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INCREASE OF *CIS*-3-HEXEN-1-OL CONTENT IN TEA LEAVES FOLLOWING MECHANICAL INJURY

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Key Word Index—*Camellia sinensis*; Theaceae; tea leaves; volatile constituents; leaf alcohol; *cis*-3-hexen-1-ol; mechanical injury.

Abstract—Dark-grown tea seedlings were exposed to $^{14}CO_2$ and incubated for 1 month. From a comparison of the radioactivities in lipid fractions obtained from both intact green seedlings and mechanically injured seedlings, it was found that the radioactivity in *cis*-3-hexen-1-ol was significantly increased by mechanical injury.

INTRODUCTION

Since *cis*-3-hexen-1-ol, the so-called leaf alcohol, was first discovered from fermented tea leaves by P. van Romburg in 1895 [1], the occurrence of the free alcohol and its esters in oils have been reported from a wide range of higher plants such as mulberry leaves [2], radish leaves [2], acacia leaves [2], Japanese mint [3], apples [4], bananas [4], oranges [4], and strawberries [4]. Because of its characteristic green leaf odour, it was considered that *cis*-3-hexen-1-ol occurred naturally in intact fresh leaves [5, 6]. However, the mechanism of its formation has not been elucidated.

We reported in a previous paper [7] that the amount of many volatile constituents of tea leaves, including *cis*-3-hexen-1-ol, markedly increased during the manufacturing process of black tea, such as withering and fermentation. Those findings indicated that fresh or intact tea leaves contained only a very small amount of *cis*-3-hexen-1-ol compared with that of mechanically injured leaves. We describe in this paper the formation of *cis*-3-hexen-1-ol from administered $^{14}CO_2$ after maceration of tea leaves.

RESULTS AND DISCUSSION

Distribution of radioactivities in lipid fractions from intact tea seedlings and injured tea seedlings which were incubated in $^{14}CO_2$

In order to determine the biosynthesis of *cis*-3-hexen-1-ol, dark-grown tea seedlings were exposed to $^{14}CO_2$ and incubated for 1 month. Lipids and volatile fractions were prepared from both intact green seedlings and mechanically injured seedlings, and the radioactivities were compared (Table 1). The radioactivities in both chloroform-soluble and methanol–water-soluble fractions of injured seedlings were lower than those in the fractions of intact seedlings. By contrast, remarkably high radioactivity was observed in the volatile fraction of injured seedlings compared with that of intact seedlings.

Formation of cis-3-*hexen*-1-*ol by mechanical injury*

Volatile fractions were subsequently analyzed by GLC; radioactivities of effluent fractions and recovery rates of authentic cold compounds were determined. Total radioactivity was calculated (Table 2) by dividing the radioactivity of the

Table 1. Comparison of radioactivities in lipids and volatile fractions between intact and injured seedlings

	Radioactivity incorporated (dpm)		
	$CHCl_3$-soluble Fraction 1	$MeOH–H_2O$-soluble Fraction 2	Volatile fraction in (1)
Intact seedlings	$13{\cdot}1 \times 10^6$	$27{\cdot}1 \times 10^6$	3430
Injured seedlings	$9{\cdot}9 \times 10^6$	$16{\cdot}6 \times 10^6$	51240

Table 2. GLC separation of the concentrated volatile fractions obtained from intact and injured seedlings

Effluent fraction (retention time, min)	Compound	Intact seedlings Radioactivity (dpm)	Intact seedlings Recovery rate of cold standard (%)	Intact seedlings Total radioactivity (dpm)	Injured seedlings Radioactivity (dpm)	Injured seedlings Recovery rate of cold standard (%)	Injured seedlings Total radioactivity (dpm)
0–9·8		16		—	304		
9·8–11·1	*cis*-3-Hexen-1-ol	6	6·10	98	928	5·43	17090
11·1–13·4	(*trans*-2-Hexen-1-ol)	0	—		576		
13·4–18·5		9	—		11		
18·5–20·8	Linalool	0	9·90	0	17	7·21	236
20·8–38·7		2	—		43		
38·7–44·0	Nerol	2	11·78	17	4	8·42	48
44·0–48·0	Geraniol	2	13·80	14	2	9·74	21
48·0–51·0	Phenylmethanol	0	3·38	0	10	2·18	459
51·0–55·0	2-Phenylethanol	0	4·87	0	1	2·83	35
55·0–		3	—		29		

effluent fractions by the recovery rate of cold standard. The amount of radioactivity in each effluent fraction from intact seedlings was low, whereas radioactivities in some effluent fractions from injured seedlings were very high, especially in *cis*-3-hexen-1-ol, that is, 98 dpm in intact seedlings and 17090 dpm in injured seedlings. The observation indicated that *cis*-3-hexen-1-ol was produced after mechanical injury. It is considered that administered $^{14}CO_2$ is not directly incorporated into free *cis*-3-hexen-1-ol during the metabolic period, but into a precursor which is then easily converted into free *cis*-3-hexen-1-ol on injury.

EXPERIMENTAL

Incubation of tea seedlings in radioactive CO_2. Tea seeds (*Thea sinensis*) were germinated and grown in the dark for 2 months at 18°. The dark-grown tea seedlings (yellow) were exposed to radioactive CO_2, which was generated by adding lactic acid to 400 μCi Na_2CO_3 in 20% NaOH soln, for 4 days in the light. Thereafter, the seedlings were incubated for 1 month in alternating 10 hr light period at 10000 lx and 14 hr dark period. The incubations were conducted at av temp of 22° in a closed box where air was circulated into 20% KOH soln outside the box to absorb the CO_2 generated. Those yellow seedlings were nearly greened within the period of 14 days photosynthesis. 7·5 g of leaves and unlignified stalks were used as intact seedlings for the extraction of lipids and the preparation of volatile fraction.

Mechanical injury to intact seedlings. 7·5 g Of a second sample of intact seedling were macerated for 0·5 hr in a mortar and allowed to brown for 2·5 hr at 18°.

Preparation of lipids and volatile fraction. The methods used were the same as described previously [7]. Lipids were extracted by means of homogenizing the tea seedlings with $CHCl_3$–MeOH (1:1) [8] which contained the authentic unlabelled *cis*-3-hexen-1-ol, linalool, nerol, geraniol, phenylmethanol and 2-phenylethanol as carriers. H_2O was added to a concn of 40% in the extracts, and the mixture was separated into $CHCl_3$-soluble and aq. MeOH-soluble fraction. The $CHCl_3$-soluble fraction was conc and steam distilled at 40° for 20 min *in vacuo*. From the distillates the volatile constituents were extracted with Et_2O, which was conc.

Separation of cis-3-*hexen-1-ol*. *cis*-3-Hexen-1-ol was separated from the conc volatile fraction by GC with thermal conductivity detector [7]: column packing, 10% Carbowax 20 M on Diasolid L (60–80 mesh); carrier gas, 30 ml/min; column temp program, 130° to 240° at a rate of 1°/min.

Determination of radioactivity. Radioactivity was measured with a liquid scintillation spectrometer. Samples containing pigments were decolorized in UV light, before radioactivity was measured. In the case of the effluent from the GC, each effluent fraction was directly trapped into 10 ml of a liquid scintillator based on toluene by observing the position of cold authentic standard, and the radioactivity was measured.

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