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PHYTOCHEMISTRY OF THE *SALICACEAE*

IV. INVESTIGATION OF THE BARK OF *SALIX PETIOLARIS* SM. (*S. gracilis* Anderss. var. *textoris* Fern.)

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SUMMARY

November bark of *Salix petiolaris* Sm. was extracted with acetone and ethanol and the extracts were subjected to chromatography on polyamide columns. The various column fractions were monitored by thin-layer chromatographic and gas–liquid chromatographic procedures. From these results and by isolation of crystalline material, the bark was shown to contain salicin, picein, vimalin, salicyloylsalicin, salireposide, grandidentatin, populin, tremulacin and/or tremuloidin, salicyloylsalicin-2-O-benzoate, (+)-catechin and β-sitosterol.

INTRODUCTION

Much of the phytochemical work to date on the *Salicaceae* has been concerned with the phenolic glycosides, of which salicin was once used as an analgesic and was the first glycoside ever isolated. The present study involves a thin-layer (TLC) and gas–liquid chromatographic (GLC) examination of bark extracts of *Salix petiolaris* Sm. for phenolic glycosides.

EXPERIMENTAL AND RESULTS

The TLC systems used were those of AUDETTE *et al.*[1] and, although no humidity control was used, salicin was chromatographed on each plate to check reproducibility. A Beckman GC-4 chromatograph and 1 mV potentiometric recorder were used for all GLC tests. Mass spectra were determined with an A.E.I. MS-2 or MS-9 spectrometer in the Mass Spectrometry Laboratory, Chemistry Dept., University of Alberta.

The November bark of young shrubs of *S. petiolaris* Sm. (up to 5 years old) was peeled off and dried as soon as possible after cutting. The dried bark strips were

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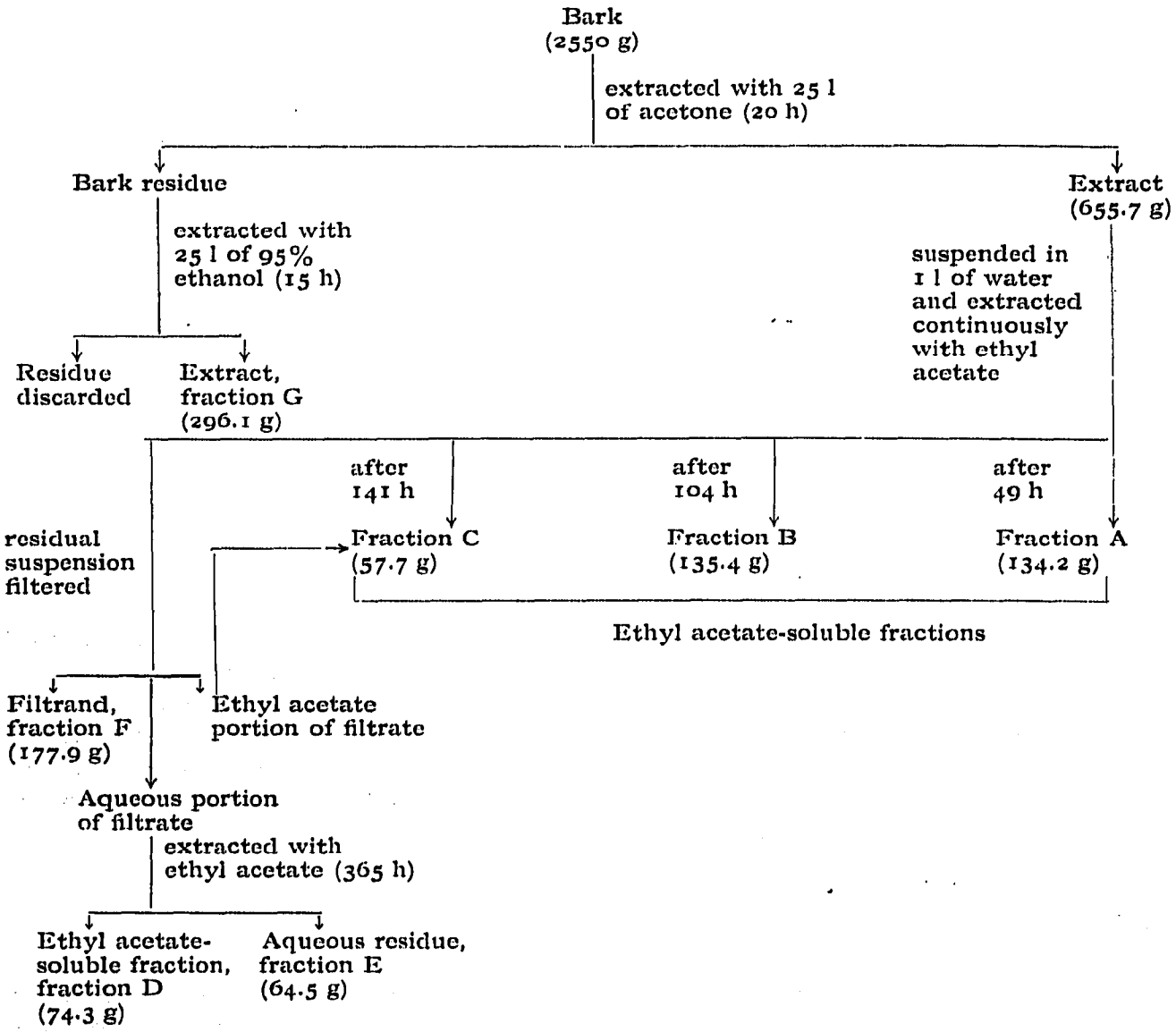

