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Stereochemistry of an Alcohol Oxidase from the Defensive Secretion of Larvae of the Leaf Beetle *Phaedon armoraciae* (Coleoptera: Chrysomelidae).

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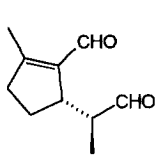
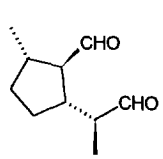
Abstract: Larvae of the leaf beetle *Phaedon armoraciae* produce the iridoids chrysomelidial **1** and plagiodial **3** from geraniol **6** by an oxidative sequence identical to that known in plants. Following ω-oxidation of geraniol the resulting 8-hydroxygeraniol **7** is oxidised *via* 8-oxogeraniol **14** and 8-hydroxygeranial **15** and to the dialdehyde **8**. In plants this transformation is achieved by NADP$^+$ dependent oxidoreductases. However, in the defensive secretion of several leaf beetles an oxygen-dependent oxidase is present. The enzyme catalyses the removal of the C(1)- and C(8)-H_R hydrogen atoms (*Re*-specificity) from the diol **7** yielding 8-oxogeranial **8**. Copyright © 1996 Elsevier Science Ltd

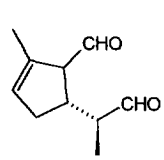
Methylcyclopentanoid monoterpenes possessing the iridane skeleton like, for example, chrysomelidial **1**, plagiodial **3**, and plagiolactone **4** are widespread constituents within the defensive secretions of phytophagous leaf beetle larvae of the subfamily Chrysomelinae.[1-3] Compounds of this class, especially nepetalactone **5** from catmint (*Nepeta cataria*) have also been shown to constitute aphid sex pheromones, for example in the damson-hop aphid (*Phorodon humuli*).[4]



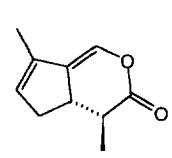
chrysomelidial **1**



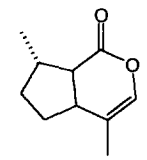
iridodial **2**



plagiodial **3**



plagiolactone **4**

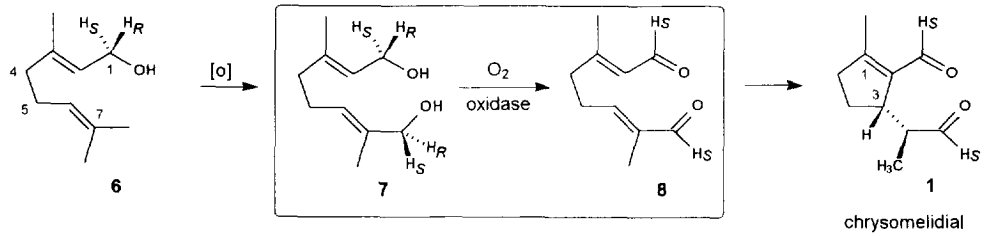


nepetalactone **5**

As shown previously, iridoid biosynthesis in plants[5,6] and insects[7,8] follows the same general route to the iridane skeleton. Studies with cell cultures of *Catharanthus roseus*,[9] *Rauwolfia serpentina*[10,11] or larvae of the leaf beetles *Phaedon armoraciae*, *Plagiodera versicolora* and *Gastrophysa viridula*[7,8] consistently support the sequence of transformations outlined in Scheme 1. Accordingly, plants, as well insects, first oxidise geraniol **6** (some plants also use nerol) at C(8) yielding 8-hydroxygeraniol **7**. The diol is subsequently oxidised to the dialdehyde **8** and, then, cyclised to the basic iridane skeleton (cf. Scheme 1), e.g. chrysomelidial **1**. Two of the enzymes involved are already known and have been isolated from plants. The ω-hydroxylation of geraniol **6** is achieved by a membrane-bound cytochrome P-450 mixed-function oxygenase. The enzyme has

been isolated from several species, cloned and studied in detail.[12,13] This enzyme catalysing the second oxidation, namely the transformation of geraniol **6** into 8-oxogeranial **8**, was first isolated from cell cultures of *R. serpentina*. The enzyme proved to be a monomeric, zinc-containing and $NADP^+$-dependent oxidoreductase with a molecular weight of 44 kD,[14] and is apparently widespread in the plant kingdom. Recently another $NADP^+$-dependent oxidoreductase was purified and characterised from *Nepeta racemosa* (formerly *N. mussinii*). This enzyme was found to be an zinc-containing αβ-heterodimer with submolecular masses of 42 and 40 kD,[15] respectively.

