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HAMAMELOSE AND ITS DERIVATIVES AS CHEMOTAXONOMIC MARKERS IN THE GENUS *PRIMULA*

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Abstract—About 550 species of vascular plants as well as a few mosses were checked for the occurrence of hamamelose, the corresponding alcohol hamamelitol and a galactoside of the latter, clusianose. Hamamelose was found in most of the species investigated. It provides therefore no useful character for chemotaxonomic purposes. Hamamelitol, however, could only be detected in the genus *Primula* and was therefore more useful. Small amounts of hamamelitol were found in the leaves of species from the subgenera *Auganthus*, *Aleuritia* and *Auricula* but not from *Primula* and *Sphondylia*. Clusianose and large amounts of hamamelitol are produced by species of 4 subsections of the section *Auricula*, but not by species of the subsections *Auricula* and *Erythrodrosum*. A taxonomic separation of the two latter from the 4 former subsections is suggested. The taxonomic position of *Primula allionii* should be reconsidered, since neither clusianose nor hamamelitol could be demonstrated in this species, whereas the other species of the subsection *Rhopsidium* do contain these carbohydrates.

INTRODUCTION

Hamamelose, a branched chain hexose (2-*C*-(hydroxymethyl)-D-ribose) is known to occur as a constituent of hamamelitannin [1, 2] (2^1, 5-digalloyl-hamamelose) in the bark of Witch Hazel, Chestnut and Oak [1, 3], as well as in the free stage in leaves of many higher plants [4]. Recently, also the corresponding sugar alcohol, hamamelitol [5] and its galactoside, clusianose [6], were detected. The concentrations of the free sugar in the leaf extracts are usually too small for normal chromatographic detection. ^{14}C-labelling by photoassimilation of $^{14}CO_2$ had to be employed to demonstrate the presence of hamamelose. Van Scherpenberg *et al.* [4] concluded from their data covering about 80 species of vascular plants that hamamelose occurs regularly in Hamamelidales, Fagales, Betulales, Cunoniales, Salicales and Primulales, but might be even more widely distributed in the plant kingdom. The present paper reports the results of the investigation of about 600 species of many families of higher plants, ferns and mosses for the occurrence of free hamamelose, hamamelitol and clusianose and evaluates their taxonomic significance.

RESULTS AND DISCUSSION

Distribution of hamamelose

For detection, hamamelose and its derivatives were usually labelled with ^{14}C by photoassimilation of $^{14}CO_2$ with excised leaves. In some cases dry leaf material from the herbarium was used. Identification was performed by paper chromatography and electrophoresis.

Hamamelose was found to occur in mosses (75% positive results, 4 species from 4 families examined), ferns (43% positive results, 28 species from 12 families examined), gymnosperms (23% positive results, 13 species from 7 families examined) and angiosperms (76% positive results, 596 species from 104 families examined)†. A failure to detect free hamamelose with the method employed, however, implies only that the rate of hamamelose synthesis was too low to label this sugar within one hour sufficiently and, therefore, does not rule out the possibility that it is still produced by species recorded here as being negative. Prolonged exposure of the leaves to $^{14}CO_2$ could have resulted in an even higher percentage of positive results. The virtually general occurrence of hamamelose is not unexpected, since hamamelose 2^1,5-bisphosphate has been detected recently as a product of the photosynthetic carbohydrate synthesis within the chloroplast [7]. At least under certain environmental conditions, part of this compound may leak out from the chloroplast into the cytoplasm, where it is dephosphorylated to free hamamelose [8] which normally does not undergo further metabolic reactions [9]. The wide distribution of hamamelose in green plants makes this monosaccharide unsuitable for chemotaxonomic purposes.

Distribution of hamamelitol and clusianose in the genus Primula

In order to synthesize clusianose, a plant must first be able to reduce hamamelose to hamamelitol. The capacity

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†A list containing the names of the species investigated is deposited with the Editor.

