Pergamon

PII: S0031-9422(97)00483-8

Phytochemistry, Vol. 47, No. 1, pp. 27–29, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

VOLATILES FROM *ZOSTERA MARINA*

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(*Received in revised form* 13 *May* 1997)

Key Word Index—*Zostera marina*; Zosteraceae; seagrass; volatile compounds; long-chain aldehyde formation; biogeneration.

Abstract—The essential oil from the shoots of the marine embryophyte, *Zostera marina* was prepared by simultaneous distillation-extraction. Volatile compounds in the oil were identified by GC and GC-mass spectrometry. The major constituents were phytol, hexadecanamide, octadecanamide, pentadecane, heptadecane, nonadecane, (8*Z*,11*Z*)-heptadecadienal (HDD), (8*Z*)-heptadecenal (HD), (9*Z*,12*Z*,15*Z*)-octadecatrienal and (9*Z*,12*Z*)-octadecadienal. HDD and HD were shown to be formed enzymatically from linoleic acid and oleic acid, respectively. © 1997 Elsevier Science Ltd

INTRODUCTION

Long chain aldehydes (LCA), such as pentadecanal, 8-heptadecenal, 8,11-heptadecadienal and 8,11,14-heptadecatrienal were found first in cucumber homogenates [1]. Subsequently, (8*Z*,11*Z*,14*Z*)-heptadecatrienal (HDT), (8*Z*,11*Z*)-heptadecadienal (HDD), (8*Z*)-heptadecenal (HD), (7*Z*,10*Z*,13*Z*)-hexadecatrienal and pentadecanal (PD) were identified as characteristic volatile compounds in species of the Ulvaceae [2]. Recently, we have demonstrated that LCA are formed enzymatically from fatty acids and that LCA-forming activity is widely distributed in marine algae, including green, brown and red types [3, 4]. However, the marine embryophyte, *Zostera marina* [5] has not been explored so far. We report herein on the volatile compounds and LCA-forming activity in this seagrass.

RESULTS AND DISCUSSION

Zostera marina is known as a seaweed bed for fish and shellfish. The seagrass were collected along the coast of an inland sea (Aio, Yamaguchi, southern Japan). Fresh shoots were subjected to simultaneous distillation extraction (SDE) to give an essential oil with a characteristic odour. The volatile compounds in the oil were identified by comparison of Kovats indices and GC-mass spectral data with those of authentic compounds (Table 1). Phytol, hexadecanamide, octadecanamide, pentadecane, heptadecane, nonadecane and LCA (5.35%), such as (8*Z*,11*Z*,14*Z*)-heptadecatrienal (HDT), (8*Z*,11*Z*)-heptadecadienal (HDD), (8*Z*)-heptadecenal (HD), (9*Z*,12*Z*,15*Z*)-octadecatrienal and (9*Z*,12*Z*)-octadecadienal were identified as major components. The oil yield (0.7%) from the seagrass was higher than that from the marine green alga, *Ulva pertusa* (≈0.007%) [2, 6, 7]. The composition of volatile components of the seagrass oil are essentially similar to that of the algal oil. However, with the seagrass oil, nitrogen compounds, such as 1-pyrroline, 4-methylpyridine and nicotine were detected as characteristic components. (7*Z*,10*Z*,13*Z*)-Hexadecatrienal, (6*Z*,9*Z*,12*Z*)-pentadecatrienal, *n*-pentadecanal, *n*-tetradecanal, (2*E*)-nonenal and (2*E*)-octenal, which were identified in the *Ulva* oil were not detected in the seagrass oil.

In a preliminary experiment, fresh shoots of *Z. marina* were homogenized in Na-Pi buffer (pH 9.0) and the homogenate incubated at 30° with oleic acid (OA), linoleic acid (LA) and linolenic acid (LNA), respectively. The reacted mixture was subjected to SDE, and the essential oil thus obtained was semi-quantitatively analysed by GC and GC-mass spectrometry. The amounts of HDT, HDD and HD in the volatile compounds increased during the incubation with the fatty acids (data not shown). Subsequently, the LCA products were converted to their 2,4-dinitrophenylhydrazone derivatives and the amounts quantified by HPLC analysis. The amounts of HDT, HDD and HD were confirmed to increase during incubation and to be produced from LNA, LA and OA, respectively. The substrate specificity for LCA-forming activity from the seagrass was shown to be similar to that from the fronds of *U. pertusa* (Table 2), while the LCA-forming activity on a fresh weight basis of the seagrass was lower than that of the green alga.

