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ASTRINGENT TANNINS OF *VIBURNUM* AND *HYDRANGEA* SPECIES*

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Key Word Index—*Viburnum*; Caprifoliaceae; *Hydrangea*; Saxifragaceae; proanthocyanidins; tannins; astringency.

Abstract—The tannins of the leaves of *Viburnum* and *Hydrangea* species consist of proanthocyanidins only, but in each genus the range is very wide. In several species of *Hydrangea* the proanthocyanidins are of the A type, otherwise they are mostly tri- or tetrameric B type. Tannin content is correlated with evolutionary advancement, the more advanced and more widely dispersed species having the less. Species with most tannin occur in E. Asia and E. N. America, but species with little or no tannin are present in both areas. The occurrence in both genera of globose inflorescences with sterile flowers is correlated neither with morphological nor with chemical characters.

INTRODUCTION

In contrast to the complicated tannins in *Acer* [1], those in *Viburnum* are simple, yielding only procyanidin when heated in HCl. There is, however, the same wide variation in tannin content and astringency, the latter ranging from a tannic acid equivalent (TAE) percent of the dry leaf of 11.5 in *V. plicatum* to 2.4 in *V. sargentii*. Except for indications of gallic acid in one species, and ellagic acid in another, the situation in *Hydrangea* is similar. It is therefore easier in these genera to evaluate the relation between tannin content and astringency, and perhaps to identify some of the factors responsible for (or at least correlated with) variation between species in astringency.

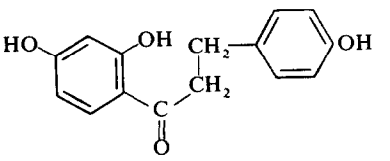
Except for records of procyanidin content in *Hydrangea* by Jay [2] and the present author [3], there appears to be no previous work on these constituents. Neither genus is regarded as rich in tannin nor remarkable for its astringent properties.

RESULTS

Determinations of anthocyanidin produced on heating with butanolic HCl (expressed as extinction, $E_{1\,cm}^{1\%}$ at 550 nm) and of TAE by haemanalysis [4] were carried out on both powdered dried leaf and on 50% aq. MeOH extracts of the leaf powder as described in [1]. Flavonoid aglycones were identified from hydrolysates in 2 N HCl by PC in Forestal and toluene–HOAc–H_2O (TAW) against marker compounds. The results of the survey of *Viburnum* and *Hydrangea* species are recorded in Table 1. The arrangement of species in the case of *Viburnum* is that of Rehder [5] and in the case of *Hydrangea* that of McClintock [6].

VIBURNUM

At first sight there seems to be little obvious connection between tannin content (E value of powder) and systematic position. However, if the species are rearranged in order of decreasing tannin content, and separated into three groups of high, medium and low tannin, as in Table 2, some kind of order may be discerned. In the first group all the species except one have a chromosome number of x = 8 (x = 9 being the usual number) and this group also includes all three of the American species examined. Most of the second group are drawn from Rehder's sections *Lantana* and *Odontotinus* and the third is made up of his section *Opulus* plus *V. henryi* (the remaining species with x = 8). *V. henryi* requires special consideration because of a number of unusual leaf constituents. Two of these have the appearance in visible and UV light of an aurone and chalcone respectively, and this is particularly interesting in view of the presence in *V. davidii* of a dihydrochalcone, davidin [7]. This compound has the most unusual feature (outside the Leguminosae and Compositae) of the absence of a hydroxyl group at position 5 (**1**). *V. henryi* also has a constituent giving the



(1)

distinctive golden yellow reaction of a dihydrochalalcone with diazotised *p*-nitroaniline, but with lower R_f in TAW than davidin. It is to be concluded that with these constituents *V. henryi* is considerably more advanced chemically than the other members of section 1, in which Rehder places it, and thus the placing of this species in a section, which, with its 'globose panicle-thyrsic' inflorescence, Troll [8], considers to be the most primitive in *Viburnum*, must now be questioned. Since Raven [9] considers x = 7 likely to be the original basic chromosome number of most of the angiosperms, it is possible that the low number x = 8 of the other members of this section, compared with x = 9 for most of the other species of *Viburnum*, may also count as evidence in favour of their primitive status. However, the peculiar, and presumably

*Part 2 in the series 'Astringency of leaves'. For part 1 see ref. [1].

