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FORMATION OF 12-OXO-*TRANS*-10-DODECENOIC ACID IN CHLOROPLASTS FROM *THEA SINENSIS* LEAVES

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Key Word Index—*Thea sinensis*; Theaceae; isolated chloroplasts; 12-oxo-*trans*-10-dodecenoic acid; 12-oxo-*cis*-9-dodecenoic acid; *trans*-2-hexenal; linolenic acid-[1-^{14}C] incorporation; leaf aldehyde.

Abstract—Linolenic acid-[1-^{14}C] was converted to 12-oxo-*trans*-10-dodecenoic acid, via 12-oxo-*cis*-9-dodecenoic acid by incubation with chloroplasts of *Thea sinensis* leaves. Thus, it was confirmed that linolenic acid is split into a C_{12}-oxo-acid, 12-oxo-*trans*-10-dodecenoic acid, and a C_6-aldehyde, *trans*-2-hexenal, leaf aldehyde, by an enzyme system in chloroplasts of tea leaves.

INTRODUCTION

Recently, 12-oxo-*trans*-10-dodecenoic acid was found in watermelon [1] and banana [2]. In a previous paper [3], the biosynthesis of leaf aldehyde, *trans*-2-hexenal, via *cis*-3-hexenal from linolenic acid in chloroplasts of tea leaves was described using a tracer experiment with linolenic acid-[U-^{14}C]. Thus linolenic acid must be split into a C_6-aldehyde and a C_{12}-oxo-acid. However, the formation of C_{12}-oxo-acids in tea leaves was still uncertain.

This paper describes the enzymatic formation of C_{12}-*trans*-10-unsaturated oxo-acid, 12-oxo-*trans*-10-dodecenoic acid (**2**), via *cis*-9-unsaturated oxo-acid, 12-oxo-*cis*-9-dodecenoic acid (**1**), from linolenic acid in the chloroplasts of tea leaves.

RESULT AND DISCUSSION

Tracer experiments show that linolenic acid added to isolated chloroplasts is converted to 12-oxo-*cis*-9-dodecenoic acid (1.4%, **1**) and 12-oxo-*trans*-10-dodecenoic acid (2.4%, **2**) as shown in Fig. 1. Of the radioactivity, 0.7% was recovered in 9-oxo-nonanoic acid and an unknown compound (1.8%). The radioactivity in **1** was transferred to **2** as the enzymatic reaction progressed (Fig. 2). This suggests that the *cis*-9-unsaturated oxo-acid (**1**) is easily isomerized to the *trans*-10-unsaturated oxo-acid (**2**) in isolated chloroplasts similar to the isomerization from *cis*-3-hexenal to *trans*-2-hexenal. With heated chloroplasts, no radioactivity was detected in C_{12}-oxo-acids. Thus, compound **2** is enzymatically generated via the *cis*-9-unsaturated oxo-acid (**1**) from linolenic acid in the chloroplasts of tea leaves.

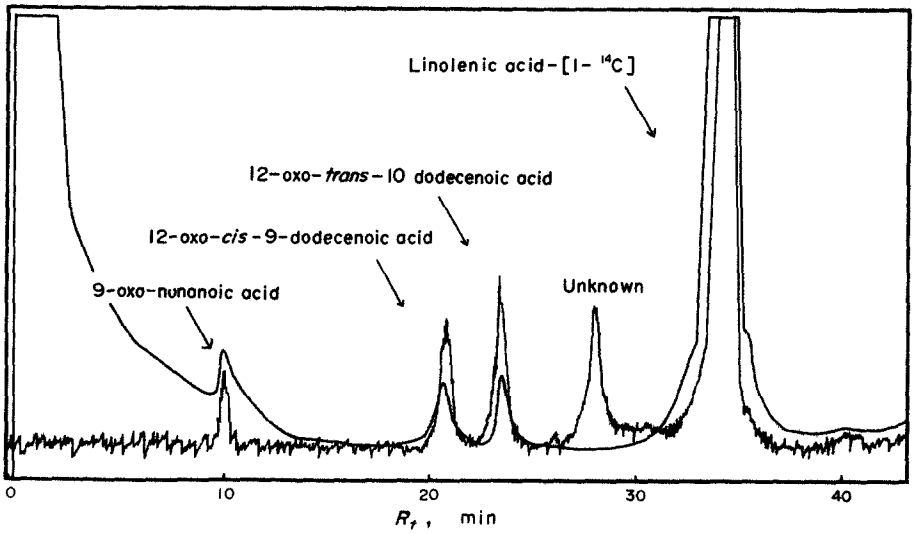


Fig. 1. Radio gas chromatogram of the oxo-acids formed from linolenic acid-[1-^{14}C] in isolated chloroplast. GC–RC traces show the radioactivity (spiked trace) and mass (smooth trace).