Scheme 1

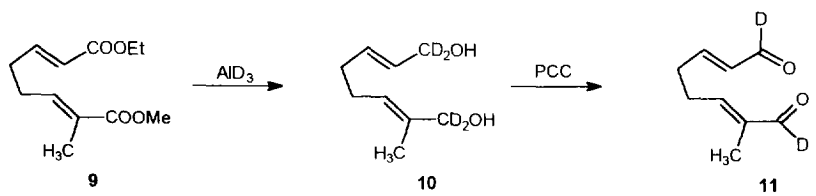


To date, none of the insect enzymes catalysing the sequence of Scheme 1 have been isolated and characterised. Our recent observation that the oxidase catalysing the formation of the dialdehyde **8**, from diol **7**, is present in the defensive secretion of several leaf beetle larvae has led to the possibility of studying the stereochemistry of the transformation **7** → **8**. In contrast to the plant derived biocatalysts this enzyme requires oxygen as a cofactor and is not inhibited by a zinc-complexing reagent like EDTA. The site specificity of the enzyme, regarding removal of one of the two enantiotopic hydrogens from the prochiral carbon atoms at C(1) and C(8) of chirally labelled precursors, indicates that both oxidation steps coincidentally remove the same hydrogen atom H_R in agreement with a *Re*-specificity of the enzyme(s) for both oxidation steps.

Enantiospecific Synthesis of Deuterium Labelled Precursors

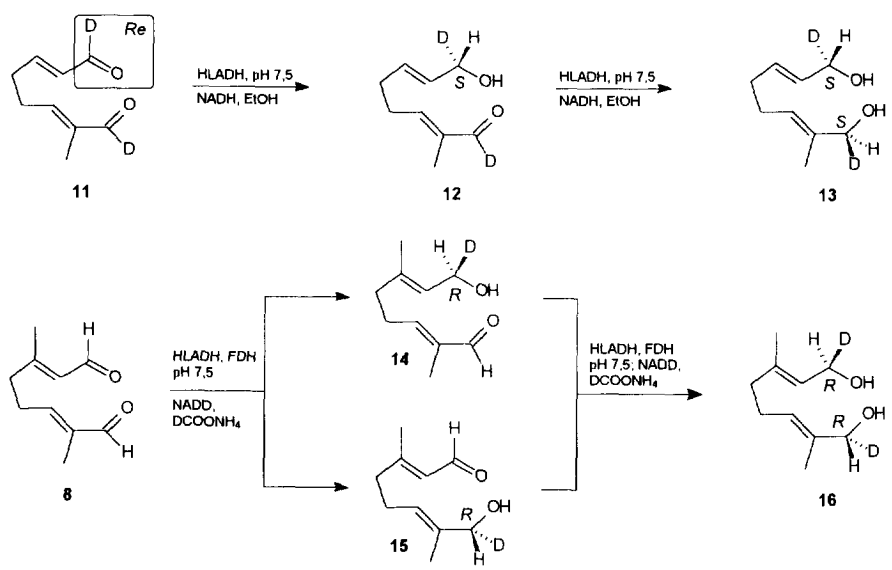
A convenient protocol to give the chirally labelled diols **13** and **16**, which were previously used for unravelling the biosynthesis of iridoids in insects,[5,6] is outlined in Scheme 2 and Scheme 3. Deuterium labelling was achieved by reduction of the diester **9** with AlD_3. The reaction proceeded without competing attack at the two double bonds.

