Volatile Composition of Oyster Leaf (*Mertensia maritima* (L.) Gray)

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Ⓢ Supporting Information

ABSTRACT: Oyster leaf (*Mertensia maritima*), also called vegetarian oyster, has a surprising oyster-like aroma. Its volatile composition was investigated here for the first time. In total, 109 compounds were identified by gas chromatography–mass spectrometry (GC-MS) and quantified by GC-FID. The use of GC–olfactometry on both polar and nonpolar columns allowed the detection of the molecules having an oyster-like, marine odor. Four compounds were identified and confirmed by synthesis: (Z)-3-nonenal, (Z)-1,5-octadien-3-ol, (Z,Z)-3,6-nonadienal, and (Z)-1,5-octadien-3-one. After evaluation of freshly prepared reference samples, these compounds were confirmed to be reminiscent of the oyster-like marine notes perceived in the tasting of cut leaves.

KEYWORDS: oyster leaf, Mertensia maritima, aroma compounds, GC-MS, oyster, marine

■ INTRODUCTION

Oyster leaf (*Mertensia maritima*) has a weak odor, but when scratched, it has first a green, slightly mushroom odor, followed by some surprising marine, oyster-like notes. When eaten, the retronasal aroma is extremely reminiscent of oyster. This explains why oyster leaf is also called vegetarian oyster.

M. maritima (L.) Gray (Boraginaceae) is a perennial, nonclonal, tetraploid herb generally found on northern exposed ocean beaches.[1] Mature plants produce flowers having bell-like shapes and blue colors. The leaves, shown in Figure 1, are ovate or elliptic, entire, usually slightly arched, with numerous chalk glands on the upper surface.

According to Scott,[2] oyster leaf is native to Britain and northern Europe. Today it is found in Scotland, northern England, and Ireland, but its population is decreasing. It is probably extinct now in northeast Denmark and absent from most of England and southern Europe. An Arctic variety, *Mertensia maritima* var. *tenella*, is found in Canada and

Spitzberg, and the variety *Mertensia maritima* var. *asiatica* is confined to the Pacific coast of northeastern Asia and North America (Alaska). *M. maritima* grows next to the sea on sandy or shingly beaches. The presence of humus resulting from seaweed degradation seems to be correlated with plant growth, although it has not been established as essential.[2]

With the increasing interest in new edible plants having unique flavors or aromas, oyster leaves are now grown in the Hebrides (northern Scotland) and in southwestern France (Bassin d'Arcachon).

To the best of our knowledge, the volatile composition of oyster leaves has never been reported in the literature. Despite the similar habitat of oyster leaves and oysters, it is not excluded that the plant may have developed different biosynthetic pathways from those of oysters, leading to a different diversity of volatile molecules. In contrast to the oyster leaf, the volatiles found in oysters have already been investigated by gas chromatography–mass spectrometry (GC-MS). All of the reported studies were, however, carried out on polar phase only. In 1985, Josephson et al. investigated the volatile compounds of fresh Pacific and Atlantic oysters (*Crassostrea gigas* and *Crassostrea virginica*, respectively).[3] (E,Z)-2,6-Nonadienal and 3,6-nonadien-1-ol were found to contribute to the pronounced melon-like flavor of Pacific oysters, and 1-octen-3-one, 1-octen-3-ol, 1,5-octadien-3-one, 1,5-octadien-3-ol, and 2,5-octadien-1-ol were reported to contribute to the heavy, green aroma of both species. In this study, the configuration of the double bond of these molecules was not determined. In 2000, Piveteau et al. identified 50 volatiles in ground fresh oysters *C. gigas* by dynamic headspace GC-MS.[4] Nine panelists detected 42 odors by GC–olfactometry, and 12 odors were attributed to identified

Figure 1. Oyster leaves analyzed in the present study. (Photo: A. Jaquier, Firmenich SA.)
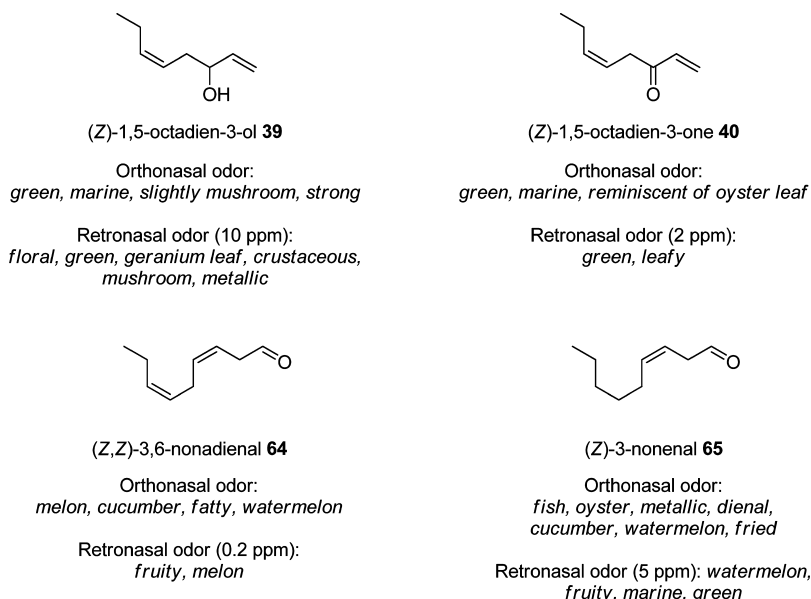
Figure 2. Odor (orthonasal and retronasal) evaluation of pure compounds **39**, **40**, **64**, and **65** with the concentration in water used for tasting.

volatiles: dimethyl sulfide, 1-penten-3-one, 2,3-pentanedione, hexanal, (E,E)-2,4-heptadienal, 1-octen-3-one, 1-octen-3-ol, 6-methyl-5-hepten-3-one, octanal, (E,Z)-2,6-nonadienal, (E)-2-octenal, and decanal. Four odors were reported during GC–olfactometry with the "marine" descriptor; however, only one could be attributed to a molecule, namely, decanal. In 2002, the analysis was repeated by extracting whole raw oyster flesh (C. gigas) by vacuum steam distillation at 20 °C.[5] As oysters are sensitive to heat and oxidation, the authors used milder conditions to avoid generating off-flavors and obtained an extract more representative of raw oyster than dynamic headspace. In total, 59 volatiles were identified. Among them, 25 were mentioned to be responsible for the overall odor of raw oyster. Four compounds in particular were attributed to the fresh and marine odors detected by GC–olfactometry: (E)-3-hexen-1-ol, decanal, 2-undecanone, and (E,Z)-3,6-nonadien-1-ol.

As the volatile composition of oyster leaf has never been reported, an in-depth GC-MS analysis was performed on an oyster leaf extract obtained by hydrodistillation under reduced pressure. GC–olfactometry on two orthogonal phases was used to identify the volatile molecules, which may contribute to the oyster-like character of the leaves. Synthetic work was performed to obtain pure standards of these molecules to confirm their identification and evaluate their organoleptic properties.