Fig. 1. Extraction scheme.

ground to a coarse powder (2550 g) and extracted in a large Soxhlet apparatus for 20 h with acetone (25 l), to yield 656 g of a viscous brown residue after removal of solvent. A second extraction of the same bark with 95% ethanol (25 l) for 15 h yielded a further 296 g of very viscous brown material.

The concentrated acetone extract was suspended in water (1 l) and subjected to continuous liquid–liquid extraction with successive 2-litre portions of ethyl acetate for a total of 365 h. The seventeen ethyl acetate extracts thus obtained were monitored by TLC[1] and bulked into six separate fractions (A–F), which weighed 134.2, 135.4, 57.7, 74.3, 64.5 and 177.9 g, respectively. These six fractions and the initial ethanol extract (G) were chromatographed on polyamide (Woelm) columns (18–20 × 2 in.) according to the method of PEARL AND DARLING[2] and PEARL AND POTTENGER[3]. The extraction scheme is shown in Fig. 1 and the polyamide chromatography scheme is shown in Tables I and II. The column eluate was monitored by TLC as before and the eluate was bulked into suitable fractions that contained up to seven glycosides but

TABLE I

POLYAMIDE CHROMATOGRAPHY FRACTIONS

Extract fraction[a]	*Column fraction*	*Number of 50-ml units collected*[b]	*Column fraction weight (g)*	*Possible components (TLC monitor)*[1]
A	A-1	44	9.83	Salicin, picein, salicyloylsalicin
	A-2	20	0.40	Salicin, picein, vimalin, populin, salicyloylsalicin
	A-3	151	3.06	Salireposide, tremulacin and/or tremuloidin
	A-4	165	0.37	Salireposide
	A-5	52	1.95	(+)-Catechin
B	B-1	14	0.09	[c]
	B-2	24	10.66	Salicin, picein, salicyloylsalicin
	B-3	27	0.89	Salicin, picein, populin, salicyloylsalicin
	B-4	25	0.37	
	B-5	14	0.30	Salicyloylsalicin, tremulacin and/or tremuloidin, salireposide, grandidentatin
	B-6	16	0.63	Salicyloylsalicin, salireposide, grandidentatin
	B-7	110	1.58	Salicin, populin, salireposide, grandidentatin
	B-8	202	0.46	Salireposide
	B-9	18	0.77	(+)-Catechin
	B-10	5	1.63	β-Sitosterol
	B-11	3	0.24	β-Sitosterol
	B-12	35	3.59	
C	C-1	18	0.15	
	C-2	10	14.25	Salicin, picein, salicyloylsalicin
	C-3	55	1.02	
	C-4	21	0.31	Salicin, picein, salicyloylsalicin, grandidentatin, tremulacin and/or tremuloidin, salireposide, salicyloylsalicin-2-O-benzoate
	C-5	96	1.44	Salireposide
	C-6	188	0.40	Salireposide
	C-7	14	0.55	(+)-Catechin
	C-8	198	2.32	β-Sitosterol
D	D-1	16	0.14	Salicin, picein
	D-2	22	21.38	Salicin, picein, salireposide
	D-3	68	0.97	Salicin, picein, salireposide, salicyloylsalicin, tremulacin and/or tremuloidin, grandidentatin
	D-4	94	3.97	Salireposide
	D-5	164	0.28	Salireposide
	D-6	22	0.36	(+)-Catechin
	D-7	214	0.76	
E	E-1	12	0.14	
	E-2	24	16.77	Salicin, picein
	E-3	426	0.88	
F	F-1	12	0.01	
	F-2	24	9.03	Salicin, picein, salicyloylsalicin, grandidentatin
	F-3	200	0.67	Salicin, picein, vimalin, grandidentatin, salireposide, salicyloylsalicin

