Phytochemistry, 1977, Vol. 16, pp. 1421–1426. Pergamon Press, Printed in England.

ASTRINGENT TANNINS OF *ACER* SPECIES*

E. C. BATE-SMITH
Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge. CB2 4AT, U.K.

(*Received* 22 *March* 1977)

Key Word Index—*Acer*; Aceraceae; maple leaves; proanthocyanins; galloyl esters; ellagitannins; astringency.

Abstract—All 25 species of *Acer* examined contain condensed (proanthocyanidins) and hydrolysable (gallo- and/or ellagi-) tannins, but each of these varies over a wide range. Except, however, for three species in which both are low, the variation is usually reciprocal, so that differences in astringency between species are relatvely small. Five species, *A. ginnala*, *A. saccharinum*, *A. tataricum*, *A. diabolicum*, and *A. rubrum*, differ from the remainder in the nature and astringency of the galloyl compounds present. The first three were placed by Haslam in a separate group, to which the last two can now be added on the strength of the present evidence. Modifications are described for the method of determination of galloyl esters suggested by Haslam.

INTRODUCTION

Acer is a genus with a more than usually complicated pattern of astringent constituents. All species contain in greater or lesser amount both condensed and hydrolysable tannins, the latter consisting of both gallo- and ellagitannins. Methods are now available for evaluating the amounts of proanthocyanidins [1] and ellagitannins [2], and advantage has been taken of these to examine the contribution which these tannins make, respectively, to the overall astringency of the leaves, as determined by precipitation of blood protein [3], of a considerable number of species. It is a reasonable assumption that this will provide an indication of astringency in the mouth (due to precipitation of the proteins of the saliva and mucosa) and of the inactivation of enzymes, and therefore of repellence to potential predators, whether animal, fungal or bacterial.

Perkins and Ushida [4] isolated a gallotannin, acertannin, from leaves of *Acer ginnala*. This was shown by Kutani [5] to have the unique constitution of a digalloylpolygalitol (the latter being an anhydro-D-glucitol). In a useful survey of the galloyl esters in 15 species of *Acer*, Haslam [6] found two other species to have the same chromatographic pattern of galloyl derivatives as *A. ginnala*—presumably these also contain acertannin—and he suggested they might form a natural group, which he called Group A. Two other groups, of 3 and 8 species respectively, had different chromatographic patterns, and one species, *A. macrophyllum*, had free gallic acid only. Except for the last, all the species had galloyl esters, in which he included esters of hexahydroxydiphenic acid, the parent of the dilactone, ellagic acid. He also suggested a method for determining galloyl esters depending on a colour reaction with iodate. Bate-Smith [7] found leucocyanidin, gallic acid and/or ellagic acid in all of 8 species, and leucodelphinidin in 3.

RESULTS

The results (Table 1) are concerned with the analysis of freshly dried and powdered leaves, and of extracts of the powder in hot 5% aqueous methanol. The species are listed according to Rehder's arrangement [8], which follows closely the earlier one of Pax. The proanthocyanidin (PA) contents of powder and extract were determined by heating with butanolic HCl and that of ellagitannin by treating the extract, after exclusion of oxygen, with nitrous acid, and measuring the absorption of the resulting blue reaction product at 600 nm. The results of the former are expressed in terms of the extinction ($E^{1\%}_{1cm}$) of the anthocyanidin produced, and of the latter as the % hexahydroxydiphenic acid ester with glucose (HH).

