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DISTRIBUTION OF AN ENZYME SYSTEM PRODUCING *cis*-3-HEXENAL AND *n*-HEXANAL FROM LINOLENIC AND LINOLEIC ACIDS IN SOME PLANTS

AKIKAZU HATANAKA, JIRO SEKIYA and TADAHIKO KAJIWARA

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

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Abstract—The activity of the enzyme system (E_2-I) producing C_6-aldehydes from C_{18}-unsaturated fatty acids was investigated using about 40 plants. Green leaves of dicotyledonous plants belonging to the Sphenopsida, Pteropsida Theaceae and Leguminosae showed a high enzyme (E_2-I) activity but edible leafy vegetables and fuits and monocotyledonous plants showed a low activity. Seasonal changes in the enzyme (E_2-I) activities were observed. The concentrations of *cis*-3-hexenol (leaf alcohol) and *trans*-2-hexenal (leaf aldehyde) and the enzyme (E_2-I) activities showed a correlation; high concentrations were observed in the summer but they were low in the winter.

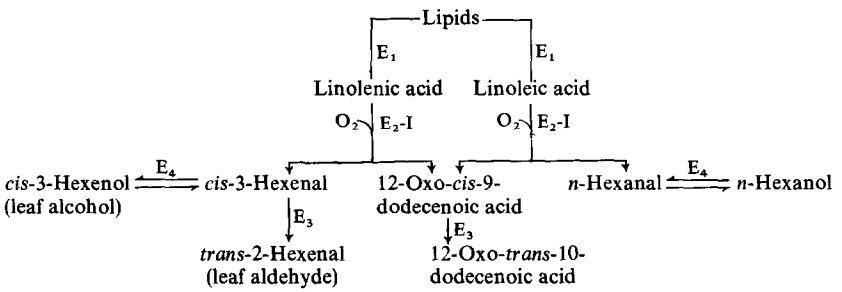
INTRODUCTION

cis-3-Hexenol (leaf alcohol) and *trans*-2-hexenal (leaf aldehyde), which are both responsible for the characteristic leaf odour, are distributed in a wide range of plants. Investigations using tea [1–9] and *Farfugium japonicum* [10, 11] clarified the biosynthesis of *cis*-3-hexenol, *trans*-2-hexenal and 12-oxo-*trans*-10-dodecenoic acid as shown in Scheme 1. Volatile C_6-aldehydes and C_{12}-oxo acid are produced by the oxidative cleavage of linolenic acids in the presence of oxygen and an enzyme system (E_2-I) which is localized in the chloroplast lamellae. It was also reported that C_6-aldehydes are formed via 13-hydroperoxides from linolenic and linoleic acids in etiolated watermelon seedling [12] and tomato fruit [13] and that C_{12}-compounds were formed from linoleic acid [12, 20, 22]. On the other hand, the distribution of linoleate-oxidizing activity in various fruits and vegetables has been reported [14–16]. However, it is not clear whether the oxidation of linoleic acid leads to volatile C_6-aldehyde formation or not. The present paper describes the distribution of the enzyme system (E_2-I) producing C_6-aldehydes from C_{18}-unsaturated fatty acids in some plants.

RESULTS AND DISCUSSION

Table 1 lists the enzyme activity producing hexenals (*cis*-3-hexenal and *trans*-2-hexenal) from linolenic acid and *n*-hexanal from linoleic acid in the homogenates of plant tissues (summer, 1976). Among the tissue homogenates of the plants tested, those from the dicotyledonous plants 2–4, 6, 11–14, 16, 17(L), 18, 21–24, 26 and 27, which were green leaf tissues, showed a high enzyme (E_2-I) activity. In contrast, the homogenates of Musci, edible leafy vegetables, fruits and monocotyledonous plants showed a low or no activity. Marine algae, *Ishigefaliacea*, *Ulva pertusa* and *Chlorella* sp., had no enzyme activity producing C_6-aldehydes.

