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BIOSYNTHETIC PATHWAY OF CUCUMBER ALCOHOL: *TRANS*-2,*CIS*-6-NONADIENOL VIA *CIS*-3,*CIS*-6-NONADIENAL

AKIKAZU HATANAKA, TADAHIKO KAJIWARA and TAKAHIRO HARADA

Department of Agricultural Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753, Japan

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Key Word Index—*Cucumis sativus*, Cucumiseae, cucumber, biosynthesis; cucumber alcohol; *cis*-3,*cis*-6-nonadienal, *cis*-3-nonenal, azelaic half aldehyde.

Abstract—*cis*-3,*cis*-6-Nonadienal and *cis*-3-nonenal in *Cucumis sativus* were identified by comparison with synthetic specimens. The identification of these compounds, combined with biochemical evidence, suggests that cucumber alcohol and *trans*-2-nonenol are biosynthesized via *cis*-3-unsaturated aldehydes from linolenic and linoleic acid, respectively.

INTRODUCTION

In earlier work on cucumber volatiles [1,2], several compounds were identified including a series of C_9-aldehydes and alcohols. The flavour of fresh cucumbers was attributed largely to aldehydes, among which 2,6-nonadienal was responsible for the characteristic aroma. Furthermore, the cucumber flavour was generated when the fruits were cut or mechanically ruptured in the presence of oxygen [3], and *trans*-2,*cis*-6-nonadienal and *trans*-2-nonenal were related to linolenic and linoleic acid, respectively, using ^{14}C-labeling techniques [4].

Recently, *cis*-3-nonenol and *cis*-3,*cis*-6-nonadienol were tentatively identified as new alcohol components in cucumbers [5].

The authors reported a biosynthetic mechanism for the formation of *trans*-2-hexenal and *cis*-3-hexenol via an important precursor, *cis*-3-hexenal, from linolenic acid in macerated tea leaves [6].

The biosynthetic pathway to *trans*-2-aldehydes from the unsaturated fatty acids in cucumbers, however, is still uncertain. If these aldehydes are generated enzymatically via biosynthetic intermediates such as *cis*-3-nonenal and *cis*-3,*cis*-6-nonadienal produced from linoleic and linolenic acid, respectively, the unsaturated fatty acids must be split into a C_9-aldehyde and a C_9-oxo acid in the cucumber. In this paper, the occurrence of *cis*-3-nonenal, *cis*-3,*cis*-6-nonadienal and azelaic half aldehyde in cucumbers has been confirmed and a biosynthetic pathway is proposed for cucumber alcohol production.

RESULTS AND DISCUSSION

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

In preliminary experiments, results reported by Fleming *et al.* [3] were quantitatively re-examined by GLC analysis. The results (Table 1) obtained were similar to those reported by Fleming *et al.* [3]. In the presence of oxygen, there was a rapid formation of aldehydes, e.g. *trans*-2-nonenal, *trans*-2, *cis*-6-nonadienal, which are responsible for the characteristic flavour of fresh

Table 1 Effect of blending, heating and oxygen on the formation of aldehydes

Condition	*trans*-2-Nonenal		*trans*-2 *cis*-6-Nonadienal	
Non-blended	trace		trace	
Blended	0·28*	(1·00)	2·01	(1·00)
Heated and then blended	0·06	(0·20)	0·23	(0·11)
Blended under N_2	0·03	(0·11)	0·16	(0·08)

* mg/kg of cucumber

Table 2 Effect of pH on the formation of *trans*-2,*cis*-6-Nonadienal

pH	*trans*-2 *cis*-6-Nonadienal	
3·5	0·79*	(0·34)
4·5	1·29	(0·56)
5·5	2·29	(1·00)
6·5	1·52	(0·66)
7·5	0·41	(0·18)

* mg/kg of cucumber

cucumbers produced during blending. The formation of aldehydes was prevented by blending under an atmosphere of nitrogen, and/or by heating whole cucumbers before blending

The optimum pH for the formation of aldehydes in cucumbers was 5·5 (Table 2) and the optimum pH value was similar in cucumber homogenates. Results clearly indicate that the aldehyde flavour components of cucumber are produced enzymatically when the cucumber is blended in the presence of oxygen.

Changes in fatty acids during blending

Grosch *et al.* [4] demonstrated that *trans*-2-nonenal and *trans*-2, *cis*-6-nonadienal were related to linoleic and linolenic acid, respectively. However, no other study has been carried out on the quantitative changes in fatty acids in relation to the formation of flavour components in cucumbers.

