Biosynthetic Pathway of Leaf Aldehyde in *Farfugium japonicum* Kitamura Leaves

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3*Z*-Hexenal, 2*E*-hexenal (leaf aldehyde) and 1-undecene were first found in *Farfugium japonicum* Kitamura (japanese silver) leaves. On blending the leaves in the presence of oxygen, 2*E*-hexenal was enzymatically generated *via* a similar biosynthetic pathway to that in *Thea sinensis* leaves: *via* 3*Z*-hexenal from linolenic acid. However, unlike *Thea sinensis* leaves neither 3*Z*-hexenol (leaf alcohol) nor 2*E*-hexenol was found in *F. japonicum* leaves.

From early times, the leaves of *Farfugium japonicum* Kitamura (japanese silver) have been used as a home remedy for suppuring erup-

tions. Leaf aldehyde, 2*E*-hexenal, was found as a biologically active substance in the leaves,¹⁾ but the biosynthesis was not explored.

We found that 2*E*- and 3*Z*-hexenal and the corresponding alcohols were formed from linolenic acid as shown in Scheme 1, when tea leaves were blended or mechanically ruptured in the presence of oxygen.^{2~12)}

To demonstrate whether or not 2*E*-unsaturated aldehydes are generally biosynthesized *via* 3*Z*-aldehydes from linolenic acid in fresh leaves, *F. japonicum* leaves were used.

RESULTS AND DISCUSSION

Identification of volatile components in *Farfugium japonicum* Kitamura leaves

Fresh *F. japonicum* leaves were blended with distilled water and the homogenates were steam-distilled. The distillate was extracted with ether and the crude essential oil was obtained from the ether extracts. Samples of the oils were quantitatively analyzed by GLC analyses. As 3*Z*-unsaturated aldehyde is easily isomerized to 2*E*-isomer in the homogenates of leaves, this aldehyde was followed by headspace vapour analysis according to the methods previously described.²⁾ 3*Z*-Hexenal, 2*E*-hexenal, *n*-hexanal and 1-undecene were first confirmed as the characteristic flavour components of the leaves by IR and MS spectral comparisons of the authentic specimens synthe-

SCHEME 1. Biosynthetic Pathway of Leaf Aldehyde in *Farfugium japonicum* Kitamura Leaves.

sized by unequivocal routes.⁵⁾ It is notable that the occurrence of 3*Z*-hexenol and 2*E*-hexenol was not confirmed in *F. japonicum* leaves unlike *Thea sinensis* leaves.²⁾

Effect of blending, heating and oxygen on the formation of aldehydes

During blending in the presence of oxygen, there was rapid formation of aldehydes, e.g. 2*E*-hexenal and *n*-hexanal (Table I). As

TABLE I. EFFECT OF BLENDING, HEATING AND OXYGEN ON THE FORMATION OF ALDEHYDES

Condition	<i>n</i> -Hexanal	2 <i>E</i> -Hexenal	1-Undecene
Non blended ^{a)}	trace	trace	trace
Blended ^{b)}			
under air	7.2 ^{d)} (100) ^{e)}	46.1 (100)	56.4 (100)
under N ₂	2.1 (29)	86.9 (188)	75.0 (133)
under O ₂	13.3 (184)	187.1 (331)	136.7 (276)
after heating ^{c)}	—	3.8 (0.6)	12.9 (28)

^{a)} Steam-distilled without blending.

^{b)} For 3 min at room temperature.

^{c)} For 3 min at 100°C.

^{d)} mg/kg of fresh leaves.

^{e)} (): percentage (%).

shown in Table I, the formation of aldehydes was prevented by blending under an atmosphere of nitrogen and/or by heating intact leaves before blending. Based on results obtained, it is concluded that the volatile aldehydes, *n*-hexanal and 2*E*-hexenal, are generated enzymatically, when fresh *F. japonicum* leaf is blended under aerobic conditions.

