Phytochemistry, 1972, Vol. 11, pp. 2503 to 2505. Pergamon Press. Printed in England.

SHORT COMMUNICATION

FORMATION OF ETHIONINE FROM HOMOCYSTEINE AND OF *S*-METHYLMETHIONINE FROM METHIONINE IN APPLE TISSUE

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(*Received* 8 *February* 1972)

Key Word Index—*Malus pumila*; Rosaceae; biosynthesis; ethionine; *S*-methylcysteine.

Abstract—Conversion of L-homocysteine into ethionine and of methionine into *S*-methylcysteine in apple tissues is demonstrated.

INTRODUCTION

ETHIONINE is a natural product of bacteria,[1] but has not been reported to occur in plants. *S*-Methylmethionine was first isolated from cabbage[2] and has been found as a product of metabolism when radioactive methionine was fed to several plant tissues,[3–5] but its presence in apple fruit tissues has not been reported. During the studies on ethylene biosynthesis and metabolism of methionine in apple tissues, we have found an active conversion of L-homocysteine to ethionine and of methionine into *S*-methylcysteine. This paper describes the isolation and radiochemical identification of these two sulfur amino acids.

RESULTS AND DISCUSSION

Identification of Ethionine

This amino acid was isolated and identified from apple tissue which had been fed with homocysteine-U-^{14}C. The conversion of homocysteine to ethionine in several experiments usually ranged from 3 to 5%. However, in one experiment, as much as 20% of the radioactivity was recovered as ethionine. The crude apple extract in 80% ethanol was first reduced with 1% mercaptoethanol and then subjected to chromatography on Whatman 3M paper in *n*-butanol–acetic acid–water (4:1:5, v/v) as the developing solvent. A spot with high radioactivity was found in the region of R_f 0·67. This radioactive spot was eluted and was identified as ethionine according to the following criteria. On paper electrophoresis at pH 2·1, 6·3, and 10·0 and on paper chromatography, the radioactivity migrated to the same distance as authentic ethionine. Upon oxidation with H_2O_2–$HClO_4$, it yielded a compound which cochromatographed on paper with ethionine sulfoxide (R_f 0·13). When the radioactive spot was eluted, reduced with 1% mercaptoethanol (100° for 1 hr) and rechromatographed on paper, the R_f increased to 0·67 where the authentic ethionine migrated. The

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compound was cocrystallized with authentic ethionine and yielded constant specific radioactivity as shown in Table 1. The trimethylsilyl derivatives were prepared by heating with bis(trimethylsilyl)acetamide for 1 hr at 85° and separated by GLC on a silicone SE30 column. Fractions were collected and their radioactivity measured. The radioactivity was found in the fraction where the known trimethylsilyl derivative of ethionine was eluted (retention time 21 min on 6 mm × 1·8 m column at 112° with a flow of 60 ml/min of He).

Similar results were also obtained when homocysteine-^{35}S was fed into the apple tissue. The data indicate that homocysteine is converted to ethionine through ethylation and not thioethylation. Since ethionine is formed in apple tissues from L-homocysteine, it is of interest to know whether *S*-ethylcysteine may also be formed from cysteine in an analogous

TABLE 1. COCRYSTALLIZATION OF THE RADIOACTIVE PRODUCT WITH AUTHENTIC ETHIONINE*

Crystallization No.	1st	2nd	3rd	4th
cpm/μmol	206	205	206	209

* L-Ethionine (68·7 mg) and the radioactive product (72 000 cpm), which was isolated from apple tissue fed with homocysteine-^{14}C and had a R_f of 0·67 on paper chromatography, were dissolved in 4 ml of hot 50% ethanol. Aliquots of the hot solution were taken for measurement of radioactivity with a scintillation counter and for determination of amino nitrogen by the ninhydrine method.[6] The specific radioactivity of the initial solution was 170 cpm/μmol. The solution was then cooled in an ice bath for 10 min and the crystals recovered by centrifugation in the cold followed by washing once in cold 95% alcohol. The crystals were then dissolved in hot 50% ethanol. These procedures were repeated four times.

