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PHENOLIC CONSTITUENTS OF *SALIX*: A CHEMOTAXONOMIC SURVEY OF FURTHER FINNISH SPECIES

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Key Word Index—*Salix*; Salicaceae; willows; chemotaxonomy; phenolic; glucosides; salicylalcohol.

Abstract—Various parts of some 30 *Salix* species were screened for 14 simple phenolic glucosides and salicylacohol. Species-specific qualitative and quantitative variation of phenolic glucosides in willow species was considerable and dependent on the part of the plant examined. Generally, there was greater diversity in glucoside composition and a higher total amount of glucosides in the twigs than in the leaves and buds. The traditional classification turned out to be inconsistent with a classification based on phenolic glucosides only. However, simple phenolic glucosides can be used for the recognition of exomorphologically similar species and hybrid forms.

INTRODUCTION

In Finland, the genus *Salix* comprises 24 native species, numerous hybrids and several introduced species. *S. phylicifolia*, *S. caprea*, *S. myrsinifolia* and *S. pentandra* are the most widespread species, and can be found all over Finland. Eleven willow species are only found in the north. Among these *S. pyrolifolia*, *S. polaris* and *S. arbuscula* are very rare and are protected species [1]. The *S. repens* group [2], containing *S. repens*, *S. arenaria* and *S. rosmarinifolia*, is mainly southern and coastal, having a scattered occurrence in the north of Finland [1].

Willows are very variable in growth form and are found in extremely diverse habitats. The smallest prostrate dwarf species have a height of a few centimeters (e.g. *S. herbacea*) while some species may reach the height of more than 10 m (*S. pentandra*). However, most of the Finnish willows are tall shrubs or small trees.

Species identification in the genus *Salix* is often problematic owing to exomorphic plasticity and ready hybridization. The hybrid forms are usually fertile so that introgression and hybrids which may carry more than two species traits are possible [3]. In some localities hybrid forms grow more abundantly than original (pure) species. It has been proposed that the phenolic glucosides, which are the dominant secondary metabolite group in *Salix* species, can be used as taxonomic indicators of morphologically variable willow species [4, 5]. Until now, however, phenolics have been screened only in a few species of northern willows [5–7]. In this study the distribution of phenolics was investigated in other willow species, indigenous to Finland or introduced and cultivated in Finland. The variation of simple phenolic glucosides was studied in current growth twigs, mature leaves, leaf buds and flower buds. In some willows the geographical within-species variation of phenolics was compared. Chemotaxonomic and evolutionary implications of the variation in secondary phenolics in closely related species and in the phylogenetically different-aged species groups is briefly discussed.

RESULTS AND DISCUSSION

The simple phenolic glucoside content of mature leaves, current growth twigs, leaf buds and flower buds are shown in Tables 1–6. The amount and composition of phenolic glucosides in each willow tissue was moderately species-specific. The glucoside composition and the total amount varied greatly within willow species. Salicin, the characteristic glucoside for the whole genus of *Salix*, was always present but mainly as a minor component, while salicortin was the most abundant salicylate in all tissues of most willow species. Only *S. triandra* did not contain salicortin. Fragilin, the third common salicylate, was frequently present as a trace component. The most rare glucosides in willows are salidroside and arbutin. The former was the characteristic component of *S. triandra*, found in quantity also in *S. xerophila*, *S. starkeana* and *S. lapponum*. Arbutin was only detected in *S. myrtilloides*, being one of the main glucosides in this low-glucoside species. 2′-*O*-acetylsalicortin was found in a few species and is the characteristic component for all tissues of *S. pentandra* and *S. fragilis*. It was also found in flower buds, leaf buds (Tables 5 and 6) and in the twigs of *S. myrsinifolia* [8] but was absent from mature leaves [5]. 2′-*O*-acetylsalicortin accompanied with salicortin was also one of the prominent glucosides in the twigs of *S. aurita* and *S. cinerea* (Joensuu chemotypes) (Table 4). Triandrin was most frequently present in twigs, leaf buds and flower buds but it was also found sporadically in leaves. Similarly, picein may be regarded as a twig glucoside but also occurs in the buds of some species. Salireposide was restricted to twigs. An appreciable amount of tremulacin, accompanied with a low content of tremuloidin, was especially common in tissues of the *S. repens* group. It was also a very abundant glucoside in the leaf buds (Table 5) and leaves of *S. myrsinites* [7] and in the leaves [8] and twigs (Table 2) [4] of *S. purpurea*. Purpurein was present in the twigs and buds but was absent in the leaf blades [8] of *S. purpurea*. Salicylalcohol, populin and 3′-*O*-acetylsalicortin were not detected in any of willow extracts screened.

Table 1. Phenolic glucosides in the mature leaves of Salicaceae spp.

