

SYSTEMATIC ASPECTS OF THE ASTRINGENT TANNINS OF *ACER* SPECIES*

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Abstract—Leaves of a further 25 species of *Acer*, mostly from Asia and N. America, show similar levels of astringency and distribution of condensed and hydrolysable tannins to those previously examined. The results are tabulated and discussed in accordance with de Jong's recent rearrangement of the genus.

INTRODUCTION

In a brief discussion of the systematic significance of the data in the first paper [1], it was concluded that 'with only 25 of the presently recognized species of *Acer* included in this survey, it is unprofitable to attempt a statistical analysis of the chemical results in relation to the classification of the species and their distribution. There must however remain some doubt as to the validity of Pax's treatment in view of the heterogeneity of his sections in both these respects'. With 48 species now available, and de Jong's recent revision of the genus [2], it seems worthwhile to embark on such an analysis. The previous results have therefore been rearranged and combined with the present ones according to de Jong's revision.

RESULTS

Table 1 includes not only the data presented in the first paper, but also the results of chromatography of the leaf hydrolysate in Forestal and the 'tannic acid equivalent' of the leaf extract in terms of the method of determination of galloyl esters introduced by Haslam [3], based on a colour reaction with sodium iodate. This value is abbreviated to TI [1] to distinguish it from the 'tannic acid equivalent' (TAE) as determined by precipitation of blood protein [4], and the ratio of TAE to TI, which is diagnostic of the type of galloyl ester present, is also given. To simplify the presentation, the TAE of the leaf powder has been omitted. De Jong's arrangement shows up to advantage in the closer grouping of species having particular features in common.

Two such groupings stand out especially clearly. The first of these is a group of five species in Section *Macrantha* Series *Tegmentosa*. All of these—and only these—have myricetin (M) and prodelphinidin (PD). On present interpretation of the significance of the occurrence of flavonoid constituents, these five species are immediately identified as the most primitive of all extant *Acer* species. It is surprising, therefore, that they are not, as

judged by their TAE values the most astringent. It is true they have high *E* values, but this is because the yield of delphinidin from PD, in terms of *E*, is so much higher than that of PCy [5]. Only one of them, *A. davidii*, also has hydrolysable tannin.

The second grouping is of five species exemplified by *A. ginnala*, which Haslam [3] distinguished by the unusual chromatographic pattern of their galloyl constituents. No further example has been encountered in the present work. The five species, separated widely in Pax's arrangement, are redistributed by de Jong in three consecutive sections: *Rubra*, *Ginnala* and *Lithocarpa*.

These two groupings are representative (with exceptional features) of two categories of *Acer* species: those with predominantly condensed tannins, and those with predominantly hydrolysable ones. As a way of expressing these differences, in Table 1 the two main categories have been allotted the symbols α and β , while a few species with little or no tannin are allotted a category γ . The qualification for category α is a high *E* value, that for β a high TI. When, as in some species, these are both of intermediate value, the species appears as $\alpha\beta$. The six species containing PD are distinguished by the symbol αD and those in the *A. ginnala* group by the symbol βG . The numbers of species allocated to these categories are: 12 in α , 19 in β and 11 in $\alpha\beta$.

Systematically it is evident that Section *Macrantha* has a preponderance of α , and Sections *Rubrum* to *Integrifolia* a preponderance of β . Most of the $\alpha\beta$ types, also those in category γ , are in Sections *Acer* and *Platanoidea*, and, since most of these have their ranges in Europe and W. Asia, this immediately suggests a scrutiny of the categories in terms of their geographical distribution (Table 2). From this it emerges that Japan, C. Asia and E. N. America have most of the α , the Himalayas most of the β . Accepting the assumption that E. Asia and E. N. America possess refugia of the earliest N. hemisphere flora, less affected by the devastation of the Ice Ages, this would suggest that the α and β categories represent separate streams out of which, subsequently, the $\alpha\beta$ forms arose, occupying for the most part areas created by the upthrust of mountains or opened for recolonization as the ice-cap receded.

The species in category αD form the best starting point

* Part 3 in the series "Astringency of leaves". For Part 2 see Bate-Smith, E.C. (1978) *Phytochemistry* 17, 267.

Table 1. Chromatography and tannin analysis of *Acer* species.

