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BIOSYNTHESIS OF *TRANS*-2-HEXENAL IN CHLOROPLASTS FROM *THEA SINENSIS*

AKIKAZU HATANAKA, TADAHIKO KAJIWARA and JIRO SEKIYA
Department of Agricultural Chemistry, Yamaguchi University, Yamaguchi 753, Japan

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Key Word Index—*Thea sinensis*; Theaceae; isolated chloroplasts; *trans*-2-hexenal; *cis*-3-hexenal; linolenic acid-[U-^{14}C] incorporation; linoleic acid-[U-^{14}C] incorporation; leaf aldehyde biosynthesis.

Abstract—The biosynthetic pathway of *trans*-2-hexenal, leaf aldehyde, in isolated chloroplasts of *Thea sinensis* leaves was examined using a tracer experiment. A high and specific incorporation of radioactivity into *cis*-3-hexenal and *trans*-2-hexenal, was observed when linolenic acid-[U-^{14}C] was incubated with the isolated chloroplasts. Thus, *trans*-2-hexenal was biosynthesized via *cis*-3-hexenal from linolenic acid in the chloroplasts.

INTRODUCTION

Leaf aldehyde, *trans*-2-hexenal (III), is formed via *cis*-3-hexenal (II) from linolenic acid (I) by an enzyme in macerated leaves of *Thea sinensis* in the presence of oxygen [1,2]. The synthetic activity for II and III was localized in the leaf chloroplasts [3]. In this paper, a biosynthetic pathway has been traced from linolenic acid to *trans*-2-hexenal (III) via *cis*-3-hexenal (II) in isolated chloroplasts.

RESULTS AND DISCUSSION

Tracer experiments show that linolenic acid (I) added to isolated chloroplasts is specifically converted to *cis*-3- and *trans*-2-hexenal (III) (Fig. 1). Hexenals are the only volatile products from (I) in isolated chloroplasts. No propanal, C_6-alcohols, C_9-aldehydes and -alcohols, or malone aldehyde were found under various conditions of GC–RC analysis. Of the radioactivity, 2% was recovered in hexenals (II and III). The radioactivity in II

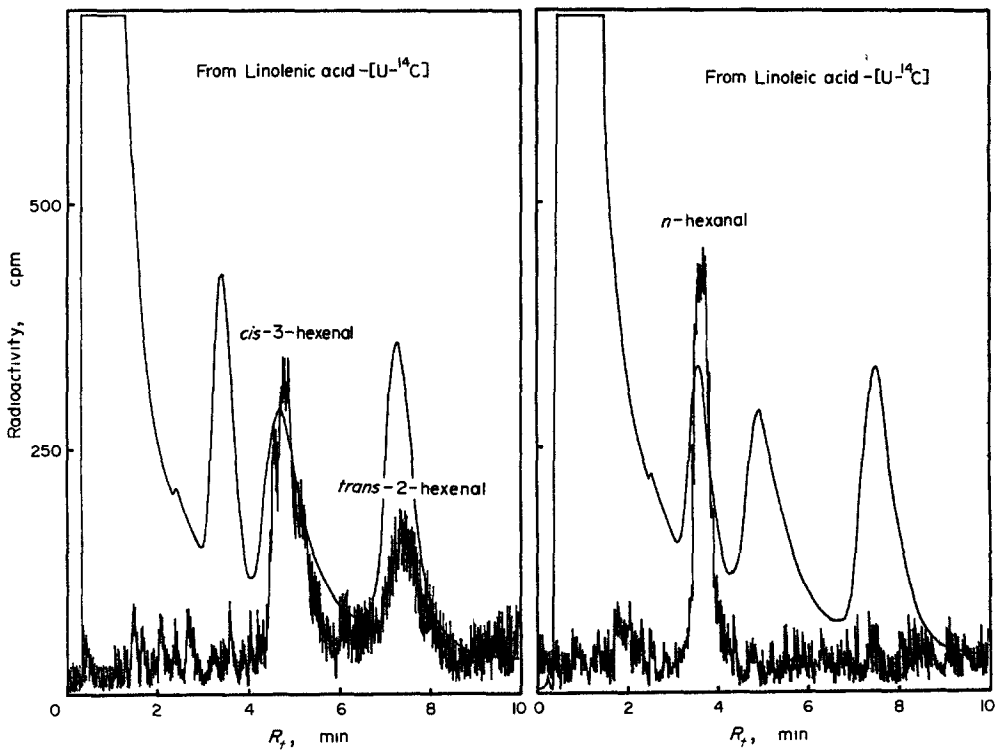

Fig. 1. Radio gas chromatograms of the aldehydes formed from linolenic acid-[U-^{14}C] and linoleic acid-[U-^{14}C] in isolated chloroplasts. GC–RC traces show the radioactivity (spiked trace) and mass (smooth trace).

was transferred to III as the reaction progressed; II being easily isomerized to III in isolated chloroplasts. With linoleic acid-[U-^{14}C], 10% of the radioactivity was recovered in *n*-hexanal; the only volatile compound, as shown in Fig. 1. On the basis of these findings, it is concluded that leaf aldehyde, *trans*-2-hexenal (III), is biosynthesized via an important intermediate, *cis*-3-hexenal (II) from linolenic acid (I) in isolated chloroplasts of *Thea sinensis* leaves.

EXPERIMENTAL

Material. Chloroplasts were prepared from leaves of *Thea sinensis* var. *Yabukita* harvested on the 6th of August 1975 according to the method of ref. [3]. The synthetic activities of the chloroplasts for hexenals (II and III) and *n*-hexanal were determined by GLC as described in ref. [3] and represented as peak areas (mm^2): 228 for hexenals from linolenic acid and 738 for hexanal from linoleic acid, respectively. Authentic samples of *cis*-3-hexenal (II) and *trans*-2-hexenal (III) were synthesized as described in ref. [2].

Radio gas chromatography (GC–RC). A GC–RC equipped with an FID-radio detector was used. The conditions of the GC–RC were: a 3 mm × 3 m stainless steel column packed with 20% PEG 20 M on Celite 545 (60–80 mesh); column temp 100°; inject. temp 150°; N_2 flow rate 60 ml/min; attenuation range of 0.16 V; sensitivity of 10 $M\Omega$ and the radio detector 1 kcpm. One nCi of toluene-[1-^{14}C] was determined as 70 counts under these conditions.

Incorporation of linolenic acid-[U-^{14}C] into hexenals. A mixture of linolenic acid-[U-^{14}C] (5 μCi, sp act 720 mCi/mmol, from New England Nuclear), linolenic acid (15 mg), and chloroplasts (0.67 g) in 20 ml of 4 × diluted McIlvaine's buffer, pH 6.3, containing 0.4 M sucrose, was vigorously shaken for 5 min at 25°. After 10 min incubation at 40°, the reaction mixture was extracted with Et_2O (10 ml × 3). The combined Et_2O extract was washed with H_2O, dried and concentrated to 0.5 ml. After unlabelled *cis*-3-hexenal, *n*-hexanal and *trans*-2-hexenal had been added to the concentrated extract as marker compounds, 50 μl of the radioactive extract (0.5 ml) was analyzed by GC–RC.

Incorporation of linoleic acid-[U-^{14}C] into n-hexanal. After linoleic acid-[U-^{14}C] (5 μCi, sp. act. 820 mCi/mmol from Applied Science Lab.) had been administered to the isolated chloroplasts, the radioactive extract (1 ml) was obtained as described above. 10 μl of the radioactive extract was analyzed by GC–RC.

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