Species	1	2	3	4	5	6	7	8	9	10	11
S. fragilis	0.802	0	0.291	0	0	0	0	0.711	0	2.801	4.605 (0.035)
S. pentandra	0.525	0	0.748	0	0	0	0	0.397	0	6.205	7.875 (0.074)
S. starkeana	0.308	0	0.156	0	0.234	0.381	0	0.958	0	0	2.037 (0.055)
S. xerophila	0.268	0	0.214	0	0.402	0.391	0	0.584	0	0	1.859 (0.155)
S. pyrolifolia	0.080	0	0.161	0	0.173	0.214	0	0.825	0	0	1.453 (0.107)
S. myrtilloides	trace	0.284	0.213	0	0	0	0	0	0	0	0.497 (0.012)
S. glauca	0.707	0	0.313	0	0	0	0	3.863	0	0	4.883 (0.295)
S. borealis	8.202	0	0	0	0	0	0	55.549	0	0	63.75 (0.57)
S. arbuscula	7.450	0	0.489	0	0	0	trace	81.894	21.802	0	111.64 (5.91)
S. repens	3.780	0	0.532	0	0	0	0	97.263	17.681	0	119.26 (1.33)
S. arenaria	4.930	0	1.215	0	0	0.238	0.512	88.129	11.414	0	106.44 (2.78)
S. dasyclados	3.015	0	0.435	0	0	0	0	9.322	0	0	12.772 (0.202)
S. acutifolia	0.710	0	0.198	0	0	0	0	4.740	0	0	5.648 (0.159)
S. daphnoides	0.789	0	0.286	0	0	0	0	15.144	0	0	16.201 (0.98)
S. reticulata	0.794	0	0.250	0.731	0	0.853	0	2.171	0	0	4.799 (0.227)
S. herbaceae	0.772	0	0.184	0	0	0	0	0.427	0	0	1.383 (0.038)
S. polaris	0.459	0	0.282	0	0	0	0	1.743	0	0	2.484 (0.125)
S. retusa	2.541	0	0.388	0	0	0	0.763	20.321	6.988	0	31.001 (0.290)

1 = Salicin; 2 = arbutin; 3 = fragilin; 4 = picein; 5 = salidroside; 6 = triandrin; 7 = tremuloidin; 8 = salicortin; 9 = tremulacin; 10 = 2′-*O*-acetylsalicortin; 11 = total glucosides (s.e.).

*Results are the means of 3 to 7 subsample runs (mg/g dry wt).

Table 2. Phenolic glucosides in the current growth twigs of Salicaceae spp.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
S. fragilis	11.762	0	0.567	0	0	1.711	0	29.570	0	2.166	0	0	45.78 (1.71)
S. pentandra	0.461	0	8.012	0	0	0.800	0	0.280	0	49.622	0	0	59.18 (2.99)
S. alba	3.650	0	0.427	0	0	1.195	0	28.202	0	0	0	0	33.47 (0.49)
S. triandra	0.189	0	0.476	0	17.597	0.390	0	0	2.471	0	0	0	21.12 (0.40)
S. starkeana	4.217	0	0.697	0	0.617	22.931	0	12.947	0	0	0	0	41.41 (0.83)
S. xerophila	3.616	0	0.469	0	1.002	11.259	0	13.837	0	0	0	0	30.18 (0.43)
S. pyrolifolia	1.752	0	trace	0.640	0	0	0	36.670	0	2.360	0	0	41.42 (2.39)
S. myrtilloides	0.160	2.123	0.406	2.901	0	2.932	0	2.156	0	0	0	0	10.68 (0.51)
S. glauca	5.296	0	0	1.753	0	0	0	44.275	0	0	0	0	51.32 (0.25)
S. borealis	0.774	0	0.628	0.671	0	0.122	0	10.811	0	0	0	0	13.01 (0.27)
S. arbuscula	8.091	0	0.498	1.365	0	0	trace	104.660	7.852	0	0	0	122.47 (6.11)
S. repens	0.701	0	0	0.530	0	0	0.906	93.981	11.050	0	0	1.830	109.00 (0.54)
S. arenaria	0.789	0	0.168	1.378	0	0	1.101	87.262	7.852	0	0	2.403	100.95 (1.48)
S. purpurea	3.363	0	0.312	0	0	0	1.021	72.213	2.351	0	10.561	12.817	102.64 (2.94)
S. dasycladös	1.122	0	0.423	3.215	0	17.132	0	23.767	0	0	0	0	45.66 (1.34)
S. daphnoides	2.722	0	0.440	0	0	0	0	87.439	0	0	0	0	90.60 (4.22)
S. reticulata	6.898	0	0	26.997	0	0	0	17.665	0	0	0	0	51.56 (0.85)
S. herbaceae	5.359	0	0	6.533	0	0	0	105.015	0	0	0	0	116.91 (3.21)
S. polaris	8.096	0	0.853	3.087	0	0	0	91.390	0	0	0	0	103.43 (1.20)
S. retusa	3.633	0	0.621	10.311	0	0	0	46.119	0	0	0	0	60.68 (0.21)

1 = Salicin; 2 = arbutin; 3 = fragilin; 4 = picein; 5 = salidroside; 6 = triandrin; 7 = tremuloidin; 8 = salicortin; 9 = salireposide; 10 = 2′-*O*-acetylsalicortin; 11 = purpurein; 12 = tremulacin; 13 = total glucosides (s.e.).