Table 1. Distribution of hamamelose, hamamelitol and clusianose in the genus *Primula*

Subgenus	Section	Subsection	Species	Hamamelose	Hamamelitol	Clusianose
Auganthus	Cortusioides		*P. polyneura* Franchet	+	+	−
			P. mollis Hooker	−	O	O
			P. kisoana Miq.	−	O	O
			P. sieboldii E. Morren	+	−	−
	Obconicolisteri		*P. obconica* Hance	+	+	−
Primula	Primula		*P. juliae* Kusn.	+	−	−
			P. elatior Hill	+	−	−
			P. veris L.	+	−	−
			P. vulgaris Hudson var. *balearica*	+	−	−
			P. garden hybrid of *vulgaris*	+	−	−
	Sredinskya		*P. grandis* Trautv.	+	−	−
Sphondylia	Sphondylia		*P. boveana* Duby	+	−	−
Craibia	Petiolares		*P. calderana* Balf.f. et Cooper	+	O	O
Aleuritia 1	Aleuritia		*P. farinosa* L.	+	−	−
			P. frondosa Janka	+	−	−
Aleuritia 2	Muscarioides		*P. vialii* Franchet	+	+	−
			P. muscarioides Hemsley	+	−	−
	Capitatae		*P. capitata* Hooker	+	−	−
Aleuritia 3	Sikkimensis		*P. reticulata* Wall.	+	−	−
			P. sikkimensis Hooker	+	+	−
			P. florindae Ward	+	−	−
	Proliferae		*P. pulverulenta* Duthie	+	+	−
			P. bulleyana Forrest	+	−	−
			P. prolifera Wall.	−	−	−
			P. aurantiaca W.W.Sm. et Forrest	+	−	−
Aleuritia 4	Minutissimae		*P. reptans* Hooker f.	+	+	−
Aleuritia 5	Crystallophlomis		*P. macrophylla* Don	+	+	−
			P. chionantha Balf. f. et Forrest	+	+	−
	Oreophlomis		*P. rosea* Royle	+	+	−
			P. elliptica Royle	+	+	−
			P. ciarkei Watt	+	O	O
	Armerina		*P. involucrata* Wall	+	O	O
			P. nutans Franchet	−	O	O
Auriculastrum	Cuneifolia		*P. cuneifolia* Ledeb.	+	−	−
			P. suffrutescens A. Gray	+	−	−
	Parryi		*P. parryi* A. Gray	+	+	−
			P. angustifolia Torrey	+	−	−
	Auricula	Cyanopsis	*P. deorum* Velen.	+	++	+
			P. glutinosa Wulfen in Jacq.	+	++	+
		Arthritica	*P. spectabilis* Tratt.	+	++	+
			P. glaucescens Moretti	+	++	+
			P. wulfeniana Schott	+	++	+
			P. clusiana Tausch	+	++	+
		(Chamaecallis)	*P. minima* L.	+	++	+
		Rhopsidium	*P. kitaibeliana* Schott	+	++	+
			P. integrifolia L.	+	++	+
			P. tyrolensis Schott	+	++	+
			P. allionii Loisel.	+	−	−
		Auricula	*P. palinuri* Petagna	+	−	−
			P. latifolia Lapeyr.	+	−	−
			P. marginata Curtis	+	+	−
			P. carniolica Jacq.	+	−	−
			P. auricula L.	+	+	−
		Erythrodrosum	*P. pedemontana* Gaudin	+	+	−
			P. apennina Widmer	+	+	−
			P. daonensis Leybold	+	−	−
			P. cottia Widmer	+	+	−
			P. hirsuta All.	+	+	−

+ = Compound detectable by the labelling technique only; + + = Large amounts of the substance in the leaft extract, which were detectable on chromatograms by alkaline $AgNO_3$; O = Only dry plant material available, which did not allow the detection of small amounts of the compounds. The species are grouped according to Wendelbo [10] and Schwarz [11].

for the synthesis of hamamelitol and clusianose is probably an additionally acquired character within those plants in which hamamelose accumulates to a significant extent. The formation of the two compounds was found to be restricted to plants of the genus *Primula*. In some cases the concentrations of hamamelose + hamamelitol in the leaves even exceeded those of sucrose [9]. Whereas hamamelose is present virtually throughout the genus *Primula*, the sugar alcohol and especially clusianose occur only in certain groups (see Table 1) and may therefore be used as a chemotaxonomic marker within this genus.

