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CHLOROGENIC ACID BIOSYNTHESIS

INCORPORATION OF [α-^{14}C]CINNAMIC ACID INTO THE CINNAMOYL AND HYDROXYCINNAMOYL CONJUGATES OF THE POTATO TUBER*

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Abstract—Experiments in which potato tuber slices were maintained on a tracer amount of [α-^{14}C]cinnamic acid and synthetic 3-*O*-cinnamoylquinic acid (pH adjusted to 6) showed that if this conjugate is a free intermediate in chlorogenic acid (3-*O*-caffeoylquinic acid) formation, then the active pool of the compound is very small and is contained within a cell compartment whose membrane is relatively permeable to phenylalanine and cinnamic acid but not to the conjugate itself. The specific activities of 3-*O*-cinnamoyl-, 3-*O*-*p*-coumaroyl-, and 3-*O*-caffeoylquinic acid; 3-*O*-*p*-coumaroyl-, and 3-*O*-caffeoylshikimic acid; and 1-*O*-*p*-coumaroyl-, and 1-*O*-caffeoyl-β-D-glucose were determined in this experiment. The results of similar experiments on which tuber slices were maintained on [α-^{14}C]cinnamic acid and quinic acid, or phenylalanine and quinic acid together with a tracer amount of [α-^{14}C]cinnamic acid, are reported. The specific activities of the isolated conjugates indicate that in each series the caffeoyl derivatives are formed by the hydroxylation of the *p*-coumaroyl derivatives. The specific activity data also suggest that cinnamic acid and the derived conjugates are formed within a compartment or compartments of the cell.

INTRODUCTION

CHLOROGENIC acid (3-*O*-caffeoylquinic acid), and a variety of closely related hydroxycinnamoyl conjugates, are widely distributed in the roots, stems, leaves, and flowers of plants.[1,2] Studies with the aid of silica gel chromatography have shown that a mixture of such conjugates accumulate in potato tuber pulp slices maintained in the dark in the presence of various substrates.[3] These conjugates include the 3-*O*-*p*-coumaroyl and 3-*O*-caffeoyl esters of quinic acid and shikimic acid, and the 1-*O*-*p*-coumaroyl, and 1-*O*-caffeoyl esters of β-D-glucose. There are thus three distinct *series* of conjugates, whose biosynthesis is to be accounted for. A series, in this sense, is defined by its non-aromatic moiety.

In an earlier study Levy and Zucker[4] obtained evidence for the presence, in trace amounts, of a conjugate of cinnamic and quinic acids in slices of tuber tissue that had been maintained in the dark on a solution of cinnamate and quinate at pH 6. They therefore proposed that chlorogenic acid is synthesized from L-phenylalanine and quinic acid by way of 3-*O*-cinnamoyl- and 3-*O*-*p*-coumaroylquinic acids (Fig. 1). The mediation of cinnamoyl-CoA in this sequence is a reasonable addition to the original hypothesis. In keeping with this proposal, the enzyme phenylalanine deaminase[5] (phenylalanine ammonia-lyase, EC 4.3.1.5) has

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[1] K. HERRMANN, *Pharmazie* **11**, 433 (1956).
[2] E. SONDHEIMER, *Botan. Rev.* **30**, 667 (1964).
[3] K. R. HANSON and M. ZUCKER, *J. Biol. Chem.* **238**, 1105 (1963).
[4] C. C. LEVY and M. ZUCKER, *J. Biol. Chem.* **235**, 2418 (1960).
[5] J. KOUKOL and E. E. CONN, *J. Biol. Chem.* **236**, 2692 (1961).

been shown to accumulate in potato tuber slices,[6] and has been partially purified from the potato in this laboratory (E. Havir, Unpublished results). Proportionality between the level of enzyme and the chlorogenic acid content of the tissue has been demonstrated under a variety of conditions.[6] The *O-p*-coumaroylquinic acid accumulating in tuber slices has been identified as the 3-isomer.[3, 7] Very low concentrations of this compound stimulated chlorogenic acid formation in tuber slices,[4] and a phenolase preparation from potato tuber was shown to oxidize the compound to chlorogenic acid.[3, 4] 3-*O*-Cinnamoylquinic acid and its isomers have been obtained by chemical synthesis[8, 9] but the hydroxylation of these compounds