*The results are the means of 5 to 7 subsamples runs (mg/g dry wt).

Table 3. The effect of growing place on phenolic glucosides in *Salix* spp.

Species place*	Plant part	1	2	3	4	5	6	7	8	9	10	11
S. rosmarinifolia												
Turku	Twigs	5.929	0	0	0	0	0	0	108.890	9.063	2.766	123.88 (6.86)
Joensuu	Twigs	1.200	0	0	0	0	0	0	84.509	8.788	2.217	96.71 (1.85)
Turku	Leafbud	2.216	0	0	0	0	0	0	41.917	0	21.916	66.05 (4.56)
Joensuu	Leafbud	8.915	0	0	0	0	0	1.179	62.917	0	19.312	92.32 (7.74)
S. lapponum												
Turku	Twigs	0.086	0.309	1.590	0	0.279	48.371	0	24.395	0	0	75.03 (6.62)
Kuusamo	Twigs	0.445	0.484	0.646	0	0	33.290	0	15.707	0	0	50.57 (0.77)
Turku	Leafbud	1.356	0.629	1.970	0	0	6.424	0	6.107	0	0	16.48 (0.15)
Kuusamo	Leafbud	1.087	0.446	1.419	0	0	13.965	0	4.251	0	0	21.17 (0.50)
S. hastata												
Kew Gardens	Twigs	2.318	0	0	0	0	0	0	75.660	0	0	77.98 (2.36)
Oulu	Twigs	1.331	0	0	0.621	0	0	0	48.755	0	0	50.71 (2.31)
Kuusamo	Twigs	3.331	0	0	1.188	0	0	0	53.136	0	0	57.66 (1.65)
Kew Gardens	Leaves	2.868	0.593	0	0	0	0	0	101.545	0	0	105.01 (3.80)
Oulu	Leaves	0.163	0	0	0	0	0	0	0.484	0	0	0.651 (0.050)
Kuusamo	Leaves	trace	0	0	0	0	0	0	trace	0	0	trace
S. lanata												
Kew Gardens	Twigs	0.390	0.615	0	1.205	0	0	0	29.197	0	0	31.41 (2.51)
Turku	Twigs	4.620	0.196	0	7.766	0	0	0	86.539	0	0	99.12 (1.21)
Kew Gardens	Leaves	0.223	trace	0	0.163	0	0	0	0.386	0	0	0.386 (0.010)
Turku	Leaves	0.169	trace	0	trace	0	0	0	0	0	0	0.169 (0.005)

1 = Salicin; 2 = fragilin; 3 = salidroside; 4 = picein; 5 = vimalin; 6 = triandrin; 7 = tremuloidin; 8 = salicortin; 9 = salireposide; 10 = tremulacin; 11 = total glucosides.

*Turku is located in the south of Finland; Joensuu is in the east of Finland, Kaavi is 80 km west of Joensuu; Kuusamo and Oulu are in the north of Finland and Kew Gardens is in the south of England.

The results are the means of 3 to 5 subsample runs (mg/g dry wt, s.e. is shown in parentheses).

Table 4. The variation of phenolic glucosides in the current growth twigs and buds of *Salix cinerea* individuals (a–c) and *S. aurita* individuals obtained from different growing places

Species place*	Plant part	1	2	3	4	5	6	7	8	9
S. cinerea										
Kaavi (a)	Twigs	1.082	0.638	7.716	0	4.028	13.430	10.776	0	37.67 (0.70)
Kaavi (b)	Twigs	1.221	0.547	9.478	0	2.095	8.053	5.721	0	27.12 (1.38)
Kaavi (c)	Twigs	1.014	0.547	6.536	0	2.017	8.388	5.793	0	24.30 (1.00)
Joensuu (a)	Twigs	0.946	1.086	3.785	0	3.885	10.264	18.694	4.352	43.02 (1.46)
Joensuu (b)	Twigs	1.563	2.309	2.036	0	4.541	7.490	4.759	14.823	37.52 (0.48)
Kaavi (a)	Leafbud	0.116	0.347	0	trace	0	1.959	0	0	2.422 (0.112)
Joensuu (a)	Leafbud	0.198	0.326	0	0	0	1.083	0	0	1.607 (0.119)
Joensuu (b)	Leafbud	trace	0.440	0	0	0	1.364	0	0	1.804 (0.221)
Kaavi (a)	Flower bud	2.746	0.405	0	0.248	trace	4.722	18.999	0	27.12 (0.46)
Joensuu (a)	Flower bud	2.759	0.240	0	0	0	2.033	9.053	0	14.09 (0.47)
S. aurita										
Helsinki	Twigs	1.119	1.498	0.230	0	5.116	7.810	10.000	13.405	39.28 (1.14)
Joensuu	Twigs	1.280	1.614	0.352	0	2.034	13.087	8.431	9.177	35.98 (1.27)
Helsinki	Leafbud	1.550	0.454	0	0.490	0	0.913	0.880	0	4.287 (0.243)
Joensuu	Leafbud	trace	0.249	0	0	0	0.479	0	0	0.727 (0.059)
Helsinki	Flower bud	7.275	0.584	0	0.583	trace	2.346	3.830	0	14.62 (0.13)
Joensuu	Flower bud	0.688	0.572	0	0.175	0	1.484	9.515	0	12.43 (0.56)