Section, series, species	M	D	Flavonoids			Extinction				Astringency			Category	Geographical Range	
			E ₁	Q	Cy	K	E _p	E _{ex}	E _p /E _{ex}	TAE	TI	TAE/TI			
I	ACER														
	A Acer														
	<i>A pseudoplatanus</i> L.	—	—	++	++	(+)	(+)	2	2	1.0	nil	—	—	Europe, W Asia	
	<i>A caesium</i> Wall. ex Brandis	—	—	+	++	—	(+)	2.5	2.5	1.0	11	22	0.5	Himalaya	
	B Monspessulana														
	<i>A monspessulanum</i> L.	—	—	+	++	+	(+)	8	7	0.9	10	13.5	0.75	Europe, W Asia	
	<i>A opulus</i> Mill	—	—	+	++	(+)	+	7.5	6.5	0.9	7.5	9	0.8	Europe	
	<i>A sempervirens</i> L. syn. <i>A orientale</i> Mill.	—	—	+	?	?	?	5.5	5	0.9	11.5	16.5	0.7	Europe	
	C Saccharodendron														
	<i>A. saccharum</i> Marsh	—	—	+	++	+	(+)	22	11	0.5	15	18	0.85	E N America	
II	PLATANOIDEA														
	A Platanoidae														
	<i>A platanoides</i> L.	—	—	—	?	?	?	2.5	2.5	1.0	2.5	—	—	Europe	
	<i>A cappadocicum</i> G/ed	—	—	—	+	++	+	23	19	0.8	4	—	—	Europe, W Asia	
	<i>A lobelii</i> Tenore	—	—	—	++	+	+	7	4.5	0.65	1	—	—	S Europe	
	<i>A campestre</i> L	—	—	+	+	+	+	9	6.5	0.7	10	14	0.7	Europe, W Asia	
	<i>A miyabei</i> Maxim	—	—	—	+	++	++	tr	tr	6	9.5	0.6	2	Japan	
	B Pubescens														
III	PALMATA														
	A Palmata														
	<i>A palmatum</i> Thunb	—	—	+	?	+	(+)	16	11	0.7	12.5	14.5	0.85	Europe, Japan	
	<i>A sieboldianum</i> Miq	—	—	—	++	+	(+)	27.5	20	0.7	15	17	0.9	Japan	
	<i>A cincinatum</i> Pursh.	—	—	+	++	(+)	+	4.5	3	0.7	12	23	0.5	W N America	
	B Sinensis														
	<i>A campbellii</i> Hook f & Thoms.	—	—	++	—	—	—	4.5	3.5	0.8	12	23	0.5	Himalaya, W China	
	<i>A wilsonii</i> Rehd.	—	—	+	+	+	(+)	12	9.5	0.8	13	21	0.6	15	China
	C Penninervia														
	<i>A laevigatum</i> Wall ‡	—	—	++	+	?	(+)	nil	nil	—	13	23.5	0.55	Himalaya, W China	
	<i>A forrestii</i> Rehd. syn. <i>A. fabri</i> Hance	—	—	++	—	—	—	tr	tr	10	14.5	0.7	1.5	China	
IV	MACRANTHA														
	A Tegmentosa														
	<i>A pensylvanicum</i> L	+	+	—	+	+	(+)	25	13.5	0.55	3.5	—	—	Europe, N America	
	<i>A tegmentosum</i> Maxim	+	++	—	++	+	+	40	32	0.8	5	—	—	Europe	
	<i>A capillipes</i> Maxim.	+	+	—	++	+	+	29	20	0.7	9	—	—	Japan	
	<i>A grosseri</i> Pax	+	++	—	++	+	++	34	25	0.75	11	—	—	C China	
	<i>A davidi</i> Franch.	++	+	(+)	+	+	(+)	39	20	0.5	13.5	17	0.8	C China	
	<i>A rufuline</i> Sieb. & Zucc.	—	—	—	++	++	+	50	31.5	0.65	3.5	—	—	Japan	
	<i>A pectinatum</i> Wall ex Nichols	—	—	—	++	++	+	21.5	18	0.85	11	—	—	Himalaya	
	<i>A hookeri</i> Miq	—	—	+	++	(+)	+	6	2.5	0.4	10.5	18	0.6	nil	Japan
	<i>A rubescens</i> Hayata	—	—	+	+	+	(+)	10	6.5	0.65	13	23.5	0.65	nil	Taiwan
	B Wardiana														
V	PARVIFLORA														
	A Parviflora														
	<i>A. nipponicum</i> Hara	—	—	+	++	+	++	19.5	14.5	0.75	16.5	40	0.4	4.5	Japan
	B Urukunduensis														
	<i>A urukunduense</i> Traut v & Meyer*	—	—	+	(+)	(+)	(+)	5	3	0.6	10	12.5	0.8	1.5	E Asia, Japan
	<i>A spicatum</i> Lam	—	—	+	+	++	+	4	3	0.75	20	25	0.8	8.5	Europe, N America
	<i>A acuminatum</i> Wall.†	—	—	+	++	+	(+)	19	11	0.6	16	26	0.6	8	Himalaya
	C Distyla														
	<i>A distylum</i> Sieb. & Zucc *	—	—	+	+	++	(+)	11	7	0.6	3	10	0.3	—	Japan
VI	TRIFOLIATA														
	<i>A griseum</i> (Pax) Franch.	—	—	+	++	+	++	8	10	1.0	11	14	0.8	4	W China
VII	RUBRUM														
	<i>A rubrum</i> L	—	—	+	++	++	(+)	20	4	0.25	7	19	0.35	4	Europe, N America
	<i>A saccharinum</i> L.	—	—	+	++	++	(+)	4.5	2.5	0.55	7.5	21	0.35	10.5	E N America
VIII	GINNALA														
	<i>A tataricum</i> L.	(+)	—	?	++	+	+	tr	tr	—	8.5	20	0.4	7	Europe, W Asia
	<i>A ginnala</i> Maxim	—	—	+	++	(+)	+	3	3	1.0	5.5	25	0.2	8	E Asia
IX	LITHOCARPA														
	A Lithocarpa														
	<i>A sterculiaceum</i> Wall. syn. <i>tilicum</i> Wall	—	—	+	+	++	—	11.5	9	0.8	5	—	—	nil	Himalaya
	<i>A diabolicum</i> Koch‡	—	—	—	++	?	++	7	2	0.3	16	31	0.5	4.5	Japan
	<i>A thomsonii</i> Miq.	—	—	+	(+)	—	++	tr	tr	—	9.5	11	0.9	1	Himalaya
	B Macrophylla														
	<i>A macrophyllum</i> Pursh.	—	—	—	+	+	(+)	26	13	0.5	4.5	—	—	nil	W N America
X	NEGUNDO														
	A Negundo														
	<i>A negundo</i> L	—	+	—	+	++	++	20	13	0.65	3.5	—	—	nil	Europe, N America
	B Cissifolia														
	<i>A cissifolium</i> Sieb & Zucc	—	—	—	++	(+)	(+)	tr	tr	—	2	—	—	nil	Japan
XI	GLABRA														
	A Glabra														
	<i>A glabra</i> Torrey	—	—	(+)	++	+	(+)	tr	tr	—	23	22	1.0	nil	W N America