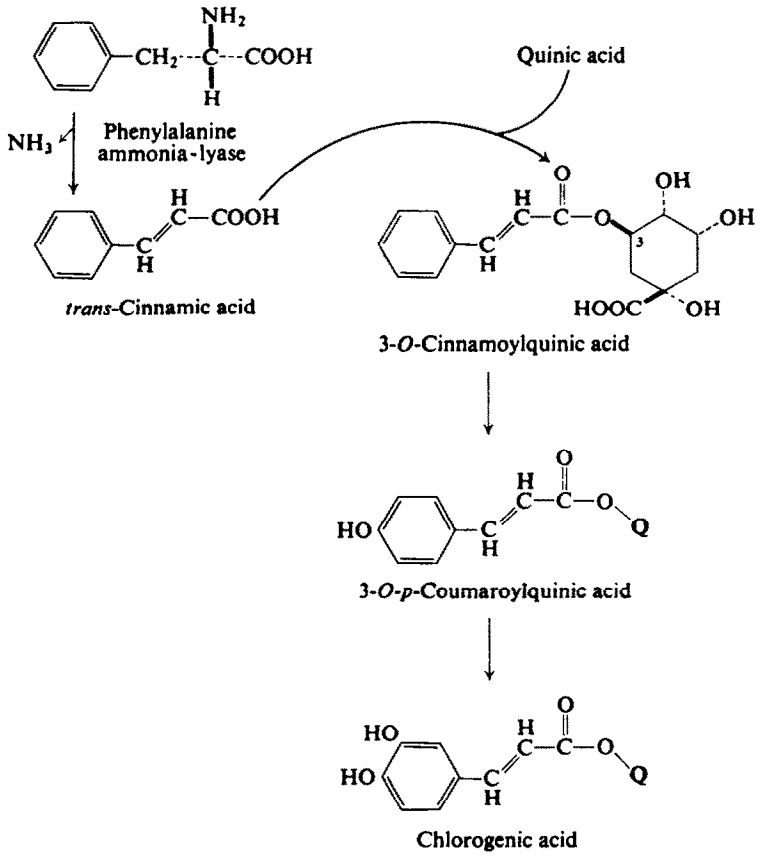

FIG. 1. POSTULATED ROUTE OF CHLOROGENIC ACID (3-*O*-CAFFEOYLQUINIC ACID) BIOSYNTHESIS.[4]

remains to be investigated.[10] In this paper it is shown that if 3-*O*-cinnamoylquinic acid is indeed a free intermediate, then exogenous cinnamoyl conjugate cannot equilibrate with the active pool of the intermediate.

It is reasonable to postulate that the conjugates of shikimic acid and quinic acid are formed by a similar series of reactions. In both series substitution is in the 3-position.[7, 11] As the

[6] M. ZUCKER, *Plant Physiol.* **40**, 779 (1965).
[7] K. R. HANSON, *Biochemistry* **4**, 2731 (1965).
[8] K. R. HANSON, *Chem. & Ind. (London)* 1691 (1963).
[9] K. R. HANSON, *Biochemistry* **4**, 2719 (1965).
[10] P. M. NAIR and L. C. VINING, *Phytochem.* **4**, 161 (1965).
[11] V. P. MAIER, D. M. METZLER, and A. F. HUBER, *Biochem. Biophys. Res. Communs* **14**, 124 (1964).

distance of the 3-, 4-, and 5-hydroxyl groups from the ionic charge of the carboxyl group is similar in quinic and shikimic acids, and as the conjugates of shikimic acid only accumulate under conditions that do not normally occur in the tissue, it is probable that the first members of the two series, whether cinnamoyl or *p*-coumaroyl, are formed by the same enzyme. *In vitro* oxidation of 3-*O*-*p*-coumaroyl- to 3-*O*-caffeoyl-shikimic acid has been demonstrated.[3]