Scheme 2



Following oxidation of the diol [1,8-$^2H_4$]-**10** with pyridiniumchloro chromate (PCC), the resulting dialdehyde [1,8-$^2H_2$]-**11** can be reduced in a stepwise, but in a stereospecific fashion to (1*S*)-[1,8-$^2H_2$]-**12** and (1S,8S)-[1,8-$^2H_2$]-**13** using an alcohol-dehydrogenase from horse liver (HLADH), with EtOH/NAD^+ as cofactors. HLADH catalyses the transfer of a hydride ion to the *Re*-face of a wide range of carbonyl compounds.[16] This invariable stereochemical course leads to (1*S*)- and (1*S*,8*S*) configuration at the two chiral centres of [1,8-$^2H_2$]-**12** and [1,8-$^2H_2$]-**13**. Reduction of the carbonyl group at C(1) proceeded much faster and, thus, allowed isolation of the intermediate (1*S*)-[1,8-$^2H_2$]-**12**. Further reduction provided the 3-*nor* derivative of 8-hydroxy-geraniol (1*S*,8*S*)-[1,8-$^2H_2$]-**13** (Scheme 3) in moderate yield (51%), but with high enantiomeric excess (≥ 95% *e.e.*) and the same absolute configuration at both chiral centres (*vide infra*). Since *in vitro* experiments with the defensive secretion and synthetic 8-hydroxygeraniol[a] **7** yield 8-oxogeranial[a] **8** and not chrysomelidial **1** (contact with the gland surface is required for cyclisation of **8** to **1**) the deuterium labelled genuine precursor (1*R*,8*R*)-[1,8-$^2H_2$]-**16** can also be used, without fear of its metabolites being masked by natural chrysomelidial **1**. The chiral diol **16** was synthesised using a route analogous to that for **13**.

Scheme 3



Accordingly, reduction of 8-oxogeranial **8** was carried out with HLADH, [$^2H_6$]-ethanol, NAD^+ and a second enzyme system (formiate-dehydrogenase, DCOONa) for *in situ* cofactor regeneration.[17,18] In this system a deuterium atom is transferred to the *Re*-face of both carbonyl groups yielding the two hydroxyaldehydes (1*R*)-**14** (70%) and (8*R*)-**15** (30%). Further reduction transformed both hydroxyaldehydes into chirally labelled (1*R*,8*R*)-[1,8-$^2H_2$]-**16**.

[a] for reasons of consistency and simplification of the nomenclature all compounds are numbered according to geraniol **6**. IUPAC nomenclature is used in the Experimental part

Enantiomeric Excess (*e.e.*) of the Labelled Alcohols

The *e.e.* of the chiral mono-hydroxy compounds **14** and **15** was conveniently determined by direct integration of the anisochronous resonances of the protons at C(1) and C(8) in the ^{1}H NMR spectra of their diastereomeric esters with (*S*)-2-methoxy-2-phenylethanoic acid (cf. Figure 1A/B). Thus, reaction of the (*S*)-α-methoxymandelic acid with the alcohols **7**, **14** and **15**, promoted by dicyclohexylcarbodiimide in the presence of 4-dimethylaminopyridine, yielded the corresponding esters **17**, **18** and **19** under non-racemizing conditions (60% overall yield).[19] As shown in Figure 1B the α-methylene protons at C(8) of the ester **17**, derived from unlabelled **7**, are 0.02 ppm anisochronous and coupled to each other (J = 4.8 Hz) forming a doublet, with the *pro-R* hydrogen resonating at higher field than the *pro-S*.[19] In agreement with the *Re*-specificity of HLADH, the transfer of the deuteride to C(8) of the dialdehyde **8** should yield the (8*R*)-hydroxyaldehyde **15** substituting the C(8)-H_R by a deuterium atom. As to be expected, the labelled ester **19** exhibits a singlet at lower field, broadened by coupling to the geminal deuterium atom. The resonance corresponding to the C(8)-H_R is absent (Figure 1A) and, hence, confirms the assignment of the absolute configuration as well as the high *e.e.* (≥ 95%) of **15**. The methylene group at C(1) of the α-methoxymandelate ester **17** (from unlabelled 8-hydroxygeraniol **7**) appears as a symmetrical eight-line multiplet, centred at 4.59 ppm, which collapses in **18** to a broad doublet (4.63 ppm) at lower field due to the substitution of the C(1)-H_R by a deuterium atom (≥ 95% *e.e.*, cf. experimental part).

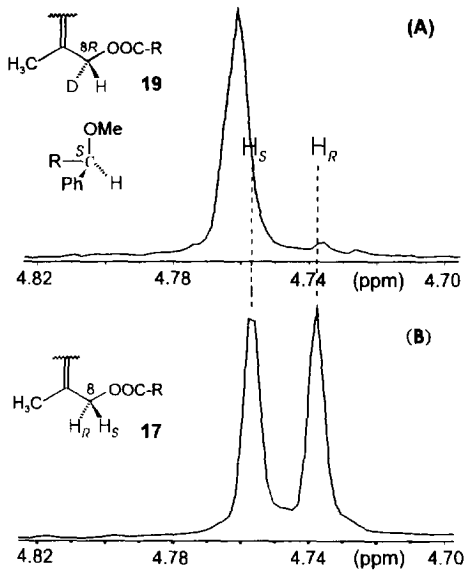


Figure 1 ^{1}H NMR spectrum of the (S)-α-methoxymandelate ester **17** derived from the hydroxyaldehydes (8*R*)-[1,8-$^2H_2$]-**15** (**A**) and unlabelled **7** (**B**).

