548. *Gallotannins. Part IX.*[1] *The Biosynthesis of Gallic Acid in* Rhus typhina

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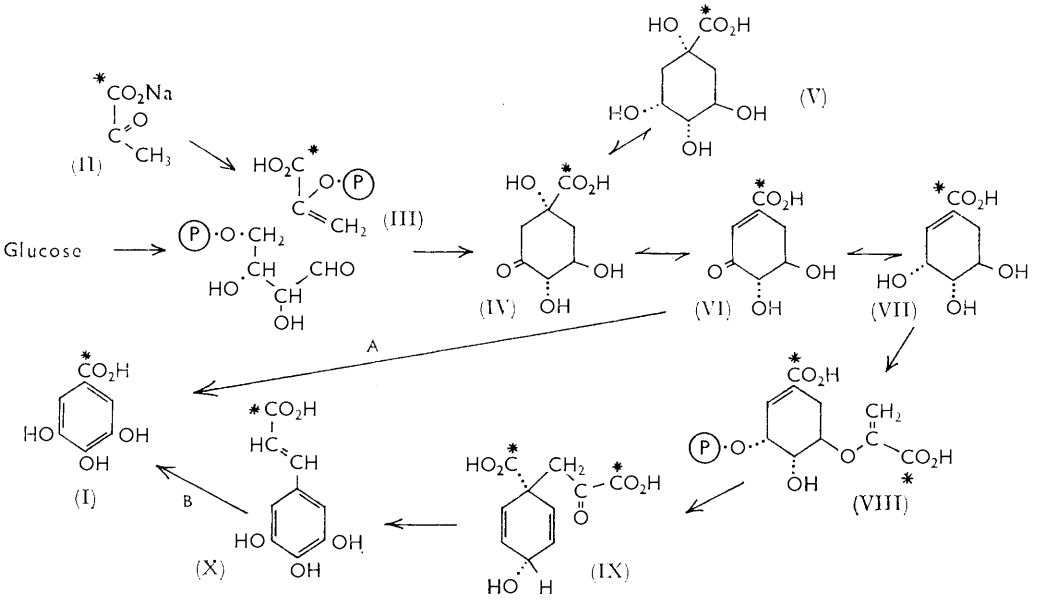
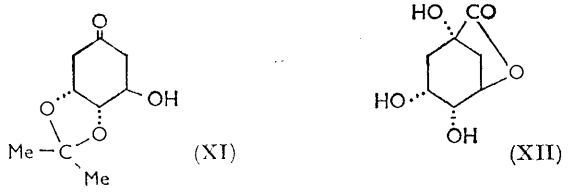
Experimental evidence has recently been obtained to support two quite different theories for the mode of biosynthesis of gallic acid (I) (see Scheme). Brücker and Hashem[2] concluded that deamination of the aromatic amino-acid tyrosine played an important role in its formation in the mould *Phycomyces blakesleeanus* and Zenk[3] proposed an analogous biogenetic scheme for sumach (*Rhus typhina*) in which the acid was formed by β-oxidation of the C_3 side-chain of 3,4,5-trihydroxycinnamic acid (X). Additional evidence in support of this pathway derives from biosynthetic studies in other fields,[4-6] in which the general

[1] Part VIII, E. Haslam, R. D. Haworth, and D. A. Lawton, *J.*, 1963, 2173.
[2] W. Brücker and M. Hashem, *Flora*, 1962, **157**, 57.
[3] M. H. Zenk, *Z. Naturforsch.*, 1964, **196**, 83.
[4] S. Z. El-Basyouni, D. Chen, R. K. Ibrahim, A. C. Neish, and G. H. N. Towers, *Phytochem.*, 1964, **3**, 485.
[5] R. J. Suhadolnik and J. Zulalian, *Proc. Chem. Soc.*, 1963, 216.
[6] A. R. Battersby, R. Binks, and D. A. Yeowell, *Proc. Chem. Soc.*, 1964, 86.

occurrence of metabolic pathways of the type $C_6{\cdot}C_3 \longrightarrow C_6{\cdot}C_1$ has been demonstrated. However, Conn and Swain,[7] as a result of feeding experiments with *Geranium pyrenaicum*, concluded that gallic acid was more likely to be formed directly from shikimic acid (VII) than by oxidation of a $C_6{\cdot}C_3$ compound, and their observations supported an earlier suggestion [8] that gallic acid was formed by dehydrogenation of 5-dehydroshikimic acid (VI). Although, in principle, it is possible that several biosynthetic routes operate for the formation of a natural product it was considered desirable to attempt to evaluate the relative importance of these two pathways to gallic acid, using a non-aromatic precursor such as (IV) or (VI) labelled in the carboxyl group. If the $C_6{\cdot}C_3$ pathway (B) were operative the labelled group should be absent from the final product whereas if the alternative route (A) is followed the labelled group would be retained. Experiments to test these hypotheses have been carried out.