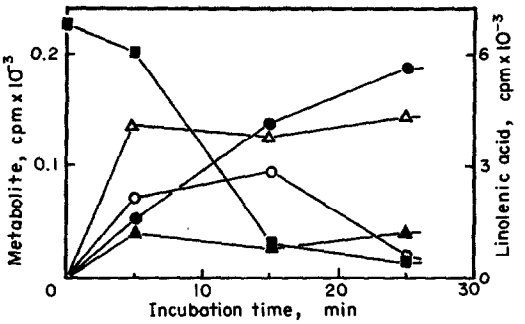
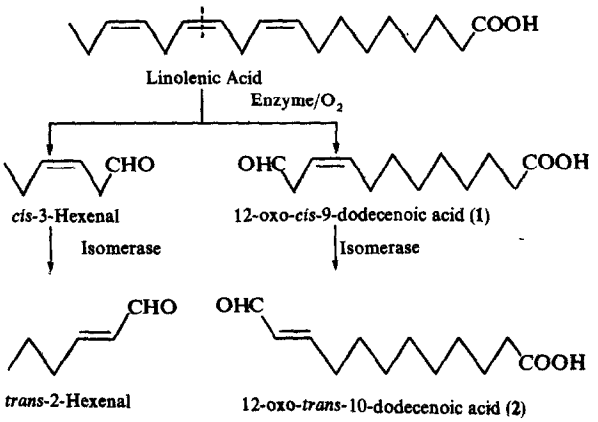


Fig. 2. Time course of C_{12}-oxo-acid formation from linolenic acid-[1-^{14}C]. Reaction mixture with linolenic acid-[1-^{14}C] was incubated at 20° for an initial 5 min and then at 40°. —■— Linolenic acid; —○—12-oxo-*cis*-9-dodecenoic acid; —●— 12-oxo-*trans*-10-dodecenoic acid; —▲— 9-oxo-nonanoic acid; —△— unknown.



Scheme 1. Biosynthetic pathway of leaf aldehyde.

Based on these findings and the results previously reported [3], the biosynthetic pathway of leaf aldehyde, *trans*-2-hexenal, and 12-oxo-*trans*-dodecenoic acid from linolenic acid was demonstrated to be as shown in Scheme 1.

EXPERIMENTAL

Material. Chloroplasts were prepared from leaves of *Thea sinensis* var. *Yabukita* harvested on the 18th August 1976 according to the method of ref. [4]. Authentic samples of 12-oxo-*cis*-9-dodecenoic acid (**1**) and 12-oxo-*trans*-10-dodecenoic acid (**2**) were synthesized by an unequivocal route [5].

Radio gas chromatography (GC–RC). A GC–RC equipped with an FID and gas phase radio detector was used. Column: 1 m × 3 mm stainless steel packed with 60–80 mesh, Chromosorb W coated with 10% Silicone GE SE-30. Column temp. 100–200° at 3°/min. Carrier gas flow rate: 60 ml N_2/min. The radio detector was 1 kcpm. One nCi of toluene-[1-^{14}C] was determined as 52 counts under these conditions.

Incorporation of linolenic acid-[1-^{14}C] *into* C_{12}*-oxo-acids.* A mixture of linolenic acid-[1-^{14}C] (5 μCi, sp. act. 50 mCi/mmol, Radiochemical Centre, Amersham), linolenic acid (9.5 mg) and chloroplasts (300 mg) in 10 ml of 4-diluted McIlvaine's buffer, pH 6.3. containing 0.4 M sucrose, was vigorously shaken for 5 min at 20°. 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 concd. Products were methylated with CH_2N_2 at −20° and unlabelled methyl-12-oxo-*cis*-9-dodecenoate, methyl-12-oxo-*trans*-10-dodecenoate and methyl-9-oxo-nonanoate added to the conc extracts as marker compounds. After the soln was made up to 1.0 ml, 50 μl of the radioactive extract was analyzed by GC–RC.

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POLYUNSATURATED COMPOUNDS OF *CENTAUREA SCABIOSA*

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Key Word Index—*Centaurea scabiosa*; Cynareae; Compositae; polyacetylenes; polyenes.

Abstract—Roots, green parts, and flower heads of *Centaurea scabiosa* were examined separately. Twenty-five polyacetylenes, 4 polyenic aldehydes, 1,8,11,14-heptadecatetraene, and the flavone apigenin were isolated and characterized. Three C_{17} hydrocarbons with from one to three isolated double bonds and a series of minor compounds were also isolated.

INTRODUCTION

Previous investigations of green parts and roots of *Centaurea scabiosa* have shown the presence of compound **2** [1, 2]. Furthermore, polyunsaturated aldehydes and compounds **2** and **8** have been detected in *C. scabiosa* subsp. *scabiosa* [3]. As no data on the flower heads of *C. scabiosa* have been published, and as UV spectra of extracts of wild flowering plants showed the presence of considerable amounts of polyunsaturated compounds, a new investigation was carried out at this laboratory.