1 = Salicin; 2 = fragilin; 3 = picein; 4 = salidroside; 5 = vimalin; 6 = triandrin; 7 = salicortin; 8 = 2′-*O*-acetylsalicortin; 9 = total glucosides.

*Helsinki is located in the south of Finland.

The results are the means of 3 to 6 subsamples runs (mg/g dry wt, s.e. is shown in parentheses).

The magnitude of the variation in total glucoside amount among willow species was considerable, being 1.1 to 12.2% in twigs and 0.05 to 11.9% in leaves. The total glucoside content in twigs was usually higher than in mature leaves (Tables 1 and 2). The majority of Caprisalix species (e.g. *S. starkeana*, *S. lanata* and *S. pyrolifolia*) contained traces of glucosides in the leaves but moderate amounts in the current-growth twigs. The distribution of glucosides in the low-growth-form Chamaetia species (*S. herbacea* and *S. polaris*) was very strongly biased towards twigs. Seventeen out of 28 species screened in this study were characterized by a high amount of salicin and its derivatives in their twigs. In these species salicylates made up more than 90% of the total glucoside amount. A similar trend in the distribution of salicylates and non-salicylates among willow species was also found in the analysis of mature leaves. *Salix myrtilloides* and *S. triandra* were exceptional in that they contained mainly non-salicylate glucosides.

The glucoside content in willow parts has been reported to be dependent on the physiological activity of the tissues, showing seasonal, diurnal and intra-species fluctuations [e.g. 4, 9]. The phenotypic expression of willow glucosides may also be influenced to a certain extent by the growing conditions [10]. Variation in the results may be increased by inconsistent sample pre-handling, extraction and different analytical methods (colour reactions, HPLC and GC) used in willow glucoside chemistry [8, 11]. Since an individual glucoside pattern may be affected by several factors, the comparison of one's results with previous glucoside analyses of the same species [9, 11, 12] is difficult. However, the glucoside patterns of most of the Central European willow species are in fairly good agreement with my results for the corresponding species in Finland. The main glucoside is mostly the same and the total composition of glucosides differs only in the minor components, which may be due to detection efficiency and/or invalid interpretation. On the other hand, northern *S. lapponum* and *S. arbuscula* twigs differ quite drastically from the closely related Central European species *S. helvetica* and *S. waldsteiniana* [3], respectively, neither of which contain salicin and its derivatives [13, 14]. Moreover, *S. hastata* obtained from Kew Gardens (U.K.), produced high contents of salicylates, both in twigs and in leaves, while Finnish *S. hastata* leaves (several individuals) contained only traces of glucosides (Table 3) [7]. Although much of the inconsistency of glucoside chemistry between northern and Central European willows may be explained by environmental, developmental and possible methodological dissimilarities, the unrelated patterns may also be a consequence of the complexity of willow species relationships and the vigorously ongoing willow speciation [3].

In several species the total amount of glucosides was higher in dormant flower buds than in leaf buds (Tables 5 and 6). An extremely high difference in glucoside concentration between flower buds and leaf buds was found in *S. caprea*. Generally, several species which contained a trace amount of glucosides in the leaves (e.g. *S. myrtilloides*, *S. xerophila*, *S. starkeana*, *S. caprea*) [5] yielded noticeably higher contents in the buds while species with high amounts of glucosides in the mature leaves (e.g. *S. repens*, *S. myrsinifolia* and *S. purpurea*) (Table 1) [5, 8] also accumulated high or moderate amounts of glucosides in the buds. The glucoside composition in flower buds was

Table 5. The phenolic glucoside content in the leafbuds of *Salix* spp.