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TABLE I *(continued)*

Extract fraction[a]	*Column fraction*	*Number of 50-ml units collected*[b]	*Column fraction weight (g)*	*Possible components (TLC monitor)*[1]
	F-4	16	0.14	Salireposide
	F-5	65	0.20	Salireposide
	F-6	15	0.08	(+)-Catechin
	F-7	268	1.53	
G	G-1	12	0.01	
	G-2	28	14.42	Picein, grandidentatin
	G-3	24	0.50	Picein, grandidentatin, tremulacin and/or tremuloidin
	G-4	40	0.26	Tremulacin and/or tremuloidin
	G-5	86	0.54	Salireposide
	G-6	62	0.33	
	G-7	18	0.56	(+)-Catechin
	G-8	53	0.50	(+)-Catechin, β-sitosterol
	G-9	95	0.85	

[a] 30 g used in each instance.
[b] See also Table II.
[c] A blank space in this column means that no indications of phenolic glycosides were found.

TABLE II

ELUTION SOLVENTS FOR POLYAMIDE CHROMATOGRAPHY

Each unit represents a 50-ml eluate fraction, bulked as indicated in Table I, to give the column fractions.

Extract fraction applied	*Distilled water*	*25% ethanol*	*50% ethanol*	*75% ethanol*	*95% ethanol*
A	1–318	319–366	367–406	—	407–432
B	1–361	362–409	410–435	—	436–493
C	1–290	291–369	370–459	460–512	513–600
D	1–280	281–348	349–436	437–519	520–600
E	1–280	281–322	323–372	—	373–462
F	1–219	220–286	287–367	368–441	442–600
G	1–228	—	229–319	320–386	387–418

were free from non-polar contaminants. The twenty-one glycosides available as reference compounds and the systems used are listed in Table III. Two components were noted in the column fractions, which did not correspond to any of the reference glycosides.

The first of these two compounds was isolated as colourless crystals (a total of 220 mg from fraction B-10 and from the corresponding fraction of a duplicate column), m.p. 135.5–136° after three recrystallisations from 95% ethanol; $[\alpha]_D^{23} = -34.6$ ($c = 2.0$ in $CHCl_3$). Accurate mass measurement gave a molecular weight of 414.3873 (the calculated value for $C_{29}H_{50}O$ is 414.3862). These results corresponded to β-sitosterol, m.p. 138–139°, $[\alpha]_D^{23} = -34.1$ ($c = 2.0$ in $CHCl_3$). The isolated compound and β-sitosterol gave identical infrared spectra, undepressed mixed melting-points and co-chromatographed on TLC and GLC systems (Table III). On acetylation

TABLE III

STANDARDS USED IN THE TLC AND GLC SYSTEMS

Compound	*TLC*[1]			*GLC*[b]
	Colour	R_F *(EXFW)*[a]	R_F *(EM)*[b]	*Retention values of TMS derivatives relative to TMS-arbutin*
Salirepin	yellow	0.19	0.14	1.73
Salicin	red	0.22	0.18	0.79
Picein	red–brown	0.24	0.22	1.38
Salidroside	yellow–brown	0.26	0.20	2.07
Triandrin	dark green	0.28	0.20	2.56
Grandidentatin	yellow	0.29	0.21	3.79
1-*p*-Coumaroyl-β-D-glucose	yellow–brown	0.32	0.25	2.83
Vimalin	dark green	0.33	0.20	2.31
Salicortin	red	0.33	0.23	2.99
Trichoside	yellow–brown	0.33	0.18	3.33
Fragilin	red	0.37	0.32	1.07
Nigracin	yellow	0.43	0.37	3.31
Salireposide	yellow	0.43	0.36	3.18
Trichocarpin	dark green	0.43	0.39	3.23
Trichocarposide	orange–brown	0.45	0.40	4.20
Populin	red	0.47	0.30	2.65
Salicyloylsalicin	red	0.49	0.41	3.07
Tremuloidin	red	0.52	0.48	2.65
Tremulacin	red	0.55	0.51	2.65
Salicyloylsalicin-2-O-benzoate	red	0.67	0.60	4.10
Salicyloylsalicin-6-O-benzoate	red	0.73	0.70	Not eluted
β-Sitosterol	purple	0.84	0.80	3.03
(+)-Catechin	orange	0.72	0.72	2.17

[a] EXFW = ethyl acetate–xylene–formic acid–water (35:1:2:2).
[b] EM = ethyl acetate–methanol (1:9).