The tannic acid equivalent (TAE) of the powder is a measure of the *effective* astringency of the leaf. This depends not only on the amount and the relative astringency of the tannins, but also on their extractability. It is sometimes less than that of the extract because exhaustive extraction with hot methanol may dissolve more of the high-molecular PA than is dissolved in a single brief treatment of the powder with cold diluted blood. The TAE of the extract is, however, nearly related to the nature and concentration of the tannin as indicated by the analytical data. With regard to the last, it is impossible from a knowledge of the *E* value of the PA and the 600 nm absorption of the HH to calculate with certainty either the amount percent of these constituents or the TAE of the leaf, because these depend on the nature and molecular complexity of the particular representatives of these classes present. Prodelphinidins have about twice the *E* value of procyanidins, the *E* values and TAE's of which vary with their structure (whether A or B types) and their molecular weights [1]. Only rough estimates, based on average values as at present known, can be made. When PD is present together with PCy, the relative amounts of each can be calculated from the λ_{max} of the anthocyanidin peak, that of cyanidin (in butanolic HCl) being 547 nm and that of delphinidin 558. The λ_{max} recorded in Table 1 gives a clear indication of those species in which PD is present, confirming the results of chromatography in Forestal solvent.

In the first two columns of figures in Table 1, the *E* values and TAE of the powdered leaves are given. It is

* Part 1 in the series 'Astringency of leaves'.

Table 1. Tannin analyses of *Acer* species

	Section	Species	Powder		Extract				Geographical range
			E	TAE	*E*	TAE	HH	λ_{max}	
I	Platanoides L.	*A. platanoides* L.	2 5	5.5	2 6	(2.5)	—	548	Europe, Caucasus
		A. cappadocicum Gled.	23	9	19	4	—	549	Caucasus, W. Asia
		A. lobelii Ten	7	1	4.5	1	—	547	Italy
II	Campestria	*A. orientale* L	5.5	7.5	5	7	9	548	E. Mediterranean
		A monspessulanum L.	8	9.5	7	10	10		S Europe, W. Asia
		A campestre L.	9	9	6.5	14	6	548	Europe, W Asia
		A opalus Mill	7 5	6	6.5	7.5	(5.5)	548	S. Europe
		A obtusatum Willd.	52	8 5	24	8.5	tr	548	S.E. Europe, Italy
III	Saccharina	*A. saccharum* Marsh	22	8	11	8	(9)	548	E. Canada, E USA.
IV	Spicata	*A spicatum* Lam.	4	18	3	16	17		Canada, E. USA
		A pseudo-platanus L.	2	0.5	2	0	—	548	Europe, W. Asia
		A. tataricum L.	2	8	—	7.5	13 5*		S.E. Europe, W Asia
		A. ginnala Maxim	3	8	3.5	—	10*		E. China, N China, Manchuria
		A. macrophyllum Pursh.	26	13	7.5	4.5	—	548	Alaska to California
V	Palmata	*A. palmatum* Thunb. (var.)	19	7.5	13.5	3.5	—	548	
		A. palmatum Thunb. (sp.)	16	7	11	6 5	—	548	Korea, Japan
VIII	Indivisa	*A. carpinifolium* S & S	61	11	16	4	—	548	Japan
IX	Macrantha	*A. davidii* Franch	39	13	20	13 5	3	554†	C. China
		A pensylvanicum L.	25	7.5	13.5	3.5	—	552†	E Canada, E. USA
		A capillipes Maxim.	29	11	20	11	6	554†	Japan
X	Argreta	*A. tetramerum* Pax.	2.5	11	1 5	12	14.5		W China
XI	Lithocarpa	*A diabolicum* K. Koch	7	16	2	20	9*		Japan
XII	Rubra	*A. saccharinum* L	4 5	10	2.5	6	21*		E. Canada, E. USA
		A. rubrum L	20.5	7	4	4	7 5*		E Canada, E. USA
XIII	Trifoliata	*A. griseum* (Franch.) Pax	8	10	10	9	8	547	W. China
XIV	Negundo	*A. negundo* L.	20	5 5	13	3.5	tr	552†	E Canada, C USA, E. USA

Key: *E*, extinction of anthocyanidin; TAE, tannic acid equivalent; HH, hexahydroxydiphenic acid glucose ester.
* Anomalous behaviour in nitrous acid reaction.
† Delphinidin present on chromatogram in Forestal solvent.

immediately obvious that the former vary over a very wide range from little more than a trace to 61 in *A. carpinifolium*. This, as PCy, represents an estimated 30% or more of the dried leaf. If the species are divided into those with high ($E > 10$) and those with low ($E < 10$) PA, it can be seen from the values in column 5 that the former have low and (except for three species in which both are low), the latter high HH. The species so divided are listed in Table 2. Except for the three species in which both are low the average TAE of the species in these divisions (and also of the five species in Haslam's Group A), is almost exactly the same, which is remarkable in view of the wide variation in the amounts of the individual tannins.