■ MATERIALS AND METHODS

Oyster Leaf Sourcing. Oyster leaves, M. maritima, grown in the Hebrides (northern Scotland) were supplied by Koppert Cress (Monster, The Netherlands).[6]

Chemicals. For the syntheses, the chemicals were supplied as follows: LiAlH₄ and tert-butyl hydroperoxide (Aldrich, Sigma-Aldrich Chemie, Steinheim, Germany); Vo(acac)₂ (Fluka, Sigma-Aldrich Chemie, Steinheim, Germany); Lindlar catalyst, Zn powder, bis-(cyclopentadienyl)titanium dichloride Cp₂TiCl₂, ZnCl₂, and Dess–Martin periodinane (Acros, Thermo Fisher Scientific, Geel, Belgium). For the identifications, **1**–**12**, **14**–**21**, **23**, **25**–**29**, **32**, **33**, **35**, **36**, **38**, **41**, **42**, **46**–**48**, **52**–**54**, **56**, **58**, **60**, **61**, **66**–**68**, **72**, **73**, **78**, **80**, **83**, **84**, **87**, **95**, **97**, **102**, **105**, **110**, and **111** are available from Aldrich (Sigma-Aldrich Chemie). **24**, **51**, **74**, and **86** were purchased from Alfa Aesar

(Karlsruhe, Germany). **31** was obtained from Acros (Thermo Fisher Scientific). **45** is an in-house ingredient, but also available via Aurora Fine Chemicals (Gratz, Austria). **76**, **77**, and **79** were purchased from Bedoukian (Danbury, CT, USA). **101** was purchased from VWR International, LLC (West Chester, PA, USA). **108** was purchased from Advanced ChemBlocks, Inc. (Burlingame, CA, USA). The following compounds were prepared as described previously: **44**,[7] **49**,[8] **57**,[7] **69**,[9] **70**,[10] **81**,[7] **94**,[7] **100**,[11] **104**,[12] and **106**.[13] **98** was synthesized according to Knochel's method via the reaction of stabilized vinyl Grignard with aldehydes.[14] **37**, **39**, **40**, **64**, and **65** were synthesized as described hereafter.

Oyster Leaf Hydrodistillation. Oyster leaves (352 g) in deionized water (800 mL) were mixed for 1 min in a blender (Krups type 577, Bassersdorf, Switzerland). The resulting puree was transferred to a round flask (3 L), and the mixer vessel was rinsed with deionized water (200 mL). The hydrodistillation was performed with a Büchi R124/E (Flawil, Switzerland) connected to a frozen trap and kept under reduced pressure with the following program: 25 mbar at 32−33 °C for 60 min, then 20 mbar at 33 °C for 45 min, 15 mbar at 33 °C for 30 min, and 10 mbar at 33 °C for 15 min. At the end of the process, the frozen trap was allowed to warm to room temperature, and the resulting liquid (46 mL) was added to the distillate (960 mL). NaCl, puriss, was added until saturation and then the solution was extracted with diethyl ether (SDS, for analysis, 3 × 120 mL). The combined organic phases were dried over anhydrous MgSO₄. The solvent was removed using a Vigreux column at atmospheric pressure, and the oyster leaf extract (80 mg) was immediately injected into GC-MS and GC–olfactometry instruments.

Odor Evaluation. The orthonasal odors of the oyster leaf extract and the synthesized (Z)-1,5-octadien-3-ol **39**, (Z)-1,5-octadien-3-one **40**, (Z,Z)-3,6-nonadienal **64**, and (Z)-3-nonenal **65** were evaluated on paper smelling strips by a panel of expert perfumers and flavorists. The retronasal odors of the synthesized compounds were described after each of them had been tasted in water (concentration given in Figure 2) by a panel of expert flavorists. All of these evaluations were performed without any information given to the assessors (context of the project, molecule structure, comments received from the other assessors).

GC-MS. A 0.5 μL aliquot of the oyster leaf extract was injected into a GC-MS 6890/5975 (Agilent, Santa Clara, CA, USA) equipped with a nonpolar SPB-1 capillary column (30 m × 0.25 mm, film thickness = 1 μm, Supelco, Bellefonte, PA, USA). The temperature program was as follows: 60 °C, 5 min isotherm, then 5 °C/min to 250 °C; injector

Table 1. Volatile Composition of Oyster Leaf Hydrodistillate

	$LRI_{exp\ nonpol}$[a]	$LRI_{ref\ nonpol}$[b]	$LRI_{exp\ pol}$[c]	$LRI_{ref\ pol}$[b]	name	%[d]	identification[e]
1	598	593	887	877	ethyl acetate	tr[f]	LRI, MS, ref
2	601	601	ND[g]	1013	trichloromethane[h]	tr	LRI, MS, ref
3	606	607	ND	1088	2-methyl-1-propanol	tr	LRI, MS, ref
4	617	622	1041	1047	(E)-2-butenal	tr	LRI, MS, ref
5	626	627	917	910	3-methylbutanal	tr	LRI, MS, ref
6	636	637	914	907	2-methylbutanal	tr	LRI, MS, ref
7	654	655	1020	1008	1-penten-3-one	tr	LRI, MS, ref
8	657	665	1158	1161	1-penten-3-ol	3.04	LRI, MS, ref
9	668	669	977	976	3-pentanone	tr	LRI, MS, ref
10	712	715	ND	1243	3-methyl-3-buten-1-ol	tr	LRI, MS, ref
11	716	725	1203	1204	3-methyl-1-butanol	0.06	LRI, MS, ref
12	720	725	ND	1218	2-methyl-1-butanol	tr	LRI, MS, ref
13	726		1109		tentative: (Z)-2-pentenal	tr	MS
14	726	723	1131	1129	(E)-2-pentenal	tr	LRI, MS, ref
15	747	746	1248	1254	1-pentanol	tr	LRI, MS, ref
16	749	750	1318	1323	(Z)-2-penten-1-ol	0.60	LRI, MS, ref
17	749		1310	1321	(E)-2-penten-1-ol	tr	LRI, MS, ref
18	773	773	1153	1156	(Z)-3-hexenal	2.08	LRI, MS, ref
19	773	773	1133	1120	4-methyl-3-penten-2-one	tr	LRI, MS, ref
20	776	780	1083	1070	hexanal	0.96	LRI, MS, ref
21	811	813	ND	1154	(E)-2-methyl-2-pentenal	tr	LRI, MS, ref
22	820		1203		tentative: (Z)-2-hexenal	0.06	MS
23	827	822	1220	1216	(E)-2-hexenal	1.61	LRI, MS, ref
24	834	836	1361	1354	(E)-3-hexen-1-ol	0.12	LRI, MS, ref
25	839	836	1384	1376	(Z)-3-hexen-1-ol	8.72	LRI, MS, ref
26	848	847	1403	1397	(E)-2-hexen-1-ol	tr	LRI, MS, ref
27	851	849	1351	1349	1-hexanol	1.45	LRI, MS, ref
28	863	865	ND	1322	1-hepten-3-ol	tr	LRI, MS, ref
29	867	873	1450	1454	3-(methylthio)propanal	tr	LRI, MS, ref
30	880		1405		tentative: (E,Z)-2,4-hexadienal	0.21	MS
31	880	884	1400	1391	(E,E)-2,4-hexadienal	0.15	LRI, MS, ref
32	896	899	ND	1609	2-ethylpyrrole	tr	LRI, MS, ref
33	906	903	1213	1213	diethyl disulfide	tr	LRI, MS, ref
34	912		ND		tentative: (E)-4-hepten-3-one	tr	MS
35	932	934	ND	1325	(E)-2-heptenal	tr	LRI, MS, ref
36	936	930	1018	1021	α-pinene	tr	LRI, MS, ref
37	954	952	1459	1457	(E)-1,5-octadien-3-ol	0.15	LRI, MS, ref
38	956	952	1303	1296	1-octen-3-one	tr	LRI, MS, ref
39	962	957	1484	1483	(Z)-1,5-octadien-3-ol	47.83	LRI, MS, ref
40	962	960	1380	1381	(Z)-1,5-octadien-3-one	tr	LRI, MS, ref
41	967	961	1452	1444	1-octen-3-ol	13.36	LRI, MS, ref
42	969	964	1256	1258	3-octanone	tr	LRI, MS, ref
43	970		1326		tentative: (Z)-5-octen-3-one	tr	MS
44	972	970	1468	1467	(E,Z)-2,4-heptadienal	tr	LRI, MS, ref
45	977	973	1339	1332	(Z)-5-octenal	tr	LRI, MS, ref
46	981	983	1233	1238	2-pentylfuran	tr	LRI, MS, ref
47	983	979	1291	1293	octanal	tr	LRI, MS, ref
48	984	984	1498	1496	(E,E)-2,4-heptadienal	tr	LRI, MS
49	988	990	ND	1289	(Z)-2-(2-pentenyl)furan	tr	LRI, MS, ref
50	991		ND		tentative: 5-ethyl-2(5H)-furanone	tr	MS
51	997	999	ND	1671	(E,E)-2,4-heptadien-1-ol	tr	LRI, MS, ref
52	1013	1007	1646	1650	phenylacetaldehyde	0.29	LRI, MS, ref
53	1017	1011	1270	1268	paracymene	tr	LRI, MS, ref
54	1027	1020	1197	1187	limonene	tr	LRI, MS, ref
55	1031		ND		tentative: (Z)-2,5-octadienal	tr	MS
56	1035	1032	1432	1433	(E)-2-octenal	0.06	LRI, MS, ref
57	1045	1044	1523	1520	(E,Z)-3,5-octadien-2-one	tr	LRI, MS, ref
58	1051	1052	1614	1610	(E)-2-octen-1-ol	2.28	LRI, MS, ref
59	1051		1674		unknown[i]	0.9	
60	1055	1059	1555	1560	1-octanol	tr	LRI, MS, ref
61	1064	1064	1442	1447	cis-linalool oxide (furanoid)	tr	LRI, MS, ref