The fatty acids constituting the lipids in intact and blended cucumbers, respectively, were quantitatively analyzed as shown in Table 3. The major fatty acids of the lipids in cucumbers were linolenic, linoleic and palmitic acid. During blending, the amounts of linoleic and linolenic acid in both the neutral fat and phospholipid fractions decreased markedly to give the aldehydes, *trans*-2-nonenal and *trans*-2,*cis*-6-nonadienal, but the decreases were not accompanied by increases in free fatty acids Therefore, these fatty acids in the neutral and phospholipid fractions must be converted to aldehydes and other compounds.

Table 3 Changes in fatty acids in each fraction during blending

Fatty acid	Free fatty acid		Fraction† Neutral fat		Phospholipid	
	0	3*	0	3	0	3
Palmitic			31·61	12·0	44·5	11·0
Oleic		-	trace		trace	
Linoleic	trace	trace	31·1	9·0	34·4	7·6
Linolenic	trace	trace	96·0	32·8	67·8	13·8

* Blending time (min) † mg/kg of cucumber

Table 4 Formation of aldehydes from unsaturated fatty acids

Fatty acid added	*trans*-2-Nonenal	*trans*-2 *cis*-6-Nonadienal
Control	0·47* (1·00)	2·54 (1·00)
Linolenic acid	0·32 (0·68)	10·47 (4·13)
Linoleic acid	9·10 (19·34)	3·39 (0·55)
Oleic acid	1·03 (2·18)	1·74 (0·69)
Arachidonic acid	0·21 (0·44)	1·73 (0·68)

* mg/kg of cucumber

Formation of aldehydes from the unsaturated fatty acids

The correlation between an increase in *trans*-2-nonenal and *trans*-2,*cis*-6-nonadienal and a decrease in linoleic and linolenic acid suggests that the acids constituting the lipids in cucumbers were converted to the flavour components during blending.

After various unsaturated fatty acids were blended with cucumbers, the carbonyl compounds were estimated and the results are shown in Table 4. The addition of linolenic or linoleic acid resulted in a large increase of C_9-aldehydes, but with arachidonic acid an increase in the aldehydes was not observed

These facts and the results demonstrating the isomerization of *cis*-3- to *trans*-2-unsaturated aldehydes (Table 5) clearly indicate that, during blending, linolenic and linoleic acid added to cucumber homogenate are specifically split into *trans*-2,*cis*-6-nonadienal and *trans*-2-nonenal via *cis*-3-unsaturated aldehydes, respectively [6,7] Moreover the structural requirement for this enzymatic oxidative cleavage is not only the *cis*,*cis*-1,4-pentadiene system of the fatty acid [8] but also the specific position of the double bonds in relation to the carboxyl group

Identification of cis-3-*nonenal*, cis-3,cis-6-*nonadienal and azelaic half aldehyde*

As *cis*-3,*cis*-6-nonadienal and *cis*-3-nonenal

Table 5 Isomerization of *cis*-3,*cis*-6-nonadienal to *trans*-2 *cis*-6-nonadienal in blended cucumbers

Standing time (min)	*cis*-3,*cis*-6-Nonadienal	*trans*-2 *cis*-6-Nonadienal	Total nonadienal
0	387·8* (1·00)	477·0 (1·00)	784·8 (1·00)
10	241·9 (0·79)	799·0 (1·68)	1040·9 (1·33)
20	196·0 (0·64)	684·0 (1·43)	880·0 (1·12)

* GLC peak area (mm^2)

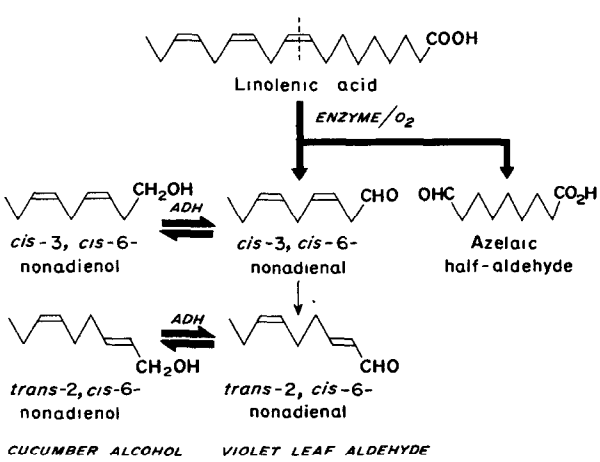
Table 6 Identification of the volatile components isolated from blended cucumber

