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INVESTIGATION OF THE CHEMISTRY AND TAXONOMY OF SUB-TRIBE QUILLAJEAE OF THE ROSACEAE USING COMPARISONS OF FRESH AND HERBARIUM MATERIAL

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Abstract—*Quillaja saponaria* Mol., contains leucodelphinidin in addition to leucocyanidin. Myricetin is, however, absent. The presence of 3′,4′,5′-trihydroxy-substituted flavonoid constituents is unusual in the Rosaceae, and suggests that the species is relatively primitive. Related species in the sub-tribe Quillajeae contain neither leucodelphinidin nor myricetin, but *Vauquelinia californica* Sarg. contains quercetagetin. Three species were examined both in the fresh condition and as herbarium material collected, in one instance, more than 100 years previously. As regards the phenolic constituents studied there was no significant difference between the fresh and preserved specimens. The latter contained traces of other fluorescent compounds easily distinguished from the substances under investigation by their mobility on paper chromatograms.

THE GENUS *Quillaja*, consisting of three temperate South American species, was placed by Focke[1] in the tribe Spiraeoideae of the Rosaceae, forming together with *Kageneckia*, *Lindleya*, *Vauquelinia*, *Exochorda* and *Euphronia* the sub-tribe Quillajeae. The Rosaceae, as a family, is distinguished by the presence in almost all its members of the flavonols quercetin and kaempferol, of leucocyanidin, and caffeic and *p*-coumaric acids. As a rule the pyrogallol-B-ring flavonoids, myricetin and leucodelphinidin, are absent, only having been recorded so far in the tribe Chrysobalanoideae (which by some authors[5] is regarded as a separate family) and in one member, *Potentilla anserina*, of the Rosoideae.[2] The occurrence of one of these trihydroxy-substituted phenolic constituents in the leaves of another member of the family is, therefore, of taxonomic and possibly also of phylogenetic interest.

The member in question, *Quillaja saponaria* Mol., was examined in the fresh condition in California, and found to contain leucodelphinidin. The result than obtained was checked in this laboratory against herbarium material obtained from the Botany Department of Cambridge University. Fresh and herbarium material of *Vauquelinia californica* Sarg., *Kageneckia angustifolia* Don and *Lyonothamnus floribundus* Gray* and herbarium material of other members of the Quillajeae (*sensu* Focke) have also been examined. *Exochorda racemosa* Rehd. has been reported upon previously.[3]

The examination consisted in the chromatography on paper of the hydrolysates of leaf material, the methods employed being the same as those used in earlier studies.[2,3] In the case of fresh leaves about 0·5 g, and in the case of herbarium material about 0·2 g (or as much as could be spared from the specimen) were hydrolysed in aqueous 2 N HCl for 20 min in a

**Lyonothamnus* is not given a definite place by Focke, but he notes that if included in the Rosaceae, it would be placed in the Quillajeae.

[1] W. O. FOCKE, in *Englerand Prantl Pflanzenfam* III, 3, Leipzig (1894).
[2] E. C. BATE-SMITH, *J. Linnean Soc.* (*Botany*) **58**, 39 (1961).
[3] E. C. BATE-SMITH, *J. Linnean Soc.* (*Botany*) **58**, 95 (1962).

TABLE 1. PHENOLIC CONSTITUENTS OF LEAVES OF FRESH AND HERBARIUM SPECIMENS

	Origin	Compound detected*								Other constituents†	
		D	Q	Cy	K	Caff.	*p*C	S	F	Forestal	TAW
(*a*) Fresh specimens											
Exochorda racemosa Rehd.	Cambridge	–	++	+	++	– ?	–	–	–	dk.–→gr. 0·86, cf. apigenin; dk.→y. 0·63, cf luteolin	
Quillaja saponaria Mol.	Berkeley	++	+	(+)	+	(+)	?	–	–		br. bl. 0·02, cf. gentisic acid; V →v. br. bl. 0·05; d. bl.–→v. br. bl. 0·10
Vauquelinia californica Sarg.	Berkeley		+	+	–	+	+	–	(+)	dk. brn →g. brn. 0·25 approx., cf quercetagetin	br bl. 0·02, cf. gentisic acid
Holodiscus discolor var. *ariaefolius* Archers and Graebn.	Berkeley	–	+	+	(+)	+	+	–	+	→g. y. 0·63 of luteolin; →dull y 0·69	
Lyonothamnus floribundus Gray	Berkeley	–	+	+	+	+					