Conversion of fatty acids to aldehydes during blending

The fatty acids constituting the lipids in intact and blended *F. japonicum* leaves, respectively, were quantitatively analyzed by GLC analyses. The major fatty acids of the lipids in the fresh leaves were linolenic, linoleic and palmitic acid, and these fatty acids were not found in free fatty acid fraction. During blending under aerobic conditions, the amounts of linolenic and linoleic acid in both the neutral fat and phospholipid fractions decreased markedly, but the decreases were not ac-

TABLE II. CONTENTS OF FATTY ACIDS IN *Farfugium japonicum* KITAMURA

Fraction	Linolenic acid		Linoleic acid	
	0 min ^{a)}	3 min ^{b)}	0 min ^{a)}	3 min ^{b)}
Free fatty acid	trace	trace	trace	trace
Neutral fat	74.3 ^{c)}	67.9	82.5	58.5
Phospholipid	67.5	41.3	87.0	42.8
Total lipid	141.8	109.2	169.5	101.3

^{a)} Blending at zero time: inactivated at 65°C for 12 min and then blended for 3 min at room temperature.

^{b)} Blended for 3 min at room temperature.

^{c)} mg/kg of fresh leaves sampled on the 3rd of July, 1976.

companied by increases in free fatty acids as shown in Table II, and the formation of 2*E*-hexenal and *n*-hexanal respectively was observed (Table I).

Formation of aldehydes from linolenic and linoleic acid

The correlation between increase in 2*E*-hexenal and *n*-hexanal, and decrease in linolenic and linoleic acid, clearly indicates that the fatty acids constituting the lipids of *F. japonicum* leaves were converted to the volatile aldehydes during blending under aerobic conditions. On blending the leaves with added linolenic or linoleic acid, 2*E*-hexenal or *n*-hexanal increased remarkably (Table III). The results clearly indicate that during blending in the presence of oxygen, linolenic acid liberated from neutral lipid and phospholipid is stereospecifically split into 2*E*-hexenal via 3*Z*-hexenal in *F. japonicum* leaves as well as *Thea sinensis* leaves. The details of the 3*Z*-hexenal-forming enzyme will be reported.¹³⁾

TABLE III. FORMATION OF ALDEHYDES FROM UNSATURATED FATTY ACIDS

Fatty acid added ^{a)}	<i>n</i> -Hexanal	2 <i>E</i> -Hexenal ^{b)}
Control	26.1 ^{c)} (100)	83.5 (100)
Linolenic acid	254.8 (997)	82.3 (101)
Linoleic acid	31.5 (121)	183.8 (225)

^{a)} Blended with each fatty acid (1.8×10^{-8} M) for 3 min at room temperature and then steam-distilled.

^{b)} 3*Z*-Hexenal was isomerized to 2*E*-isomer during steam-distillation.

^{c)} mg/kg fresh leaves.

On the other hand, neither 3*Z*-hexenol nor 2*E*-hexenol was found in *F. japonicum* unlike *Thea sinensis* leaves.²⁾ The alcohol dehydrogenase activity was not detected according to the method in tea ADH.⁴⁾ Thus, it seems that the redox systems involving alcohol dehydrogenase which convert 3*Z*-hexenal and 2*E*-hexenal to the corresponding alcohols do not occur in *F. japonicum* leaves.

EXPERIMENTAL

IR spectra were taken on a Hitachi EPI-G2. MS spectra were recorded on a Japan Spectroscopic Model TAM-01SG spectrometer. Gas chromatography was performed using a Shimadzu GC-4B equipped with FID. GLC analyses of the essential oil were carried out on a 3 m × 3 mm stainless steel column packed with 20% PEG-20 M on celite 545, 60~80 mesh. Analyses of fatty acids were performed on a 1 m × 3 mm stainless steel column packed with 20% PEG-adipate on Chromosorb W, 60~80 mesh.