Chemical syntheses of isotopically labelled precursors such as (VI) have been unsuccessful. Thus, the cyanohydrin reaction on the ketone [9,10] (XI) led, under the most favourable conditions of catalysis and after hydrolysis of the intermediate nitrile, to a 6% yield of quinic acid (V) and its 1-C-epimer. The failure of this reaction was due to the unusual lability of the ketone (XI) to both acids and base when it readily decomposed to give



hydroquinone (90%). Cleavage of the lactone ring of quinide (XII) with [^{18}O]-enriched water (6%) gave [*carboxy*-^{18}O]quinic acid with 5% ^{18}O-enrichment in the carboxyl group. This form of isotopic labelling was, however, readily lost by exchange with water (50% loss after 3 days at 15°, 100% after 30 min. at 100°) and was in consequence unsuitable for the projected biosynthetic studies.

[7] E. E. Conn and T. Swain, *Chem. and Ind.*, 1961, 592.
[8] E. Haslam, R. D. Haworth, and P. F. Knowles, *J.*, 1961, 1854.
[9] G. Zemplen, L. Mester, and I. Dory, *Acta Chim. Acad. Sci. Hung.*, 1954, **4**, 151.
[10] H. O. L. Fischer, *Ber.*, 1921, **54**, 775; H. O. L. Fischer and G. Dangschat, *ibid.*, 1932, **65**, 1009.

Biological methods of preparation of a suitable $C_6 \cdot C_1$ precursor were more successful. Uniformly labelled [^{14}C]glucose fed to cut rose blooms just prior to senescence [11] gave [^{14}C]quinic acid (V) which was converted into (IV) by catalytic oxidation.[8,12] The percentage of isotopic labelling (12·4) in the carboxyl group of (IV) was determined by conversion into protocatechuic acid whose specific activity was compared to that of its decarboxylation product catechol. When sodium [1-^{14}C]pyruvate (II) was fed to cut rose blooms the quinic acid isolated contained 48% of the isotopic labelling in the carboxyl group. Although the sodium pyruvate is most probably taken into the carbohydrate pool, the increased proportion of radioactivity in the carboxyl group must be due in the main to the direct incorporation of some unmodified C_3 fragments into the shikimic acid pathway [13] (see Scheme). The quinic acid isolated from both these experiments, however, possessed relatively low specific activities and its use in further biosynthetic experiments was not considered.

When sodium [1-^{14}C]pyruvate was fed to sumach (*R. typhina*) leaf discs the gallic acid (I) subsequently isolated had 60% of its isotopic labelling in the carboxyl group whereas the shikimic acid (VII) isolated from the same experiments had 80% of its radioactivity in the same functional group. Clearly these results do not support unequivocally either biosynthetic pathway previously outlined [routes (A) and (B)] since, if there is a direct relationship between gallic (I) and shikimic (VII) acids, the distribution of isotopic labelling should be the same in both molecules (A) or, if the alternative route (B) were followed, the isotopic labelling would be expected to be predominantly in the C_6 fragment. However, until further information relating to the metabolism of these acids in sumach becomes available, these results, showing a relatively high incorporation of radioactivity into the carboxyl group of the phenolic acid, are most rationally interpreted as indicating that some if not all the gallic acid is derived under these conditions by dehydrogenation of a non-aromatic precursor such as (VI) or (VII). Further work is in progress on this problem.

Experimental.—Radioactive counting of samples was carried out using a Packard tricarb automatic counter. Solutions were prepared by dissolving the sample (0·01 g.) in ethanol (1·0 c.c.) and adding a solution of a liquid scintillator in ethanol (9·0 c.c.).

[^{18}O-*Carboxyl-labelled quinic acid.*] Quinide (3·0 g.) was added to 6% [^{18}O]water (1·0 c.c.) and the mixture sealed in a Pyrex tube (15 × 1 cm.), heated at 80° for 4 days. The pale yellow solution was reduced to dryness, and the residual gum crystallised from anhydrous ethanol or ethyl acetate as small prisms (1·5 g.), m. p. 172°. Measurement of the ^{18}O-enrichment was carried out using minor modifications of the method of Rittenberg and Pontecorvo.[14]

