Phytochemistry, 1965, Vol. 4, pp. 495 to 498. Pergamon Press Ltd. Printed in England

GALLOYL ESTERS IN THE *ACERACEAE*

E. HASLAM

Department of Chemistry, Sheffield University, Sheffield

(*Received* 15 *November* 1964)

Abstract—The phenolic constituents of the leaves of fifteen *Acer* species have been examined paper chromatographically and a suggestion is put forward for subdivision of the plant family on the basis of the particular form in which gallic acid is bound in the leaves.

INTRODUCTION

ESTERS and glycosides of a number of hydroxy and methoxy benzoic acids have recently been reported as occurring in all higher plants.[1] Detailed evidence of their distribution is however not available and in consequence their use as taxonomic tracers has not been developed. Preliminary investigations suggest that esters of gallic acid may have some interest in this respect and their use in the subdivision of the plant family *Aceraceae* is described below.

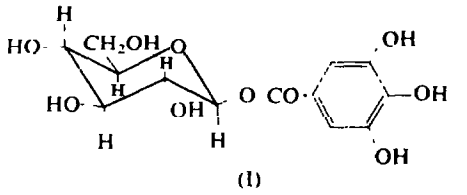
Gallic acid is normally encountered in plant tissues in ester form, the complexity of these esters varying from that of β-D-glucogallin[2] (I) to the gallotannins[3]-polygalloylglucose derivatives in which the acid is bound not only in aliphatic ester linkages but also in depside form (II). More often than not the acid is found in association with D-glucose but its esters with anhydro and branched chain sugars and with quinic acid have been recorded.[3] Closely related to gallic acid, and probably biogenetically derived from it by oxidative coupling,[4] is the readily lactonized hexahydroxydiphenic acid (III). This acid occurs in fresh plant tissues again almost invariably in ester form as an important structural unit of the ellagitannins.[4]

RESULTS AND DISCUSSION

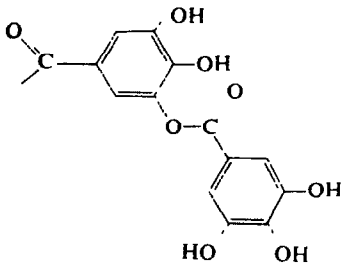
In 1922 Perkin and Uyeda[5] isolated *Acer* tannin from the leaves of the Korean maple (*Acer ginnale*) and in later work Kutani[6] assigned to it the structure of 3,6-di-*o*-galloyl-1,5-anhydro-D-glucitol (IV). Paper chromatographic analysis of the phenolic constituents of the leaves of *A. ginnale* gave the pattern shown in Fig. 1. Analysis of the leaves of a further fourteen species of *Acer* showed that another two (*A. tartaricum* and *A. saccharinum*) gave rise to a phenolic pattern very similar to that of *A. ginnale* with IV (Fig. 1a) as the predominant component containing gallic acid. These three species have been arranged together to form group A. Another three species (group B: *A. platanoides*, *A. campestre* and *A. rubrum*) showed a different distribution of phenolic compounds containing a type of galloyl ester whose u.v. absorption and chromatographic properties resemble closely those of sumach and chinese

[1] S. Z. EL-BASYOUNI, D. CHEN, R. K. IBRAHIM, A. C. NEISH and G. H. N. TOWERS, *Phytochem.* 3, 485 (1964).
[2] E. GILSON, *Compt. rend.* 136, 385 (1903).
[3] E. HASLAM and R. D. HAWORTH, *Progr. Org. Chem.* 6, 1 (1964).
[4] O. TH. SCHMIDT, *Fortschr. Chem. Org. Naturstoffe* 14, 71 (1956).
[5] A. G. PERKIN and Y. UYEDA, *J. Chem. Soc.* 66 (1922).
[6] N. KUTANI, *Chem. Pharm. Bull. (Tokyo)* 8, 72 (1960).