The conjugates of glucose encountered are esters of a hemi-acetal,[12, 7] and as such are chemically distinct from the esters of the quinic and shikimic series. The finding by Corner and Swain[13] that, in the presence of extracts of geranium leaves, 1-*O*-hydroxycinnamoyl esters of β-D-glucose are formed from uridine diphosphate-glucose and *p*-coumaric or caffeic acid suggests that conjugates of glucose may be formed in this way in the potato. It is possible, however, that the normal substrates for hydroxylation are the conjugates, rather than the free acids. In this study it is shown that 1-*O*-cinnamoyl-β-D-glucose can also be formed by the potato tissue, and evidence is presented that in all three series, caffeoyl conjugates are primarily formed from *p*-coumaroyl conjugates.

RESULTS AND DISCUSSION

Attempted Use of the Mono-O-cinnamoylquinic Acids as Trapping Substances

Slices of tuber pulp tissue were maintained in the dark on 3-*O*-cinnamoylquinic acid, together with tracer amounts of [α-^{14}C]cinnamic acid, and the incorporation of radioactivity into the various conjugates of the tissue determined (Table 1, Experiment 1). The chromatographic separation on the analytical column of the compounds studied is illustrated in Fig. 2 (3-*O*-Cinnamoylquinic acid has almost the same R_{cg} as caffeic acid (peak 5)). Extensive incorporation into the 3-*O*-*p*-coumaroyl and 3-*O*-caffeoyl esters of quinic acid took place, whereas the isolated 3-*O*-cinnamoylquinic acid contained very little radioactivity. Essentially similar results were obtained when tracer amounts of L-[U-^{14}C]phenylalanine was employed in place of [α-^{14}C]cinnamic acid.

Attempts were made to establish that radioactivity was indeed incorporated into 3-*O*-cinnamoylquinic acid. The initial low specific activity of the isolated conjugate was considerably diminished on paper chromatography. When the value was constant (value given in Table 1) the compound was hydrolyzed by alkali (5 N) and the cinnamic acid was chromatographed on the silica gel column with cyclohexane–chloroform (50: 50 by volume, equilibrated with acid) as the eluting solvent. Radioactivity was associated with the cinnamic acid peak.

In addition to the 3-*O*-cinnamoylquinic acid a small amount of a cinnamoyl conjugate, or a mixture of such conjugates, was obtained when the fractions from the region R_{cg} 0·6–0·7 (between peaks 7 and 8, Fig. 2) were chromatographed on paper. This material had a low specific activity similar to that of the 3-isomer, and may have arisen from the 3-isomer by the migration of the cinnamoyl group.[9]

When the same experiment was carried out with 1-*O*-cinnamoylquinic acid as a trapping substance, the chlorogenic acid was again highly radioactive, whereas the cinnamoyl ester isolated from the tissue had very low or zero activity.

The fate of the 3-*O*-cinnamoylquinic acid not recovered from the tissue is unknown, but it would appear, from a consideration of the related experiments shown in Table 1, that the missing ester was not hydrolyzed to a significant extent. Had an appreciable amount of

[12] J. B. HARBORNE and J. J. CORNER, *Biochem. J.* **81**, 242 (1961).
[13] J. CORNER and T. SWAIN, *Nature* **207**, 634 (1965).