manner. We have therefore fed L-cysteine-^{35}S to apple tissue. The ethanol extract was first chromatographed on paper. Since *S*-ethylcysteine and methionine migrated very closely on paper chromatography, the region with R_f 0·40–0·55 was eluted. The trimethylsily derivatives were prepared and subjected to GC. Under the conditions described above, *S*-ethylcysteine (R_t, 11·3 min) was completely separated from methionine (R_t, 14·7 min). We found that the radioactivity moved with trimethylsilyl methionine but none with trimethylsilyl *S*-ethylcysteine. The data indicate that the methylation reaction in the apple tissue is specific for homocysteine. The nature of the ethyl donor in this conversion has not been studied. Although ethionine has not been shown to be present naturally in apple tissue, the present results suggest the possibility that ethionine may be a natural amino acid in this fruit.

Identification of S-*Methylmethionine*

In studying metabolites of L-methionine-methyl-^{14}C in apple tissue by paper chromatography in a butanol–acetic acid–water system, about 3·5% of the radioactivity was detected near the origin. In the same solvent system, methionine has an R_f of 0·47. This radioactivity was dialysable against water, and in electrophoresis at pH 6·5 moved towards the cathode, suggesting that it was a non-protein cation.

For isolation of this compound, an ethanol extract of apple tissue which had been fed with L-methionine-methyl-^{14}C was concentrated, adsorbed on a cation exchange resin

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(Dowex 50, H^+ form) and then eluted with 2 N NH_4OH. After concentration, the eluate was then passed through an anion exchange resin (Dowex 2, OH^- form). These procedures removed organic acids, neutral compounds and the acidic and neutral amino acids. The effluent solution was then concentrated and the radioactive compound in this fraction was identified as *S*-methylmethionine according to the following criteria. It cochromatographed on paper with authentic *S*-methylmethionine in *n*-butanol–acetic acid–water (4:1:4, v/v) solvent system. On paper coelectrophoresis at pH 2·5 and 7·0, the compound migrated with an authentic sample and finally, upon heating in 20% KOH, radioactive dimethylsulfide was detected by gas radiochromatography on two different columns, Porapak Q at 150° and diethylene glycol succinate at 20°. Both columns separated dimethylsulfide from methanethiol and dimethyldisulfide. No radioactivity was found in methanethiol and dimethyldisulfide. The formation of dimethylsulfide from *S*-methylmethionine in alkali has been documented.[4] The enzymic synthesis of *S*-methylmethionine from methionine and *S*-adenosylmethionine has also been reported.[3,7]

EXPERIMENTAL

Plant materials and chemicals. Apples (cv. Golden Delicious) were brought from a local market. L-Methionine-methyl-^{14}C was obtained from International Chemical and Nuclear Corporation. Radioactive L-homocysteine was prepared from L-methionine-U-^{14}C and L-methionine-^{35}S. L-Methionine (10 μCi) was reacted in 0·1 ml of 48% HI for 48 hr at 30°, yielding homocysteine thiolactone. After evaporation, the resulting homocysteine thiolactone was separated from HI and I_2 by paper electrophoresis (40 V/cm for 30 min) at pH 6·3. After the thiolactone was eluted and evaporated, it was subjected to alkaline hydrolysis,[8] yielding homocysteine. The radiopurity of the substrates was verified by paper and TLC.

Feeding of radioactive substrates and extraction procedures. Apple plugs (1 cm in dia. and 2 cm in length) were cut with a cork borer and razor blade and the radioactive substrate in 2% KCl solution were introduced into the plugs by a vacuum injection technique described previously.[9] After incubation for 4 hr, the tissues were ground and extracted with 80% EtOH.

Acknowledgements—This investigation was supported by Research Grant GB-20336 from the National Science Foundation.

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