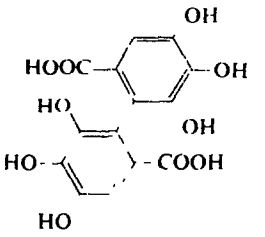
gallotannins,[3] (Fig. 2a). Methyl gallate was rapidly formed when crude extracts from these species were subject to methanolysis[7] at pH 5·9 thus lending support to the suggestion that in these extracts compounds are present in which gallic acid is bound in the depside form (II). The major group (group C) of eight species (*A. spicatum*, *A. pennsylvanicum*, *A. rotundilobum*, *A. griseum*, *A. monspessulanum*, *A. saccharum*, *A. palmatum*, and *A. pseudoplatanus*) produced in small quantity a series of galloyl esters of unknown structure but distinct paper chromatographic pattern (Fig. 3a, b, c). The structures of these substances are under scrutiny but on the



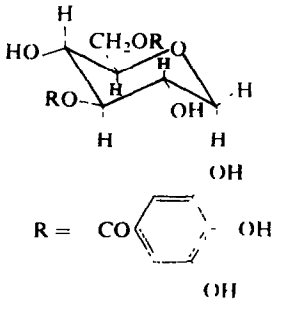
(I)



(II)



(III)



(IV)

basis of other workers observations[8] these may be of an ellagitannin type. Careful analysis of *A. macrophylla* at various stages of growth showed the leaves of this species to produce only free gallic acid. Significantly the formation of hydroxycinnamoyl esters by this species was considerably greater than in the others analysed.

An additional relationship which emerged from this study is the correlation between the form of gallic acid derivative in the leaves of a particular *Acer* species and the concentration of galloyl esters present. Mature leaves of species from group A contained on average the

[7] R. ARMITAGE, G. S. BAYLISS, J. GRAMSHAW, E HASLAM, R. D. HAWORTH, K. JONES, H. J. ROGERS and T. SEARLE, *J. Chem. Soc.* 1842 (1961).
[8] W. E. HILLIS and A. CARLE, *Biochem. J.* **82**, 435 (1962).

equivalent of 40–50 mg of gallic acid per leaf whereas those of groups B and C contained only 5–15 mg of gallic acid. This observation suggests that quantitative as well as qualitative measurements are probably of importance in determining taxonomic relationships by chemical methods.

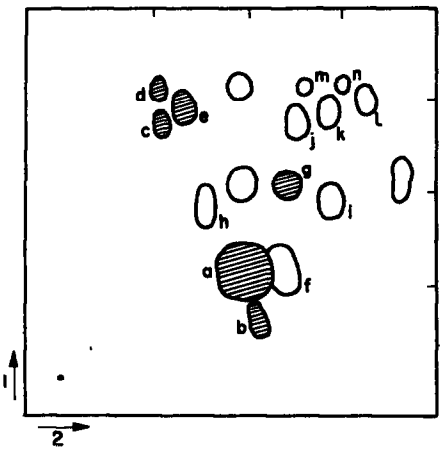


FIG. 1.

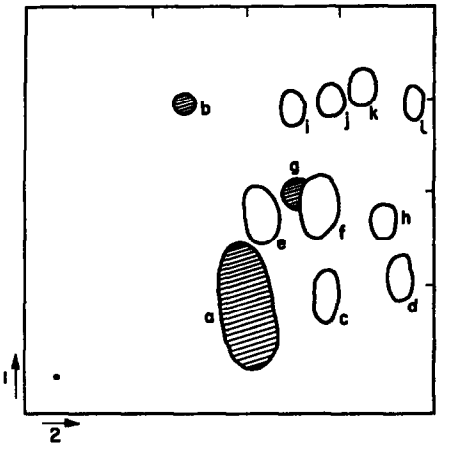


FIG. 2.

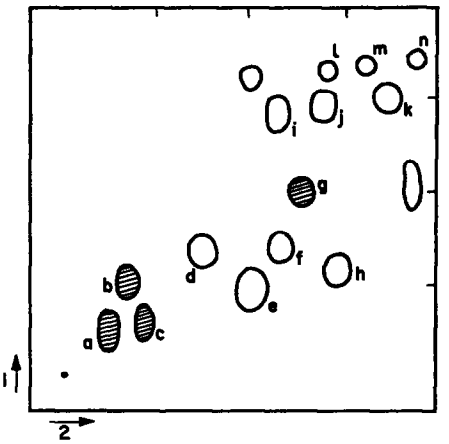


FIG. 3.