Table 1. continued

	$LRI_{exp\ nonpol}$[a]	$LRI_{ref\ nonpol}$[b]	$LRI_{exp\ pol}$[c]	$LRI_{ref\ pol}$[b]	name	%[d]	identification[e]
62	1069		1710		unknown[i]	0.30	
63	1071		1647		unknown[i]	0.06	
64	1074	1070	1519	1520	(Z,Z)-3,6-nonadienal	0.26	LRI, MS, ref
65	1074	1073	1452	1454	(Z)-3-nonenal	tr	LRI, MS, ref
66	1077	1077	1472	1470	trans-linalool oxide (furanoid)	tr	LRI, MS, ref
67	1085	1082	1396	1388	nonanal	tr	LRI, MS, ref
68	1088	1083	1910	1896	phenylethyl alcohol	0.48	LRI, MS, ref
69	1090	1090	1421	1419	perillene	tr	LRI, MS, ref
70	1093	1093	1602	1602	2-hydroxy-2,6,6-trimethyl-1-cyclohexanone	tr	LRI, MS, ref
71	1119		1576		tentative: (Z,Z)-2,6-nonadienal	tr	MS
72	1129	1130	1589	1585	(E,Z)-2,6-nonadienal	0.76	LRI, MS, ref
73	1138	1143	1523	1520	(E)-2-nonenal	0.37	LRI, MS, ref
74	1139	1137	1682	1694	(Z)-3-nonen-1-ol	1.65	LRI, MS, ref
75	1139		1739		tentative: (Z,E)-3,6-nonadien-1-ol	tr	MS
76	1139	1137	1730	1729	(E,Z)-3,6-nonadien-1-ol	tr	LRI, MS, ref
77	1139	1139	1749	1748	(Z,Z)-3,6-nonadien-1-ol	2.56	LRI, MS, ref
78	1146	1146	1765	1768	(E,Z)-2,6-nonadien-1-ol	0.17	LRI, MS, ref
79	1151	1150	1710	1718	(E)-2-nonen-1-ol	tr	LRI, MS, ref
80	ND	1160	1657	1662	1-nonanol	tr	LRI, MS, ref
81	1172	1171	1666	1656	(E,Z)-2,4-nonadienal	tr	LRI, MS, ref
82	1180		ND	1800	tentative: (E)-ocimenol	tr	MS
83	1187	1187	ND	1503	decanal	tr	LRI, MS, ref
84	1190	1192	1706	1694	(E,E)-2,4-nonadienal	0.07	LRI, MS, ref
85	1199		1887		tentative: (E,E)-2,4-nonadien-1-ol	tr	MS
86	1206	1208	1602	1602	2,6,6-trimethyl-1-cyclohexene-1-carbaldehyde	tr	LRI, MS, ref
87	1219	1213	1595	1591	methyl thymol ether	tr	LRI, MS, ref
88	1234		ND		tentative: 2,4,6-nonatrienal (isomer 1)	tr	MS
89	1237		1871		tentative: 2,4,6-nonatrienal (isomer 2)	tr	MS
90	1243		1880		tentative: 2,4,6-nonatrienal (isomer 3, E,E,E)	0.24	MS
91	1253		1899		tentative: 2,4,6-nonatrienal (isomer 4)	0.21	MS
92	1259		2072		unknown[i]	0.10	
93	1267		ND		tentative: 2,4,7-decatrienal (isomer 1)	0.05	MS
94	1272	1271	1770	1769	(E,Z)-2,4-decadienal	tr	LRI, MS, ref
95	1289		ND	1598	undecanal	tr	LRI, MS, ref
96	1291		ND		tentative: 2,4,7-decatrienal (isomer 2)	tr	MS
97	1293	1289	1816	1814	(E,E)-2,4-decadienal	tr	LRI, MS, ref
98	1304	1307	ND	2076	2-nonen-4-olide	tr	LRI, MS, ref
99	1310		2265		tentative: 4-hydroxy-2-nonenal	tr	MS
100	1341	1342	1987	1994	cis-4,5-epoxy-(E)-2-decenal	tr	LRI, MS, ref
101	1347	1347	2008	2011	trans-4,5-epoxy-(E)-2-decenal	tr	LRI, MS, ref
102	1368	1369	2593	2586	vanillin	tr	LRI, MS, ref
103	1427		2141		tentative: dimethylquinoline	tr	MS
104	1472	1473	1996	1995	4,5-epoxy-β-ionone	tr	LRI, MS, ref
105	1474	1472	1942	1941	β-ionone	0.19	LRI, MS, ref
106	ND	1485	1989	1994	3,4-epoxy-β-ionone	tr	LRI, MS, ref
107	1510	1512	2355	2351	cassia lactone	tr	LRI, MS, ref
108	1600	1594	2360	2368	dill apiole	tr	LRI, MS, ref
109	1731		1959		tentative: butyl dodecanoate	tr	MS
110	1810	1820	2036	2017	isopropyl tetradecanoate[g]	tr	LRI, MS, ref
111	1937	1943	ND	2800	hexadecanoic acid	tr	LRI, MS, ref
112	2129		2368		tentative: butyl hexanoate	tr	MS
113	2329		2573		tentative: butyl octadecanoate	tr	MS

[a]LRI_{nonpol}, LRI on SPB-1. [b]LRI obtained with a reference sample. [c]LRI_{pol}, LRI on Swax. [d]Percentages were determined by GC-FID on nonpolar phase with the use of an internal standard and application of correction factors. [e]LRI, MS, ref: identification done by comparison of the mass spectrum and the linear retention indices of a reference compound. If only one method was applied (MS alone), the identification was tentative. [f]tr, <0.05%. [g]ND, not detected. [h]Possible contaminant. [i]**59**: MS (EI, 70 eV) 108 (24); 93 (30); 91 (23); 79 (100); 77 (32); 67 (30); 55 (21); 41 (21). **62**: MS (EI, 70 eV) 124 (11); 109 (3); 95 (57); 81 (93); 74 (42); 69 (34); 57 (31); 55 (100); 43 (83); 41 (53). **63**: MS (EI, 70 eV) 109 (9); 101 (43); 99 (9); 85 (8); 83 (100); 71 (21); 55 (100); 43 (50) (1-nonen-4-ol?). **92**: MS (EI, 70 eV) 138 (96); 123 (17); 109 (16); 105 (19); 95 (43); 91 (46); 81 (43); 79 (100); 77 (44); 67 (74); 55 (33); 41 (25) (2,4,6-nonatrienol?).