(*b*) Herbarium specimens

Kageneckia angustifolia Don	C: Bridges, Chile	−	+	−	+	+	+	−	(+)	y. 0·15; f. y. 0·95	f. bl. 0·02
K. crataegoides Don	C: Gillies, Chile, before 1852	−	−	−	−	+	+	−	(+)	y. 0·15; f. y. 0·95	f. bl. 0·02
Lindleya mespiloides H.B.K.	K: E. Palmer, Mexico	−	+	+	+	++	+	−	(+)		
Lyonothamnus floribundus Gray	K: ?	−	+	+	+	++	+	−	(+)		f. bl. 0·02; y. 0·05
Quillaja saponaria Mol.	C: H. Cuming, Chile, 1831	+	++	−	+	−	+	−	(+)	v. br. bl. 0·85	br. bl. 0·02; V.→v. br. bl. 0·05, 0·09; V.→d. V. 0·17; V. 0·67
Q. sellowiana Thuill.	C: H. L. Lindley, Brazil, before 1866	−	+++	(+)	+	−	+	−	−	y. 0·15; v. br. bl. 0·85; br. apr.→y. 0·95	br. bl. 0·02; V.→v. br. bl. 0·05; f. y. trail to 0·69; →f. V. 0·7
Vauquelinia californica Sarg.	K: A. Rehder, Arizona, 1914	−	++	+	+	++	+	−	(+)	dk. brn.→g. brn. 0·28, cf. quercetagetin	
V. Corymbosa Cor.	K: Pringle, Mexico, 1885	−	+	(+)	+	(+)	++	−	(+)		

* D = delphinidin, Q = quercetin, Cy = cyanidin, K = kaempferol, Caff. = caffeic acid, *p*C = *p*-coumaric acid, S = sinapic acid, F = ferulic acid. The symbols +++, ++, +, (+), and − refer to the presence, and in what relative strength, or the absence of the particular constituent.

† Observed in u.v. light: apr. = apricot, bl. = blue, brn. = brown, g. = golden, gr. = green, V = violet, y. = yellow, br. = bright, d. = deep, dk. = dark, f. = faint, v. = very, → = after fuming with ammonia vapour.

boiling water bath, the hydrolysates extracted with a small volume of isoamyl alcohol, and the extracts chromatographed on No. 1 Whatman paper in two solvent systems (Forestal solvent (acetic acid:conc. HCl:water; 30:3:10 by vol.); and toluene-acetic acid (TAW: top phase of toluene; acetic acid:water; 4:1:5 by vol.)).

The results of the examination of fresh leaves are recorded in Table 1*a* and those of the herbarium specimens in Table 1*b*.

DISCUSSION

Quillaja saponaria is the soap-tree of Chile, so-called because of the presence of saponin in the bark. According to Reiche[4] the trunk may reach a height of 10 m or more and a thickness of 1 m. In Chile it grows between latitudes 31° and 38° from sea level to 2000 m altitude. *Q. saponaria* was the only one of the genus examined which contained either of the two tri-hydroxy-substituted derivatives; *Q. sellowiana*, had neither. This, like most of the other species examined, had the typical Rosaceous pattern of quercetin, leucocyanidin, kaempferol, and caffeic and *p*-coumaric acids, with small amounts of ferulic acid. *Vauquelinia californica*, but not *V. corymbosa* had a constituent tentatively identified as quercetagetin.

Quercetagetin has a very characteristic appearance in u.v. light and, with its low R_f in Forestal solvent, can be identified with some confidence. Its occurrence is sporadic and irregular* and has no apparent systematic significance except as an indication of the ability, not previously recorded in the Rosaceae, to hydroxylate the 6 position of the flavonoid nucleus.

The presence of leucodelphinidin in *Q. saponaria* is an indication that it is a relatively primitive plant standing apart in this respect from the great majority of the members of the Rosaceae so far examined. It differs from these both in its geographical position and in being, for the Rosaceae, an exceptionally large tree, a character it has in common with the Chrysobalanoideae. A further distinction is the property from which it derives its name, the presence of a saponin, a feature so unusual in the Rosaceae that this alone might suggest an isolated position for it in the taxonomy of the family. This is to some extent provided in the very recent revision of the family by Schulze-Menz in the 12th edition of Engler's Syllabus.[5] This author now restricts the Quillajeae to the three genera *Quillaja*, *Kageneckia* and *Vauquelinia*. *Exochorda* and *Lindleya* form a new sub-tribe, Exochordieae, and *Lyonothamnus* is included in a new sub-tribe Sorbarieae, taken out of Focke's sub-tribe Spiraeeae.

