Phytochemistry, 1967, Vol. 6, pp. 1067 to 1073. Pergamon Press Ltd. Printed in England

AROMATIC METABOLISM IN PLANTS—IV.

THE INTERCONVERSION OF SHIKIMIC ACID AND QUINIC ACID BY ENZYMES FROM PLANT CELL CULTURES*

OLUF L. GAMBORG

Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan, Canada

(*Received* 20 *December* 1966)

Abstract—The interconversion of shikimic and quinic acids has been demonstrated with enzymes from cell suspension cultures of mung bean (*Phaseolus aureus* Roxb.). The enzymes were extracted after disruption of the cells by sonic oscillation. Spectrophotometric assays of the extracts demonstrated the presence of quinate dehydrogenase (EC 1.1.1.24), dehydroquinate dehydratase (EC 4.2.1.10) and shikimate dehydrogenase (EC 1.1.1.25). The products were identified by gas–liquid chromatography. The results showed that 5-dehydroquinate and 5-dehydroshikimate were intermediates.

INTRODUCTION

SHIKIMIC acid is a key intermediate in the biosynthesis of many aromatic compounds in plants and microorganisms.[1–3] Although quinic acid is related structurally to shikimic acid it is not an essential intermediate in the formation of aromatic compounds.[4] However, Weinstein, Porter and Laurencot[5] have shown that plants can convert quinic acid to shikimic acid, phenylalanine, and tyrosine, and Goldschmid and Quimby have demonstrated the interconversion of the two acids in shoots of hemlock.[6]

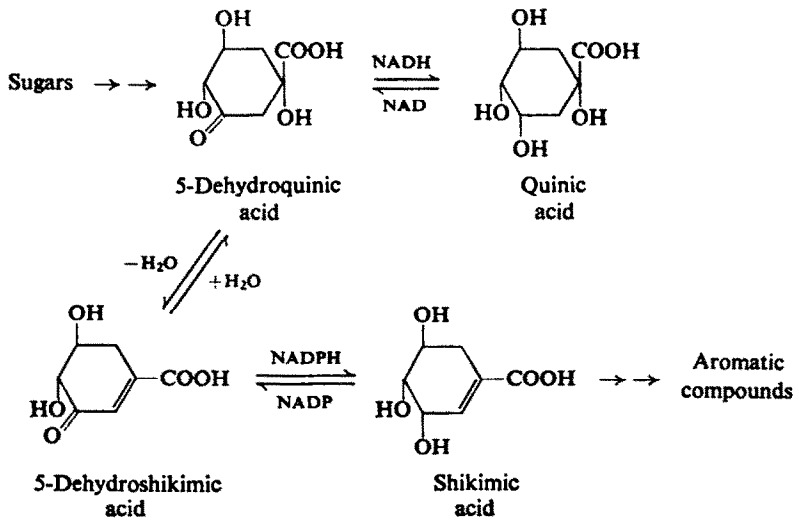
Shikimic acid is derived biosynthetically from 3-deoxy-D-arabinoheptulosonic acid 7-phosphate.[7] The first intermediate which is stable is 5-dehydroquinic acid.[8] The 5-dehydroquinic acid may then be converted to 5-dehydroshikimic acid and shikimic acid or to quinic acid.[1] The reactions are shown in the diagram below.

5-Dehydroquinate dehydratase (EC 4.2.1.10) and shikimate dehydrogenase (EC 1.1.1.25) catalyze the formation of shikimic acid from 5-dehydroquinic acid. These enzymes occur in plants.[9, 10] Quinate dehydrogenase (EC 1.1.1.24) which catalyzes the formation of quinic acid from 5-dehydroquinic acid has been isolated recently in this laboratory from a plant source.[11]

Issued as N.R.C. No. 9540.

* Presented in part at the 17th Annual Meeting of the American Institute of Biological Sciences, College Park, Maryland, 14–19 August, 1966.