Table 2. GC-MS–Olfactometry Analysis of Oyster Leaf Extract on Nonpolar Column

LRI$_{nonpolar}$	odor description	odorant	literature descriptors[a,b,c]
657	fruity, pungent	1-penten-3-one 7	"pungent, fish-like"[b]
774	green, grass	(Z)-3-hexenal 18	"leaf, green"[a]; "leaf-like"[b]
827	almond, green	—[d]	—
842	green, cut grass, (Z)-3-hexenol-like	(Z)-3-hexenol 25	"grass"[a]; "leaf-like"[b]
868	boiled potato, strong	3-(methylthio)propanal 29	"cooked potato"[a]; "potato-like"[b]
890	cereal, popcorn, baked cake	—[d]	—
931	metallic, mushroom, aldehydic	(E)-2-heptenal 35	"soap, fat, almond"[a]; "fatty, almond-like"[b]
934	gassy, metallic, fruity, green	—[d]	—
954–962	metallic, mushroom, seaweed, green, marine, strong	zone 1: (E)-1,5-octadien-3-ol 37 +1-octen-3-one 38 + (Z)-1,5-octadien-3-ol 39 + (Z)-1,5-octadien-3-one 40	37: "earth, herb"[a,e]; 38: "mushroom, metal"[a,b]; 39: "earth, herb"[a,e]; 40: "geranium-like, metallic"[b]
968	mushroom, strong	1-octen-3-ol 41	"mushroom"[a,b]
1014	floral	phenylacetaldehyde 52	"hawthorn, honey, sweet"[a]; "honey-like, flowery"[b]
1037	fatty, fruity, aldehydic	(E)-2-octenal 56	"green, nut, fat"[a]; "fatty, nutty"[b]
1074	marine, typical oyster, green, melon	zone 2: unknown 62 + unknown 63 + (Z,Z)-3,6-nonadienal 64 + (Z)-3-nonenal 65	64: "soapy"[b]; 65: "cucumber-like"[b]
1090	floral, leafy	phenylethyl alcohol 68 + 2-hydroxy-2,6,6-trimethyl-1-cyclohexanone 70	68: "honey, spice, rose, lilac"[a]; "honey-like, spicy"[b]; 70: "weak, camphor, cellar"[c]
1120	cucumber, green, fatty	tentative: (Z,Z)-2,6-nonadienal 71	"cucumber, wax, green"[a]
1131	fatty, green, cucumber	(E,Z)-2,6-nonadienal 72	"cucumber, wax, green"[a]; " fatty, nutty"[b]
141	green, fatty, strong	(Z)-3-nonen-1-ol 74 + (Z,Z)-3,6-nonadien-1-ol 77	74: "melon, green, vegetal"[c]; 77: "violet, cucumber"[c]
1192	fatty, decadienal-like	(E,E)-2,4-nonadienal 84	"fatty"[b]
1248	fatty, decadienal-like	2,4,6-nonatrienal (tentative (E,E,E)) 90	"fatty, oily"[c]
1292	fatty, citrusy	undecanal 95	"oil, pungent, sweet"[a]
1305	acidic, pyrazinic, slightly burnt, leathery	2-nonen-4-olide 98	"cinnamic, aggressive, plastic, hay"[c]
1336	aldehydic, green, metallic, fatty, strong	cis-4,5-epoxy-(E)-2-decenal 100	"metal, green"[a]
1347	aldehydic, metallic, green, fatty, strong	trans-4,5-epoxy-(E)-2-decenal 101	"metal, green"[a,b,e]
1366	vanillin-like, nutty	vanillin 102	"vanilla"[a,b]
1509	woody, metallic, sinensal-like	—[d]	—
1563	pyridine-like, wet dog, unpleasant	—[d]	—

[a]Acree, T.; Arn, H. Flavornet. http://www.flavornet.org, 1997 (accessed August 2012). [b]Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Technical Universität Müchen: Munich, Germany, 2000. [c]Descriptor from Firmenich data bank built from authentic samples. [d]Compound not detected by MS due to its low concentration. [e]Isomer cis or trans not defined.

Table 3. GC-MS–Olfactometry Analysis of Oyster Leaf Extract on Polar Column

LRI$_{pol}$	odor description	odorant	literature descriptors[a,b,c]
1152	green, grass	(Z)-3-hexenal 18	"leaf, green"[a]; "leaf-like"[b]
1298	mushroom	1-octen-3-one 38	"mushroom, metal"[a,b]
1378	green, cut grass, marine, seaweed	(Z)-3-hexen-1-ol 25 + (Z)-1,5-octadien-3-one 40	25: "grass"[a]; "leaf-like"[b]; 40: "geranium-like, metallic"[b]
1412	green, fatty	tentative: (E,Z)-2,4-hexadienal 30	"green"[a]
1449	mushroom, 1-octen-3-ol-like	1-octen-3-ol 41 + (Z)-3-nonenal 65	41: "mushroom"[a,b]; 65: "cucumber-like"[b]
1460	3-(methylthio)propanal-like, earthy	3-(methylthio)propanal 29	"cooked potato"[a]; "potato-like"[b]
1485	marine, green, metallic, seaweed	(Z)-1,5-octadien-3-ol 39	39: "earth, herb"[a,e]
1522	green, marine, grass, fatty	(Z,Z)-3,6-nonadienal 64 + (E,Z)-3,5-octadien-2-one 57	57: " lactonic, mushroom"[c]; 64: "soapy"[b]
1546	aldehydic, green, fatty, waxy	(E)-2-nonenal 73	"orris, fat, cucumber"[a]; "tallowy, cucumber-like"[b]
1592	watermelon, cucumber, green, fatty	(E,Z)-2,6-nonadienal 72	"cucumber, wax, green"[a]; "fatty, nutty"[b]
1647	honey, floral, nutty	phenylacetaldehyde 52	"hawthorne, honey, sweet"[a]; "honey-like, flowery"[b]
1685	waxy, black tea	(Z)-3-nonen-1-ol 74	"melon, green, vegetal"[c]
1708	green, leafy, fatty	(E,E)-2,4-nonadienal 84 + unknown 62	84: "fatty"[b]
1754	green, marine, oyster, seaweed	(Z,Z)-3,6-nonadien-1-ol 77	"violet, cucumber"[c]
1772	cucumber, green, fatty	(E,Z)-2,6-nonadien-1-ol 78	"cucumber"[a]; "cucumber-like"[b]
1909	floral	phenylethyl alcohol 68	"honey, spice, rose, lilac"[a]; "honey-like, spicy"[b]
1990	green, waxy, strong	cis-4,5-epoxy-(E)-2-decenal 100	"metal, green"[a,e]
2023	fatty, metallic, green	—[d]	—

[a]Acree, T.; Arn, H. Flavornet; http://www.flavornet.org, 1997 (accessed August 2012). [b]Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Technical Universität Müchen: Munich, Germany, 2000. [c]Descriptor from Firmenich data bank built from authentic samples. [d]Compound not detected by MS due to its low concentration. [e]Isomer cis or trans not defined.

temperature, 250 °C; transfer line temperature, 280 °C; carrier gas, helium at a constant flow rate of 1 mL/min; split ratio, 1:50.