The enzyme (E_2-I) activity in a 4000 *g* pellet, corresponding to the chloroplast rich fraction of green leaf tissues, showed the same distribution as that of the whole tissue homogenates. The same results were observed in



Scheme 1. Biosynthetic pathway for production of C_6- and C_{12}-compounds from linolenic and linoleic acids. E_1: lipolytic acyl hydrolase. E_2-I: enzyme system producing C_6-aldehydes E_3: enzymic and/or nonenzymic isomerization factor. E_4: alcohol dehydrogenase.

Table 1. Distribution and activity of enzyme system producing C_6-aldehydes in summer

Plant no. and common name	Class	Family	Genus and species	Organ*
1. Hair moss	Musci	Polytrichaceae	*Pogonatum inflexum*	L
2. Horsetail	Sphenopsida	Equisetaceae	*Equisetum arvense*	L
3. Osmund	Pteropsida	Osmundaceae	*Osmunda japonica*	L
4. Bracken		Polypodiaceae	*Pteridium aquilinum*	L
5. Ginkgo	Ginkgopsida	Ginkgoaceae	*Ginkgo biloba*	L
6. Chinese black pine	Coniferopsida	Podocarpaceae	*Podocarpus macrophylla*	L
7. Melon	Dicotyledoneae	Cucurbitaceae	*Cucumis melo*	L
8. Cucumber			*Cucumis sativus*	F
				L
9 Cabbage		Cruciferae	*Brassica oleracea* var. *capitata*	L(green)
				L(pale green)
10. Cauliflower			*Brassica oleracea* var *botrytis*	floret
11. Radish			*Raphanus sativus*	L
12. Sakaki		Theaceae	*Cleyera japonica*	L
13. Tea			*Thea sinensis*	L
14. Camellia			*Camellia japonica*	L
15. Sasanqua			*Camellia sasanqua*	L
16. Japanese persimmon		Ebenaceae	*Diospyros kaki*	L
17 Strawberry		Rosaceae	*Fragaria grandiflora*	F(red)
				L
18 Japanese apricot		Amygdalaceae	*Prunus mume*	L
19. Peach			*Prunus persica*	L
20 Japanese wistaria		Leguminosae	*Wisteria floribunda*	L
21. False acacia			*Robinia pseudoacacia*	L
22. Alfalfa			*Medicago sativa*	L
23. White clover			*Trifolium repens*	L
24. Soybean			*Glycine max*	L
25. Kidney bean			*Phaseolus vulgaris*	L
26. Mulberry		Moraceae	*Morus bombycis*	L
27. Japanese maple		Aceraceae	*Acer palmatum*	L(green)
				L(red)
28. Spinach		Chenopodiaceae	*Spinacia oleracea*	L
29. Potato		Solanaceae	*Solanum tuberosum*	tuber
30. Egg plant			*Solanum melongena*	L
31. Tomato			*Lycopersicon esculentum*	F(Pink)
				L
32. Lettuce		Compositae	*Lactuca sativa*	L
33. Banana	Monocotyledoneae	Musaceae	*Musa paradisiae*	F
34. Onion		Liliaceae	*Allium cepa*	L
35. Duckweed		Lemnaceae	*Lemna polyrhiza*	L
36 Rice		Gramineae	*Oryza sativa*	L
37. Wheat			*Triticum aestivum*	L

*L: leaf. F: fruit.
†Plant materials were harvested in late May–July 1976, except for alfalfa in September.
‡Total hexenals: *cis*-3- and *trans*-2-hexenal.

the experiment performed in June–July 1977. The result obtained in green leaves is similar to that obtained in tea leaves; the enzyme system (E_2-I) is localized in chloroplast lamellae of green leaves [1–9].

Ginkgo leaf, strawberry fruit and banana fruit showed only a slight enzyme activity, though these tissues were often used for experiments on C_6-aldehyde formation [17–20].