Table 1.—Continued

Section, series, species	M	D	Flavonoids			Extinction	Astringency	Category	Geographical Range			
			El	Q	Cy							
B Arguta												
<i>A. tetrapterum</i> Pax	—	—	+	++	(+)	+	2.5	1.5	0.65	12	26	0.5 7
XII INTEGRIFOLIA												
<i>A. Trifida</i>												
<i>A. oblongum</i> Wall ex DC**	—	—	+	(+)	—	+	tr	tr	12.5	16.5	0.75	3
<i>A. lanceolatum</i> Mollhard	—	—	+	+	(+)	+	tr	tr	17	36	0.5	5.5
<i>A. albo-purpurascens</i> H. T. Tsai	—	—	—	++	+	(+)	30	20	0.65	8	—	nil α
B Pentaphylla												
<i>A. pentaphyllum</i> Diels	—	—	+	—	—	++	tr	tr	10	14	0.7	4.5
XIII INDIVISA												
<i>A. carpinifolium</i> Sieb & Zucc	—	—	—	+	++	—	61	16	0.25	4	—	nil α

Key (to Table 1): M, myricetin; D, delphinidin (from prodelphinidin); El, ellagic acid, Q, quercetin; Cy, cyanidin (from procyanidin); K, kaempferol; E_p , E_{ex} , extinction of anthocyanidin from powder and extract, respectively; TAE, tannic acid equivalent (precipitation of blood protein); TI, tannic acid equivalent (iodate reaction); HH, hexahydroxydiphenic acid glucose ester; Category, see Text.