Table 1. Flavonoid and Tannin analysis of *Viburnum* and *Hydrangea* leaves

Genus, section, subsection§ species	Flavonoids*				Powder		Extract		Geographical range
	Q	Cy	K	L	*E*	TAE	*E*	TAE	
Viburnum L.									
1 *Thyrsosma*									
V. fragrans Bge.	+++	++	+		44	10	34	9	N. China
V. grandiflorum Wall. syn. *V. foetens* Dcne.	+	+	+		23	6	27	5	Himalayas
V. henyri Hemsl.	+	−	(+)		3	2.5	2	<2	C. China
2. *Lantana*									
V. carlesii Hemsl.	+++	++	(+)	+	42	5	30	8.5	Korea
V. cotinifolium D.Don	++	++	(+)	+	24	5.5	16	5	Himalayas
V. buddleifolium C. H. Wright	++	++	(+)	+	15	4	8	<1.5	
V. lantana L.	++	++	(+)	+	17	3	9	<2	Europe, W. Asia
V. mongolicum (Pall.) Rehd.	(+)	++	(+)		30	6.5	23	7	E. Siberia, N. China
V. rhytidophyllum Hemsl.	+	+	+	+	13	3.5	9	1	C. and W China
V. macrocephalum Fort.	++	+	(+)	+	9	4	7	<3	China
3 *Pseudotinus*									
V. sympodiale Graebn.	+	(+)	+		26	5.5	16	7	China
4. *Pseudopulus*									
V. plicatum Thunb.	++	++	++	+	52	11.5	43	10.5	China, Japan
5. *Lentago*									
V. lentago L.	++	++	(+)		38	9	27	7.5	E. N. America
6. *Tinus*									
V. tinus L.	++	+	(+)		27	8.5	18	6	Mediterranean
V. davidii Franch.	+++	+	+		11	3	7	4	W. China
V. cinnamomifolium Rehd.	++	(−)	+	+	11	2.5	8	2	
7. *Megalotinus*									
V. cylindricum D.Don	++	++	(+)		40	10	32	8	W. China, Himalayas
8. *Odontotinus*									
V. foetidum Wall.	++	+	(+)		25	7	27	6.5	W. China
V. corylifolium Hook f. & Thoms.	+	++	(+)		34	10	15	5	C. and W. China
V. lobophyllum Graebn.	+	+	+		16	4.5	13	4	C. and W. China
V. dentatum L.	++	++	(+)	+	40	10	36	9	E. N. America
V rafinesquianum Schult.	++	++	(+)	+	31	7.5	17	5.5	E. and C. N. America
9. *Opulus*									
V opulus L.	++	(+)	+		9	<5	6	2	Europe, N. Africa, N Asia
V. sargentii Koehne	++	(+)	+		4	2.5	4	1.5	N. E. Asia
Hydrangea McClint.									
I. *HYDRANGEA*									
1. *Americanae*									
H. arborescens L. subsp. *arborescens*	++	−	+		nil	nil	nil	nil	E. U.S.A.
H quercifolia Bartram (June)					58	13.5	50	18	E U.S.A.
" " (Dec.)	++	++	−		52	12	52	21	
2. *Asperae*									
H. aspera D.Don‡	(+)	(+)	+		16.5	3	10	3.5	E. Himalaya to Formosa and Java
subsp. *aspera*	++	+	+	A†	18	<3	10	<2	
subsp. *robusta* (Hook. f. & Thom.) McClint.	++	(+)	+		5	<2	2	<2	E. Himalaya to Formosa
subsp. *sargentiana* (Rehder) McClint.	+++	+	++	A	tr.	nil	nil	nil	China
3. *Calyptranthae*									
H anomala D.Don‡ subsp. *anomala*	++	++	−		41	7.5	44	>8.5	E. Himalaya to Japan
									E. Himalaya, W. & C. China
subsp. *petiolaris* (Sieb & Zucc.) McClint.	++	++	−		38	9	38	11	Sakhalin, Japan, Formosa
4. *Petalanthae*									
5. *Heteromallae*									
H. paniculata Sieb	++	(+)	(+)		9.5	7.5	5	8	China, Japan
H. heteromalla D.Don	++	+	+	A	11.5	nil	11.5	nil	China, India, Tibet