Rhee *et al.* [14] and Pinsky *et al.* [16] reported that some plants belonging to Leguminosae and Solanaceae had a high linoleate-oxidizing enzyme activity but that most of the leafy vegetables and fruits had little or no activity. Holden [15] also described a linoleate-oxidizing activity in some green leaves. Although the distribution of the enzyme (E_2-I) activity producing C_6-aldehydes was partially similar to that of linoleate-oxidizing activity (e.g. in Leguminosae, edible leafy vegetables and fruits) the plants with a high linoleate-oxidizing activity (e.g. Solanaceae) did not always show a high enzyme (E_2-I) activity. Thus the enzyme (E_2-I) activity producing C_6-aldehydes appears to be distributed in a different manner to the linoleate-oxidizing activity. However, it is not clear yet whether green leaf tissues with a moderate to high enzyme activity producing C_6-aldehydes involve lipoxygenase in the enzyme system (E_2-I) or not. The fact that the enzyme system (E_2-I) is localized in the 4000 *g* pellet suggests that most of these enzyme systems are a similar type of enzyme system to that in tea chloroplasts.

Seasonal changes in the activities of enzyme system (E_2-I) in tea leaves were described previously; they were high in summer and low in winter [6, 7]. As shown in Table 2, the enzyme activity of radish and Theaceae

Activity of enzyme system producing C_6-aldehydes†										
Fresh tissue homogenate (μg/g fr. wt)				4000 *g* precipitate fraction (μg/g ppt.)					Isomerization rate‖	
cis-3-hexenal	*trans*-2-hexenal	total hexenals‡	*n*-hexanal	*cis*-3-hexenal	*trans*-2-hexenal	total hexenals‡	*n*-hexanal	Degree of activity	Fresh tissue homogenate	4000 *g* pellet
0	0	0	19	90	16	106	230	L	0	2
1004	57	1061	1507	1776	481	2257	1877	H	5	21
214	107	321	966	3816	54	3870	2298	H	33	1
85	321	406	702	290	458	748	1580	H	79	61
0	24	24	6	132	63	195	48	L	100	32
577	165	742	109	1710	284	1994	388	H	22	14
2921	316	3237	430	—	—	—	—	H	10	—
26	14	40	42	162	27	189	62	L	35	14
0	0	0	88	24	8	32	23	L	0	25
532	123	655	52	150	71	221	117	M	19	32
555	33	588	36	600	347	947	184	M	6	37
49	7	56	12	48	19	67	48	L	13	28
1012	93	1105	357	216	101	317	781	H	8	32
854	14	868	292	2544	189	2733	1403	H	2	7
382	279	661	318	3000	134	3134	1785	H	42	4
389	5	392	150	498	0	498	604	M	2	0
105	7	112	61	792	102	894	209	L	6	11
621	79	700	141	0	1712	1712	3427	H	11	100
11	21	32	55	108	115	223	101	L	66	52
692	61	753	256	1512	95	1607	1137	H	8	6
623	85	708	342	3000	521	3521	1115	H	12	15
386	14	400	52	—	—	—	—	M	4	—
68	9	77	154	288	27	315	56	L	1	9
1037	263	1300	439	5376	499	5875	1124	H	20	8
1029	127	1156	509	—	—	—	—	H	11	—
566	104	670	110	36	324	360	250	M	16	90
299	70	369	1375	552	402	954	995	H	19	42
1025	410	1435	61	360	324	684	209	M	29	47
820	58	878	124	576	133	709	144	M	7	19
769	9	778	368	2820	101	2921	1510	H	2	3
555	2	557	287	3372	303	3675	3014	H	0	8
179	86	265	73	96	0	96	94	L	33	0
0	0	0	27	96	16	112	41	L	0	14
224	38	262	30	—	—	—	—	M	15	—
26	0	26	6	240	38	278	71	L	0	14
171	203	374	81	348	0	348	174	L	54	0
0	0	0	6	60	8	68	17	L	0	12
0	175	175	84	360	177	537	115	L	100	33
38	0	38	30	12	8	20	38	L	0	40
54	0	54	36	510	55	565	200	L	0	10
26	0	26	1	300	8	308	17	L	0	3
288	57	345	15	0	55	55	27	L	17	100

§H, M, L show high, moderate and low enzyme activity, respectively.