TABLE 1. RESULTS OF MAINTAINING SLICES OF POTATO TUBER PULP TISSUE ON [α-^{14}C]CINNAMIC ACID TOGETHER WITH OTHER SUBSTRATES

Peak* (Fig. 2)	R_{cg}	Compound	Compounds supplied to the tissue†					
			Experiment 1		Experiment 2		Experiment 3	
			3-*O*-Cinnamoylquinate and [α-^{14}C]cinnamate		Quinate and [α-^{14}C]cinnamate		L-Phenylalanine, quinate, and [α-^{14}C]cinnamate	
			(mμmole/g)	(counts/min/μmole)	(mμmole/g)	(counts/min/μmole)	(mμmole/g)	(counts/min/μmole)
		L-Phenylalanine supplied	—		—		12,500	
		Quinic acid supplied	—		12,500		12,500	
		3-*O*-Cinnamoylquinic acid supplied	1250		—		—	
		absorbed	1000		—		—	
		[α-^{14}C]Cinnamic acid supplied	21	(2×10^6)	1250	(65×10^3)	34	(2×10^6)
		absorbed‡	20		1200		33	
			Compounds from the analytical column					
	0·42	3-*O*-Cinnamoylquinic acid	300	(<80)	....		—	
8	0·72	3-*O*-*p*-Coumaroylquinic acid	50	(80,000)	20	(145)	19	(360)
10	1·00	3-*O*-Caffeoylquinic acid (chlorogenic acid)	150	(20,000)	171	(94)	460	(340)
7	0·56	3-*O*-*p*-Coumaroylshikimic acid	10	(6000)	7	(145)	13	(15)
9	0·77	3-*O*-Caffeoylshikimic acid	25	(5000)	8	(180)	19	(30)
	0·80	1-*O*-Cinnamoyl-β-D-glucose	—		10	(140)	—	
12	1·28	1-*O*-*p*-Coumaroyl-β-D-glucose	2	(120,000)	182	(130)	90	(85)
15	1·81	1-*O*-Caffeoyl-β-D-glucose	0·4	(120,000)	13	(145)	22	(75)
6	0·52	(Caffeic acid, quinic acid)	1	(80,000)	11	(125)	37	(136)
11	1·09	(Ferulic acid, quinic acid)	—		15	(90)	20	(88)
13	1·49	*O*-Glucosylcaffeic acid	—		16	(147)	34	(25)
3	0·23	*p*-Coumaric acid	—		10	(210)	50	(100)
5	0·42	Caffeic acid	—		8	(200)	34	(440)

* The numbers assigned to the various peaks are those used previously.[3] Structural assignments have been considered elsewhere.[3,7] The parentheses about conjugates 6 and 11 indicate that the products of hydrolysis are listed. These compounds had zero mobility on electrophoresis at pH 7.

† Tuber slices (40 g) were maintained in the dark at 24° for 16 hr on solutions of the compounds indicated (10 ml, pH adjusted to 6). The tissue was analyzed as described in the Experimental section. The amounts of the various compounds listed are minimal values uncorrected for losses. A dash signifies that insufficient material was obtained for examination. In Experiment 1 the trace of caffeic acid was masked by the 3-*O*-cinnamoylquinic acid.

‡ The radioactivity in the ethanol extract and the radioactivity applied to the analytical column, expressed as a percentage of the absorbed radioactivity, were, for Experiment 1, 50 and 48 per cent; 2, 60 and 50 per cent; 3, 55 and 48 per cent.

cinnamic acid been released in a part of the cell where it could equilibrate with the absorbed tracer, an accumulation of glucose conjugates (Experiment 2), and a greater uniformity in the specific activities of the various conjugates isolated (compare Experiment 2 with 1 and 3) would be expected.