The injection was repeated with 0.5 μL of the extract in a GC-MS 6890/5973 (Agilent) equipped with a polar SWax capillary column (30 m × 0.25 mm, film thickness = 0.25 μm, Supelco). The temperature program was as follows: 50 °C, 5 min isotherm, and then 5 °C/min to 240 °C; injector temperature, 250 °C; transfer line temperature, 280 °C; carrier gas, helium at a constant flow rate of 1 mL/min; split ratio, 1:50.

Mass spectra were generated at 70 eV at a scan range from m/z 27 to 350. Linear retention indices (LRIs) were determined after injection of a series of n-alkanes (C_5–C_{28}) under identical conditions.

Identification of Components. The compounds listed in Table 1 were identified by comparison of their mass spectra and LRIs obtained from a proprietary database. If only one method was applied (MS data alone or LRI alone), the identification was tentative. This database is composed of analytical data obtained from synthesized or commercially available compounds that were unequivocally characterized by GC-MS and NMR spectroscopy. The commercial sources or synthetic procedures of all the identified compounds are given above under Chemicals.

GC−Olfactometry. The sniffing experiments were performed by three trained panelists, who were asked to use free vocabulary to describe odors perceived at the sniffing port. The panelists repeated the experiments on nonpolar and polar columns. Only the odors detected by at least two of the three panelists and described with very similar descriptors are reported in Tables 2 and 3. Odorants were identified by comparison of their LRIs, mass spectra, and odor characteristics with the data of the reference compounds.

GC−Olfactometry on Nonpolar Column. A 1 μL aliquot of extract was injected into a GC-MS 6890-5973N (Agilent) equipped with a HP-1 capillary column (60 m × 0.32 mm, film thickness = 1 μm, Agilent) and a sniffing port. The temperature program was as follows: 50 °C, 5 min isotherm, and then 3 °C/min to 120 °C followed by 5 °C/min to 250 °C; injector temperature, 250 °C; carrier gas, helium at a constant flow rate of 2.7 mL/min; split ratio, 1:1. The column effluent was split 1:5 into an MS detector and a heated sniffing port.

GC−Olfactometry on Polar Column. A 1 μL aliquot of extract was injected into a GC-FID Varian 380 equipped with a DBWax megabore column (30 m × 0.53 mm, film thickness = 1 μm, J&W Scientific, Folsom, CA, USA) and a sniffing port. The temperature program was as follows: 50 °C, 5 min isotherm, and then 5 °C/min to 240 °C; injector temperature, 250 °C; carrier gas, helium at constant flow rate of 5 mL/min; split ratio, 1:10. The column effluent was split 1:10 into a flame ionization detector (FID) and a heated sniffing port.

Quantitation by GC. The oyster leaf extract was injected into a GC 6890 (Agilent) equipped with a double injector and two nonpolar DB-1 capillary columns (60 m × 0.25 mm, film thickness = 0.25 μm, J&W 122-1062). One column was connected to an MS 5973N (Agilent) for identification and the other to an FID for quantitation. The temperature program was as follows: 50 °C for 5 min, increased to 120 °C at a rate of 3 °C/min, then increased to 250 °C at a rate of 5 °C/min, 5 min isothermal, then increased to 300 °C at a rate of 15 °C/min, and then 20 min isothermal; split ratio, 1:10; injection volume, 0.2 μL; injector and detector temperatures, both 250 °C; carrier gas, helium at constant flow rates of 1.8 and 2.1 mL/min, respectively. For the quantitative data given in Table 1, the oyster leaf extract (17.0 mg) was diluted 10 times in dichloromethane with methyl octanoate (6.0 mg) as an internal standard. The major components (>0.05 FID %) were quantified. Their percentages were obtained from the FID area corrected with the use of the response factors, previously measured with pure standards under the same conditions or calculated according to the method of de Saint Laumer et al.[15] The relative response factors (RRFs) were calculated as follows: RRF = ($m$compound × $area$ISTD)/(mISTD × $area$compound), where $m$compound and $area$compound are the mass and corresponding GC peak area of the analyte and mISTD and $area$ISTD are the mass and GC peak area of the internal standard. With this instrument configuration, the simultaneous injection of the extract on both columns allowed us to assign each "FID peak" to an

"MS peak." In the case of coelutions, the ratios of the coeluting compounds were estimated via the acquisition done on polar phase. Minor peaks were noted as trace amounts (tr) in Table 1.

Authentic Samples: Preparation of (±)-(Z)-1,5-Octadien-3-ol 39. *(E)-Oct-2-en-5-yn-1-ol 115.* A solution of **114** (17 g, 139.1 mmol) in Et_2O (30 mL) was added dropwise to a suspension of $LiAlH_4$ (4.5 g, 118.7 mmol) in Et_2O (380 mL) at 0 °C. After 18 h, H_2O (4.5 mL), 15% aqueous NaOH (4.5 mL), and H_2O (45 mL) were added successively, and the reaction mixture was filtered through a Büchner funnel and then dried (Na_2SO_4) and concentrated. Bulb-to-bulb distillation afforded pure **115** in an 81% yield: bp, 81 °C/0.4 mbar; IR, 3321, 2975, 2936, 2918, 2878, 1672, 1455, 1421, 1374, 1321, 1270, 1223, 1092, 1061, 995, 968, 892, 781; ^1H NMR, δ 1.14 (t, J = 7.5 Hz, 3H); 1.68 (br s, 1 OH); 2.20 (tq, J = 2.2, 7.5 Hz, 2H); 2.92−2.95 (m, 2H); 4.13 (dd, J = 1.2, 5.9 Hz, 2H); 5.66−5.73 (m, 1H); 5.87−5.95 (m, 1H); ^{13}C NMR, δ 12.4 (t); 14.2 (q); 21.8 (t); 63.2 (t); 76.0 (s); 84.2 (s); 127.3 (d); 130.3 (d); MS (EI, 70 eV), 124 (2, M$^{+•}$); 123 (8); 109 (35); 95 (79); 91 (66); 81 (18); 79 (100); 78 (22); 77 (77); 67 (34); 65 (30); 57 (32); 55 (17); 53 (22); 51 (19); 41 (29); 39 (30).