Compound	Evidence	Relative proportions by GLC
n-Hexanal	IR, 2,4-DNPH* GLC	20
trans-2-Hexenal	IR, 2,4-DNPH, GLC	45
cis-2-Pentenol	IR GLC	41
n-Hexanol	IR, GLC	07
cis-3-Hexenol	IR, GLC	24
cis-3-Nonenal	MS IR NMR, 24-DNPH GLC	22
cis-3,*cis*-6-Nonadienal	MS, IR, NMR, 2,4-DNPH, GLC	32
trans-2-Nonenal	NMR, IR 2,4-DNPH, GLC	98
trans-2,*cis*-6-Nonadienal	NMR IR, 2,4-DNPH, GLC	526
cis-3-Nonenol	MS, IR, GLC	22
trans-2-Nonenol	MS, IR GLC	28
cis-3,*cis*-6-Nonadienol	MS, IR, GLC	47
trans-2 *cis*-6-Nonadienol	MS, IR, GLC	28
Azelaic half-aldehyde	IR, 2,4-DNPH, GLC	–

* 2,4-DNPH = 2,4-dinitrophenyl hydrazone derivative

were very unstable and readily isomerized to *trans*-2-aldehydes in cucumber homogenates (Table 5) they were immediately extracted with organic solvents from the homogenates and purified by high vacuum-distillation and preparative GLC. *cis*-3-Nonenal, *cis*-3,*cis*-6-nonadienal and azelaic half aldehyde were converted to the 2,4-dinitrophenylhydrazone derivatives [7] and their structures were fully substantiated by IR and NMR-spectral comparison with the authentic specimens synthesized by unequivocal routes [9].

The main components identified are shown in Table 6. These results suggest that the unsaturated fatty acids are first split into *cis*-3-aldehydes and oxo-acids, and subsequently *trans*-2-aldehydes are formed by the isomerization of *cis*-3-aldehydes.



Scheme 1 Proposed biosynthetic pathway for cucumber alcohol production

On the basis of these findings, the authors propose a possible biosynthetic pathway which may account for the formation of *trans*-2,*cis*-6-nonadienal via *cis*-3,*cis*-6-nonadienal and the corresponding alcohol, cucumber alcohol, from linolenic acid in cucumbers as shown in Scheme 1.

EXPERIMENTAL

NMR spectra (60 MHz) were obtained in $CDCl_3$ containing TMS as an internal reference

Materials Fresh cucumbers (*Cucumis sativus*, variety "chojituochiai V"), approximately 3 cm in diam, were used Authentic samples of *cis*-3-nonenal and *cis*-3,*cis*-6-nonadienal were synthesized [9]

Preparation of essential oil Fresh cucumbers (250 g) were blended with 250 ml H_2O in a Waring blender for 3 min and then the mixture was steam-distilled until 300 ml of distillate was collected After the distillate was saturated with NaCl, it was extracted 3× with 100 ml Et_2O The combined extract was dried over dry Na_2SO_4 After removal of Et_2O, the crude essential oil was made up to 2 ml with CCl_4.

Preparation of fatty acids A crude lipid fraction was obtained from 1 kg cucumbers by blending and subsequent extraction with 1 l of $CHCl_3$–MeOH (2 1) Crude lipid was separated into Me_2CO-soluble and Me_2CO-insoluble fractions Concentrate of the Me_2CO-soluble fraction was dissolved in Et_2O–petrol (1 1) Free fatty acids were extracted with 1% Na_2CO_3, acidified and extracted with Et_2O The material not extracted with Na_2CO_3 was saponified by alcoholic KOH and the unsaponifiable matter was removed by extraction with Et_2O The soln of residual potassium salts was acidified and fatty acids from the neutral fat fraction were extracted with Et_2O The phospholipid fraction was repeatedly extracted from the Me_2CO-insoluble fraction with $CHCl_3$–MeOH (1 1) and then the concentrate of the combined extracts was treated with 160 ml 20% HCl H_2O bath (100°C) for 14 hr under N_2 Fatty acids liberated from the phospholipid fraction were extracted with Et_2O Fatty acids from the various lipid fractions were esterified with CH_2N_2

Preparation of cis-3-unsaturated aldehydes Fifty 250 g portions of cucumbers were blended with 250 ml H_2O and 100 mg of either linolenic or linoleic acid in a Waring blender for 2 min Immediately the homogenate was filtered through two layers of gauze, then the filtrate was extracted with Et_2O After the combined extracts were concentrated, the concentrate was vacuum-distilled to give an aldehyde fraction without isomerization The aldehyde fraction was separated by preparative GLC on a PEG-20M column and the purified compounds were submitted to spectral analysis