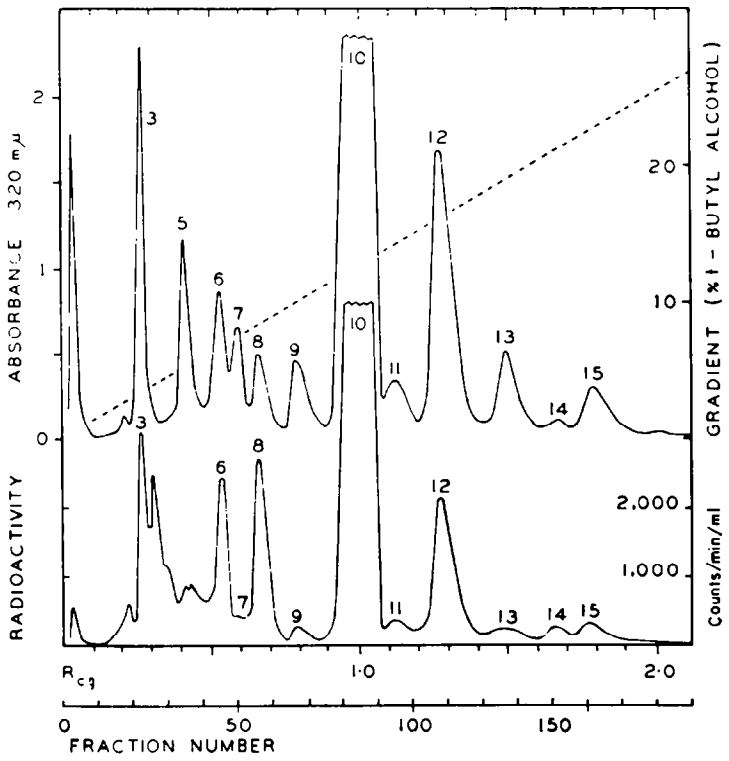

FIG. 2. CONJUGATES FROM POTATO TUBER SLICES MAINTAINED ON L-PHENYLALANINE AND QUINATE IN THE PRESENCE OF A TRACER AMOUNT OF [α-^{14}C]CINNAMATE.

The figure shows the elution record for Experiment 3, Table 1. Chromatography on the analytical silica gel (7 g) column was performed as described previously[3,9]: rectilinear gradient; mixing vessel 800 ml of cyclohexane–chloroform, equilibrated with acid (10:90 v/v); reservoir 800 ml of *t*-butyl alcohol–chloroform, equilibrated with acid (30:70 v/v); fraction volumes 7 ml. The numbers given to the various peaks correspond to those used in Table 1 and elsewhere[3]; the several peak substances have been assigned the following structures[3,7]: 3, *p*-coumaric acid; 5, caffeic acid; 6, conjugate of caffeic and quinic acids, not identical with 1-*O*-caffeoylquinide[7]; 7, 3-*O*-*p*-coumaroylshikimic acid; 8, 3-*O*-*p*-coumaroylquinic acid; 9, 3-*O*-caffeoylshikimic acid; 10, 3-*O*-caffeoylquinic acid (chlorogenic acid); 11, conjugate of ferulic and quinic acids; 12, 1-*O*-*p*-coumaroyl-β-D-glucose; 13, 3 or 4-*O*-glucosylcaffeic acid; 14, unknown; 15, 1-*O*-caffeoyl-β-D-glucose. The identity of the compound of high specific activity, or low absorbancy, emerging after *p*-coumaric acid (peak 3) has not yet been established.

Active pool size. If the hypothesis is accepted that 3-*O*-cinnamoylquinic acid is indeed a free intermediate in chlorogenic acid biosynthesis, then it follows that the active pool of this material is retained within a membrane which is permeable to phenylalanine and cinnamic acid, but not to the cinnamoyl conjugate. Were the membrane permeable to the conjugate, then the pool would have been increased by the added unlabelled compound and incorporation of radioactivity into 3-*O*-*p*-coumaroyl- and 3-*O*-caffeoylquinic acid would have been

argely prevented. If the specific activity of the precursor cinnamoyl conjugate corresponded to that of the 3-*O*-*p*-coumaroylquinic acid isolated, then the pool size was of the order of 0·3 μmole/kg of tissue—an amount of material which could only be detected under the most favourable circumstances.