[1] A. C. NEISH, In *Biochemistry of Phenolic Compounds* (Edited by J. B. HARBORNE), p. 295. Academic Press, New York (1964).
[2] B. A. BOHM, *Chem. Rev.* **65**, 435 (1965).
[3] M. I. GIBSON and F. GIBSON, *Biochem. J.* **90**, 248 (1964).
[4] B. D. DAVIS and U. WEISS, *Arch. Exptl. Path. Pharmakol.* **220**, 1 (1953).
[5] L. H. WEINSTEIN, C. A. PORTER and H. J. LAURENCOT, JR., *Contrib. Boyce Thompson Inst.* **21**, 201 (1961).
[6] O. GOLDSCHMID and G. R. QUIMBY, *Tappi* **47**, 528 (1964).
[7] C. H. DOY and K. D. BROWN, *Biochim. Biophys. Acta* **104**, 377 (1965).
[8] P. R. SRINIVASAN, J. ROTHSCHILD and D. B. SPRINSON, *J. Biol. Chem.* **238**, 3176 (1963).
[9] D. BALINSKY and K. D. DAVIES, *J. Exptl Botany* **13**, 414 (1962).
[10] O. L. GAMBORG, *Can. J. Biochem.* **44**, 791 (1966).
[11] O. L. GAMBORG, *Biochim. Biophys. Acta* **128**, 483 (1966).



The results in the present report demonstrate the interconversion of shikimic acid and quinic acid by enzymes from cell suspension cultures derived from mung bean plants.

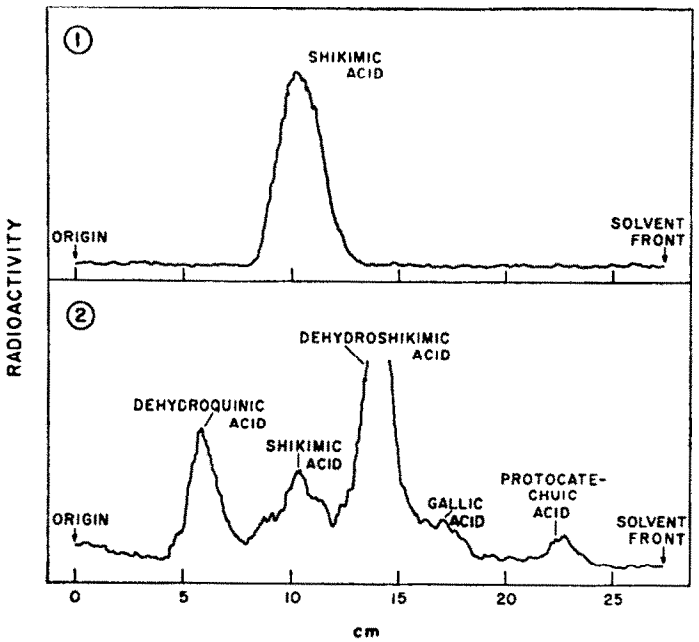


FIG. 1. THE CONVERSION OF SHIKIMIC ACID TO DEHYDROSHIKIMIC ACID AND DEHYDROQUINIC ACID BY ENZYMES FROM CELL SUSPENSION CULTURES OF MUNG BEAN. (1) TRACING OF SCANNER RECORD OF (^{14}C)SHIKIMATE. (2) TRACING OF THE SCANNER RECORD OF THE REACTION MIXTURE AFTER INCUBATION WITH (^{14}C)SHIKIMATE AS SUBSTRATE.