In the third and fourth columns the *E* and TAE values of the extracts are given. The *E* of the extract is nearly always lower than that of the powder, the ratio giving a measure of the extractability of the tannin. *A. carpinifolium*, the species with the highest *E* value of the powder, is an outstanding example of inextractability of PA. The TAE's of the extract and the powder are usually about the same. In the fifth column, the estimated hexahydroxydiphenoylglucose (HH) content of the extract is given. This is assumed to be also the HH content of the powder, because the constituents responsible for the reaction with HNO_2 appear to be completely extracted.

Table 2. *Acer* species belonging to different tannin classes

PH and HH both low	PA high	HH high
lobelii	*capillipes*	*campestre*
platanoides	*cappadocicum*	*griseum*
pseudoplatanus	*carpinifolium*	*monspessulanum*
	davidii	*opalus*
	macrophyllum	*orientale*
	negundo	*tetramerum*
	palmatum	
	pensylvanicum	
	obtusatum	
	saccharum	
	spicatum	

While PA and HH account for much of the astringency, most species also contain gallotannin in the form of acertannin or otherwise. There is at present no recognised method of determining galloyl residues, but Haslam [6] described a reaction for galloyl esters which showed promise of providing such a method. This consisted in treating the ester, or the aqueous acetone extract of the plant tissues, with KIO_3 at 0°, and measuring the absorption of the red colour produced at 550 nm. The red product is actually an intermediate in the formation, in due course, of a yellow product, and the conditions of the determination have, therefore to be precisely specified. Galloyl groups in the form of depsides and digalloyl compounds such as HHDPG also react, and Haslam includes these in his general description 'galloyl esters'. Further observations have now been made on tannic acid and other model compounds and as a result the following procedure has been adopted. Instead of reacting for the specified time (40 min) at 0°, the reaction is allowed to proceed (when sufficient galloyl ester is present) until a maximum is reached. Other phenolic constituents, including gallic acid itself, react to form yellow products which interfere increasingly as the relative concentration of the galloyl derivatives decreases, so that, as Haslam points out, the results are less reliable when these are present in lower concentration. The results of these preliminary experiments were sufficiently promising for a more detailed exploration of the reaction to be undertaken.

Reaction of galloyl esters with iodate

The most readily available sources of galloyl esters were the 'tannic acids' of commerce. These may be either Chinese or Turkish, and vary in composition accordingly.

Chinese, from galls or leaves of *Rhus* species (Anacardiaceae) consists mainly of hepta-octagalloylglucose; Turkish, from galls of *Quercus infectoria* (Fagaceae), mainly of hexa-heptagalloylglucose in which certain hydroxyls on the glucose ring may be variously unesterified (E. Haslam, personal communication).

In addition to these tannic acids, small amounts of gallotannins prepared from them and a number of synthetic model compounds have been available (See Table 3).

The course of the reaction with Chinese tannic acid under varying conditions was first studied. At 0°, in aqueous solution, the reaction proceeded for 90 min before the maximum of absorption was reached. As recommended by Haslam, absorption was measured at 550 nm, although the peak is actually close to 500 nm.

With increasing temperature, the maximum was reached in progressively shorter time, without significant change in magnitude up to 15°, but thereafter, the absorption at the maximum fell. The optimum temperature for routine procedure seemed, therefore, to be 15°. Under these conditions the $E^{1\%}_{1cm}$ at 550 nm for this sample of tannic acid was 15.5. When others of the available compounds were examined, however, it was found that some of them produced a precipitate before the maximum was reached. Haslam's leaf extracts were made with 70% aqueous acetone, and his reaction mixture contained acetone up to 20%. In tests on these substances with this concentration of acetone no ppt was formed, nor with MeOH at concentrations as low as 10%, but the E at the maximum varied with the concentration of organic solvent. Thus with 20% acetone at 15° E = 20, with 25% MeOH E = 26.5, with 20% 21.4, with 10% 20.6. Turkish tannic acid with 12.5% MeOH had E = 20.