An alternative interpretation must be considered. El-Basyouni *et al.*,[14] on the basis of studies of lignin formation in wheat, suggest that an active metabolic pool of "ethanol insoluble" cinnamoyl and hydroxycinnamoyl esters occurs in plant tissues, and that hydroxylations of the "insoluble" cinnamoyl and *p*-coumaroyl esters take place. The "ethanol soluble" conjugates of quinic and shikimic acids may be formed by trans-esterification from the "insoluble" conjugates. The data of Table 1 suggest that "soluble" caffeoyl conjugates are not formed in this way (see below: pairing of specific activities), but the failure to obtain a significant amount of labelling of 3-*O*-cinnamoylquinic acid may be explained if the true intermediate in chlorogenic acid biosynthesis is an "insoluble" cinnamoyl conjugate and the "soluble" *p*-coumaroyl conjugates are formed by trans-esterification.

The compartment hypothesis: The notion that conjugate formation takes place within a compartment, or compartments, of the cell receives some support from a consideration of the specific activities of the conjugates isolated in Experiments 1, 2, and 3. The dilution of the added [α-^{14}C]cinnamic acid in forming 3-*O*-*p*-coumaroylquinic acid was, in Experiment 1, 25-fold, in 2, 450-fold; and in 3, 5600-fold. In each case endogenous cinnamic acid appears to be a more efficient precursor of conjugate formation than exogenously supplied acid: e.g. if in Experiment 2 all of the supplied quinic acid were converted to cinnamic acid the calculated further dilution would still be 40-fold. Quinic acid is, in fact, a poor precursor of the aromatic moiety of chlorogenic acid (M. Zucker, Unpublished results). It is possible that phenylalanine ammonia lyase and the enzymes responsible for conjugate formation are confined to the developing plastid. At the present time the only grounds for such speculation is the observation that continuous exposure to light both markedly stimulates the formation of this enzyme and leads to the appearance of green chloroplasts.[6]

Conjugates Accumulating in the Presence of Cinnamate and Quinate

When greater than trace amounts of cinnamic acid are supplied to the tissue, glucose conjugates of this acid and its hydroxy derivatives accumulate in quantity. In Experiment 2 ([α-^{14}C]cinnamate, 0·005 M; quinate, 0·05 M) more 1-*O*-*p*-coumaroyl-β-D-glucose accumulated than chlorogenic acid. 1-*O*-Cinnamoyl-β-D-glucose[7, 12] did not accumulate in detectable amounts in Experiments 1 and 3, but was found in Experiment 2. In all experiments the *p*-coumaroyl conjugate predominated in the glucose series, whereas the caffeoyl conjugate was dominant in the quinic acid series.

The conditions of Experiment 2 correspond to those employed by Levy and Zucker.[4] At that time only paper chromatographic methods were available. It seems clear from the present study that these investigators were working with mixtures of conjugates of glucose, shikimic acid, and quinic acid and that at different times they handled mixtures in which different conjugates predominated. Thus the R_F values given for a conjugate of cinnamic and quinic acids, and for a conjugate of *p*-coumaric and quinic acids, correspond to those of the glucose esters: System D (*n*-butyl alcohol, ammonium hydroxide, ammonium carbonate) Whatman 3 mm paper, descending: cinnamic acid, 0·48 (0·44 reported); *p*-coumaric acid, 0·24 (0·27 reported), 1-*O*-cinnamoyl-β-D-glucose, 0·72 (0·67 reported for the cinnamoyl conjugate), 1-*O*-*p*-coumaroyl-β-D-glucose, 0·45 (0·49 reported for the *p*-coumaroyl conjugate).

[14] S. Z. EL-BASYOUNI, A. C. NEISH, G. H. N. TOWERS, *Phytochem.* 3, 627 (1964).

In contrast, the synthetic mono-*O*-cinnamoylquinic acids moved more slowly than cinnamic acid: 1-isomer, 0·26; 3-, 4-, and 5-isomers, 0·36 (migration occurs between the 3-, 4-, and 5-positions on chromatography in this system[9]). The mono-*O*-*p*-coumaroylquinic acids have even lower R_F values.[9] Despite this finding, it is hardly possible that the reported stoichiometry measurements (quinic:cinnamic, and quinic:*p*-coumaric) were performed on conjugates of glucose. Attempts to isolate the *O*-cinnamoylquinic acid studied by Levy and Zucker have been unsuccessful, but this failure may be the result of physiological and genetic differences between the 1959 Kennebec potatoes used in the original study and those used in these experiments.