Species	1	2	3	4	5	6	7	8	9	10	11	12
S. pentandra (b)	1.075	0	9.315	0	0	0.554	0	0.922	28.262	0	0	40.13 (1.96)
S. triandra (a)	0	0	0	0.448	22.138	0.320	0	0	0	0	0	22.91 (0.08)
S. fragilis (b)	4.250	0	0.361	0	0	1.457	0	10.987	1.897	0	0	18.95 (0.50)
S. alba (a)	0.393	0	0.418	0	0	0.549	0	1.493	0.553	0	0	3.40 (0.14)
S. starkeana (a)	0.387	0	0.791	0	0.575	3.270	0	1.678	0	0	0	6.70 (0.05)
S. xerophila (a)	trace	0	trace	0	0.570	2.579	0	0.694	0	0	0	3.84 (0.18)
S. myrtilloides (b)	trace	1.243	0.594	0	0	1.423	0	0	0	0	0	3.26 (0.15)
S. myrsinifolia (a)	12.654	0	0.317	0	0	0.674	0	79.976	0.604	0	0	94.23 (1.54)
S. myrsinifolia (b)	6.326	0	0.238	0	0	1.234	0	46.409	0	0	0	54.21 (1.63)
S. caprea (a)	0.178	0	0.206	0	0	1.429	0	0.994	0	0	0	2.81 (0.13)
S. caprea (b)	0.246	0	0.256	0	0	0.908	0	0.408	0	0	0	1.82 (0.05)
S. repens (a)	1.334	0	0.186	0.501	0	0	0	40.287	0	0	6.761	49.07 (1.89)
S. arenaria (b)	1.450	0	0.389	1.569	0	0	0	32.336	0	0	5.437	41.18 (0.51)
S. purpurea (b)	10.855	0	0.382	0	0	0	trace	77.396	0	2.593	12.296	103.52 (1.79)
S. dasyclados (a)	0.965	0	0.194	1.666	0	4.194	0	8.185	0	0	0	15.20 (0.41)
S. myrsinites (a + b)	5.061	0	1.237	0.709	0	0	2.665	59.377	0	0	18.681	87.75 (0.91)

1 = Salicin; 2 = arbutin; 3 = fragilin; 4 = picein; 5 = salidroside; 6 = triandrin; 7 = tremuloidin; 8 = salicortin; 9 = 2′-*O*-acetylsalicortin; 10 = purpurein; 11 = tremulacin; 12 = total glucosides.

(a) = male; (b) = female

Results are the means of 3 to 5 subsample analysis (mg/g dry wt, s.e. is shown in parentheses).

Table 6. Phenolic glucosides in the flowerbuds of *Salix* spp.

Species	1	2	3	4	5	6	7	8	9	10	11
S. triandra (a)	0	0	0	23.763	0.420	0	0	0	0	0	24.18 (0.38)
S. fragilis (b)	4.336	0.339	0	0	1.513	0	11.048	1.897	0	0	19.13 (0.73)
S. alba (a)	0.245	0.355	0	0	0.551	0	2.034	0.323	0	0	3.51 (0.12)
S. starkeana (b)	0.475	0.848	0	0.739	4.464	0	1.974	0	0	0	8.50 (0.06)
S. lanata (a)	2.530	0.154	2.216	0	0	0	22.173	0	0	0	27.07 (1.15)
S. myrsinifolia (a)	3.108	0.564	0	0	1.003	0	63.692	3.487	0	0	71.85 (0.46)
S. myrsinifolia (b)	2.693	0.320	0	0	1.806	0	43.420	1.545	0	0	49.78 (0.77)
S. caprea (a)	6.866	0.391	0	0	8.326	0	60.478	0	0	0	76.06 (0.99)
S. caprea (b)	15.160	0.342	0	0	3.728	0	15.552	0	0	0	34.78 (0.63)
S. arenaria (b)	1.417	0.397	1.236	0	0	1.210	31.869	0	0	3.675	39.80 (0.89)
S. purpurea (b)	12.569	0.394	0	0	0	0.720	83.164	0	2.376	13.523	112.75 (0.99)
S. dasyclados (a)	6.264	0.271	0.834	0	7.127	0	27.801	0	0	0	42.30 (1.19)
S. myrsinites (a + b)	2.340	0.574	0.860	0	0	1.014	55.659	0	0	11.677	74.49 (1.58)

1 = Salicin; 2 = fragilin; 3 = picein; 4 = salidroside; 5 = triandrin; 6 = tremuloidin; 7 = salicortin; 8 = 2′-*O*-acetylsalicortin; 9 = purpurein; 10 = tremulacin; 11 = total glucosides.

(a) = male; (b) = female.

Results are the means of 3 to 5 replicates (mg/g dry wt, s.e. is shown in parentheses).

quite similar to that found in twigs of the same species. This may be mainly explained by tissue similarities: a considerable part of the phytomass in willow winter-dormant inflorescences consists of receptacle tissues which resemble the primary stem tissues. Moreover, preliminary analyses of separate flower organs showed that the glucoside composition of receptacles was similar to that of the twigs, while the bracts of pistils and the bracts of stamens were similar to the foliage leaves. On the other hand, pistils, stamens, scales and nectar extract did not contain glucosides in detectable amounts (unpublished results).

The existence of exceptionally high amounts of phenolic glucosides in buds, especially flower buds, might indicate their contribution to winter hardiness. At the same time, the glucosides may partly function as carbon storage for those willows which will start to flower before leaf burst early in the spring. Nor should one forget the possible role of willow phenolic glucosides in pollination via salicylate metabolites and in plant growth regulation by maintaining dormancy. However, one of the main functions of the bitter-tasting simple glucosides may be to provide the willows with resistance against pathogens and certain generalist herbivores [16–18].