The reaction mixture consisted of 5 μmoles (G^{14}C)shikimate (0·56 μc), 2 μmoles NADP, 2 ml of the extract, and 0·05 M 2-amino-2-methyl-1,3-propanediol buffer, pH 8·5, in a total volume of 3·5 ml. After incubation for 16 hr at 24° the mixture was lyophilyzed and analyzed by paper chromatography as described previously.[11]

RESULTS

Preliminary Experiments

The products of the reactions were identified initially by paper chromatography of the reaction mixtures in which ^{14}C-labelled substrates were employed. A crude dialyzed extract obtained after sonic oscillation of mung bean cell cultures served as the source of enzymes.[11] The results in Fig. 1 indicate that shikimic acid was converted to dehydroshikimic acid and dehydroquinic acid. The reaction mixture also contained small quantities of two components which were identified as gallic acid and protocatechuic acid. Tests were not performed to determine if these acids were formed by enzyme action or chemically from dehydroshikimic acid.[12]

Spectrophotometric Analyses

Each of the three enzymes which carry out the reactions were assayed by measuring the reduction or oxidation of the respective nicotinamide adenine dinucleotide. The quinate dehydrogenase reaction is shown in Fig. 2. The data indicate the reversibility of the reaction.

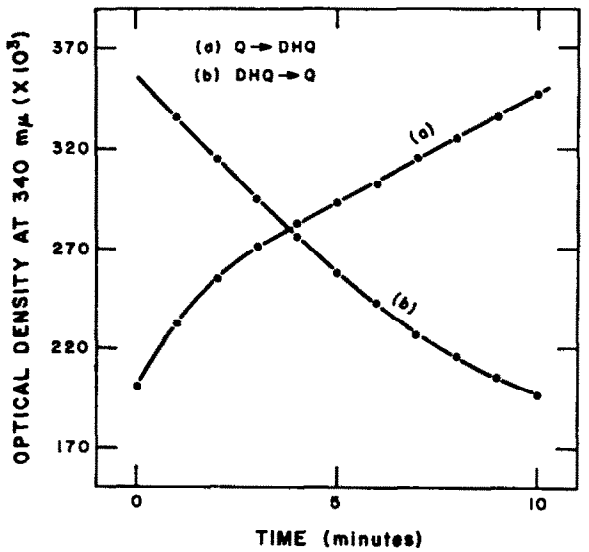


FIG. 2. THE INTERCONVERSION OF QUINATE AND DEHYDROQUINATE CATALYZED BY QUINATE DEHYDROGENASE FROM MUNG BEAN CELL CULTURES.

The reaction mixture consisted of 70 μmoles propanediol buffer, pH 9·0, 0·06 ml of enzyme, substrate and coenzyme in a total volume of 1·0 ml. The temperature was 28°. The substrates were: (*a*) 5 μmoles quinate + 1 μmole NAD; (*b*) 4 μmoles dehydroquinate + 0·1 μmoles NADH. Abbreviations: Q = Quinic acid; DHQ = 5-dehydroquinic acid.

The shikimate dehydrogenase and the dehydroquinate dehydratase reactions are shown in Fig. 3. The conversion of dehydroquinate to shikimate was followed by measuring the oxidation of NADPH. The data in Fig. 4 show the direct conversion of dehydroquinic acid to dehydroshikimic acid by dehydroquinate dehydratase.

[12] S. R. GROSS, *J. Biol. Chem.* 233, 1146 (1958).

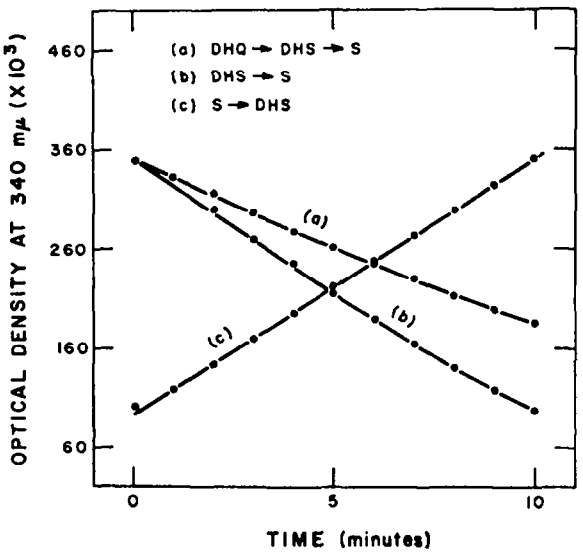


FIG. 3. REACTIONS OF THE DEHYDROQUINATE DEHYDRATASE AND SHIKIMATE DEHYDROGENASE FROM MUNG BEAN CELL CULTURES. THE CONDITIONS WERE THE SAME AS IN FIG. 2.