‖Isomerization rate (%) = $\frac{\textit{trans}\text{-2-hexenal}}{\text{total hexenals}} \times 100$.

decreased in winter. Spinach exhibited little activity both in summer and winter. However, that of chinese black pine almost doubled in winter. Thus enzyme (E_2-I) activity in most plants changes during the seasons.

As shown in Scheme 1, *cis*-3-hexenal is at first produced from linolenic acid, then rapidly isomerized to *trans*-2-hexenal. The isomerization rate varied widely both in the tissue homogenate and 4000 *g* pellet (Table 1).

Table 3 shows the concentrations of *n*-hexanal, *trans*-2-hexenal, and *cis*-3-hexenol in tissue homogenates in summer (*cis*-3-hexenal was not detected because of complete isomerization to *trans*-2-hexenal during steam-distillation). A high concentration of *trans*-2-hexenal was observed in leaf homogenates (plants 6, 11–13, 16, 18, 21 and 27) which also showed a high enzyme (E_2-I) activity. A high concentration of *cis*-3-hexenol was found in plants 12, 13, 21 and 27. But in plants 6, 11 and 16 little or no *cis*-3-hexenol was detected, although the plants contained a large amount of *trans*-2-hexenal. This was probably due to lack of alcohol dehydrogenase (E_4) as found with *F. japonicum* [10]. In winter, the concentration of *trans*-2-hexenal was decreased and *cis*-3-hexenol was barely detectable. This seemed to be due to the decrease in the activity of enzyme system (E_2-I) and/or lipolytic acyl hydrolase (E_1) which catalyses the hydrolysis of lipids. Takei *et al.* [21] also reported the wide distribution of *cis*-3-hexenol and *trans*-2-hexanal with high concentrations in summer and low amounts in winter. *n*-Hexanal showed similar seasonal changes to that of *trans*-2-hexenal, though its concentration was low.

The fact that the enzyme (E_2-I) activity and the concentrations of *trans*-2-hexenal and *cis*-3-hexenol are very low in edible leafy vegetables and fruits suggests

a reason for the selection of these vegetables and fruits during a long period of cultivation.

Table 2. Activity of the enzyme system producing C_6-aldehydes in the winter

		Enzyme activity producing C_6-aldehydes*			
		Fresh tissue homogenate (μg/g fr. wt)		4000 g pellet (μg/g ppt.)	
Plant no.	Organ	Total hexenals†	*n*-hexanal	Total hexenals†	*n*-hexanal
6	L	1524	153	4877	804
11	L	999	104	458	366
12	L	157	91	856	1109
13	L	200	75	376	456
14	L	283	95	424	811
23	L	434	660	139	304
28	L	80	36	108	192

*Plant materials were harvested in November–December 1976.
†Total hexenals: *cis*-3- and *trans*-2-hexenal.

Table 3. Concentrations of *n*-hexanal, *trans*-2-hexenal and *cis*-3-hexenol in the essential oil of tissue homogenates in the summer

		Concentration (μg/g fr. wt)		
Plant no.	Organ	*n*-hexanal	*trans*-2-hexenal	*cis*-3-hexenol
2	L	0.97	1.65	0
6	L	0.07	121.0	tr
9	L(green)	tr	3.30	tr
11	L	0.14	20.70	0
12	L	tr	8.36	5.94
13	L	tr	6 47	3 87
14	L	0.10	7 70	1.78
16	L	tr	16.0	tr
18	L	1.20	41.20	1.85
21	L	tr	26 10	2.82
27	L(green)	2.38	208.20	6.34
	L(red)	1.30	39 07	2.86
28	L	tr	0	0
31	F	tr	tr	tr
	L	0 94	29.26	tr

tr = trace.

EXPERIMENTAL

Fresh plant materials were harvested near the University immediately before use except for plants 29 and 30 which were obtained from a local market Enzyme activity (E_2-I) producing C_6-aldehydes in a crude tissue homogenate was assayed with linolenic or linoleic acid substrate by method I previously described [4, 7]. Enzyme activity (E_2-I) in a 4000 g pellet prepared by the method of [4, 7], was assayed by method II previously described [4, 7]. Preparation of essential oil and quantitative analysis of C_6-compounds were the same as previously described [6].

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