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PRODUCTION OF LINALOL AND GERANIOL BY HYDROLYTIC BREAKDOWN OF BOUND FORMS IN DISRUPTED TEA SHOOTS*

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Key Word Index—*Camellia sinensis*; Theaceae; tea shoots; volatile constituents; *trans*-2-hexenal; linalol; geraniol; mechanical injury.

Abstract—Linalol and geraniol were produced in tea shoots, disrupted by mechanical means, by hydrolytic breakdown of non-volatile compounds. The formation of linalol and geraniol was inhibited by Hg^{2+} and glucono-1,4-lactone, a specific inhibitor of β-D-glucosidase. On adding exogenous β-D-glucosidase to the homogenate of heat-treated shoots, linalol and geraniol were released from their bound forms.

INTRODUCTION

Monoterpene alcohols such as linalol and geraniol and their oxides have been identified in the aroma of tea by GC/MS [1, 2]. Those compounds which determine tea aroma the most are produced in the tea shoots during tea manufacture, especially in black tea fermentation [3, 4]. However, the mechanism of terpene alcohol formation during black tea processing is not clear. It has been assumed that terpene alcohols in the aroma of black tea are probably produced from oxygenated isoprenoid hydrocarbons [4].

This paper shows that monoterpene alcohols in mechanically disrupted shoots are produced by hydrolytic breakdown of what appear to be the related non-volatile β-D-glucosides.

RESULTS

Preliminary experiments

The pentane-soluble volatiles produced immediately after blending tea shoots and after incubation for 30 min at 40° are compared in Fig. 1. The main volatiles present are *trans*-2-hexenal, linalol and geraniol. An increase in all volatiles can be clearly observed following incubation.

The time course of changes in hexenal, linalol and geraniol in the pentane extract is shown in Table 1. The amounts of the volatiles increased proportionally with the time of incubation at 40°. However, no changes occurred at 0°.

Effects of oxygen and polyphenol in the reaction system

Aerobic conditions were effective in the production of hexenal in the homogenate, while the presence of oxygen in the reaction system slightly inhibited the production of linalol and geraniol (Table 2). Polyphenol present in the homogenate inhibited the production of volatiles; adsorption of the polyphenol with Polyclar AT restored volatile production (Table 2).

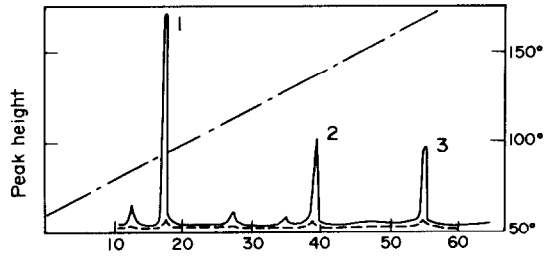

Fig. 1. GLC analysis of volatiles in tea shoots. ----, Gas chromatogram of volatiles in tea shoots incubated for 30 min at 0°. ———, Gas chromatogram of volatiles in tea shoots incubated for 30 min at 40°. – – –, Column temperature: 1—*trans*-2-hexenal, 2—linalol, 3—geraniol.

Effects of inhibitors

As shown in Table 3, the production of hexenal was inhibited by CN^- and also Hg^{2+}. The production of linalol and geraniol was not inhibited by CN^-, F^- and I^-. However, Hg^{2+} and glucono-1,4-lactone, a specific inhibitor of glucosidase, showed distinctive inhibitory effects on the production of linalol and geraniol.

Production of linalol and geraniol from the steamed tea shoots after adding glucosidase

The pentane-soluble volatiles were absent from the homogenates made from the steamed tea shoots before and after incubation. However, when almond glucosidase (EC 3.2.1.21) was added to the homogenate of the steamed shoots which were then incubated for 120 min at 40°, the same amounts of linalol and geraniol were produced, as with the homogenate of fresh shoots (Table 4).

* Part 1 in a projected series "Black Tea Aroma and its Formation".

Table 1. Time course of changes of volatiles in the pentane extract (ng/g fresh tissue, Benihomare)

Incubation period (min)	*trans*-2-Hexenal		Linalol		Geraniol	
0	30	36	1.0	1.1	3.6	3.8
	42		1.2		4.0	
15	227	244	3.0	3.3	11.0	12.3
	261		3.6		13.6	
30	504	513	4.3	4.5	17.0	17.6
	522		4.7		18.2	

Details are given in the Experimental.

Table 2. Effects of oxygen and polyphenol on the formation of volatiles (ng/g fresh tissue, Yabukita)

Treatment	*trans*-2-hexenal	Linalol	Geraniol
A	1023 ± 102	3.9 ± 0.5	3.7 ± 0.3
B	1320 ± 115	4.2 ± 0.4	4.0 ± 0.3
C	506 ± 35	4.6 ± 0.5	4.3 ± 0.4

A: Control—the homogenate was made without Polyclar AT and incubated aerobically. B: Without polyphenol—the homogenate was made with Polyclar AT and incubated aerobically. C: Without polyphenol and oxygen—the homogenate was made with Polyclar AT and incubated anaerobically.