***In Vitro* and *in vivo* Oxidation of Deuterium Labelled 8-Hydroxygeraniol (16)**

Feeding the chiral diol (1*S*,8*S*)-[1,8-$^2H_2$]-**13** to 14/16-day-old larvae of the leaf beetle *Phaedon armoraciae*, led to a rapid incorporation and metabolisation of the precursor. Analysis of the defensive secretion, withdrawn as a small droplet from the evertible glands of the larva, by GC-MS indicated the formation of a 1-nor-chrysomelidial **1**, still containing the two deuterium atoms of the precursor **13**. Thus, oxidation of the diol **13** proceeds at both centres, C(1) and C(8), coincidentally with removal of the H_R and is in agreement with a *Re*-specificity of the enzyme. If (1*R*,8*R*)-[1,8-$^2H_2$]-**16** is fed to the larvae, deuterium labelled chrysomelidial **1** is formed, but the correct determination of the molecular mass of the artificial metabolite **1** (<<

1%) is not possible due to the coelution with the unlabelled natural product (>> 99%). However, if the defensive secretions from about six larvae are collected and pooled in a chilled phosphate buffer (0.1 M, pH 7.5), a stock solution is obtained which can be used for *in vitro* oxidations of the diols **13** and **16** without competing cyclisation of the intermediary aldehydes. The progress of the reaction is easily followed by UV spectroscopy at 243 nm due to the absorption of the resulting α,β-unsaturated (di)aldehydes **11**, **12**, **14**, **15** or **8**. Alternatively, the products can be extracted and analysed by GC-MS. Initial *in vitro* experiments clearly showed that the conversion of 8-hydroxygeraniol **7** to 8-oxogeranial **8** was dependent on the presence of molecular oxygen as a cosubstrate. Degassing of the solution with helium stopped the reaction, while flushing the system with oxygen restarts the oxidation. Moreover, the addition of nicotinamide cofactors like NAD^+ and $NADP^+$ has no visible effect on the enzymatic activity. Obviously, the enzyme from *P. armoraciae* is not a $NADP^+$ dependent oxido-reductase, but instead an O_2-dependent oxidase like, for example, the oxidase from *Chrysomela populi* which catalyses the oxidation of salicylalcohol to salicylaldehyde.[20] Since the addition of EDTA did not inhibit the oxidation of 8-hydroxygeraniol **7**, the enzyme apparently also does not contain a Zn^{2+} atom and is, thus, different from all hitherto isolated plant enzymes involved in iridoid biosynthesis.[21]

Interestingly, the *in vitro* conversion of (1*R*,8*R*)-[1,8-$^2H_2$]-hydroxygeraniol **16** by the secretion *P. armoraciae* is non-selective and leads to the accumulation of the two mono-hydroxyaldehydes **14** and **15** in virtually equal amounts (cf. Fig. 2). The compounds were identified by their mass spectra and by comparing their retention times with the synthetic references (*vide supra*). In both compounds the oxidation proceeds with exclusive loss of the deuterium label from C(1) or C(8) and, thus, corroborates the stereochemical course (*Re*-specificity) observed after feeding of the diol (1*S*,8*S*)-[1,8-$^2H_2$]-**13** to larvae of *P. armoraciae*.