Table 1–*continued*

Genus, section, subsection, species	Flavonoids* Q	Cy	K	L	Powder *E*	TAE	Extract *E*	TAE	Geographical range
6. *Macrophylla*									
H. macrophylla (Thunb.) Seringe									
subsp. *macrophylla*									
subsp. *serrata* (Thunb.) McClint	+ +	–	+		nil	nil	nil	nil	Japan, Korea
II. *CORNIDIA*									
1. *Monosegia*									
H. seemannii Riley	(+)	(–)	+ +		3.5	3	2	2	Mexico
H. intergrifolia Hayata	(+)	(–)	+		4	3.5	2	2.5	Philippines, Formosa
2. *Polysegia*									
Only herbarium material available									S. America

* Q = quercetin; Cy = cyanidin; K = kaempferol; L = luteolin, as reported by Bohm and Glennie [12] or detected by chromatograms in Forestal.
† A = contains procyanidin type A.
‡ Subspecies not specified.
§ Applicable only to the genus *Hydrangea*.

advanced, feature of sterile peripheral flowers in the umbels of *V. macrocephalum*, *V. opulus* and *V. sargentii* which, with their low tannin content might also support this correlation, is also a feature of *V. plicatum* (Section *Pseudopulus*), which has the highest tannin content of all.

The species which synthesise luteolin cut across several of Rehder's sections and are so widely separated both geographically and in tannin content that this character is not very helpful from the taxonomic point of view.

Table 2. *Viburnum* species arranged in decreasing order of proanthocyanidin content

Species	*E*	Chromosome number
Group 1 (High *E*)		
V. plicatum	52	18
V. fragrans	44	16, 32
V. carlesii	42	18
V. cylindricum	40	18
V. dentatum	40	36, 54
V. lentago	38	18
V. corylifolium	34	?
V. rafinesquianum	31.5	36
V. mongolicum	30.5	18
V. tinus	27	36
V. sympodiale	26.5	18
V. foetidum	25	16, 18
V. cotinifolium	24.5	18
V. foetens	23	16
Group 2 (Medium *E*)		
V. Lantana	17	18
V. lobophyllum	16	18
V. buddleifolium	15	18
V. rhytidophyllum	13	18
V. cinnamomifolium	11.5	18
V. davidii	11	18
Group 3 (Low *E*)		
V. macrocephalum	9	18
V. opulus	8.5	18
V. sargentii	4	18
V. henryi	2.5	32, 48

Extractability and relative astringency (RA) of Viburnum *species*

The ratio of *E* extract to *E* powder is a rough indication of the extractability of the tannin. In most cases, extraction has been about 75%. Occasionally, however, the extraction has been as much as 100% and occasionally less than 50%. The RA, caculated from the ratio of TAE/*E* of either the powder or the extract, is usually so close to 0.25 as to indicate a considerable degree of constancy in the molecular structure and complexity of the procyanidins throughout the genus. The magnitude of the RA suggests a molecular size of the order of tri- or tetrameric [4], having an $E_{1\,cm}^{1\%}$ of about 150. This allows the actual percentage of procyanidin of the leaf to be estimated at two-thirds of the quoted *E* value. This would mean for example, in the case of *V. plicatum* that procyanidins make up about 34% of the dry weight of the leaf!

HYDRANGEA

As in *Viburnum*, there is the same wide range of tannin content from very high to low and in two species, the *E* value is in fact nil. The species with the highest *E* value is one of the two N. American species, *H. quercifolia*, but the other, *H. arborescens*, contains no tannin. The Mexican *H. seemannii* and the Philippine-Formosan *H. integrifolia* also have very little tannin and the commonly cultivated 'hydrangeas', varieties of the Japanese-Chinese *H. macrophylla*, have none.