The dioecious nature of willow plants may complicate the investigation of the ecological importance [18] and chemotaxonomical utilization of phenolic glucosides. Previous results on the distribution of twig and leaf glucosides between willow sexes do not indicate any clear-cut trend of sex-biased abundance [4, 9, 19]. On the other hand, the staminate catkins of Central European willow individuals have been shown to contain appreciably higher amounts of glucosides than the carpel catkins [9]. In the present study, *S. caprea* and *S. myrsinifolia* also showed a strong difference in bud glucosides between the sexes (Tables 5 and 6): the leaf buds and flower buds of male individuals contained higher total amounts of glucosides but the qualitative composition was identical in both species. The individuals analysed were in a winter-dormant phase so a substantial proportion of the glucoside variation between sexes may be real and not induced by different developmental stages, as has been suggested with respect to the Central European early spring catkins [9].

The glucoside compositions of *S. rosmarinifolia*, *S. lapponum* and *S. aurita* grown under different environmental conditions and in different localities (Turku, Helsinki, Joensuu, Kuusamo) were essentially identical (Tables 3 and 4). The intra-species differences between local willow stands were mostly quantitative, while qualitative differences, when they occurred, involved minor components and may actually be quantitative. On the other hand, *S. cinerea* individuals (in the Joensuu and in Kaavi populations) turned out to be slightly different chemotypes. None of the Kaavi individuals contained 2′-*O*-acetylsalicortin but they did contain a fairly high amount of picein, while both Joensuu plants yielded 2′-*O*-acetylsalicortin and lower amounts of picein. It is possible that *S. cinerea* individuals grown in the Kaavi population may be some kind of hybrid with other Caprisalix species (like *S. phylicifolia* or *S. caprea*), which are very abundant in this area. The failure of Kaavi individuals to flower, might also suggest the incompetent genetic combination of a hybrid origin. This chemotypic variation which is found also in polymorphic *S. phylicifolia* and *S. myrsinifolia* individuals [19], may be an indication of the genetic instability and temporary speciation lines of these actively evolving species [3].

The numerical classification of all Finnish willow species based on leaf glucoside analysis, in this study and in previous papers [5–8], forms several clusters (Fig. 1). Eighteen of the 33 species analysed, mostly of northern origin, belong to a group containing a low amount of several leaf glucosides, which are mainly non-salicylates. The phylogenetically aged species, *S. pentandra* (with *S. fragilis*) and *S. triandra* form a group of their own, in which the predominant components are 2′-*O*-acetylsalicortin and salidroside, respectively. The closely related species, *S. repens*, *S. arenaria* and *S. rosmarinifolia* form a group which also includes northern *S. myrsinites* and *S. arbuscula*, with the introduced species, *S. purpurea* and *S. retusa*. Typical of these species is a high amount of glucosides, with the most prominent salicylate being tremulacin. *Salix myrsinifolia*, together with its northern counterpart *S. borealis*, forms the next group, which also includes the introduced species, *S. dasyclados* and *S. daphnoides*.

The clustering of willow species on the basis of the glucoside content in their twigs showed a higher average dissimilarity between species than on the basis of their leaf glucosides (Fig. 2). As with the classification of leaf glucosides *S. repens*, *S. arenaria* and *S. rosmarinifolia* comprise a group which also includes northern *S. arbuscula*. This group is characterized by its high glucoside content, with an appreciable amount of tremulacin and salireposide. The morphologically close species *S. aurita* and *S. cinerea* form a group with a moderate glucoside content, especially of triandrin and 2′-*O*-acetylsalicortin. *Salix starkeana* and its northern counterpart *S. xerophila* form a group, characterized by the glucoside triandrin. The exomorphically dissimilar species, *S. caprea*, *S. phylicifolia* and *S. lapponum* are also connected with this group, chiefly because of their complementary triandrin content. *S. myrtilloides*, *S. purpurea*, *S. triandra* and *S. pentandra* each form a group of their own, all of which contain one predominant marker glucoside: arbutin, purpurein, salidroside and 2′-*O*-acetylsalicortin, respectively.

A grouping of willow species on the basis of phenolic glucosides does not completely coincide with the morphological and phylogenetical classifications. The glucoside profile of *S. arbuscula*, which is often difficult to distinguish, morphologically, from *S. phylicifolia* [3] is totally different from that of *S. phylicifolia* [5, 8]. Also notable is the extreme dissimilarity in leaf glucoside chemistry between the morphologically similar *S. phylicifolia* and *S. myrsinifolia* [5]. On the other hand, the twig glucoside spectra of the species pairs like *S. repens*–*S. arenaria* (*S. rosmarinifolia*), *S. xerophila*–*S. starkeana*, *S. aurita*–*S. cinerea* and *S. polaris*–*S. herbaceae* strongly support the morphological classification. Moreover, the tissues of northern *S. myrsinites* [7] of the subgenus Chamaetia and northern *S. arbuscula* of the subgenus Caprisalix have the same secondary chemical profile as southern *S. repens* of the subgenus Caprisalix, although these species are morphologically quite distinct. However, these are all low-growth-form willows. *Salix triandra*, *S. pentandra*, *S. fragilis* and *S. alba* of the subgenus *Salix* and *S. reticulata* of the subgenus *Chamaetia* are considered to be more primitive and older species, which existed before glaciation [20]. The twigs of *S. triandra*, *S. pentandra* and *S. reticulata* differ distinctly from the common glucoside