DISCUSSION

Recent work on the production of volatile carbonyl compounds in disrupted plant tissues has revealed that they are produced by the enzymic oxidation of polyunsaturated fatty acids from lipids [5–14]. The results of this investigation have also confirmed that hexenal found in tea shoots was produced by macerating the shoots in the presence of air and the metal-containing enzyme, lipoxygenase. The role of this enzyme in hexenal formation was indicated by the inhibitory effect of CN^- on the process. Also, the inhibitory effect of Hg^{2+} on hexenal formation is in line with its known inactivation of the hydrolytic breakdown of lipids [15–18].

By contrast, the production of linalol and geraniol occurred under anaerobic conditions and was not inhibited by CN^-. From these results, it appears that linalol and geraniol found in disrupted tissues are not produced by oxidative breakdown of isoprenoid hydrocarbons. Furthermore, the lack of inhibition by fluorine ions rules out the simple release of terpene alcohols from the related pyrophosphates.

The production of linalol and geraniol was inhibited by Hg^{2+} and glucono-1,4-lactone, a specific inhibitor of glucosidase [19]. Furthermore, by adding exogenous glucosidase to the steamed tea homogenate, linalol and geraniol were released. Thus, an endogenous β-glucosidase appears to be responsible for the release of

Table 4. Production of linalol in fresh and steamed shoots (Yabukita)

Sample	Treatment	Incubation period 60 min	120 min	240 min
Fresh shoots	A	100*	—	—
Steamed shoots	B	0	0	0
	C	0	Trace	Trace
	D	33	73	85

A: Control—details of preparation are given in Experimental. B: Without glucosidase—the homogenate of steamed shoots was prepared as same as A. C: With glucosidase and without Polyclar AT—the homogenate was prepared by using the buffer containing glucosidase without Polyclar AT. D: With glucosidase and Polyclar AT—the homogenate was prepared with glucosidase and Polyclar AT.

* The content of linalol in the homogenate made from fresh shoots (7.5 ng/g fresh tissue) for 60 min is set at 100.

Table 3. Effects of inhibitors on the formation of volatiles

Inhibitor	Concentration (M)	*trans*-2-Hexenal (%)	Linalol (%)	Geraniol (%)
CN^-*	10^{-1}	91	9	8
	10^{-2}	56	2	1
	10^{-3}	5	0	0
F^-*	10^{-1}	19	0	0
I^-*	10^{-1}	23	9	7
	10^{-2}	19	8	10
Hg^{2+}*	5×10^{-2}	55	72	80
	5×10^{-3}	15	8	10
Glucono-1,4-lactone†	1	0	47	81
	10^{-1}	0	21	45

* Benihomare.
† Yamakai.
%: Inhibition ratio.
Each inhibitor was added to the homogenation buffer before treatment. The pH of the homogenation buffer was readjusted after adding each inhibitor.

linalol and geraniol in disrupted shoots. Glucosides of monoterpene alcohols are known to occur in many plants [20–25] and are hydrolysable by glucosidase [20]. It is, therefore, reasonable to assume that linalyl and geranyl-β-D-glucosides occur in tea shoots.

EXPERIMENTAL

Material. Fresh shoots, consisting of a bud and two leaves, (*Camellia sinensis* L.) were plucked from the clonal garden in National Research Institute of Tea. Steamed shoots were prepared by steaming tea shoots for 1 min at 100°. All enzymic activities in tea shoots were inactivated by this procedure.

Preparation of volatile fractions. The tissue (2 g equiv of fresh shoot) was blended in a 100 ml flask with 1 g Polyclar AT and 20 ml 0.2 M acetate buffer, pH 4.5, for 2 min at 0°. The tissue and all others were precooled at 0°. The triturated material was kept in anaerobic conditions by evacuating the flask and incubated for 30 min at 40°. At the end of this period, the flask was cooled immediately in an ice-water bath and *n*-pentane (3 ml) was added to the homogenate. The mixture was shaken for 1 min and centrifuged. The clear pentane layer was removed and used for the assay of volatile compounds. These separations were carried out at 0°.

GC and peak identification. A Shimazu model 4B GLC equipped with a FID was used. GC conditions were as follows: column packing, 5% Carbowax 20 M on Chromosorb G (80 × 100 mesh); He flow rate, 30 ml/min; column temp. program 60–180° at the rate of 2°/min. GC analysis was carried out by using 20-μl pentane extracts. The amount of each volatile compound was estimated from the peak area (cm^2). The identities of the volatiles were established by a Hitachi model RMU-6MG GC-MS.

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