The amounts of enzyme and substrates were: (*a*) 0·2 ml enzyme, 4 μmoles 5-dehydroquinate and 0·1 μmole NADPH; (*b*) 0·03 ml enzyme, 4 μmoles 5-dehydroshikimate and 0·1 μmole NADPH; (*c*) 0·01 ml enzyme, 5 μmoles shikimate and 1 μmole NADP. Abbreviations: S = Shikimic acid; DHS = 5-Dehydroshikimic acid.

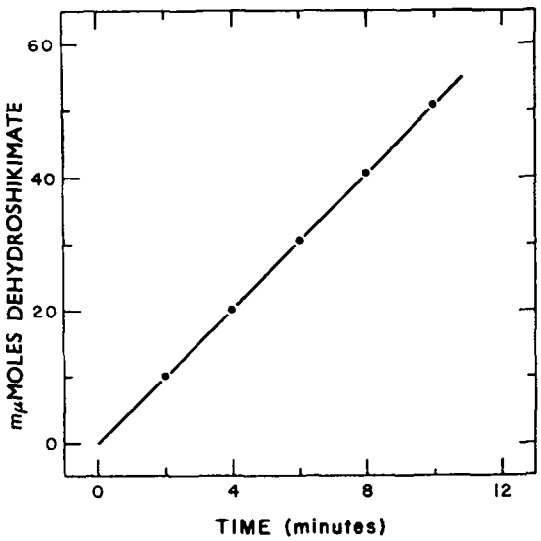


FIG. 4. THE CONVERSION OF DEHYDROQUINATE TO DEHYDROSHIKIMATE BY DEHYDROQUINATE DEHYDRATASE FROM MUNG BEAN CELL CULTURES.

The reaction mixture consisted of 0·2 ml enzyme, 2·5 μmoles 5-dehydroquinate, 0·1 M propanediol buffer, pH 7·5, in a total volume of 2·5 ml. The temperature was 30°. The enzyme was assayed by recording the increase in absorption at 240 mμ.

Analyses of Products

The paper chromatographic techniques failed to separate quinate and dehydroquinate, and a gas chromatographic method was developed for the identification of the products.[13] The acids were isolated from reaction mixtures by column chromatography with Dowex 1-X8 (acetate),[14] and analyzed by GLC as their trimethylsilyl ethers.

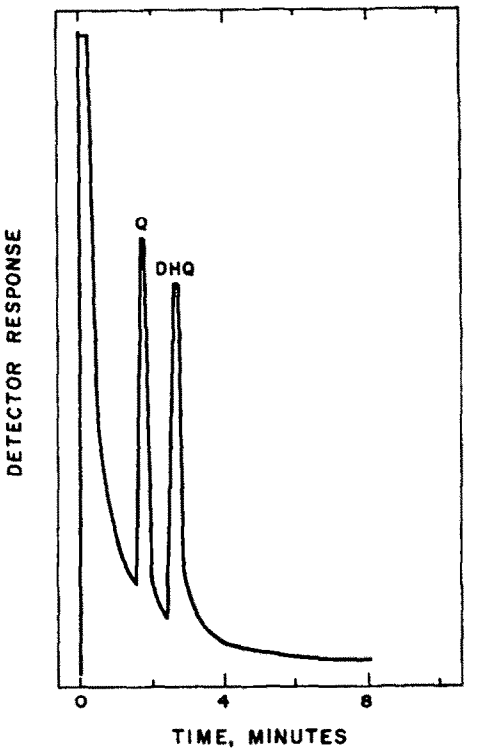


FIG. 5. ANALYSIS BY GLC OF THE FORMATION OF DEHYDROQUINIC ACID FROM QUINIC ACID CATALYZED BY QUINATE DEHYDROGENASE FROM CULTURED MUNG BEAN CELLS. THE ACIDS WERE ANALYZED AS THEIR TRIMETHYLSILYL ETHERS.[13]