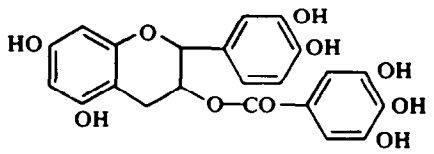
In Table 3 the results with model galloyl esters are compared with those given by the commercial tannic acids. With 5 or more galloyl residues per molecule of glucose the extinction is the same, indicating that only 5 residues are open to attack. The extinction per residue is then 20% of the whole. With 2 residues the extinction per residue is, however, twice as much. With the galloylquinic acid, the extinction per residues is less. In tara gallotannin, since one of the four quinic hydroxyls is uncombined, there will be only 3 accessible galloyl groups. The extinction per residue is therefore nearly the same as it is in the synthetic digalloylquinic acid.

The formation of a precipitate during the course of the reaction appears to be due to the absence of free hydroxyls on the parent residue. This was met with in gallotannin fractions prepared from both Chinese and Turkish tannic acids and in synthetic pentagalloylglucose, necessitating an increase in MeOH in the reaction mixture to 25%, and conspicuously in tetragalloylmethylglucose, which still formed a ppt at 25% MeOH. Furthermore, solutions of the low-molecular isolates and preparations, when mixed with diluted blood, would not settle in the centrifuge. The two digalloyl compounds formed neither a cloud nor a precipitate at the concentration which the limited amount of material allowed. Cloudiness in natural extracts when mixed with diluted blood and centrifuged therefore probably indicates the presence of low-molecular tannins.

Table 3. Iodate reaction of natural tannins and model compounds

	$E^{1\%}_{1cm}$	$E^{1\%}_{1cm}$ per galloyl group (accessible)
Chinese tannic acid (mainly hepta-octagalloylglucose)	21	4.2
Turkish tannic acid (mainly hex-heptagalloylglucose)	20	4.0
Sumach gallotannin (hepta-octagalloylglucose)	22	4.4
Pentagalloylglucose	20	4.0
Digalloylglucose	18	9.0
Tara gallotannin (tetra-pentagalloylquinic acid)	10	3.3
Digalloylquinic acid	6	3.0

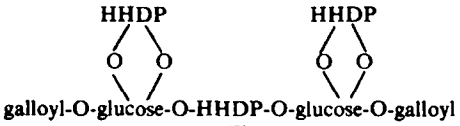
An interesting case is that of the catechin gallates (**1**).



1

Instead of reaching a maximum in 20 min, the absorption continued to rise slowly for several hr, with so much yellow absorption that the flat 'peak' was at 475 nm instead of the usual 520. This seems likely to be due to the reaction of the C_{15} catechin moiety, since extracts of leaves containing flavonoids but not galloyl derivatives, when treated with iodate, give yellow reaction products similarly increasing in absorption up to several hours.

Only one ellagitannin model compound has been available for examination, and that of uncertain structure, viz; Seikel and Hillis's [10] fraction D3 of *Eucalyptus delegatensis* tannin (**2**). In addition to the usual red



