Phytochemistry, 1978, Vol. 17, pp 1011–1012. © Pergamon Press Ltd. Printed in England. 0031-9422/78/0501-1011 $02 00/0

OXYGEN FIXATION IN THE BIOSYNTHESIS OF FERULIC ACID BY GREEN *ZEA MAYS**

GEORGE J. FRITZ† and BRIAN D. ANDRESEN
Departments of Agronomy and Pharmaceutical Chemistry, University of Florida, Gainesville, FL 32611. USA

(*Received* 17 *October* 1977)

Key Word Index—*Zea mays*; Gramineae; biosynthesis; oxygen fixation; phenylpropanoids; ferulic acid.

Abstract—The results of experiments in which seedlings of *Zea mays* were grown in the light in an atmosphere enriched with oxygen-18 indicate that the hydroxyl and methoxyl oxygen atoms in ferulic acid are derived from molecular oxygen.

INTRODUCTION

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a phenolic widely distributed in plants [1], which appears to have more than one role in metabolism. It has been identified as a constituent of suberin and cutin [2]. Chloroplasts have been shown to catalyze a light-sensitized oxidation of ferulic acid to an acid dimer [3] and the possible role of these dimers as links in cell wall structure has been discussed [4]. Ferulic acid has been reported to synergize IAA-induced growth by counteracting IAA decarboxylation [5], and to inhibit the activity of IAA oxidase [6, 7]. Ferulic acid can also serve as a substrate for isoperoxidases [8]. Evidence has also been presented that ferulic acid is an intermediate in the biosynthesis of coumarins and phenylpropanoid moieties of flavonoids and lignins[9–11].

No definitive experiments concerning the source of oxygen atoms for the hydroxylation reactions have been reported. This paper is concerned with the demonstration that the hydroxyl and methoxyl oxygen atoms in ferulic acid synthesized by green *Zea mays* are derived from molecular oxygen.

RESULTS AND DISCUSSION

In preliminary experiments, ferulic acid was isolated from green *Z. mays* grown in an atmosphere containing the natural abundance of oxygen-18. The identity of the isolated compound was confirmed by TLC and MS comparisons with that of authentic material.

Ferulic acid isolated from the shoot–root axes of green *Z. mays* grown in an atmosphere enriched with 10.6 at.% oxygen-18 was analyzed by mass spectrometry. The MS analysis of the average of 11 scans, when corrected for natural abundance isotopes, indicated that the carboxyl oxygen atoms in ferulic acid were not labelled and that the phenolic oxygen atoms were labelled to the extent of 9.3 ± 0.6 at.%. The incorporation of ^{18}O atoms into the molecule was apparent by comparison of the labelled and unlabelled mass spectra. The presence of oxygen-18 in the phenolic oxygen atoms only was confirmed by comparing the abundance of ^{18}O in the molecular ion region (*m/e* 194, 196) with that in the $M - CO_2^+$) ion region (*m/e* 150, 152). Absolute isotopic incorporation determinations were made by averaging four mass spectra and subtracting background noise. Atom per cent enrichment was calculated by standard methods [12].

These results suggest that the phenolic oxygen atoms of ferulic acid are derived from molecular oxygen and support the previously reported findings for the role of oxygen fixation in the biosynthesis of caffeic acid [13] and *p*-coumaric acid [14]. The small discrepancy in the labelling of phenolic oxygen atoms in ferulic acid and atmospheric oxygen (9.3 ± 1.0 at.% vs 10.6 at.%) is very close to the limits of experimental error, and may be due to preformed ferulic acid in the tissue, or to an ^{18}O isotope effect, or to dilution of the oxygen-18 enrichment of the atmosphere by photosynthetic evolution of $^{16}O_2$ of natural abundance.

To exclude the possibility that the observed enrichment in ferulic acid was an indirect effect caused by the reduction of $^{18}O_2$ to H_2O during respiration followed by subsequent incorporation of $H_2{}^{18}O$ into ferulic acid, tissue water samples were recovered in successive fractions by lyophilization of *Z. mays* seedlings grown in an atmosphere enriched with 10.6 at.% oxygen-18, and were analyzed by mass spectrometry. These data have been reported [14]; the enrichment values are very small, between 0.0 and 0.2 at.% excess ±0.1 at.%. Therefore, it can be concluded that the hydroxyl and methoxyl oxygen atoms in ferulic acid are derived exclusively from molecular oxygen.

EXPERIMENTAL

Maize grains (*Zea mays* L., Wf9 × 38-11, fertile version) were grown in a sealed 1000 cc flask in a room lighted during daylight hours and seedlings were harvested at 7.5 days. Details of the conditions of plant growth in an atmosphere enriched with 10.6 at.% oxygen-18, harvesting of tissues, removal of tissue water from shoot–root axes and cotyledons, and MS have been described [14]. Only the shoot–root tissue was used for the isolation of ferulic acid from seedlings grown in an oxygen-18 atm, because preliminary investigation showed that cotyledonary tissue contained relatively large quantities of ferulic acid on the first day of germination. Although ferulic acid was found to be present in both the EtOH-soluble and EtOH-insoluble fractions [14], only the EtOH-insoluble fraction was recovered when

* Florida Agricultural Experiment Station Journal Series No. 652.

† To whom requests for reprints should be addressed.

plants were grown in an oxygen-18 atmosphere. The isolation of ferulic acid from plant tissues and its purification for MS were performed in a manner similar to that described for *p*-coumaric acid [14].

Acknowledgement—We are grateful to Harold Snellen for his technical assistance and expertise in the isolation procudure.

REFERENCES

1. Robinson, T. (1963) *The Organic Constituents of Higher Plants* p. 49. Burgess, Minneapolis.
2. Riley, R. G. and Kolattukudy, P. E. (1975) *Plant Physiol.* **56**, 650.
3. Stafford, H. A. and Brown, M. A. (1977) *Plant Physiol.* **59**, 94.
4. Stafford, H. A. and Brown, M. A. (1976) *Phytochemistry* **15**, 465
5. Tomaszewski, M. and Thimann, K. V. (1966) *Plant Physiol.* **41**, 1443.
6. Gortner, W. A. and Kent, M. J. (1958) *J. Biol. Chem.* **233**, 731
7. Gelinas, D. A. (1973) *Plant Physiol.* **51**, 967
8. Pickering, J. W., Powell, B. L., Wender, S. H. and Smith, E. C. (1973) *Phytochemistry* **12**, 2639.
9. Neish, A. C. (1965) in *Plant Biochemistry* (Bonner, J. and Varner, J. E. eds) 2nd edn, pp. 581–617. Academic Press, New York.
10. Brown, S. A. (1966) *Ann. Rev Plant Physiol.* **17**, 223.
11. Freudenberg, K. (1968) in *Constitution and Biosynthesis of Lignin* (Freudenberg, K. and Neish, A. C. eds) pp. 45–122. Springer-Verlag, Berlin.
12. Roboz, J. (1968) *Introduction to Mass Spectrometry Instrumentation and Techniques* pp. 455–456. John Wiley New York.
13. Fritz, G. J., King, R. W. and Dougherty, R. C. (1974) Phytochemistry **13**, 1473.
14. Fritz, G. J. and Andresen, B. D. (1978) *Phytochemistry* **17**, 581.