The reaction mixture consisted of 50 μmoles of 5-dehydroquinic acid, 40 μmoles NAD, 1·2 ml enzyme,* and propanediol buffer at pH 8·5 in a total volume of 4·2 ml. The temperature was 28° and the incubation period was 7·5 hr. Abbreviations: see Fig. 2.

* The enzyme was a 30–60% ammonium sulphate fraction[11] containing 12·3 mg protein, and 0·11 units of quinate dehydrogenase per ml.

The data in Fig. 5 support the spectrophotometric evidence for the formation of dehydroquinic acid from quinic acid. The products formed in a reaction mixture fortified with the necessary enzymes and coenzymes and with shikimic acid as substrate are shown in Fig. 6. The results show the formation of quinic acid from shikimic acid.

[13] J. P. SHYLUK, C. G. YOUNGS and O. L. GAMBORG, *J. Chromatog.* **26**, 268 (1967).
[14] E. HASLAM, R. D. HAWORTH and P. F. KNOWLES, *Methods in Enzymology*, Vol. 6. Academic Press, New York (1963).

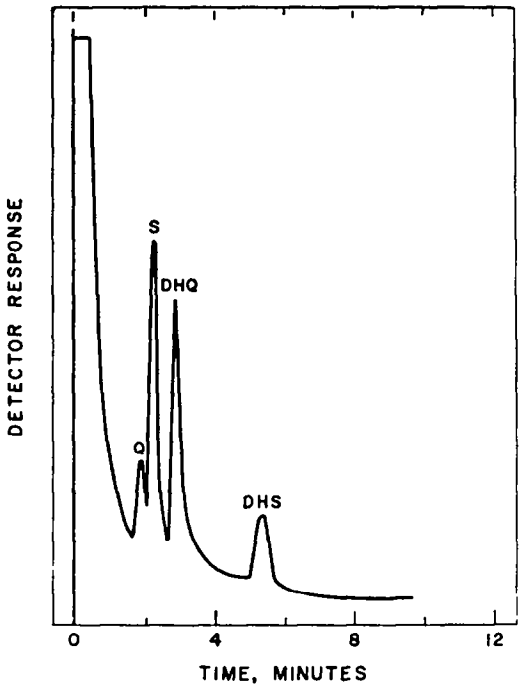


FIG. 6. ANALYSIS BY GLC OF THE PRODUCTS OBTAINED WITH SHIKIMATE AS SUBSTRATE IN THE PRESENCE OF NADH, NADP AND ENZYMES FROM MUNG BEAN CELLS.

The reaction mixture consisted of 50 μmoles shikimate, 40 μmoles NADP, 25 μmoles NADH, 1·4 ml enzyme* and propanediol buffer at pH 8·5 in a total volume of 3·2 ml. The temperature was 28° and the incubation time was 8·5 hr. Abbreviations: see Figs. 2 and 3.

*The enzyme was a 30–60% ammonium sulfate fraction and contained the following: 12·1 mg protein, 1·66 units of shikimate dehydrogenase, 0·26 units of 5-dehydroquinate dehydratase, and 76 munits of quinate dehydrogenase per ml.