* Leaves senescent, results unreliable. *A. caudatum* Wall, also examined, is not included by de Jong. According to Bean most of this in cultivation is *A. acuminatum*, with which the present analysis agrees.

† Regarded by de Jong as synonym of *A. urukunduense*.

** Three samples of this species examined: one from Himalaya (that recorded here); a second from Messrs. Hillier and a third from Kew, both probably original Wilson collection from China, differing from the first in amounts of α and β constituents. *A. lanceolatum* is regarded by de Jong as probably a form of *A. oblongum*.

‡ These spp. contain a dark-absorbing constituent at R_f 0.50 in Forestal interfering with the detection of Cy.

for a more detailed analysis. Except for *A. negundo*, these all have M as well as PD, confirming their primitive flavonoid status. Except for *A. davidii*, none of them has hydrolysable tannin, so that the remaining four are prototypes of the postulated ancestral α stream. One has its range in E. Asia, one in Japan, one in C. China and the fourth in E. N. America. If they do in fact have a common origin, this must presumably be holarctic. The presence of ellagitannin in *A. davidii* suggests C. China as one of the meeting-points of the α and β streams. *A. hookeri*, which Rehder quotes as a related species, has its range in E. Himalaya and is placed in category β , so that *A. davidii* is intermediate both chemically and geographically between this species and, for instance, *A. tegmentosum*, with its range in Korea and Manchuria.

There is a precedent within the Sapindaceae for the track suggested here. *Xanthoceras sorbifolium* Bunge is the only species on record [6] in the Sapindales having the PD-M pattern. This is placed (EP) in the tribe Harpullieae of the Sapindaceae, with which Leenhouts [7] suggests *Acer* has the closest affinities. Its range is N. China.

Besides the presence of M, the relative amounts of Q and K are useful indices of evolutionary status. *A. negundo*, for instance, has more K than Q, confirming the relatively advanced condition of this species indicated by the absence of M, whereas *A. tegmentosum*, *A. pensylvanicum*, and *A. capillaris* all have more Q than K. *A. davidii*, as well as differing from them all in the presence of El, has two unidentified constituents absorbing in the UV at R_f 0.56 and 0.7, similar to constituents also observed in *A. wilsonii* which occurs in the same region.

Nature and origin of the β constituents

While the α constituents, the condensed tannins, are commonly present in all classes of vascular plants, the β constituents, the hydrolysable tannins, are present only in dicots and only in certain orders of these. Ellagitannins are more common than gallotannins, so that the affinities of *Acer*, which contains both, must be looked for in one of those orders which also contain both. Several of the families in the Sapindales are, in fact, outstanding in this respect: *Rhus* species (Anacardiaceae) are the source in commerce of tannic acid, the best known of all tannins; *Coriaria* species (Coriariaceae) are rich sources of tannin containing both ellagic and gallic acid; and *Melianthus* species (Melianthaceae) are rich in ellagitannin. None of these species contains proanthocyanidins, so that there are analogies, within the Sapindales, of the β type of *Acer*.

Of the actual constitution of these gallo-ellagitannins, very little is known. Haslam's reaction does not distinguish between galloyl and hexahydroxydiphenol residues, both being included in his 'galloyl esters', but in the 15 species he examined he observed three different patterns of these esters by 2D chromatography, one associated with the ginnala group (β G), one with *A. platanoides*, *A. campestre* and *A. rubrum* resembling the pattern observed in *Rhus* gallotannins, and the third, the most frequent, in the remaining eight species, probably more complex ellagitannins. It was remarked previously [1] that the fairly constant relation of TAE/TI indicated

Table 2. Geographical distribution of *Acer* species belonging to different tannin categories

	No. spp. in tannin category				Total
	α	$\alpha\beta$	β	γ	
Japan	3	3	2	1	9
E. Asia	1	2	1	0	4
C. China	2	1	0	0	3
W. China	1	2	1	1	5
Himalaya	1	2	5	1	9
Europe	1	4	1	3	9
E. N. America	2	1	3	0	6
W. N. America	1	0	2	0	3
Total	12	15	15	6	48

an overall similarity in the hydrolysable tannins of the different species, perhaps even a constancy of composition of these tannins. The 'acertannin' of Perkin and Ushida [8], a digalloyl-polygalitol, was not identified in Haslam's chromatographic studies.