(2)

reaction product, this developed transient absorption between 600 and 650 nm, but eventually gave the usual peak at about 500 nm, with E = 11.2. However, two species of plants containing exclusively ellagitannin in high concentration have been examined: *Melianthus minor* and *Francoa ramosa*. These enabled the relationship between the TAE as determined by haemanalysis and the E of the 550 nm absorption to be explored. As in the case of blood precipitation, a 'tannic acid equivalent' of the iodate reaction can be calculated as the E of the sample per cent of that of a typical tannic acid. This iodate TAE will be distinguished from the blood TAE by use of the abbreviation TI. The results with the above two species, and also with *Cotinus coggyria*, which contains sumach tannin (similar to, or even maybe identical with Chinese tannic acid) were: *Melianthus minor* L. TI 40, TAE 20, TAE/TI 0.5; *Francoa ramosa* D. Don TI 48, TAE 19, TAE/TI 0.4; and *Cotinus coggygria* Scop. TI 35, TAE 34, TAE/TI 1.0. The gallotannin present in *Cotinus* gives the same tannic acid equivalent for both the iodate reaction and the blood precipitation, confirming that it is indeed a typical tannic acid, but the ellagitannin from the other two species has only half the astringency that

would have been expected from the iodate reaction. Determination of HH provides helpful information. In *Melianthus* this was 22%, in *Francoa* 23.5%, in each case close to half the TI value. Obviously, it is necessary to isolate more ellagitannins and determine the relationships between the analytical data, before it will be possible to evaluate the individual contributions of the galloyl and hexahydroxydiphenoyl residues to the iodate absorption in species such as *Acer*, in which both are present, and their respective contributions to astringency. The situation is further complicated in *Acer* by the contribution of proanthocyanidins to astringency, but means are available of determining this [1].

Iodate reaction of Acer *species*

In Table 4 the tannic acid equivalent of the iodate reaction (TI) of sixteen species of *Acer* which have appreciable amounts of ellagitannin are given. These fall into two groups, each in alphabetical order, depending on the relationship between TI and the astringency, TAE. In the first group the ratio of TAE to TI is 0.5 or less, in the second, 0.7 or greater. The first group corresponds with Haslam's Group A, differing in the chromatographic pattern of the galloyl esters, and also in other respects mentioned earlier. It was from *A. ginnala* that Perkin and Ukeda isolated ascertannin, and it would be interesting if it is a distinctive feature of this group, correlated with the lesser astringency per iodate TI, and associated with the unidentified constituent on the chromatogram in Forestal given by all the members of the group (see Experimental).

Considering the wide variation in ellagitannin content, and the appreciable amounts of PA in many of the species, the TAE/TI ratio of the members of the second group is remarkably constant. This suggests that the galloyl esters (in which HHDPG is for this purpose included) play a dominant role in determining astringency in these species. It suggests, further, that the hydrolysable tannin might be all of one kind, containing both galloyl and HHDP residues in the same molecule, as for instance, in corilagin. This would give a value for TAE/TI intermediate between those for gallotannin (1.0) and ellagitannin (0.5) given by *Cotinus* and *Melianthus*, respectively. In this connection an analytical method specific for galloyl groups, comparable with that now available for HH is needed.

Table 4. Tannic acid equivalents and astringency of *Acer* species

Species	TI	TAE	TAE/TI
diabolicum	31	16	0.5
ginnala	25	5.5	0.22
rubrum	19	7	0.37
saccharinum	21	7.5	0.35
tataricum	20	8.5	0.43
campestre	14	10	0.72
capillipes	11	8.5	0.77
griseum	14	11	0.8
monspessulanum	13.5	10	0.76
opalus	9	6.4	0.71
orientale	16.5	11.5	0.7
platanoides	7.5	5.7	0.75
spicatum	25	20	0.8
tetramerum	26	20	0.77
macrophyllum	*nil*		
pseudoplatanus	8.5	0.6	0.07

Three species of *Acer* which contain no ellagitannin were also examined. *A. macrophyllum*, which Haslam found to contain gallic acid not combined in ester form, did not produce a peak with iodate, but gave a slowly increasing yellow absorption. *A. platanoides* gave an iodate peak TI 7.5, had TAE 5.7, TAE/TI 0.75. *A. pseudoplatanus* gave an iodate peak TI 8.5, but only TAE 0.6, i.e. virtually non-astringent, it presumably contains a low-molecular galloyl ester.