Pairing of Specific Activities

In Experiment 1 where a tracer amount of [α-^{14}C]cinnamic acid was employed, notable variations in the specific activities of the various conjugates were observed. Similar variations were found in Experiment 3, in which slices of pulp tissue were maintained on quinic acid and phenylalanine in the presence of tracer amounts of [α-^{14}C]cinnamic acid. In both cases the specific activities of the *p*-coumaroyl and caffeoyl esters of the same series (quinic acid, shikimic acid, or glucose) tended to be similar. The exception to this is in Experiment 1 where 3-*O*-caffeoylquinic acid has an appreciably lower specific activity than 3-*O*-*p*-coumaroylquinic acid. This difference may well arise from the fact that 3-*O*-caffeoylquinic acid is present at the start of the experiment. A similar dilution effect is observed in Experiment 2. In Experiment 3 much more 3-*O*-caffeoylquinic acid was formed and the contribution by the material initially present is therefore masked.

The simplest explanation of the observed pairing is that, for the three series of conjugates, the caffeoyl esters are formed from the corresponding *p*-coumaroyl esters. The differences in specific activities arise from differences in the time course of synthesis of the three series of conjugates. This interpretation excludes the following possibilities:

(i) That the *p*-coumaroyl or caffeoyl esters of quinic and shikimic acids are formed to a significant extent by trans-esterification from the 1-position of glucose (a thermodynamically favorable process).

(ii) That the *p*-coumaroyl and caffeoyl esters of shikimic acid are formed to a significant extent from those of quinic acid by the net elimination of water.

(iii) That the caffeoyl esters are formed to a significant extent from free caffeic acid. (It is known that exogenous caffeic acid will give rise to 1-*O*-caffeoyl-β-D-glucose in *Solanum* species.[12] The present inference is merely that this is not an important route under the conditions studied.)

(iv) That the caffeoyl esters are formed to a significant extent from an "insoluble" caffeoyl conjugate of the type postulated by El-Basyouni *et al.*[14]

Although it is possible to oxidize *p*-coumaroyl to caffeoyl conjugates by phenolase preparations *in vitro*, and although the enzymes of the phenolase complex may be purified[15], this does not establish that this route is of importance *in vivo*. Alternative explanations can be given for the present findings, but the combination of *in vivo* and *in vitro* evidence for oxidation at the "soluble" conjugate level carries more weight than either argument alone.

Alternative Precursors of the Aromatic Moiety

Tyrosine is enzymatically converted into *p*-coumaric acid in many tissues by the enzyme tyrase (tyrosine ammonia-lyase) but this enzyme appears to occur with high activity only in

[15] S. PATIL and M. ZUCKER, *J. Biol. Chem.* **240**, 3938 (1965).

grasses.[16] When potato slices were maintained on a tracer amount of DL-[β-^{14}C]tyrosine only one radioactivity peak was observed: R_{cg} 0·96. The chlorogenic acid peak (u.v. light absorbtion) and the radioactivity peak did not coincide, whereas in Experiments 1, 2, and 3 the radioactivity and light-absorption peaks coincided. The radioactive compound is thus not chlorogenic acid, and exogenous tyrosine is not a precursor of either *p*-coumaric acid or of chlorogenic acid.

The results of maintaining potato slices on *p*-[α-^{14}C]coumaric acid were less clear. The bulk of the recovered radioactivity was in the *p*-coumaric acid peak, but a small following peak of high specific activity was observed (cf. peak 3, Fig. 2). A small amount of radioactivity was spread over the entire conjugate region, (R_{cg} 0·4–1·8) with no more than trace amounts associated with any one conjugate.