(acetic anhydride–pyridine), both yielded acetates, m.p. 127–128°, undepressed on mixing the two products. The entire mass spectrum of the isolated material agreed closely with that reported for authentic β-sitosterol[4].

The second compound was noted in column fractions A-5, B-9, C-7, D-6, F-6, G-7 and G-8 (Table I) and was isolated as a dark brown solid (1.59 g from B-9 and the corresponding fraction of a duplicate column). The solid was purified by passing it down a polyamide column (water as eluant) followed by three recrystallisations from water to yield 316 mg of pale brown crystals. Passage down another polyamide column produced a pale buff solid (200 mg), m.p. 171–173°, $[\alpha]_D^{23} = +14.4$ ($c = 2.0$ in acetone–water, 1:1), $\lambda_{max.}$ 280.5 nm ($E_{1\,cm}^{1\%} = 230.6$; $c = 0.00098$ in 95% ethanol). The $\lambda_{max.}$ shifted to 296 nm upon the addition of *N* sodium hydroxide solution. Found: C, 61.97; H, 4.93%. Calculated for $C_{15}H_{14}O_6$: C, 62.06; H, 4.86%. Authentic (+)-catechin had m.p. 174.5–175.5°, undepressed upon mixing with the isolated compound. The two samples gave identical infrared spectra and co-chromatographed on TLC and GLC systems; the mass spectra were virtually identical and agreed closely with that of (+)-catechin[5]. The colours produced by three reagent sprays[6], the optical rotation[7] and the ultraviolet spectra[8] also agreed with literature data for (+)-catechin.

The column fractions referred to in Table I were checked for constituents on the

0.3% OV-1 column of BOLAN AND STEELE[9]. The only change from their systems was that the temperature programme began isothermally at 190° for 10 min, then increased at 6°/min to 250° and was maintained at that temperature until all components were eluted. All samples were injected as the trimethylsilyl (TMS) ethers and retention times were recorded relative to TMS-arbutin. The results from the GLC monitor were very similar to those listed in Table I from the TLC monitor and therefore they are not given in detail. The retention values for the reference compounds are listed in Table III.

DISCUSSION

The bark was extracted with acetone because this solvent is known to cause little, if any, hydrolysis of glycosides[10]. However, this initial extraction was followed by an ethanol extraction step to ensure complete removal of glycosides from the bark. The presence of a considerable amount of grandidentatin in the ethanol extract (G) justified this precaution.

Despite extraction with ethyl acetate for 365 h, the aqueous residue (fraction E) still contained glycosides. This finding is in agreement with earlier work[10], in which it was found that ethyl acetate was only partially effective in extracting some of these relatively polar phenolic glycosides.

After application of the TLC and GLC methods, the only glycosides that remained unresolved were tremuloidin and tremulacin. When a positive result was obtained, it is therefore reported in this study as tremulacin and/or tremuloidin. The compounds thus shown to be present in the various extract fractions from the bark were salicin, picein, vimalin, salicyloylsalicin, salireposide, grandidentatin, populin, tremulacin and/or tremuloidin, salicyloylsalicin-2-O-benzoate, (+)-catechin and β-sitosterol. In general, GLC was superior to TLC, but when large numbers of samples were involved (during column monitoring), the relative speed of TLC made it the method of choice.

Catechin is one of the most widely occurring flavonoids and, with the leucoanthocyanidins, forms the economically important condensed tannins[11]. It has been reported earlier in leaf galls of *S. fragilis*[12,13], in leaves and wood of unspecified *Salix* species[14], in leaves of several *Salix* species[15] and in the bark of *S. purpurea*[16].

The differences in the glycosides reported in this study and in earlier work can be accounted for mainly on the basis of seasonal variation[17], as AUDETTE *et al.*[1] used June bark while November bark was used by the present authors. In addition, some glycoside reference compounds were not available for the earlier study.

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