Systematic significance of the chemical data

The above results give ample support for Haslam's separation of a *ginnala* group from the remaining species. They also provide the link between *A. saccharinum* and *A. rubrum* which, as he suggests, the morphological relationships of the two species pre-suppose. The five species at present included in the group fall into three sections in Pax's arrangement, and are equally widely separated geographically, and it must remain for further chemical information to decide whether they do, in fact, represent a natural group.

Of the three species in Haslam's Group B, *A. rubrum* is now assigned to Group A, *A. platanoides* is one of a number of species each with particular individual characteristics, and *A. campestre* is no different from the majority of the species in his Group C. These differ considerably among themselves, but *A. pseudoplatanus* is outstanding amongst them in having practically no astringency, no HH, very little PA, but quite appreciable iodate reaction. Another species with highly individual characteristics is *A. macrophyllum*. This, as Haslam pointed out, has no galloyl esters, but contains uncombined gallic acid. Its astringency is due to a rather high concentration of PA.

If a division is to be sought of the large group of species without these particular features, it is likely to be found in the predominance of one or other of the two classes of tannins. Referring to Table 1, this division gives results shown in Table 2. There is little agreement between these lists and Pax's arrangement: *lobelii* and *platanoides* are together in Platanoides; *capillipes*, *davidii* and *pensylvanicum* in Macrantha. The last three, and *negundo*, do have prodelphinidin in common. Four of the species in the third column are, however, in Section Campestria. There seems to be no firm connection between the systematic relationships of the species and the kind of tannin they contain. Moreover, a calculation of the average TAE of the species in the last two columns shows that the overall astringency of the two groups is exactly the same. Since every species has some PA, and, as pointed out by Haslam, some galloyl ester or free gallic acid, the ability to synthesise either kind of tannin is present in all species. Except for the three species with little or no tannin, it does seem as if the objective is a certain level of astringency, and the way this level is attained is unimportant.

In a number of other cases [11, 13, 15] the geographical distribution of species has been helpful in interpreting the chemical data in its systematic aspects. In the present instance there is one clear lead in that the species with least tannin are all European in their range, this being a tendency also in *Iris*, *Geranium* and others of the genera studied. The species with high HH are also European or Western Asian, but this may be an accident of sampling. Species with high PA, on the other hand, are widely

scattered in their distribution, *A. carpinifolium*, for instance, in Japan, *obtusatum* in S.E. Europe, *spicatum* in Eastern N. America, and this is characteristic of the distribution, section by section, of the members of the genus as a whole. Few of Pax's sections are homogeneous either as regards the nature of their tannins or their geographical ranges. In his description of the genus, he paid particular attention to the past and present distribution. Its fossil record is unusually rich and, on account of the unique characters of leaf and fruit, exceptionally reliable. Maples are among the commonest plants of the tertiary strata, their origin is identified in the lower tertiary with certainty, spreading widely in the miocene and developing further right up to the present day. "Originally circumpolar, the distribution of sections Spicata and Rubra was more northerly, that of Campestria and Platanoides more southerly." Unfortunately these sections are about the most heterogeneous of them all! In one respect, however, Pax's observations anticipated the most recent views on biogeographical distribution: "the extant species of N. Japan have affinities with those of E. Asia and W. America, those of S. Japan with those of C. China and the Himalayas, those of the W. Himalayas with those of the Mediterranean region."

With only 25 of the 150 presently recognised 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.

DISCUSSION

Of the 25 species of *Acer* examined here, all but three are moderately astringent, but the astringency can be due to condensed tannins, hydrolysable tannins, or both together. All the species have the ability to synthesise both kinds, so that the problem is what determines which of the two is produced preferentially.

Phylogenetically, PA's are present in the most primitive vascular plants, gallo- and ellagitannins appearing for the first time in the Dicots [12]. In *Ribes*, it was suggested [13], that ellagitannins were produced in certain species at the expense of the trihydroxy-flavonoids myricetin and prodelphinidin. If the same principle applies in the present case, the species with high HH are likely to be more advanced than those with high PA, in which case their ranges should be further from the area of origin of the genus. There is support for this to the extent that the species with high HH are for the most part found in Europe. Those with high PA have their ranges variously in E. Asia and E. N. America, and those with little or no tannin of either kind in extreme W. Europe.