DISCUSSION

The results of these experiments have shown that quinic acid and shikimic acid may be interconverted by quinate dehydrogenase, dehydroquinate dehydratase and shikimate dehydrogenase in mung bean cells in culture. The latter two enzymes function in the formation of aromatic compounds by the shikimic acid pathway in plants and microorganisms.[2, 9,10] Quinate dehydrogenase has been anticipated in plant tissues because of the frequent occurrence of quinic acid in plants,[2] but there are no reports at present that the enzyme occurs in any plant organ, although the cotyledons of mung bean contain detectable amounts.[15]

The importance of quinic acid in plant metabolism is not known.[1, 16] It occurs free or as an ester in many plants. The amounts in a given tissue may vary considerably during the growing period.[6, 16] The results of experiments with labelled quinic acid have shown that shikimic acid, phenylalanine and tyrosine became heavily labelled,[5, 6] and quinic acid was a precursor of pelargonidin, although it was less efficient than shikimic acid.[17] Umbarger

[15] O. L. GAMBORG, Unpublished results.
[16] M. HASEGAWA, *Wood Extractives*, p. 263. Academic Press, New York (1962).
[17] H. Co and P. MARKAKIS, *Phytochem.* 5, 755 (1966).

and Davis[18] have suggested that the formation of quinic acid in microorganisms, when it occurs, is a catabolic process. Information on the pathways of degradation of quinic acid in plants is scarce. The results of Weinstein, Porter and Laurencot[5] indicate that some of the labelled quinate fed to leaves of various plants was converted into aliphatic acids and amino acids associated with the Tricarboxylic Acid Cycle, but the percentage incorporation into the aliphatic compounds was considerably lower than that converted into such compounds as shikimic acid, phenylalanine and tyrosine. These findings suggest that quinic acid supplied exogenously is preferentially converted into shikimic acid and aromatic compounds derived from shikimic acid. There is not sufficient information on the metabolism of quinic acid to determine if quinic acid and quinate dehydrogenase may have a regulatory role in the biosynthesis of aromatic compounds.

EXPERIMENTAL

Chemicals

Generally labelled shikimic acid was isolated from shoots of *Ginkgo biloba* L. after exposure to radioactive carbon dioxide. The 5-dehydroquinic acid was prepared from quinic acid by the method of Haslam, Haworth and Knowles.[14] The 5-dehydroshikimic acid was prepared biologically.[19]

Source of Enzymes

The enzymes were obtained from batches of cells originating from the roots or the cotyledons of mung bean (*Phaseolus aureus* Roxb.). The cells were cultured in 200 ml of the PRL-4-C-CM medium in 1 l. Erlenmeyer flasks as described previously.[10] The cells were disrupted by sonic oscillation and the extracts subjected to ammonium sulfate precipitation and treatment on hydroxylapatite columns as described elsewhere.[11] The shikimate dehydrogenase and the 5-dehydroquinate dehydratase were eluted from the column by the 40 mM phosphate buffer at pH 7·5, and appeared in the fractions immediately succeeding those containing the quinate dehydrogenase.

Assays

The enzymes were assayed by measuring the change in absorbance at 340 mμ in a Gilford automatic spectrophotometer.

Chromatography

The products were chromatographed on Whatman No. 1 paper with *n*-butanol–formic acid–water (50: 2·5:10), and benzyl alcohol–isopropanol–*tert*-butanol–water (3:1:1:1) as the solvents.[16] The compounds were identified by the periodate–nitroprusside–piperazine reagent,[20] and 2,4-dinitrophenyl hydrazine.

The trimethylsilyl ethers of the respective acids were prepared and analyzed by gas–liquid chromatography as described previously.[13] The column packing was 4% QF-1 on Chromosorb P.

Acknowledgements—The author is indebted to Dr. E. W. Underhill for the sample of ^{14}C-shikimic acid, and to Mr. M. Chisholm for the preparation of 5-dehydroshikimic acid. The excellent technical assistance by Mr. J. P. Shyluk is gratefully acknowledged.

[18] E. Umbarger and B. D. Davis, *The Bacteria*, Vol. III, p. 167. Academic Press, New York (1962).
[19] I. I. Salamon and B. D. Davis, *J. Am. Chem. Soc.* **75**, 5567 (1953).
[20] R. A. Cartwright and E. A. G. Roberts, *Chem. Ind.* 230 (1955).