Gallic and ellagic acids are both visible on chromatograms in Forestal solvent, but while the latter is readily identified by its R_f and violet UV fluorescence, the former's R_f is in a position where other common constituents occur, absorbs in UV, and although it darkens when fumed with NH_3 it can only be identified with confidence when present in sufficient quantity. Most species appear to contain gallic acid in however small amount [3], but because of the uncertainty of its identification on Forestal papers it has not been included in Table 1. In *A. macranthum* it is not combined in ester form [3], and it may therefore be present to some extent as the free acid in other species.

Category γ and the level of astringency

It was remarked earlier [1] that except for three species, *A. lobelii*, *A. platanoides* and *A. pseudoplatanus* in which the TAE was exceptionally low, there seemed to be remarkably little difference in this respect between *Acer* species in spite of the different kinds of tannin they contained. These three are European species, of which in the sample then examined the representation was preponderant. This has now been corrected by examining a larger number of Asian and W. N. American species. As a result one more, *A. cissifolium*, a Japanese species, has been found to have low TAE, and is placed with the first three in category γ . In all these cases the reason seems to be impoverishment of the α constituent. Several species in this second sample have high astringencies, but in a majority the TAE lies in a narrow range between 10 and 15; only 5 of the 50 species have TAE higher than this. Four of these are in category β , one $\alpha\beta$. They are widely spread geographically. The W. N. American *A. glabrum*, with TAE = 23, is placed by de Jong in a monotypic series. Its tannin appears to consist almost entirely of gallotannin (TAE/TI = 1.0) and in this respect it is unique. Of the other two species in W. N. America, *A. macrophyllum* contains gallic acid which is unesterified [3] and considerable PA ($E = 26$) but its TAE is low. *A.*

circinatum a vine, also appears to contain considerable gallic acid, but unusually in the genus it contains a coumarin (scopoletin?). All three species in this area therefore have exceptional properties. With the European species in category γ , they can be regarded as 'outliers' from the main concentration of the genus in China and Japan.

The species with the second highest TAE, *A. spicatum*, is also in category β . This was made the type species of a large section by Pax, who considered it to be one of the earliest. It was, however, one of the least homogeneous sections from the chemical point of view [1] and was split by Pojarkova [10]. De Jong now places *A. spicatum* in an isolated series in Section *Parviflora*. Its range is in E. N. America, one of the areas likely to harbour ancient relict species. It has much ellagitannin and some PA.

The third highest, *A. lanceolatum*, is S. Chinese, placed by de Jong in Section *Integrifolia* along with *A. oblongum*, with which he thinks it may be synonymous. In its chemistry it resembles the Himalayan form of this species, but with higher tannin content. The tannin appears to be wholly ellagitannin, TAE/TI = 0.5, HH = 5.5.

The next, *A. nipponicum*, is almost equally α and β . *A. diabolicum*, also Japanese, is one of the ginnala group, with modified ellagitannin and some PA. It seems, therefore, that a high level of astringency is associated in the main, but not exclusively, with hydrolysable tannins. Like that of low tannin, the incidence of high tannin is not associated with any particular geographical range nor with any of the morphological features currently used in taxonomic arrangements of the genus.

REFERENCES

1. Bate-Smith, E. C. (1977) *Phytochemistry* **16**, 1421.
2. De Jong, P. C. (1976) *Meded. Landbouwhogeschool Wageningen* **76**, 1.
3. Haslam, E. (1965) *Phytochemistry* **4**, 495.
4. Bate-Smith, E. C. (1973) *Phytochemistry* **12**, 907.
5. Bate-Smith, E. C. (1975) *Phytochemistry* **14**, 1107.
6. Bate-Smith, E. C. (1962) *J. Linn. Soc. (Botany)* **58**, 95.
7. Leenhouts, P. W., quoted in [2].
8. Perkin, A. G. and Ushida, Y. (1922) *J. Chem. Soc.*, 66.
9. Bate-Smith, E. C. (1972) *Phytochemistry* **11**, 1755.
10. Pojarkova, A. I. (1933) *Acta Inst. Bot. Akad. Sci. USSR, Ser. 1* **1**: 143, 223.