(±)-(3-(Pent-2-ynyl)oxiran-2-yl)methanol 116. *tert*-Butyl hydroperoxide (70%, 13.8 g, 107.2 mmol) was added dropwise at 25 °C to a solution of vanadyl acetylacetonate (VO(acac)$_2$), 208 mg, 0.78 mmol) and **115** (6.5 g, 52.33 mmol) in toluene (160 mL). After 5 h, the reaction mixture was diluted with Et_2O, washed with 15% aqueous $NaHCO_3$ and then with H_2O to neutrality, dried (Na_2SO_4),and concentrated. Bulb-to-bulb distillation afforded pure **116** in a 90% yield: bp, 70 °C/0.3 mbar; IR, 3416, 2976, 2919, 2878, 2211, 1681, 1455, 1420, 1364, 1320, 1294, 1195, 1083, 1016, 941, 918, 857, 777, 703, 639; ^1H NMR, δ 1.11 (t, J = 7.6 Hz, 3H); 1.25 (m, 1 OH); 2.16 (tq, J = 2.5, 7.6 Hz, 2H); 2.54 (qquint, J = 2.5, 17.4 Hz, 2H); 3.10−3.14 (m, 2H); 3.64 (dd, J = 4.1, 12.5 Hz, 1H); 3.94 (dd, J = 2.5, 12.5 Hz, 1H); ^{13}C NMR, δ 12.4 (t); 14.0 (q); 21.7 (t); 53.7 (d); 58.0 (d); 61.3 (t); 73.4 (s); 84.2 (s); MS (EI, 70 eV), 140 (0, M$^{+•}$); 121 (10); 111 (21); 109 (100); 107 (24); 95 (29); 93 (12); 91 (22); 83 (21); 81 (42); 79 (69); 77 (59); 67 (66); 65 (43); 57 (36); 55 (32); 53 (54); 51 (29); 43 (26); 41 (78); 39 (52).

(±)-(Z)-(3-(Pent-2-enyl)oxiran-2-yl)methanol 117. A solution of **116** (5 g, 35.66 mmol) in toluene (100 mL) was hydrogenated over a Lindlar catalyst (1.0 g). After absorption of 870 mL, the reaction mixture was filtered and concentrated. Bulb-to-bulb distillation afforded **117** in a 96% yield: bp, 73 °C/0.25 mbar; IR, 3412, 3011, 2963, 2933, 2874, 1654, 1462, 1403, 1374, 1309, 1282, 1209, 1079, 1023, 977, 940, 908, 858, 814, 794, 719, 640; ^1H NMR, δ 0.97 (t, J = 7.7 Hz, 3H); 2.05 (quint, J = 7.7 Hz, 2H); 2.28−2.44 (m, 3H); 2.95−3.01 (m, 2H); 3.61 (dd, J = 4.5, 12.8 Hz, 1H); 3.90 (dd, J = 2.5, 12.8 Hz, 1H); 5.32−5.39 (m, 1H); 5.50−5.58 (m, 1H); ^{13}C NMR, δ 14.2 (q); 20.7 (t); 29.2 (t); 55.3 (d); 58.2 (d); 61.7 (t); 122.3 (d); 134.9 (d); MS (EI, 70 eV), 142 (0, M$^{+•}$); 127 (1); 111 (15); 95 (16); 93 (17); 91 (10); 83 (21); 82 (35); 81 (40); 79 (22); 77 (10); 69 (38); 68 (45); 67 (100); 65 (10); 61 (12); 57 (23); 56 (11); 55 (68); 54 (18); 53 (21); 43 (31); 41 (75); 39 (32).

(±)-(Z)-1,5-Octadien-3-ol 39. In a 250 mL flask under N_2, Zn powder (7.3 g, 0.112 mmol) was added to a solution of $(C_5H_5)_2TiCl_2$ (bis(cyclopentadienyl)titanium dichloride, 6.7 g, 27 mmol) in tetrahydrofuran (THF) (150 mL), followed by $ZnCl_2$ (3.68 g, 27 mmol). After 40 min at 20 °C, a solution of the epoxy alcohol **117** (3.2 g, 22.5 mmol) in THF (15 mL) was added to the green solution. After 45 min at 20 °C, the blue reaction mixture was quenched by the addition of 1% aqueous H_2SO_4 (100 mL) and then extracted with Et_2O (3 × 100 mL). The organic phase was washed to neutrality with H_2O, dried (Na_2SO_4), concentrated, and purified by bulb-to bulb distillation (bp, 78 °C/8 mbar) to afford 1.31 g of **39** (94% purity by GC-MS, containing 2.4% of (E)-1,5-octadien-3-ol **37**) in a 46% yield: IR, 3349, 3080, 3011, 2963, 2934, 2875, 1645, 1456, 1423, 1405, 1305, 1203, 1126, 1034, 989, 919, 866, 792, 715; ^1H NMR, δ 0.97 (t, J = 7.7 Hz, 3H); 1.77 (br s, 1 OH); 2.04−2.11 (m, 2H); 2.29−2.34 (m, 2H); 4.09−4.18 (m, 1H); 5.12 (dt, J = 1.4, 10.5 Hz, 1H); 5.25 (dt, J = 1.4, 16.5 Hz, 1H); 5.32−5.40 (m, 1H); 5.54−5.60 (m, 1H); 5.90 (ddd, J = 5.6, 10.5, 16.5 Hz, 1H); ^{13}C NMR, δ 14.2 (q); 20.7 (t); 35.0 (t); 72.5 (d); 114.7 (t); 123.7 (d); 135.3 (d); 140.5 (d); MS (EI, 70 eV), m/z

(%) 126 (1); 108 (1); 93 (2); 79 (3); 70 (38); 69 (15); 57 (100); 55 (35); 53 (5); 42 (14); 41 (40); 39 (17).

LRI (SPB-1) 957; in natural extract, LRI (SPB-1) 962. LRI (SWax) 1483; in natural extract, LRI (SWax) 1484.

(±)- (E)-1,5-Octadien-3-ol **37**. MS (EI, 70 eV), *m/z* (%) 126 (1); 108 (3); 93 (2); 79 (6); 70 (47); 69 (25); 57 (100); 55 (35); 53 (6); 42 (12); 41 (35); 39 (15).

LRI (SPB-1) 952; in natural extract, LRI (SPB-1) 954. LRI (SWax) 1457; in natural extract, LRI (SWax) 1459.

(Z)-1,5-Octadien-3-one **40**. Dess−Martin periodinane (15% solution in CH_2Cl_2 (22.8 g, 8 mmol)) was added at 20 °C to a solution of **39** (600 mg, 4.7 mmol) in CH_2Cl_2 (1 mL). After 45 min, the reaction mixture was quenched by the addition of 5% aqueous NaOH (6 mL) and then extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was washed to neutrality with H_2O, dried (Na_2SO_4), concentrated, and purified by bulb-to-bulb distillation (bp, 80 °C/12 mbar) to afford 295 mg of pure **40** in a 50% yield: IR, 3021, 2959, 2930, 1872, 1683, 1635, 1615, 1457, 1399, 1325, 1208, 1186, 1084, 984, 890, 872, 858, 710; ^{1}H NMR, δ 0.99 (t, *J* = 7 Hz, 3H); 2.07 (quint, *J* = 7 Hz, 2H); 3.35 (d, *J* = 6.5 Hz, 2H); 5.51−5.65 (m, 2H); 5.84 (d, *J* = 10.5 Hz, 1H); 6.26 (d, *J* = 18 Hz, 1H); 6.39 (dd, *J* = 10.5, 18 Hz, 1H); ^{13}C NMR, δ 13.9 (q); 20.9 (t); 38.7 (t); 120.1 (d); 128.5 (t); 135.5 (d); 136.0 (d); 198.6 (s); MS (EI, 70 eV), *m/z* (%) 124 (1); 109 (9); 95 (7); 83 (4); 82 (3); 81 (5); 69 (8); 68 (3); 67 (6); 55 (100); 41 (21).