[^{14}C]*Quinic acid.* A cut rose bloom (var. Lydia) just prior to senescence was placed with the stem in a solution (2·5 c.c.) of sodium [1-^{14}C]pyruvate (0·25 mc., 22·5 mg.) and stood in a gentle draught and full light at room temperature. Solution adsorbed by the rose was replaced with tap water as required and after 3 days the rose petals were collected and ground with crushed glass and water (5 × 5 c.c.). Quinic (0·15 g.) and shikimic (0·15 g.) acids were added to the aqueous extract which, after filtration through a Seitz filter, was applied to an Amberlite C.G. 400 (Ac^-) column (32 × 2 cm.) and the cyclohexane acids isolated as previously described.[8] [^{14}C]-Quinic acid (0·071 g.) was obtained as small prisms, m. p. and mixed m. p. 171—172° (from ethanol) and gave a count of 5800 c.p.m./mg. The proportion of isotopic labelling in the carboxyl group was determined by conversion [8] into [^{14}C]protocatechuic acid whose activity was compared to that of [^{14}C]catechol obtained by heating the protocatechuic acid in water (10 c.c.) in a Carius tube at 200° for 14 hr. Evaporation of the water gave [^{14}C]catechol which was crystallised from benzene.

Uniformly labelled [^{14}C]glucose (0·025 mc.) was fed to a cut rose in an identical manner and the quinic acid (0·05 g.) isolated had a count of 1873 c.p.m./mg. The proportion of labelling in the carboxyl group was determined as above.

Growth of sumach leaf discs on sodium [1-^{14}C]*pyruvate.* Leaf discs (1 cm. diam., 2·0 g.) cut from the freshly gathered terminal shoots of a sumach (*R. typhina*) bush in July were floated on a solution of sodium [1-^{14}C]pyruvate (22·5 mg., 0·5 mc., 20 c.c.) in a Petri dish which was

[11] L. H. Weinstein, C. A. Porter, and H. J. Laurencot, *Contrib. Boyce Thomson Inst.*, 1959, **20**, 121.
[12] K. Heyns and H. Gottschalck, *Chem. Ber.*, 1961, **94**, 343.
[13] D. B. Sprinson, *Adv. Carbohydrate Chem.*, 1960, **15**, 235.
[14] D. Rittenberg and L. Pontecorvo, *Internat. J. Appl. Radiation Isotopes*, 1956, **1**, 208.

placed in the dark in a box through which a gentle stream of air was passed. After 24 hr. at room temperature the leaves were ground in a mortar with glass and water (5 × 10 c.c.) and after filtration of the plant tissues the aqueous filtrate was extracted with ethyl acetate (5 × 100 c.c.). The resultant aqueous and ethyl acetate fractions were treated separately.

Shikimic acid (0·1 g.) was added to the aqueous layer and the same acid reisolated (0·09 g.) from the mixture as described previously.[8] The [^{14}C]shikimic acid (283 c.p.m./mg.) was converted by catalytic oxidation [11] and acid treatment [15] into [^{14}C]protocatechuic acid (0·065 g.) and the proportion of isotopic labelling determined as described above. Evaporation of the ethyl acetate extract gave a yellow gum which was dissolved in 2N-sulphuric acid (20 c.c.) and heated under reflux for 14 hr. when it was diluted with water and extracted with ether (5 × 100 c.c.). The ether extract was shaken with sodium hydrogen carbonate solution (2 × 25 c.c.) and the latter extract acidified with 2N-sulphuric acid and re-extracted with ether (5 × 100 c.c.). The gum remaining on removal of the ether was applied equally to twenty silica gel plates previously developed with methanol–10N-hydrochloric acid (9 : 1). The plates were developed in two dimensions with (*a*) benzene–methanol–acetic acid (45 : 8 : 4) and (*b*) chloroform–ethyl acetate–formic acid (5 : 5 : 1) and the gallic acid, R_F (*a*) 0·3, (*b*) 0·5, isolated by elution with acetone. Removal of the acetone gave a gummy solid to which authentic gallic acid (0·1 g.) was added before crystallisation thrice from water. The gallic acid (0·088 g.) crystallised as needles, m. p. and mixed m. p. 262—264°, and gave 970 c.p.m./mg., unchanged on further crystallisation. The proportion of radioactivity in the carboxyl group was determined as for protocatechuic acid.

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[*Added in Proof.*—Several workers have suggested that the principal fate of pyruvate in plant tissues is oxidative decarboxylation (*e.g.*, C. J. Coscia, M. I. Ramirez, W. J. Schubert, and F. F. Nord, *Biochemistry*, 1962, **1**, 447). Thus Nord and his colleagues interpret the results of feeding ^{14}C-pyruvate to lignin-producing tissues in terms of an oxidative decarboxylation followed by incorporation of the isotopic carbon dioxide into the carboxyl group of (III) *via* the "dicarboxylate shuttle." The same rationalisations applied in this case lead to identical conclusions regarding the biosynthesis of gallic acid.]

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[15] R. Grewe and J. P. Jeschke, *Chem. Ber.*, 1956, **89**, 2080.