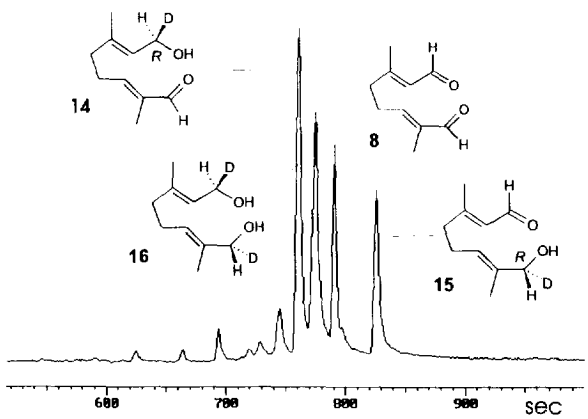


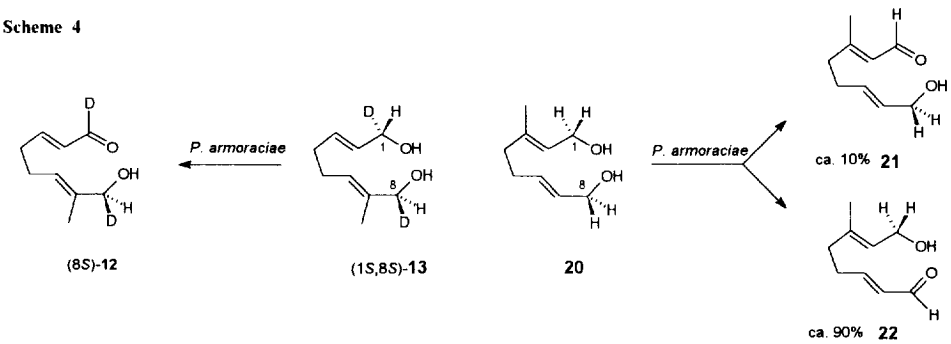
Figure 2 Section of the gas chromatogram of the *in vitro* metabolisation of (1*R*,8*R*)-[$^2H_2$]-**16**. For conditions see experimental section.

It is interesting to note that a similar *non*-selective oxidation of unlabelled 8-hydroxgeraniol **7** to 8-oxogeraniol **14** and 8-hydroxygeranial **15** has been reported by *Uesato* et al.[11] and *Ikeda* et al.[14] for a functionally comparable dehydrogenase isolated from *Rauwolfia serpentina* cells. The phytogenic enzyme is a monomer

with a molecular weight of 44.000 *Da* and contains a zinc ion. The substrates enter the enzyme by an ordered *Bi-Bi*-mechanism with $NADP^+$ binding first.[14]

Interestingly, if 3-*nor* hydroxygeraniol (1*S*,8*S*)-[1,8-$^2H_2$]-**13** is used instead of **16** as the substrate for the *in vitro* oxidation with the *Phaedon* enzyme, oxidation becomes highly regioselective. The oxidation occurred almost exclusively at C(1) yielding (8*S*)-**12** as an intermediate (cf. Scheme 4). Accordingly, the corresponding 7-*nor* 8-hydroxygeraniol **20** was selectively oxidised at C(8) (cf. Scheme 4), indicating that the methyl groups at C(3) and C(7) of (8*S*)-**13** and **20** govern the positioning of the substrate, preferentially exposing the less substituted allylic alcohol to the active site of the enzyme for hydride transfer. In the case of **13**, absolute regioselectivity was observed due to the proximity of the methyl group to C(8), whereas with **20** the regioselectivity was slightly diminished, probably because the methyl group is not on the adjacent carbon to C(1). The hydroxy-aldehydes **12**, **21** and **22** were then slowly further oxidised to the corresponding dialdehydes.

Scheme 4



The preference of the enzyme to remove the C(1)- or the C(8)-H_R of 8-hydroxygeraniol 7 appears to be rather general, since the enzymes present in the defensive secretions of several other chrysomelid larvae exhibit the same *Re*-specificity in *in vitro* experiments (cf. Table 1).

Table 1 Stereochemical course of the 10-hydroxygeraniol-oxidase from chrysomelid larvae

insect species	stereochemistry
Gastrophysa viridula	*Re*
Hydrothassa glabra	*Re*
Phaedon armoraciae	*Re*
Plagiodera versicolora	*Re*
Phratora vitellinae	*Re*
Prasocuris phellandri	*Re*

Besides of the terpenoid diols **13**, **16** and **20**, the hydroxyaldehydes **12**, **14**, **15**, **21** and **22** the enzymes from *P. armoraciae* and *Phratora* spp. also oxidise some aromatic alcohols, *e.g* salicylalcohol to salicylaldehyde with the same stereochemistry. Taking into account the fact that, among leaf beetles, a shift away from monoterpenoid (iridoid) defences towards plant derived defences has apparently occurred several times and

independently,[2,22,23] it will be interesting to see whether or not the oxidases employed for oxidation of terpenoid alcohols and aromatic precursors in closely and distantly related leaf beetles follow the same stereochemical course as described here. Such a finding could strongly support the idea that these enzymes may have evolved and diversified from an archetypal 10-hydroxygeraniol oxidase by adaptation to novel plant (bio)chemistry, after the insect moves to another host plant as a result of environmental stresses.[2]