Preparation of azelaic half aldehyde The fraction containing azelaic half aldehyde was extracted with $CHCl_3$ from the homogenate of fresh cucumbers The combined extracts were concentrated and the concentrate was esterified with CH_2N_2 Azelaic half aldehyde methyl ester was separated by GLC on a PEG-adipate column and the purified compound was submitted to spectral analysis

GLC Essential oil from cucumber was analyzed by GLC with a 3 mm × 3 m stainless steel column packed with 20% PEG-20M on celite 545, 60–80 mesh The column temp was programmed from 100 to 180° at 2° min GLC analyses of

fatty acids were performed on a 3 mm × 1 m stainless steel column packed with 20% PEG-adipate on Chromosorb W, 60–80 mesh. The column temp. was isothermal at 180°.

Effect of blending, heating, oxygen and pH on the formation of aldehydes. (1) Effect of blending: Volatile components of cucumbers were prepared without blending and other volatile components were prepared after blending for 3 min. (2) Effect of heating: Fresh cucumbers were immersed in a 60° water bath for 20 min. (3) Effect of oxygen: Fresh cucumbers were blended under N_2 or in the presence of air. (4) Effect of pH: Fresh cucumbers were blended with each pH of McIlvain's buffer. The essential oil from each sample was prepared as described above and analyzed by GLC.

Changes in fatty acids in each fraction during blending. The fatty acids for zero blending time were prepared from cucumbers inactivated by heating at 80° for 15 min; other samples were prepared after blending for 3 min according to the method described above.

Fatty acids derived from each fraction were analyzed by GLC.

Formation of aldehydes from unsaturated fatty acids. A soln of each fatty acid (200 mg), dissolved in a small amount of MeOH and H_2O, was blended with fresh cucumbers for 3 min. The essential oil was then isolated and analyzed by GLC.

Identification of cis-3,cis-6-*nonadienal, cis-3-nonenal and azelaic half aldehyde. cis*-3,*cis*-6-Nonadienal was purified as described above. IR ν_{max}^{film} cm^{-1}: 2710 (H–C=O), 1730 (C=O), 730 (*cis*, –CH=CH–). 2,4-DNPH: IR ν_{max}^{KBr} cm^{-1}: 3300 (–NH–), 1610 (–C=N–), 725 (*cis*, –CH=CH–). NMR ($CDCl_3$): δ 0·95 (3H, *t*, $C\underline{H}_3$–CH_2–), 2·03 (2H, *p*, Me–$C\underline{H}_2$–CH=), 2·80 (2H, *t*, =CH–$C\underline{H}_2$–CH=), 3·15 (2H, *t*, =CH–$C\underline{H}_2$–CH=N–), 5·28, 5·45 (4H, *m*, –$C\underline{H}$=$C\underline{H}$–CH_2–$C\underline{H}$=$C\underline{H}$–), 7·36 (1H, *t*, –CH_2–$C\underline{H}$=N–), 7·75 (1H, *d*, Ho), 8·13 (1H, *dd*, Hm'), 8·95 (1H, *d*, Hm), 10·88 (1H, *s*, =N–NH–). *cis*-3-Nonenal was purified in the same manner. IR ν_{max}^{film} cm^{-1}: 2710 (H–C=O), 1730 (C=O), 730 (*cis*, –CH=CH–). 2,4-DNPH: IR ν_{max}^{KBr} cm^{-1}: 3300 (–NH–), 1610 (–C=N–), 725 (*cis*, –CH=CH–). NMR ($CDCl_3$): δ 0·90 (3H, *t*, $C\underline{H}_3$–CH_2–), 1·48 (6H, *m*, Me–($C\underline{H}_2$)$_3$–CH_2–), 2·11 (2H, *q*, –CH_2–$C\underline{H}_2$–CH=), 3·20 (2H, *t*, =CH–$C\underline{H}_2$–CH=), 5·58 (2H, *m*, –$C\underline{H}$=$C\underline{H}$–), 7·51 (1H, *t*, –CH_2–CH=N–), 7·93 (1H, *d*, Ho), 8·34 (1H, *dd*, Hm'), 9·13 (1H, *d*, Hm), 11·08 (1H, *s*, –N=NH–). The IR-spectra of these aldehydes showed no absorption at 970 cm^{-1} for the *trans*-form. The IR and NMR-spectra of the natural compounds were identical with those of synthetic specimens. Azelaic half aldehyde methyl ester was purified as described above. IR ν_{max}^{film} cm^{-1}: 2710 (H–C=O), 1725 (C=O).

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