There are several species which, although they produce cyanidin when heated with HCl, do not, at the concentration present in the extract, precipitate blood proteins. These appear to have procyanidins type A or D, the RA of which is much less than that of the more usual types B or C [10]. The anthocyanidin formed from them is partly dimeric, has the same λ_{max}, 547–549 nm in BuOH–HCl, as cyanidin, but has R_f 0.3 in Forestal and can therefore be mistaken for delphinidin.

So far as the 13, mostly N. temperate, species so far examined are concerned, the present results are not systematically meaningful in terms of McClintock's arrangement. Although fresh specimens of the S. American species in Section *Cornidia*, Sub-section *Polysegia* were not available, herbarium specimens (which are of no use for quantitative analysis) showed no qualitative difference chromatographically from those species in Section *Hydrangea*. This supports McClintock's conclusion that "there is not a single floral structure that can be found to separate *Hydrangea* and *Cornidia*".

The genus appears to contain few of the less usual flavonoid compounds, but the coumarins umbelliferone and hydrangetin (8-*O*-methyldaphnetin) were present in all the species examined by Billek and Kindl [11]. Hydrangenol, a dihydroisocoumarin, is uniquely present in *H. macrophyllum*. Gallic acid was observed on the chromatogram of *H. quercifolia* and ellagic acid on that of *H. paniculata*, but they were not present in extracts in ester form and presumably do not, therefore, contribute to astringency. *H. quercifolia*, a native of E. N. America, is the species with the highest tannin content. The next highest, *H. anomala*, is Himalayan-C. Chinese. Thus the species with most tannin are native in areas which have been continuously available for plant life since long before the beginning of the Tertiary period and are refugia for many disjunct E. Asian-E. N. American genera [6]. Conversely, the species with the least tannin and the most individual and differentiated phenolic constituents, *H. macrophylla*, with its often completely sterile-flowered globose inflorescence, occurs at the extreme E. Asian limit of the range. Other species of *Hydrangea* produce inflorescences of completely sterile flowers in cultivation, but only *H. macrophylla* and *H. arborescens* are known to produce them in the wild. Thus in *Hydrangea*, unlike *Viburnum*, the character is associated with absence of tannin in both species which exhibit it. In any case, in spite of the superficial resemblance, there is no close analogy between the globose, sterile inflorescences in the two genera, because in *Viburnum* the enlarged sterile flowers are petaloid and in *Hydrangea* sepaloid; the former abscind, the latter persist.

In a personal communication, Dr. McClintock has pointed out the strong resemblance between the Mexican *H. seemannii* and the Philippine-Formosan *H. integrifolia*, and this is borne out by the chemical data. The low tannin content and the preponderance of K over Q are both to be regarded as indications of evolutionary advancement, strongly contrasting with the opposite situation in *H. anomala* and *H. quercifolia*.

Extractability and RA of Hydrangea *species*

Except in *H. aspera* and the *Cornidia* species, the tannin, when present, appears to be completely extracted by boiling aqueous MeOH. TAE/*E*, as in *Viburnum*, is usually about 0.25, except in those species thought to contain A type procyanidin, in *H. aspera* subsp. *aspera*, where it is 0.2, and in *H. aspera* subsp. *sargentiana* and *H. heteromalla*, where it is apparently zero. This is probably due to the much higher yield of cyanidin from A type proanthocyanidin [10]. With this exception the tannins in the two genera appear to be very similar in constitution and properties.

EXPERIMENTAL

Plant material. The leaves, harvested at maturity, were obtained from the Cambridge University Botanic Garden or from the Royal Botanic Gardens, Kew. Usually only one sample of each species was examined, but in the case of *H quercifolia* samples were taken in June and December, the results agreeing very closely Drying and powdering of leaves, preparation of extract, determination of anthocyanidin, astringency and chromatography were carried out as described previously [1].

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