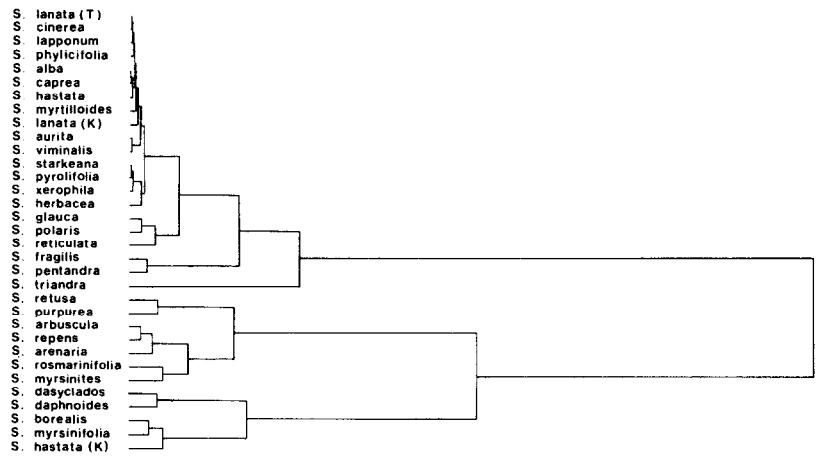

Fig. 1. The clustering of the *Salix* species based on the composition of simple phenolic glucosides in the mature leaves (T and K refer to the growing place in Turku and Kew Gardens, respectively).

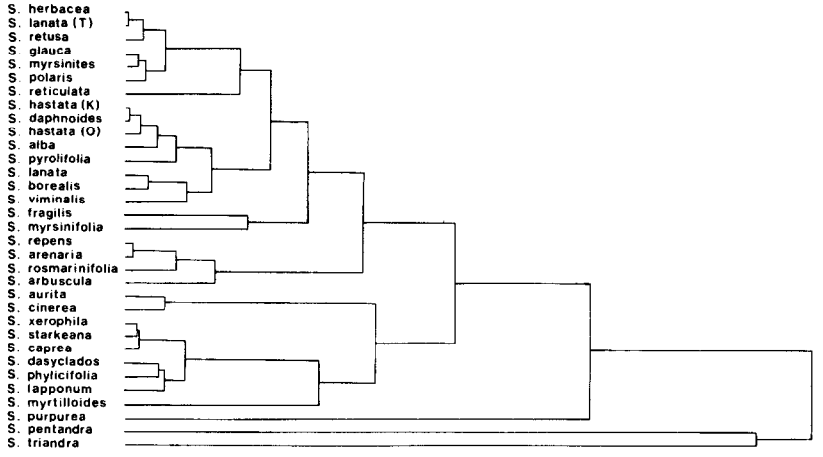

Fig. 2. The clustering of the *Salix* species based on the composition of simple phenolic glucosides in the current-growth twigs (T, O and K refer to the growing place in Turku, Oulu and Kew Gardens, respectively).

trend. All of them have the special main glucoside (salidroside, 2′-*O*-acetylsalicortin and picein, respectively), while the twigs of *S. fragilis* and *S. alba* rank with the common group of willows. However, on the basis of leaf glucosides as well as on leaf exomorphic traits *S. fragilis* is close to *S. pentandra*. Consequently, in these older willows and in some younger willow groups (*S. myrsinifolia*-*S. phylicifolia*), the evolution rate of their chemical characteristics may have been higher than the evolution rate of their exomorphic features, so that changes in their chemistry occur before changes in their morphology.

Finally, in several cases the use of simple phenolic glucosides, as a tool for the recognition of morphologically similar willow species or to distinguish between hybrids is appropriate and useful when it is used together with exomorphic and cytological features. On the other hand, the traditional morphologic-taxonomic classification of willows differs quite considerably from the chemotaxonomical classification of willows based only on phenolic glucosides. There are also marked variations in the glucoside patterns of the different plant parts.