EXPERIMENTAL

General: Reactions were performed under Ar. Solvents and reagents were purified and dried prior to use. Anh. $MgSO_4$ was used for drying. Alcohol-dehydrogenase from horse liver (HLADH), as a solution in KH_2PO_4 buffer (0.02 M, pH=7.0, 100 mg/10 ml), was purchased from Boehringer, Mannheim. Formiate dehydrogenase from *Candida boidinii* (FDH, 35 mg/ml glycerine solution) was a gift from Prof. C. Wandrey (IET/IBT Forschungszentrum Jülich). ^1H- and ^{13}C NMR: *Bruker Cryospec WM 250* and *Bruker WM 400*; IR: *Bruker IFS 88*. GC-MS: *Finnigan ITD 800* combined with a *Carlo-Erba* gas chromatograph, model *Vega*, equipped with a fused-silica capillary *CP-SIL-8-CB (Chrompack, 30m x 0.25 mm)*; carrier gas, He at 30cm/s; scan range: 35-249 Dalton/s. Analytical GLC: *Carlo-Erba* gas chromatograph, *HRGC 5300, Mega* series, equipped with fused silica capillaries, *SE 30* (10m x 0.32mm); H_2 at 30 cm/s as carrier. Silica gel, *Si 60*, (0.040-0.063 mm, *E. Merck*, Darmstadt, Germany) was used for liquid column chromatography.

(2*E*,6*E*) -1,2-Dimethyl-8-ethyl-octa-2,6-dienoate (9)

A chilled solution of triethylphosphono acetate (9.20 g, 40.80 mmol) in THF (180 ml) was metalled with *n*-BuLi (22 ml, 15% soln. in hexane, 41.00 mmol). Stirring was continued for 20 min and, then, a solution (2*E*)-2-methyl-6-oxo-hex-2-enoic acid methyl ester (4.80 g, 31.00 mmol)[8] in THF (15 ml) was added. The solution was stirred for 3 h at rt. prior to hydrolysis with H_2O (200 ml). Extractive work-up with ether (3 x 40 ml) afforded the crude product **9** which was further purified by chromatography on SiO_2 using ether/pentane (15:85, v:v) for elution. Yield: 4.00 g (59%). ^1H NMR (250 MHz, $CDCl_3$): δ [ppm] 1.29 (t, 3H, $-CH_3$), 1.83 (s, 3H, $-CH_3$), 2.2-2.4 (m, 2 H-C(4), 2 H-C(5)), 3.73 (s, 3 H, $-OCH_3$), 4.18 (quart., 4 H, $-OCH_2-$), 5.83 (d, J=15 Hz, 1 H-C(7)), 6.7 (t, 1 H-C(3)), 6.86-7.0 (m, 1 H-C(6)). IR (neat): 2983, 2952, 1717, 1653, 1436, 1388, 1367, 1315, 1265, 1237, 1197, 1163, 1124, 1086, 1043, 976, 854, 747 cm^{-1}. MS (%): 226 ($M^{+\bullet}$,1), 195(26), 194(73), 181(39), 180(5), 166(24), 152(9), 148(8), 137(6), 128(12), 121(14), 120(14), 114(100), 113(79), 93(32), 91(12), 86(56), 85(34), 82(15), 81(25), 79(10), 77(11), 69(19), 68(41), 59(25), 55(28), 53(27), 45(11), 43(12), 41(27), 39(21). HR-MS: *m z* calcd. for $C_{12}H_{18}O_4$: 226.1205, found: 226.1222.