As shown in Table 1, clusianose was found only in the species of 4 subsections of the section *Auricula*, namely *Cyanopsis*, *Arthritica*, *Chamaecallis* and *Rhopsidium*, *Primula allionii* being the only exception. All the clusianose-producing species contained large amounts of hamamelitol as well.

The latter compound was also found in several other species scattered over almost all subgenera from which more than 1 or 2 species have been investigated. In contrast to the clusianose-containing species, these species contained significantly less hamamelitol. Often only traces of this compound were detected. As with hamamelose, the detection of hamamelitol was also limited by the rate of its labelling during one hour of photosynthesis in $^{14}CO_2$ which may not always have achieved the threshold accumulation necessary for a positive result. However, one might assume from the data shown in Table 1 that the ability to form small amounts of hamamelitol is a widespread property within the genus *Primula*. The single exception may be the subgenus *Primula*, the 5 (plus 1 hybrid) species examined of which yielded consistently negative results. This finding, as well as other characteristics, e.g. the occurrence of a certain type of stephanocolpate pollen grains which is otherwise very rare in *Primula* [10, 13], indicate that this subgenus is rather distinctive. In addition, hybridization experiments suggested that this group comprises closely allied species [12]*.

The accumulation of hamamelitol and the formation of clusianose is obviously restricted to the above mentioned four allied subsections of the section *Auricula* and therefore serves as a new taxonomic marker within the genus *Primula*. The division of the section *Auricula* into species containing or lacking these two saccharides is in good agreement with the former concepts of Schott [14] and Widmer [15]. Schott [14] segregated the European species of Duby's [16] sections *Auricula* and *Arthritica* from the non-European ones and combined them in his subgenus *Auriculastrum* forming two groups, *Saniculina* and *Nothobritanica*. *Saniculina* comprised the sections *Auricula* and *Erythrodrosum*, *Nothobritanica* the sections *Arthritica*, *Rhopsidium*, *Chamaecallis* and *Cyanopsis*. Widmer [15] did not follow Schott's classification but put the species of *Auriculastrum* in a new order; her *Purpureae-Longibracteae* correspond to *Nothobritanica* and her *Purpureae-Brevibracteae* plus *Luteae* correspond to *Saniculina*. Later, Schott's sections of *Auriculastrum* were classified as subsections. While Pax [17] did not keep the subsections of the groups *Saniculina* and *Nothobritanica* separate, other recent authors [18–20] have done so. The phytochemical data reported here support the division of the section *Auricula* into the groups *Nothobritanica* and *Saniculina*.

The only anomaly is provided by *P. allionii*. This species was included by Duby [16] in his section *Auricula*, most of the European species of which were transferred by Schott [14] to *Saniculina*; *P. allionii*, however, was transferred to *Nothobritanica*, being the type species of his section *Rhopsidium*. Widmer, [15] on the contrary, grouped *P. allionii* within *Purpureae-Brevibracteae* which correspond together with *Luteae* to Schott's *Saniculina*. The other 3 species of *Rhopsidium* were included in the *Purpureae-Longibracteae* which correspond to Schott's *Nothobritanica*. Later authors [17–20] have returned to Schott's system for this species. Our data, however, support Widmer's concept, since *Primula allionii* does not contain hamamelitol or clusianose, whilst all other species of *Rhopsidium* do.

The accumulation of hamamelitol and the formation of clusianose characterize *Nothobritanica* as an advanced taxon. As indicated by chromosome numbers [20] as well as by morphological characters, however, *Saniculina* seems to comprise the more specialized species within the advanced section *Auricula*. This may be the consequence of diverging evolution.