It is perhaps significant that in *Q. saponaria* myricetin does not appear to accompany leucodelphinidin as it does in the great majority of plants containing the tri-hydroxy-substituted flavonoids. This might mean that in *Q. saponaria* leucodelphinidin is a rather specialized survival of the primitive metabolic pattern. It is less likely that it occurs as the result of the insertion of a hydroxyl group in the 5′ position of a leucocyanidin residue, although the occurrence of quercetagetin in the supposedly related species *Vauquelinia californica* is presumably due to an acquired ability to hydroxylate the 6-position of the flavonoid skeleton. The two cases are, in fact, quite different. There is no known instance of the hydroxylation of a 3′4′-dihydroxy-flavonoid to a 3′,4′,5′-trihydroxy-flavonoid, but the circumstances in which

* Quercetagetin has been isolated from the Leguminosae, Papaveraceae, Ericaceae, Primulaceae and Compositae (Dr J. B. Harborne, personal communication) and has been demonstrated by the author in *Empetrum nigrum*.

[4] C. REICHE, *Flora de Chile*, Vol. 2, p. 209, Imprenta Cervantes, Santiago (1898).
[5] G. K. SCHULZE-MENZ, in A. ENGLER'S *Syllabus der Pflanzenfamilien*, (12th Edition), Vol. 2, Borntraeger, Berlin (1964).

6- or 8-hydroxy- and methoxy-substituted flavonoid compounds occur leaves little doubt that this is due to an acquired ability for nuclear substitution of the A ring.

Exochorda racemosa is indicated by the presence in it of the flavones apigenin and luteolin as less closely related to any of the other species examined. In this respect it agrees more closely with *Holodiscus discolor*, placed by Focke in his sub-tribe Holodisceae. The presence of flavones is to be regarded as an advanced character, and the sequence of sub-tribes now proposed by Schulze-Menz. viz. Quillajeae, Exochordieae (e.g. *Lindleya* but not *Exochorda*) Gillenieae, Sorbarieae (e.g. *Lyonothamnus*), Neillieae, Spiraceae, Holodisceae is consistent with the phylogenetic implications of the present data.

Comparison of Fresh with Herbarium Material

Where comparison was possible, the results indicated no qualitative differences between fresh and herbarium specimens in the constituents under investigation. Some of the herbarium specimens contained fluorescent substances not present in the fresh plants: some blue fluorescent constituents with $R_f > 0{\cdot}5$ in toluene–acetic acid and some yellow fluorescent material forming an indistinct trail in this solvent. It is surprising that such readily oxidizable constituents as the polyphenols should survive both the treatments to which the plants are subjected during preservation, and storage under herbarium conditions for over 100 years without either complete deterioration of the constituents themselves or the appearance of interfering products of oxidation. It has of course to be remembered that the constituents studied were the aglycones and not the original glycosides; it would be beyond expectation that these would have remained in their native condition during such treatment and after so long a time. Some loss of leuco-anthocyanin is indicated by the colour of the preserved material. In most cases where LA was present in the fresh leaves, and some, usually a lesser amount, in the preserved specimen, the latter had a more or less deep tan colour. (This is characteristic also of the dead leaves of such species.) The "phlobaphenes" responsible for this colour will, however, still produce a certain amount of anthocyanidin when digested with mineral acid, so that the qualitative reactions characteristic of the species can still as a rule be elicited in the herbarium specimen. This has been amply demonstrated by Cain *et al.*[6] in their phytochemical surveys of New Zealand plants.

It seems that a study of the conditions most favourable to preservation of the phenolic constituents in herbarium material would be well worth while, so that such material can be used for the study of the phenolic chemistry when fresh specimens, as is so often the case, cannot be obtained. The present results are to be regarded as no more than an indication, as chance offered, of the potential usefulness of herbarium material for such studies. It is especially important to note what small quantities were required for the present work. As little as 0·05 g would have been sufficient in most cases, although 0·2 g or more was usually available.

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[6] B. F. Cain, S. Scannell and R. C. Cambie, *New Zealand J. Sci.* **4**, 3, 604, 707, 731 (1961); **5**, 537 (1962).