bark of *S. caprea*, which contained eight times the concentration found in the old bark. However, certain species show the opposite pattern in the amount of each glycoside with respect to age. The old bark of *S. myrsinifolia* contained much higher amounts of fragilin and vimalin and lower amounts of salicortin and triandrin than the younger. The same trend was found in *S. phylicifolia* with picein, which increased, while triandrin decreased. However, *S. caprea* bark did not show the pattern found in the species in Central Europe[4,16]. There the levels of salicortin and salicin have been reported to decline, and those of triandrin and picein to increase, with age.

The qualitative glycosidic composition declines slightly with age only in the bark of *S. caprea* and *S. myrsinifolia*. This decline concerns only the minor glycosides and is not very important. This chemical reduction may simply be due to the fact that the concentration of minor glycosides falls below the detection limits of the analytical procedure.

TABLE II

DISTRIBUTION OF PHENOLIC GLYCOSIDES IN THE WOOD OF TWIGS OF *SALIX* sp. AND *POPULUS TREMULA*

The twigs are the same as in Table I. Results are expressed as means of two subsample analyses (mg/g on the dry weight basis).

Species	*Salicin*	*Fragilin*	*Picein*	*Salidrosid*	*Vimalin*	*Triandrin*	*Tremuloidin*	*Salicortin*	*Total*
S. caprea									
young	0.177	0.034	0	0	0	0.052	0	*	0.263
old	0.019	0	0	0	0	0.132	0	*	0.151
S. pentandra									
young	0.463	0.896	0	0	0	0.271	0	0.419	2.049
S. myrsinifolia									
young	0.366	0	0.154	0	0	0.521	0	0.889	1.940
old	0.262	0.086	0.339	0	0	0.277	0	0.335	1.488
S. phylicifolia									
young	0.208	0	0.051	0	0	0.968	0.060	0	1.401
old	0.061	0.086	0.263	0	0	0.449	0.077	0	0.937
S. viminalis									
young	0	0	0	0	0	0	0	0	0
S. cv. aquatica									
young	0.090	0	0.209	0	0	0.641	0.064	0	1.004
S. lapponum									
old	0.020	0	0	0	0	1.692	0	0	1.712
P. tremula									
old	0.203	0	0	0	0	0	0	2.427	2.630

* Positive result, but not calculated.

TABLE III

DISTRIBUTION OF PHENOLIC GLYCOSIDES IN THE WHOLE TWIG OF *SALIX* sp. AND *POPULUS TREMULA*

Twig samples were taken in May 1982, except *S. cinerea* in October 1983. Results are expressed as means of two subsample analyses (mg/g on the dry weight basis).

Species	*Salicin*	*Fragilin*	*Picein*	*Salidrosid*	*Vimalin*	*Triandrin*	*Tremuloidin*	*Salicortin*	*Total*
S. caprea									
young	4.438	0	0	0	0.671	3.743	0	1.271	10.123
old	1.389	0	0	0	0.085	3.262	0	1.546	6.282
S. pentandra									
old	1.247	3.626	0	0	0	0	0	1.805	6.678
S. phylicifolia									
young	1.055	0.329	1.146	0	0	5.724	0.147	3.996	12.397
old	1.939	0.166	0.571	0	0	3.545	0.384	2.331	8.935
S. cv. aquatica									
young	0.790	0.060	0.621	0	0	2.458	0	0.949	4.878
S. triandra									
young	0.050	0	0	1.404	0	0.120	2.527	0	4.101
old	0.157	0	0	2.043	0	0.152	1.926	0	4.278
S. cinerea									
old	1.789	0.681	3.076	0	2.223	*	0	1.829	9.598
P. tremula									
young	2.155	0	0	0	0	0	0	10.777	12.932
old	1.696	0	0	0	0	0	0	7.515	9.211

* Positive result, but not calculated.

Phenolic glycosides accumulate in the outer part of the twigs and only very small amounts are detected in wood tissues (Table II). The total yields in wood are around 5% of the total glycoside concentration in bark (Table I), with the exception of *S. pentandra*, *Populus tremula* and *S. lapponum*. In the wood of *S. pentandra* and *P. tremula* the glycoside content reaches 10 and 12%, respectively, while *S. lapponum* yields as much as 40% of that found in the bark. *S. viminalis* is exceptional, containing no glycosides in wood. Generally, the glycosidic composition of wood seems to be almost the same as that of bark. However, salicortin, which is one of the major components in bark, is absent in many species and the relative amount of triandrin is considerably higher in wood than in bark.

When the whole twig samples were analysed (Table III) they did not reveal any marked qualitative differences compared with the bark, although the whole twig and bark samples were taken at different times from different habitats and clones. However, the total glycoside amounts, in many samples, remained at a level which was generally less than a half of that in the bark. That is expected, because most of the secondary phenolics in twigs are concentrated in the bark and nearly 50% of the dry phytomass of these twigs consists of wood. When the contents of total phenolics in the bark and wood of these species were compared, the Folin–Ciocalteu[19] reactive phenolics never exceeded 3% in wood[18].

Certain willow species used in this study had been examined earlier in Central Europe for the presence of phenolic glycosides (*e.g.*, refs. 3, 11, 20, 21). My results are qualitatively in a fairly good agreement with those. Most of the reported compounds have been detected in northern species, too. However, some distinct differences exist with respect to the main glycosides (*e.g.*, *S. pentandra*) and the amounts of the total glycosides (*S. caprea* and *S. myrsinifolia*). Some northern species (*e.g.*, *S. myrsinifolia*) yielded glycosides which are not reported in central European species. The concentrations were, however, very low, but still detectable by capillary gas chromatography.

The total amounts of glycosides in cultivated *S. viminalis* were only one tenth of those found in Central Europe[11]. *S. viminalis* is not native to Finland, and, consequently, may not be adapted physiologically to the climatic conditions existing outside the region of its geographical distribution. Its ability to synthesize or accumulate chemicals may have been disturbed due to unfavourable environmental conditions, *e.g.*, the shortened growing season, the long, cold winter. Furthermore, cultivation practices, *e.g.*, fertilization, have been shown to affect the quantity of the phenolics in the leaves[18].

The differences in the glycoside content between northern (Finnish) and southern (Central European) willows may be a real phenomenon rather than a consequence of the different extraction or analysis procedure. Environmental factors (temperature, light, moisture, etc.) may considerably effect the state of a plant and its ability to synthesize secondary chemicals. It is also possible that the capacity to produce certain compounds, which is genetically determined, varies among the local willow populations. Also, even if every effort is made to select "pure" species for analysis, there is always a possibility of some degree of hybridization, which has not induced visible morphological differences.

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