The small group of species typified by *A. ginnala* seem to have suppressed the astringency of their leaves by modification of their hydrolysable tannins; they still have a high iodate reaction but a low TAE. This suggests that besides the economy effected by not producing tannin, there is an evolutionary advantage, under certain modern conditions, in the elimination of astringency, as evidenced by the great majority of herbaceous plants which contain no tannin.

When and where astringency was, or still is, advantageous to a plant, hydrolysable tannins are more effective, weight for weight, than condensed tannins, and gallotannins more than ellagitannins. This seems, therefore, to have been the trend of evolution: ferns and gymnosperms have condensed tannins but no hydrolysable tannins, ellagitannins are much more common in dicots than gallotannins, and this is the order of increasing efficiency as astringents. The hydrolysable tannins have a further advantage over the condensed, in that they are, by definition, hydrolysable. The phenolic residues so released are themselves fungistatic, and as Dix has shown [14], gallic acid from gallotannin in *A. platanoides* can diffuse on to the surface of the leaf and act as an inhibitor of the germination of fungal spores. Ellagitannins may not be so effective in this respect because ellagic 'acid' (actually a lactone) is very insoluble. This is a further argument in favour of the gallotannins being phylogenetically more advanced than the ellagitannins.

Any application of these generalisations to the particular case of *Acer* needs far more evidence in detail than is at present available as to the susceptibility of different species to fungal invasion in different ecological situations. *Acer* is essentially a genus of N. temperate distribution and although, in some primitive, probably non-*Acer*, form it must have emerged from tropical forest conditions, it is no longer exposed to their particular hazards.

EXPERIMENTAL

Plant material. The leaves, harvested at maturity, were obtained from the Cambridge University Botanic Garden or from the Royal Botanic Gardens, Kew. They were dried at 40°, powdered, and sieved through a 100-mesh sieve. Usually only one sample of each species was examined.

Preparation of extract. A weighed sample of the leaf powder was extracted × 3 with boiling 50% aq. MeOH, and the extract filtered through fibreglass. In the case of species containing mucilage (*A. capillipes, cappadocicum, davidii, saccharinum, griseum*) the extract foamed during boiling and was difficult or impossible to filter. This could be overcome by increasing the MeOH content of the extractant, but the mucilage in the extract also interfered with the estimation of astrigency (see below). The combined extracts were concd *in vacuo* to remove MeOH. For the determination *E*, HH and TAE a convenient concn was found to be the equivalent of 1% referred to the powdered leaf.

Determination of anthocyanidin. Extract (0.5 ml) was heated for 2 hr at 95° with 4 ml of 5% conc HCl in *n*-BuOH. The visible spectrum was recorded and absorptivity measured at the peak, which lay between 547 nm and 558 nm depending on the relative amounts of cyanidin and delphinidin present. Results were recorded as $E^{1\%}_{1cm}$ of the powdered dried leaf. Anthocyanidin production in the solid powder was determined in the same way, after first boiling the sample of powder for 4–5 min in 0.5 ml of 50% MeOH.

Determination of ellagitannin (HH). 0.5 ml of extract (= 20 mg fresh leaf) was measured in a 1 cm dia. test-tube drawn into a capillary. 1.5 ml 50% aq. MeOH and 0.16 ml of 6% aq. AcOH were added, the temp. was adjusted to 25–30° as convenient, and N_2 bubbled for 15 min; 0.16 ml of 6% aq. $NaNO_2$ was then added, N_2 passed for a further 0.25 min, and the capillary sealed off. Spectrophotometric measurements were begun immediately. The first peak to appear was at 500 nm which reached a maximum in 2–3 min; the blue reaction (600 nm) and a yellow reaction (400–430 nm) developed more slowly. At 30° the blue reaction reached a max in 30–40 min. and the absorptivity at this time was used for calculation of HH ($E^{1\%}_{1cm} = 51.5$). The blue colour faded to *ca* 0 at 24 hr.