FIGS. 1–3. TWO-DIMENSIONAL CHROMATOGRAMS OF *A. ginnale*, *A. platanoides*, AND *A. saccharum*. Solvents: 1, 6% acetic acid; 2, butan-2-ol:acetic acid:water 14:1:5.

Shaded areas on the chromatograms represent galloyl esters.

Figure 1: a–e, gallic esters; g, gallic acid; f, h, i, "flavanoid" compounds; j–n hydroxycinnamoyl esters. Figure 2: a, b, gallic esters; g, gallic acid; c–h, "flavanoid" compounds; i–l, hydroxycinnamoyl esters. Figure 3; a–c, gallic esters; g, gallic acid; d–h, "flavanoid" compounds; i–n, hydroxycinnamoyl esters.

Certain area of agreement between this preliminary subdivision of the *Aceraceae* based on particular phenolic constituents and classifications based on morphological characteristics are apparent. Thus in the classification of Pojarkova[9] *A. campestre* and *A. platanoides* are

[9] A. POJARKOVA, *Acta Inst. Bot. Acad. Sci.* (*U.S.S.R.*) I, 225 (1933).

grouped under Platanoidea as are *A. ginnale* and *A. tartaricum* in the sub-group *Trilobata* and both of these relationships also follow from the chemical analysis outlined above. Clearly however on this limited sample of *Acer* species (over 150 are recorded)[9] the chemical method described is as yet not sufficiently refined to bring out for instance the differences between individual members of group C and show the relationships between *A. rubrum* and *A. saccharinum* to which morphological methods draw attention.

EXPERIMENTAL

Examination of Phenolic Constituents

Extraction. Leaves (3 g) were crushed in a mortar with powdered glass (1 g) and water (5 ml) to give a fine paste. Water (50 ml) chilled to 10° was added and after 5 min the suspension filtered through a pad of iron-free cellulose (10 g) in a Hirsch funnel. The pad was washed with water (4 × 20 ml), and the combined extract acidified with N H_2SO_4 (1–2 ml) and extracted with ethyl acetate (8 × 100 ml). Removal of the ethyl acetate at 30° gave the crude extract.

Quantitative determination of the galloyl esters. The crude extract was dissolved in acetone (20 ml) and water (15 ml) and diluted to 100 ml. Aliquots were put in test tubes, made up to 3·5 ml with water and cooled in an ice bath for 30 min. Potassium iodate solution (saturated, 1·5 ml) was added to each tube, and after 40 min at 0° the absorptivity determined at 550 mμ. The number of equivalents of gallic acid present as galloyl ester was then determined by reference to β-penta-*o*-galloyl-D-glucose.[7] The method of assay is more reliable for extracts containing a high concentration of galloyl esters (group A) than for others (groups B and C) where the higher concentration of different phenols leads to inaccuracies.

Qualitative analysis. Two-dimensional paper chromatographic analysis was carried out with (1) 6% acetic acid and (2) butan-2-ol:acetic acid:water (14:1:5). Galloyl esters were revealed as brown to mauve areas using Gibbs reagent,[10] as pink areas using a spray of saturated KIO_3 solution, and by their violet fluorescence or strong absorption under u.v. light and in the presence of ammonia vapour. Gallic acid was identified on all chromatograms. Provisional identification of other compounds were made by the usual methods.[11,12]

Acknowledgements—The author thanks Mr. Hart (Curator, Botanical Gardens, Sheffield) for assistance in the provision and identification of samples.

[10] F. E. KING, T. J. KING and L. C. MANNING, *J. Chem. Soc* 563 (1957).
[11] J. B. HARBORNE, *J. Chromatog.* **2**, 581 (1959).
[12] J. B. HARBORNE and J. J CORNER, *Biochem. J.* **81**, 242 (1961).