LRI (SPB-1) 960; in natural extract, LRI (SPB-1) 962. LRI (SWax) 1381; in natural extract, LRI (SWax) 1380.

(Z,Z)-3,6-Nonadienal **64**. (Z,Z)-3,6-Nonadienal **64** was prepared as described by Frerot et al:[16] MS (EI, 70 eV), *m/z* (%) 138 (6); 123 (12); 110 (11); 109 (20); 95 (30); 94 (13); 84 (29); 82 (20); 81 (35); 79 (44); 70 (30); 69 (29); 68 (23); 67 (100); 55 (60); 53 (24); 41 (90).

LRI (SPB-1) 1070; in natural extract, LRI (SPB-1) 1074. LRI (SWax) 1520; in natural extract, LRI (SWax) 1519.

(Z)-3-Nonenal **65**. (Z)-3-Nonen-1-ol **74** (19.88 g, 0.140 mol, 1 equiv) was converted into (Z)-3-nonenal **65** (1.6 g, 97% purity by GC-MS; yield, 8% not optimized) following the oxidation procedure described for the preparation of (E)-3-nonenal in Frerot et al.:[15] MS (EI, 70 eV), *m/z* (%) 140 (1); 122 (5); 111 (5); 98 (16); 97 (18); 96 (32); 85 (16); 84 (69); 83 (52); 81 (25); 70 (35); 69 (94); 67 (28); 57 (25); 56 (27); 55 (100); 54 (35); 41 (73).

LRI (SPB-1) 1073; in natural extract, LRI (SPB-1) 1074. LRI (SWax) 1454; in natural extract, LRI (SWax) 1452 (in coelution with 1-octen-3-ol).

To confirm the presence of (Z)-3-nonenal in the extract, (E)-3-nonenal was prepared with 99% purity by GC-MS, as described in Frerot et al.,[16] and was reinjected for MS and LRI comparison: MS (EI, 70 eV), *m/z* (%) 140 (1); 122 (4); 111 (6); 98 (16); 97 (15); 96 (28); 85 (12); 84 (56); 83 (51); 81 (24); 70 (41); 69 (83); 67 (25); 57 (26); 56 (25); 55 (100); 54 (25); 41 (86). LRI (SPB-1) 1075; LRI (SWax) 1447.

■ RESULTS AND DISCUSSION

Sample Preparation and Volatile Composition. A hydrodistillation under reduced pressure was performed on fresh-cut leaves. The resulting extract was analyzed by GC-MS. The major volatile compounds were quantified by GC-FID with the use of an internal standard and response factors.[17] The complete listing of identified volatiles is given in Table 1. In total, 109 compounds were identified, showing the high complexity of the extract. The major compound was (Z)-1,5-octadien-3-ol (**39** in Table 1), accounting for up to 47.83% of the overall chromatogram. 1,5-Octadien-3-ol has been identified as a major compound in Pacific oyster by Josephson et al.,[3] but the configuration of the double bond was not determined at that time. In our study, the major compound was the cis-isomer **39**, whereas only a trace amount of the trans-isomer **37** was detected. The other major volatile compounds were 1-octen-3-

ol **41** (13.36%), (Z)-3-hexen-1-ol **25** (8.72%), 1-penten-3-ol **8** (3.04%), (Z,Z)-3,6-nonadien-1-ol **77** (2.56%), (E)-2-octen-1-ol **58** (2.28%), (Z)-3-hexenal **18** (2.08%), (Z)-3-nonen-1-ol **74** (1.65%), (E)-2-hexenal **23** (1.61%), 1-hexanol **27** (1.45%), and hexanal **20** (0.96%).

A high number of unsaturated alcohols and unsaturated aldehydes were identified on nonpolar phase. As the cis- and trans-isomers of these molecules generally coelute on nonpolar phase, their double-bond configuration could be determined only after reinjection of reference compounds on polar phase.

GC−Olfactometry of the Oyster Leaf Extract. The hydrodistillate was evaluated on a paper smelling strip by perfumers and flavorists. Marine oyster-like notes were perceived together with green, grass, and leafy notes. GC−olfactometry analysis was performed by a panel of experts on both a nonpolar and a polar phase to identify the volatiles that contribute to the oyster leaf aroma. As shown in Tables 2 and 3, the green odor of the extract was attributed to (Z)-3-hexenal **18** (green, grass), (Z)-3-hexen-1-ol **26** (green, cut grass), and (E,Z)-2,6-nonadienal **72** (green, cucumber). Floral notes were attributed to phenylacetaldehyde **52** and phenylethyl alcohol **68**. Fatty, metallic, and aldehydic notes were attributed to unsaturated aldehydes, in particular (E)-2-octenal **56**, (E)-2-nonenal **73**, (E,E)-2,4-nonadienal **84**, cis-4,5-epoxy-(E)-2-decenal **100**, and trans-4,5-epoxy-(E)-2-decenal **101**. The mushroom character was mainly attributed to 1-octen-3-one **38** and 1-octen-3-ol **41**.

Two zones were detected on the nonpolar column and described by all of the panelists with the "*marine*" descriptor (Table 2). Zone 1 was perceived as a strong odor with "*metallic, mushroom, seaweed, green, marine*" descriptors and lasted from LRI 954 to 962. Four compounds eluted in this zone, the major compound (E)-1,5-octadien-3-ol **37** (LRI 954), with trace amounts of 1-octen-3-one **38** (LRI 956), (Z)-1,5-octadien-3-ol **39** (LRI 962), and (Z)-1,5-octadien-3-one **40** (LRI 962). As we could not exclude that one of the trace compounds **37**, **38**, and **40** had a very low odor threshold and could therefore contribute more than **39** to the overall odor of the zone, GC−olfactometry was repeated with the same panelists on a polar column. Under these conditions, the four compounds were well separated, and their odor evaluation at the sniffing port was therefore more reliable. As shown in Table 3 (Z)-1,5-octadien-3-ol **39** was perceived by all of the assessors with similar descriptors: "*green, marine, metallic, seaweed, fresh*". (Z)-1,5-Octadien-3-one **40** was detected in a zone described as "*green, marine, seaweed, grass*". Interestingly, (Z)-1,5-octadien-3-one **40** was found in trace amount in coelution with (Z)-3-hexen-1-ol **25** and identified thanks to its typical fragments *m/z* 124 and 109. As a marine, seaweed note could be perceived by the panelists on top of the green, grass odor of (Z)-3-hexen-1-ol, GC−olfactometry tended to indicate that the unsaturated ketone **40** is a powerful constituent. (E)-1,5-Octadien-3-ol **37** was not detected, probably due to its occurrence in trace compared with the cis-isomer **39**. 1-Octen-3-one **38** was clearly associated with its typical mushroom odor.