Materials

Fresh leaves of *Farfugium japonicum* Kitamura were used. Linoleic and linolenic acid (99% purity) were purchased from Wako Pure Chemical Co., Osaka, Japan. Other chemicals were synthesized through unequivocal routes and were proved to be of 99% purity.^{7,9)}

Preparation of essential oil

After *F. japonicum* leaves (40 g) were blended with distilled water (200 ml) in a Waring blender for 3 min at room temperature, the mixture was steam-distilled to give 500 ml of distillate. The distillate was saturated with NaCl and extracted three times with 50 ml of ether. The combined ether solutions were dried over anhydrous Na₂SO₄. Evaporation of solvent gave the crude essential oil.

Preparation of fatty acids

A crude lipid fraction was prepared from fresh *F. japonicum* leaves (100 g) by blending and subsequent extraction with CHCl₃-MeOH mixture (2:1, v/v, 1 liter). The crude lipid thus obtained was separated into acetone-soluble and acetone-insoluble fractions. The acetone-soluble fraction was evaporated and the residue dissolved in ether-petrol (1:1). The free fatty acids in the organic solution were extracted with 1% sodium carbonate and the lower was acidified with HCl. The mixture was extracted with ether and free fatty acids were obtained after evaporation of ether. The fraction not extracted with sodium car-

bonate was saponified with alcoholic potassium hydroxide, unsaponified material being extracted with ether and discarded. The solution of potassium salts was acidified with HCl and fatty acids derived from the neutral fat fraction were extracted with ether. The phospholipid fraction was repeatedly extracted from the acetone-insoluble fraction with CHCl₃-MeOH (1:1) and the concentrate of combined extracts was hydrolyzed with 20% HCl (160 ml) in boiling water for 14 hr under N₂. The fatty acids liberated from the phospholipid fraction were extracted with ether. Fatty acids from each fraction were esterified with CH₃N₂ and aliquots were analyzed by GLC.

Isolation and identification of 1-undecene and 3*Z*-hexenal

1-Undecene: the essential oil prepared as described above was fractionated to give 1-undecene by preparative GLC: column; 20% PEG-20M (3 m × 5 mm), column temp.; 170°C. The IR and MS spectra of the natural compound were identical with those authentic specimens; IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹; 1825 (CH₂=CH-), 1640 (CH₂=CH-), 995 (CH₂=CH-), 915 (CH₂=CH-). MS (*m/e*); 42 (19.8%), 56 (78.2%), 70 (100.0%), 83 (80.2%), 97 (57.4%), 111 (26.7%), 126 (18.8%), 157 (19.8%).

3*Z*-Hexenal: as 3*Z*-hexenal was easily isomerized in the homogenates of leaves, the blended leaves were extracted immediately with ether. The aldehyde fraction including 1-undecene was prepared by preparative GLC and then the 2, 4-dinitrophenylhydrazone derivative was obtained according to the method reported previously.⁶⁾ The purified derivative of natural aldehyde was identified with that of 3*Z*-hexenal obtained by synthesis, mp 100~102°C (from ethanol); IR $\nu_{\text{max}}^{\text{KBr}}$: 3300 (-NH-), 1620 (-C=N-), 730 (*Z*, -CH=CH-).

Effect of blending, heating and oxygen on the formation of volatile components

Effect of blending: volatile components of intact *F. japonicum* leaves (non-blended sample) were prepared without blending, and other volatile samples were prepared after blending for 3 min at room temperature.

Effect of heating: fresh *F. japonicum* leaves were immersed in a water bath kept at 100°C for 3 min and then blended.

Effect of oxygen: fresh *F. japonicum* leaves were blended in nitrogen, oxygen or air for 3 min at room temperature. The essential oil from each sample was prepared by the method described above and analyzed by GLC under the above conditions.

Conversion of fatty acid to aldehydes during blending

For blending time 0, fatty acids were prepared from *F. japonicum* leaves inactivated at 65°C for 12 min.

Other fatty acids were prepared after blending fresh leaves with distilled water for 3 min according to the preparation method described above. Fatty acids in each fraction were quantitatively analyzed by GLC under the above conditions.

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