Runeckles found in tobacco leaf discs that *p*-[α-^{14}C]coumaric acid is incorporated into chlorogenic acid,[17] and the formation of 1-*O*-*p*-coumaroyl-β-D-glucose from the free acid has been demonstrated in a number of tissues.[12, 13] It is possible, therefore, that the incorporation is limited because the supplied *p*-coumaric acid is unable to reach the site of synthesis. If this is the case, then the matter to be determined is whether the "soluble" cinnamoyl conjugates in any or all of the three series or "insoluble" cinnamoyl conjugates are significant intermediates, or whether they are by-products that occur only when the system is presented with abnormal amounts of cinnamic acid.

The representative experiments described in this paper suffer from the limitations associated with most experiments in which radioactive substrates are added to intact cells or tissues: the localization of the enzymes acting on the substrate is not known and alternative interpretations of the results are possible according to the nature and number of membranes and pools said to be present in the cell. Such experiments are, however, an advance on studies in which natural products are simply catalogued. A full description of conjugate formation will necessarily be concerned with both the enzymes—their induction and feedback inhibition—and with the structure of the cell in which the conjugates are formed.

EXPERIMENTAL

Radioactivity was measured with a Nuclear-Chicago 720 liquid scintillation counter. For counting, aqueous and non-aqueous samples were added to a mixture (10 ml) of toluene and ethanol (2:1 by vol), containing 2,5-diphenyloxazole (6 g/l.) and *p*-bis[2-(5-phenyloxazolyl)]-benzene (100 mg/l.). The counting efficiency for the aqueous samples (0·2 ml) was 62 per cent. All measurements were corrected to this aqueous sample basis by use of an internal standard ([1-^{14}C]toluene, New England Nuclear). All critical determinations were performed to a standard deviation of less than 2 per cent.

[α-^{14}C]Cinnamic acid was prepared from benzaldehyde and [2-^{14}C]malonic acid (New England Nuclear) by the method of Knoevenagel[18] and was purified by paper chromatography. *p*-[α-^{14}C]Coumaric acid was prepared from *p*-hydroxybenzaldehyde and [2-^{14}C] malonic acid by Vorsatz' modification of Knoevenagel's method.[19] The reactants in a sealed tube were left at room temperature for three weeks, and the product was purified with the aid of the analytical silica gel column. L-[U-^{14}C]Phenylalanine and DL-[β-^{14}C]tyrosine were obtained from New England Nuclear.

[16] A. C. NEISH, *Phytochem.* **1**, 1 (1961).
[17] V. C. RUNECKLES, *Can. J. Biochem. Physiol.* **41**, 2249, 2259 (1963).
[18] E. KNOEVENAGEL, *Deut. Ber. Chem. Ges.* **31**, 2596 (1898).
[19] F. Vorsatz, *J. Prakt. Chem.* **145**, 265 (1936).

Experiments with Tuber Slices

In the three experiments shown in Table 1, solutions of the compounds listed (40 times amount indicated per gram) were prepared and adjusted to pH 6 with $KHCO_3$ (final volume 10 ml). Slices (40 g, 1 mm thick) of the pulp region of potato tubers (*Solanum tuberosum* L. "Kennebec") were washed several times in distilled water, moistened with the appropriate solution, and maintained in the dark at 24° for 16 hr. The slices were then washed with water, the tissue was extracted with ethanol, and the extract was applied to the preliminary and analytical silica gel columns.[3] The apparatus described previously had been modified, in that the effluent from the column passed through Teflon rather than polyethylene tubing.[9] The absorbancy of each effluent fraction at 280 and 320 mμ was determined and also the radioactivity in each fraction (see Fig. 2). The specific activities calculated for the fractions associated with a given peak were fairly constant. The peak fractions were either combined and the material was chromatographed on paper until constant specific activity was obtained, or the fraction corresponding to the peak maximum was treated in this way. The paper chromatography systems[3, 9] used were: System *A*: *n*-butyl alcohol–acetic acid–water (4:1:5 by vol, upper phase); System *B*: 5% acetic acid. Except for 3-*O*-cinnamoylquinic acid (see above) the specific activities calculated after paper chromatography did not differ greatly from those originally estimated.

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