Observations on the Biosynthesis of Gallic Acid and Caffeic Acid

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ANALYSIS of a range of leaf extracts suggests a relationship between the metabolism of the hydroxycinnamic acids and gallic acid. Esters or glycosides of the former occur widely[1,2] whereas the corresponding derivatives of gallic acid have a more limited distribution. The two metabolic functions appear to be mutually exclusive in many plants but in some families (*e.g. Aceraceae*[3]) a balance probably exists.

Recent work has predicted two pathways for the biosynthesis of gallic acid (I) which differ in the origins of the carbon skeleton and the phenolic hydroxyl groups. The first,[4] in which the carboxyl group of gallic acid is derived from the β-carbon atom of L-phenylalanine, follows established pathways for the conversion of $C_6{\cdot}C_3$ to $C_6{\cdot}C_1$ compounds.[5] The second[6] is a direct dehydrogenation of 5-dehydroshikimic acid (II). Further evidence has been obtained for the operation of both of these pathways in *Rhus typhina* (Sumach) and *Acer saccharinum* (Silver maple). Substrates (0·001—0·3 mM.) were added to leaf discs (*ca.* 5 g.) and gallic acid was isolated after enzymic hydrolysis of the extract. Radioactivity at C-7 was determined by nitration of trimethylgallic acid,[7] and the combined activity at C-2 and C-6 by successive treatment of methyl 2-nitrotrimethylgallate[8] with hydrogen bromide and barium hypobromite to give bromopicrin (isolated after reduction to methylamine[9] as *N*-methylphthalimide). The results obtained in *R. typhina* are shown in Table 1. A parallel series of results was obtained in *A. saccharinum* which suggest similar, if not identical, pathways for gallic acid biosynthesis in both plant species. Myricetin, isolated in experiments with *R. typhina*, after feeding labelled (—)-shikimic acid gave on methylation and oxidation trimethylgallic acid with no activity at C-7. This is in agreement with the conversion of the shikimic acid to a $C_6{\cdot}C_3$ derivative and loss of the carboxyl group prior to incorporation into the flavanoid structure.[10]

The study of metabolic pathways *in vivo* by tracer techniques suffer from a number of recognized limitations. Experiments using [1-^{14}C]-D-glucose and [^{14}C]carbon dioxide have been used to explore the possibility that the administered substrate influences the ultimate course of biosynthesis. Sprinson's interpretation of the incorporation of D-glucose into shikimic acid[12] predicts (see Scheme) that for [1-^{14}C]-D-glucose as substrate gallic acid, derived from L-phenylalanine, should lead to labelling in the carboxyl group whereas synthesis from (II) should not. The results with *R. typhina* and *A. saccharinum*, allowing for some randomisation of the label which occurs, are more consistent with the second of these possibilities. Indeed in *A. saccharinum* with short feeding times (< 24 hr.) the activity at C-7 is less than 0·03. In addition (—)-shikimic acid also isolated from *R. typhina* after feeding [1-^{14}C]-D-glucose showed, after standard degradations,[11,13] an identical distribution of activity to the gallic acid. Conversely in very young tissue of *A. saccharinum* brief exposure (2 and 15 min.) to [^{14}C]carbon dioxide gave gallic acid with 0·27 and 0·23 of the activity at C-7; these results may be rationalised in terms of the second pathway to gallic acid and the initial formation of hexose precursors[14] with carbon-14 predominantly at C-3 and C-4 of the sugar.

It has not yet been possible to adequately compare gallic and hydroxycinnamic acid biosynthesis in the same plant but the results of an examination of caffeic acid biosynthesis contrasts with those described above. Experiments were carried out with *Vaccinium vitis-idaea*, *Nicotiana tabacum*, and *Hydrangea macrophylla* and the

TABLE 1

Biosynthesis of gallic acid in Rhus typhina

Substrate	Time (hr.)	Fraction of activity C-7	C-2, C-6	Incorp. (%)
[^{14}C]-(—)-Shikimic acid (0·25 in carboxyl[11])	65	0·25	—	0·85
[3-^{14}C]-DL-Phenylalanine	24	0·97	—	0·05
[U-^{14}C]-L-Phenylalanine†	90	0·16	—	0·03
[1-^{14}C]-D-Glucose	56	0·08	0·67	0·02

† U, uniformly labelled.

P = Phosphate
● = labelled C-atom

SCHEME

TABLE 2

Biosynthesis of caffeic acid

Substrate	Time (hr.)	Fraction of activity C-8, C-9	C-7	C-[1·6]	C-2	Incorp. (%)
[1-^{14}C]-D-Glucose	36[a]	0·77	0·23			0·01
[1,6-^{14}C]-(−)-Shikimic acid	90[a]	0·90	0·10			0·02
[U-^{14}C]-D-Ribose†	100[a]	0·92	0·08			0·05
[^{14}C]-CO_2	0·25[b]	0·57	0·35	0·08		
[^{14}C]-CO_2 plus						
,, D-ribose	72[b]	0·69	0·16	0·15	0·02	
,, D-erythrose	72[b]	0·72	0·16	0·11	0·01	
,, L-serine	72[b]	0·78	0·12	0·09	0·01	

† *U*, uniformly labelled; [a] *Nicotiana tabacum*; [b] *Vaccinium vitis-idaea*.

caffeic acid (III) degraded to determine the activity at C-2, C-6, and C-7. The principal locus of radioactivity is the aliphatic C-3 side-chain (Table 2). This observation is explicable in terms of a ready entry of the precursors (in some cases after degradation) only into the third carbohydrate fragment (VI) which contributes to the carbon skeleton of the acid.

Whilst the converse may appertain in the biosynthesis of gallic acid in *R. typhina* and *A. saccharinum* most of the observations suggest that during normal modes of metabolism the carbon skeleton and phenolic hydroxyl groups of gallic acid are derived directly from shikimate, probably *via* (II). These results also permit the suggestion of a way in which gallic and hydroxycinnamic acid metabolism are linked to the synthesis of phenylalanine in the plant. The situations examined are those in which a steady state of phenol synthesis has been attained and when, by analogy with mechanisms observed in micro-organisms,[15] the synthesis of phenylalanine may be controlled by feedback inhibition at the chorismate mutase (a) or D-arabino-2-deoxy-7-heptulosonic acid synthetase (b) steps. In the case of gallic acid metabolic control is exerted at the chorismate

mutase step and labelled precursors are incorporated into (IV) and (V) whereas for the hydroxycinnamic acid control of the heptulosonic acid synthetase predominates and entry of labelled precursor is only readily accomplished into (VI).

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