Determination of astringency (TAE). Finger blood (0.1 ml) was diluted with 5 ml H_2O. To 1 ml of dil. blood was added 1 ml of the extract (or a lesser vol. dil. to 1 ml) and rapidly mixed, and centrifuged at 3000 rpm. The %pptn was calculated from the difference between the absorptions at 578 nm of the experimental

and a control sample consisting of 1 ml of dil. blood plus 1 ml H_2O. The equivalent tannic acid content of the extract was calculated from the relation % tannic acid = 0.015 + 0.00024 × % pptn; and that of the leaf sample from the amount of leaf powder extracted. The astringency of the solid powder was determined by vigorously shaking 10 mg of powder with 3 ml (or more as necessary) of 1:100 dil. blood, centrifuging, and measuring the difference of the absorptions at 578 nm as above. The determination is usually simple and rapid, but occasionally difficulties arise, and this especially so in the case of *Acer*. (1) When saponins are present, as is often the case in *Acer* [16], the supernatant, after the usual period of centrifugation, is cloudy, requiring a longer period of centrifugation at a higher speed. If the supernatant is still not clear it may be necessary to approach the point of complete pptn by gradual approximation. (2) Extracts of leaves sometimes react with haemoglobin to form methaemoglobin. (*Luzula pilosa* (L) Willd. is an outstanding example of this). It is then necessary to boil the extract, since the factor responsible seems to be enzymic. The extract of *A. negundo* behaved in this way.

Determination of galloyl ester groups. See above.

Chromatography was carried out in Forestal solvent on the hydrolysate of the leaves in 2N aq. HCl. Delphinidin had R_f 0.30, ellagic acid 0.33, cyanidin 0.50 and gallic acid 0.65. An unusual constituent was present in the five species in the *ginnala* group. This had a slightly higher R_f than gallic acid, but whereas the latter darkens when fumed with NH_3, the *ginnala* constituent appeared intensely deep blue in UV when so treated.

Acknowledgements—The author wishes to thank the Directors of the Cambridge University Botanic Garden and The Royal Botanic Gardens, Kew, for providing him with plant material and Dr. E. Haslam for supplying samples of galloyl esters.

REFERENCES

1. Bate-Smith, E. C. (1975) *Phytochemistry* **14**, 1107.
2. Bate-Smith, E. C. (1972) *Phytochemistry* **11**, 1153.
3. Bate-Smith, E. C. (1973) *Phytochemistry* **12**, 907.
4. Perkin, A. G. and Ushida, Y. (1922) *J. Chem. Soc.* 66.
5. Kutani, N. (1960) *Chem. Pharm. Bull. (Tokyo)* **8**, 72.
6. Haslam, E. (1965) *Phytochemistry* **4**, 495.
7. Bate-Smith, E. C. (1962) *J. Linn. Soc. (Botany)* **58**, 95.
8. Rehder, A. (1958) *Manual of Cultivated Trees and Shrubs*, 2nd edition. Macmillan, New York.
9. Pax, F. (1902) in *Das Pflanzenreich* (Engler, A. ed.) Vol. IV. 163 W. Engelmann, Leipzig.
10. Seikel, M. E. and Hillis, W. E. (1970) *Phytochemistry* **9**, 1115.
11. Bate-Smith, E. C. (1973) *J. Linn. Soc. (Botany)* **67**, 347.
12. Bate-Smith, E. C. (1973) in *Chemistry in Botanical Classification*. Academic Press, New York.
13. Bate-Smith, E. C. (1976) *Biochem. Syst. Ecol.* **4**, 13.
14. Dix, N. J. (1974) *Ann. Botany* **38**, 505.
15. Bate-Smith, E. C. (1977) *Biochem. Syst. Ecol.* in press.
16. Heine, E. W. (1966) *Pharmazie* **21**, 493.