EXPERIMENTAL

Plant sources. Most of the fresh leaf samples were obtained from the Botanische Garten München and from the Staudensichtungsgarten of the Technische Universität München. In a few cases dry material of *Primula* species from the Bayerische Staatsherbarium was used.

Detection of hamamelose, hamamelitol and clusianose. Labelling with ^{14}C was performed by $^{14}CO_2$ fixation by excised leaves according to the method of van Scherpenberg *et al.* [4]. For preparation of the leaf extract and its separation by PC we followed the method of the same authors. Hamamelose and hamamelitol were separated from each other and from ribitol by paper electrophoresis in Na borate and Na germanate buffers [6]. Larger amounts of unlabelled compounds were detected by spraying the paper chromatograms or pherograms with alkaline $AgNO_3$ [21]. For the detection of labelled substances radioautography with Agfa X-ray paper or Currix CP film was employed. Authentic hamamelose was prepared from hamamelitannin according to the method described earlier [22] and authentic hamamelitol was produced by reduction of hamamelose with $NaBH_4$ [23]. Clusianose was prepared from the leaves of *Primula clusiana* [6].

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REFERENCES

1. Mayer, W., Kunz, W., Loebich, F. (1965) *Ann. Chem.* **688**, 233.
2. Ezekiel, A. D., Overend, W. G., Williams, N. R. (1969) *Carbohyd. Res.* **11**, 233.
3. Mayer, W., Kunz, W. (1959) *Naturwissenschaften* **46**, 206.
4. Van Scherpenberg, H., Gröbner, W., Kandler, O. (1965) Festschr. K. Mothes 65. Geburtstag, pp. 287–406. VEB Gustav Fischer, Jena.
5. Sellmair, J., Beck, E., Kandler, O. (1968) *Z. Pflanzenphysiol.* **59**, 70.

**Primula grandis*, however, was not included in this study.

6. Beck, E. (1969) *Z. Pflanzenphysiol.* **61**, 360.
7. Beck, E., Stransky, H., Furbringer, M. (1971) *FEBS Letters* **13**, 229.
8. Gilck, H., Beck, E. (1974) *Z. Pflanzenphysiol.* **72**, 395.
9. Sellmair, J., Kandler, O. (1970) *Z. Pflanzenphysiol.* **63**, 65.
10. Wendelbo, P. (1961) Arb. Univ. Bergen, Mat.-Nat. Ser. No. **11**, 1.
11. Schwarz, O. (1968) Wiss. Z. d. Fried. Schiller Univ. Jena, Math.-Naturw. Reihe **17**, 307.
12. Valentine, D. H. (1961) in *A Darwin Centenary* (Wanstall, P. J. ed.) Vol. 71. Buncle, Arbroath.
13. Spanowsky, W. (1962) *Feddes Rept.* **65**, 149.
14. Schott, H. (1851) *Die Sippen der österreichischen Primeln.* Verl. K. Gerold. u. Sohn, Wien.
15. Widmer, E. (1891) *Die europ. Arten der Gattung Primula.* Oldenburg, München.
16. Duby, E. (1844) in *Prodr.* (de Candolle, A. ed.) **8**, 33.
17. Pax, F., Knuth, R. (1905) *Primulaceae* in *Das Pflanzenreich* (Engler, A. ed.) IV, 17. Engelmann, Leipzig.
18. Lüdi in Hegi (1927) *Illustrierte Flora von Mitteleuropa V/3*, 1715.
19. Smith, W. W., Fletcher, H. F. (1942) *Transact. Roy. Soc. Edinburgh* **61**, 631.
20. Kress, A. (1963) *Österr. Bot. Z.* **110**, 53.
21. Trevelyan, W. E., Procter, D. D., Harrison, J. S. (1950) *Nature* **166**, 444.
22. Gilck, H., Thanbichler. A., Sellmair, J., Beck, E. (1975) *Carbohyd. Res.* **39**, 160.
23. Wolfrom, M. L., Thompson, A. (1963) in *Methods in Carbohyd. Chem. II* (Whistler, R. L., Wolfrom, M. L., eds.) p 65. Academic Press, New York.