EXPERIMENTAL

Material The willow species sampled are listed in Table 7. Twenty to 40 current growth-twigs with mature leaves exposed to the sun and 30 to 40 winter-dormant twigs with buds were collected and put in plastic bags from individuals or clones of more than three-years-old. The samples were kept at 0° while being transported to the laboratory. Immediately, after separation of the plant parts, composite samples were freshly analysed after prehomogenization in liquid nitrogen when needed. The rest of the material was over-dried at 45–48°, as described in a previous paper [21]. The twigs and mature leaf samples were collected in August or in early September (1986 and 1987) and the bud samples were collected in late October to January (1987 and 1988). As the growth intensity of willow species varies, no

Table 7. The specifications of the willow species analysed in this study (a, b and c refer to different individuals)

Species*	Growth place	An individual = 1 Clonal = 2	Indigenous = 1 Introduced = 2	Wild stand = 1 Cultivated stand = 2	Tissues: twigs = 1; leaves = 2; flower-buds = 3; leafbuds = 4
Subgenus: *Amerina*					
S. fragilis L.	Joensuu	2	2	2	1,2
S. fragilis L.	Joensuu	2	2	2	3,4
S. pentandra L.	Joensuu	1	1	1	1,2
S. pentandra L.	Kaavi	1	1	1	3,4
S. alba subsp.	Kaavi	1	2	2	1,2,3,4
S. triandra L.	Joensuu	2	1	2	1,2,3,4
Subgenus: *Caprisalix*					
S. aurita L.	Helsinki	1	1	2	1,3,4
S. aurita L.	Joensuu	2	1	1	1,3,4
S. cinerea (a-c) L.	Kaavi	1	1	1	1,3,4
S. cinerea (a-b) L.	Joensuu	1	1	1	1,3,4
S. starkeana Willd.	Joensuu	2	1	1	1,2,3,4
S. xerophila B. Flod.	Kuusamo	2	1	1	1,2,4
S. pyrolifolia Ledeb.	Oulu	1	1	2	1,2
S. myrtilloides L.	Kuusamo	2	1	1	1,2,4
S. glauca L.	Saariselkä	2	1	1	1,2
S. hastata L.	Kuusamo	1	1	1	1,2
S. hastata L.	Oulu	2	1	2	1,2
S. hastata L.	Kew Gardens	1	...	2	1,2
S. lanata L.	Turku	1	1	2	1,2
S. lanata L.	Kew Gardens	2	...	2	1,2
S. lapponum L.	Kuusamo	2	1	1	1,4
S. lappomun L.	Turku	1	1	2	1,4
S. caprea (a-b) L.	Kaavi	1	1	1	3,4
S. myrsinifolia (a-b) Salisb. (=S. nigricans)	Kaavi	1	1	1	3,4
S. borealis Fries	Kilpisjärvi	2	1	2	1,2
S. arbuscula L.	Kew Gardens	2	...	2	1,2
S. repens L.	Helsinki	1	1	2	1,2,4
S. arenaria L.	Helsinki	1	1	2	1,2,3,4
S. rosmarinifolia L.	Joensuu	2	1	1	1,4
S. rosmarinifolia L.	Turku	2	1	1	1,4
S. purpurea L.	Joensuu	2	2	2	1,3,4
S. ×dasyclados Wimmer	Joensuu	2	2	2	1,2,3,4
S. daphnoides Vill.	Joensuu	2	2	2	1,2
S. acutifolia Willd.	Oulu	2	2	2	2
Subgenus: *Chamaetia*					
S. myrsinites L.	Tervola	2	1	1	3,4
S. reticulata L.	Kilpisjärvi	2	1	1	1,2
S. herbacea L.	Saariselkä	2	1	1	1,2
S. polaris Wahlenb	Oulu	2	1	2	1,2
S. retusa L.	Oulu	2	2	2	1,2

*The classification (and nomenclature) used by Rechinger (1964) and the herbarium voucher specimens of the willow species available at the University of Joensuu, Department of Biology.

more than 25 cm from the shoot tip of twigs were taken for analysis. All mature and unblemished leaves, i.e. including a leaf blade, a petiole and a leaf base were selected from each current-growth internode. The twigs and leaves of *S. herbaceae*, *S. polaris*, *S. reticulata*, *S. glauca* and *S. arenaria* were analysed only in oven-dried form. The tissues of all other species were screened both from fresh and oven-dried material.

The phenolics were extracted by two separate extraction procedures, as previously described [7,21], i.e. oven-dried or fresh samples were extracted with MeOH, C_{18}-octadecyl column purified, concd and GC-analysed as TMSi-derivatives. The phenolics were extracted from dried material with aq. Me_2CO and EtOAc, polyamide column purified, conc. and GC-analysed as TMSi-derivatives. This long extraction method was used especially for the validation of quantification (impurity reduction and salireposide calculation) and qualification (separation of salireposide from the main glucoside fraction, because it overlapped with salicortin) [21]. The quantification was based on

standard components, which were in elution order: salicin, arbutin, fragilin, picein, salidroside, vimalin, triandrin, tremuloidin, populin, salicortin, salireposide, 2′-*O*-acetylsalicortin, 3′-*O*-acetylsalicortin and purpurein. Salicylalcohol, an aglycone of salicin, was also screened. The existence of the components was confirmed by TLC, by glucoside hydrolysis and in a few cases by GC-MS [22, 23].

Cluster analysis was performed in order to group the willow species (UPGMA-method with ln-transformation [24]). In some cases, (marked as 'trace' in tables) the trace was replaced by 0.01, since the results for some willow glucosides was otherwise unquantifiable.

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