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Table 1. Volatile components in shoots of *Zostera marina*

Compound	Peak area (%)
⟨Acetogenins⟩	
Hexanal	0.4
(2*Z*)-Hexenal	0.9
2,4-Heptadienal	Trace
Tridecanal	0.2
(8*Z*, 11*Z*)-Heptadecadienal	0.7
(8*Z*, 11Z, 14*Z*)-Heptadecatrienal	0.5
(8*Z*)-Heptadecenal	1.1
(9*Z*, 12*Z*, 15*Z*)-Octadecatrienal	1.3
(9*Z*, 12*Z*)-Octadecadienal	1.8
3-Penten-2-ol	0.2
2-Pentanol	0.3
2-Pentenol	Trace
(3*Z*)-Octenol	0.6
Pentadecanol	Trace
Nonadecanol	Trace
2,3-Butanedione	Trace
2-Heptadecanone	Trace
Hexanoic acid	Trace
Tetradecanoic acid	Trace
Hexadecanoic acid	0.3
Hexadecanamide	1.1
Octadecanamide	1.2
Tridecane	5.0
Tetradecane	0.2
Pentadecane	19.4
Hexadecane	0.7
Heptadecane	9.3
Octadecane	Trace
Nonadecane	3.0
Methyl palmitate	Trace
Methyl linoleate	Trace
⟨Mevalogenins⟩	
2-Methyl-3-buten-2-ol	0.3
Epicubenol	Trace
δ-Cadinol	Trace
Isophytol	0.3
Phytol	2.3
Geranylacetone	Trace
β-Ionone	1.0
6,10,14-Trimethyl-2-pentadecanone	Trace
δ-Cadinene	Trace
γ-Muurolene	Trace
1-Pyrroline	0.4
4-Methylpyridine	2.3
Nicotine	Trace

Trace: less than 0.1%.

LCA-forming activity has been reported for 46 species of brown, red and green algae [8]. On the other hand, α-oxidation activity producing pentadecanoic acid via PD and PA is reported in terrestrial plants, such as cucumber fruits [9, 10], germinated peanut cotyledons [11], pea leaves [12], potato tubers [13], rice seedings [14] and tobacco leaves [15]. Thus, LCA-forming activity might occur widely in the plant kingdom, including marine and terrestrial plants [16, 17]. The activity in the marine embryophyte will be of interest from an evolutionary point of view from ancestral green algae to the earliest land plants [5].

EXPERIMENTAL

Materials. Fresh shoots of *Z. marina* and fresh fronds of *U. pertusa* were collected along the Aio coast in Yamaguchi, southern Japan, in June 1996. Fatty acids (over 98% purity) (Wako) were used as substrates after purification by CC.

Identification of volatile components. Fr. shoots (10 g) were homogenized with distilled H_2O (500 ml) and the homogenate immediately subjected to SDE. Extraction of the distillate (700 ml) with pentane–CH_2Cl_2 (200 ml × 3, 1:1) gave an essential oil (70 mg: yield 0.7%). Volatile compounds in the oil were identified by comparison of Kovats R_I and GC-MS data with those of authentic specimens. FID-GC was performed using a fused-silica capillary column 0.28 mm (i.d.) × 50 m coated with SF-96. The column temp. was held at 70° for 5 min and programmed to increase at 2° min^{-1} from 70 to 220°. GC-MS were recorded using a fused-silica capillary column 0.28 mm (i.d.) × 40 m coated with SF-96. The column temp. was programmed to increase from 75 to 190° at 3° min^{-1} (25 min hold at 190°) and then 190 to 210° at 3° min^{-1}. The ionization energy was 20 eV.

Assay of LCA-forming activity. Fresh shoots (15 g) were homogenized with 30 ml of 0.1 M Na-Pi buffer (pH 9) containing 0.2% Triton X-100. The homogenate was filtered through four layers of cheesecloth. The fatty acid substrate (5 μmol) was added to the filtrate (3 ml) and the mixt. incubated at 25° for 1 hr. The reaction mixt. was subjected to SDE to give an essential oil. The oil was analysed by GC (SF-96 capillary column, 0.28 mm × 50 m) and GC-MS (SF-96, 0.28 mm × 40 m, 20 eV). Aldehydes formed during the incubation were analysed quantitatively by HPLC as 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives. Each reaction mixt. was added to a 0.1% EtOH–HOAc soln of 2,4-dinitrophenylhydrazine (3 ml, pH 4). The hydrazone derivatives thus obtained were extracted with hexane (20 ml × 3) and the combined extracts washed with satd NaCl soln and then dried (Na_2SO_4). The extract was evapd *in vacuo* to leave the solid 2,4-DNPH derivatives. These were dissolved in MeCN (0.3 ml) and a portion (5 μl) was analysed by HPLC with a UV detector at 349 nm using a Zorbax ODS column 4.6 mm (i.d.) × 150 mm MeCN–H_2O–THF (90:9:1), flow rate 1 ml min^{-1} and 70 kg cm^{-2}.

Acknowledgements—This study was supported in part by a Grant-in-Aid (no. 07660140) from the Ministry of Education, Science and Culture, Japan. The authors thank Dr Naomichi Banba, Faculty of Agriculture, Okayama University, for critically reading the manuscript.

Table 2. Comparison of LCA-forming activities in *Zostera marina* and *Ulva pertusa*

Substrate	Product	Aldehyde forming activity (n mol g^{-1} fr. wt) *Z. marina*	*U. pertusa*
Palmitic acid	Pentadecanal (PD)	4.0	180.2
Oleic acid	(8*Z*)-Heptadecenal (HD)	2.4	77.3
Linoleic acid	(8*Z*, 11*Z*)-Heptadecadienal (HDD)	3.0	142.8
Linolenic acid	(8*Z*, 11*Z*, 14*Z*)-Heptadecatrienal (HDT)	3.9	160.9

The fatty acid substrate (5 μmol) was added to the crude enzyme solution (3 ml) and the mixture incubated at 25° for 1 hr.

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