Zone 2 was described as "*marine, typical oyster, green, melon*" (Table 2, LRI 1074). In this zone, four compounds were detected in trace amount: two unknown compounds, **62** and **63**, (Z,Z)-3,6-nonadienal **64**, and (Z)-3-nonenal **65**. According to GC−olfactometry on polar phase, **64** and **65** most likely contributed to the marine oyster note, because **62** was found on polar phase in a zone with a different description ("*green, leafy, fatty*") and **63** was not perceived (Table 3). Interestingly,
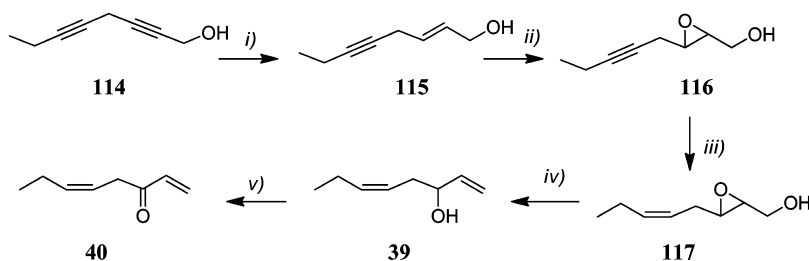
Figure 3. Synthesis of (Z)-1,5-octadien-3-ol **15** and (Z)-1,5-octadien-3-one **77**: (*i*) LiAlH$_4$, Et$_2$O; (*ii*) tBuO$_2$H, VO(acac)$_2$; (*iii*) H$_2$, Lindlar; (*iv*) (C$_5$H$_5$)$_2$TiCl$_2$; (*v*) Dess−Martin.

(Z,Z)-3,6-nonadienal **64** and (Z)-3-nonenal **65**, which coeluted on nonpolar phase, were also found in coelution on polar phase, but with other compounds. (Z,Z)-3,6-Nonadienal **64** coeluted with (E,Z)-3,5-octadien-2-one **57**, and the peak was described as "*green, grass, aldehydic, marine.*" (Z)-3-Nonenal **65** was found in trace amount in coelution with a high amount of 1-octen-3-ol **41**. When sniffing this peak, panelists could perceive only the strong mushroom-like odor of 1-octen-3-ol **41**, which may have covered the odor of **65**. To confirm the possible marine character of (Z,Z)-3,6-nonadienal **64** and (Z)-3-nonenal **65**, the preparation of pure standards was performed as described hereafter.

An additional zone with "*oyster, marine*" descriptors was identified during GC−olfactometry on polar phase (Table 3, LRI 1754), but was much weaker that those described above. This odor was attributed to the trace amount of (Z,Z)-3,6-nonadien-1-ol **77**.

Synthesis and Evaluation of Pure Samples. To confirm the marine character of the four volatile molecules highlighted by GC−olfactometry, pure standards of (Z)-1,5-octadien-3-ol **39**, (Z)-1,5-octadien-3-one **40**, (Z,Z)-3,6-nonadienal **64**, and (Z)-3-nonenal **65** were synthesized, as described under Materials and Methods. The descriptions of their orthonasal and retronasal odors by perfumers and flavorists are given in Figure 2.

The synthesis of (Z)-1,5-octadien-3-ol **39** and (Z)-1,5-octadien-3-one **40** has already been reported in the literature. Compound **39** was prepared either by Lindlar reduction of the intermediate oct-1-en-5-yn-3-ol[18,19] or by Grignard addition of vinyl magnesium bromide on (Z)-3-hexenal.[20] In the present study, the preparation of **39** and **40** was completed as described in Figure 3. Selective reduction of the propargylic bond of **114**[21] with LiAlH$_4$ in diethyl ether[22] afforded the allylic alcohol **115** in good yield. Epoxidation of **115** into **116**[23] followed by the Lindlar reduction afforded **117**.[24] Reduction of the 2,3-epoxy alcohol **117** into the alk-1-en-3-ol **39** was obtained following the procedure reported by Yadav et al.[25] Dess−Martin oxidation of **39** afforded pure **40**.[26] Compared with previous synthetic work, which placed the Lindlar reduction of oct-1-en-5-yn-3-ol as the final step,[18,19] this synthetic pathway could avoid the reduction of the terminal double bond, as well as the isomerization of the cis double bond, which may occur when (Z)-3-hexenal is used as an intermediate.[20] Moreover, the formation of optically active **39** could potentially be obtained by using an asymmetric Sharpless-type epoxidation.[27,28]

As shown in Figure 2, (Z)-1,5-octadien-3-ol **39** has a strong green, marine odor with a slightly mushroom note as well, which is in good agreement with the descriptors obtained by GC−olfactometry. The marine crustaceous character is also perceived in the mouth in association with some floral, green, geranium leaf, and mushroom, metallic notes. Recently, it was

tentatively identified by Piveteau et al. in fresh oysters, but its contribution to the oyster aroma was not observed.[4] Indeed, a marine odor was detected by GC−olfactometry at a retention index close to that of 1,5-octadien-3-ol, but the authors attributed the marine odor to decanal. Considering the odor of pure decanal (fatty, orange peel), it is highly probable that the authors have not assigned the marine character correctly. In a previous study, (Z)-1,5-octadien-3-ol **39** was reported to be important in the aroma of crustaceans, but described only as "*metallic*".[19]

The odor of the pure corresponding ketone (Z)-1,5-octadien-3-one **40** was described by the panelists as "*green, marine, reminiscent of oyster leaf*". To the best of our knowledge, it has never been reported in oyster, probably because it is very difficult to detect, as it can coelute with (Z)-3-hexen-1-ol **25** on polar phase and with (Z)-1,5-octadien-3-ol **39** on nonpolar phase. It was already identified in the headspace of boiled cod and boiled trout[29] and evaluated by GC−olfactometry as "*geranium-like*". Its odor detection threshold in water was previously reported to be 0.0012 ppb, being 100 times more potent than 1-octen-3-one.[20]

(Z,Z)-3,6-Nonadienal **64** and (Z)-3-nonenal **65** have never been reported in oyster, perhaps because both compounds generally coelute on nonpolar phase and (Z)-3-nonenal may coelute with 1-octen-3-ol on polar phase. As shown in Figure 2 both compounds have in common a melon, watermelon, slightly cucumber orthonasal and retronasal odor, but (Z)-3-nonenal **65** is more associated with a marine, oyster character. Both have been previously reported in watermelon[30] and tentatively in cucumber.[31]

This analysis showed the importance of using two orthogonal phases to understand the complexity of the volatile composition of oyster leaf. In particular, the combination of GC-MS and GC−olfactometry on nonpolar and polar phases was crucial to identify the molecules having an oyster-like character. After evaluating the organoleptic properties of pure compounds obtained by synthesis, we confirmed that (Z)-1,5-octadien-3-ol **39**, (Z)-1,5-octadien-3-one **40**, (Z,Z)-3,6-nonadienal **64**, and (Z)-3-nonenal **65** were reminiscent of the oyster-like marine notes perceived when tasting cut leaves. To the best of our knowledge, among these four molecules, only (Z)-1,5-octadien-3-ol **39** was previously tentatively identified in oyster, and its marine character was not determined. The biosynthetic pathway of these molecules was beyond the scope of this study. The formation of these molecules may be initiated by hydroperoxidation of fatty acids by lipoxygenase, as previously reported for watermelon[30] and mushroom,[32,33] even though attempts to detect lipoxygenase enzyme activity with oysters on linolenic acid remain unsuccessful.[34] The ability of these unsaturated compounds to undergo oxidation, or double-bond isomerization and polymerization, may explain, at

least in part, the "fragility" of the oyster aroma. A slow release of these molecules could be of interest to impart marine, oyster-like notes into fragrances or flavours.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

GC profile of the oyster leaf hydrodistillate on nonpolar and polar columns. GC-profile of zones 1 and 2 with descriptors obtained by GC−olfactometry on nonpolar and polar columns. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ ABBREVIATIONS USED

EI, electron ionization; FID, flame ionization detector; GC-MS, gas chromatography−mass spectrometry; LRIs, linear retention indices; RRF, relative response factor; THF, tetrahydrofuran

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