(2*E*,6*E*)-2-Methyl-[1,8-$^2H_4$]-octa-2,6-dien-1,8-diol (10)

A well stirred, chilled solution of $LiAlD_4$ (3.86 g, 94 mmol) in ether (120 ml) was gradually treated with $AlCl_3$ (4.50 g, 33.60 mmol). After stirring at rt. for 30 min a solution of the diester **9** was slowly added. Stir-

ring was continued for 3 h, the mixture was cautiously hydrolysed with dil. NaOH (30 ml, 2N), the precipitate filtered off and the aq. layer carefully extracted with ether (3 x 50 ml). Chromatography on SiO_2, using ether/pentane (30/70) for elution yielded **10** as a viscous oil. Yield: 2.30 g (80%). ^1H NMR (250 MHz, $CDCl_3$): δ [ppm] 1.56 (s, 2 H, -OH), 1.65 (s, 3 H, $-CH_3$), 2.08-2.18 (m, 2 H-C(4), 2 H-C(5)), 5.38 (t,br. 1 H-C(3)), 5.62-5.7 (m, 1 H-C(7), 1 H-C(6)). IR (neat): 3327, 3024, 2985, 2917, 2854, 2184, 2083, 1705, 1668, 1437, 1381, 1247, 1190, 1148, 1113, 1082, 1065, 972, 905, 792, 743, 667 cm^{-1}. MS (%): 142 ($M'^{\bullet}$-H_2O,2), 124(5), 85(22), 84(5), 82(7), 81(6), 73(5), 71(8), 70(14), 69(8), 59(11), 58(6), 57(8), 56(62), 55(6), 45(9), 44(5), 43(100), 42(15), 41(8), 40(5), 39(6). HR-MS: *m z* calcd. for $C_9H_{10}{}^2H_4O$: 142.1295, found: 142.1300.

(2*E*,6*E*)-2-Methyl-[1,8-$^2H_2$]-octa-2,6-dien-1,8-dial (11)

A soln. of the diol **10** (1.50 g, 9.60 mmol) in CH_2Cl_2 (10 ml) was slowly added to a suspension of pyridiniumchloro chromate (6.25 g, 28.00 mmol) in CH_2Cl_2 (175 ml),. After 3 h stirring at rt. a mixture of ether/water (1:1, v:v, 150 ml) was added. Insoluble chromium salts were filtered off. The org. layer was extracted with ether (3 x 60 ml), and $MgSO_4$ was added to adsorb the last traces of chromium salts. Removal of solids and chromatography on SiO_2 with ether/pentane (70:30, v:v) yielded **11** as a faint yellow liquid. Yield: 0.75 g (52%). ^1H NMR (250 MHz, $CDCl_3$): δ [ppm] 1.77 (s, 3 H, $-CH_3$), 2.52-2.62 (m, 2 H-C(4), 2 H-C(5)), 6.17 (d, J=15Hz, 1 H-C(7)), 6.4-6.5 (t,br. 1 H-C(3)), 6.77-6.9 (m, 1 H-C(6)). IR (neat): 3471, 2928, 2076, 1712, 1675, 1645, 1441, 1393, 1364, 1252, 1147, 1054, 979, 809, 778 cm^{-1}. MS (%): 124 $M'^{\bullet}$-O=CH_2,8), 114(5), 113(6), 109(14), 97(6), 96(10), 86(6), 85(9), 84(35), 83(8), 82(22), 81(5), 74(5), 73(13), 72(13), 71(100), 70(30), 69(20), 68(17), 67(7), 58(12), 57(21), 56(92), 55(19), 54(13), 53(13), 45(22), 44(9), 43(79), 42(60), 41(31), 40(25), 39(35). HR-MS: *m z* calcd. for $C_8H_{10}{}^2HO$ (M'-COD): 124.0873, found: 124. 0862.

(2*E*,6*E*,8*S*)-8-Hydroxy-2-methyl-[1,8-$^2H_2$]-octa-2,6-dien-1-al (12)

A soln. of the dialdehyde **11** (0.75 g, 4.90 mmol) in phosphate buffer (130 ml, 0,1 M KH_2PO_4, pH=7.5) was shaken at rt., and EtOH (51 ml, 89 mmol), NAD^+ (200 mg, 0,3 mmol) and HLADH suspension (2 ml, 54 U) in KH_2PO_4 buffer (20 mM) were added. After 2 h a second portion of the HLADH suspension (1 ml, 26 U) was added and the reaction monitored by GC-MS. After 18 h the soln. was extracted with ether (3 x 40 ml), and the hydroxyaldehyde was purified by chromatography on SiO_2 using ether/pentane (70:30, v:v) for elution. The product **12** was obtained as a faint yellow, viscous oil. Yield: 0.27 g (35%). ^1H NMR (250 MHz, $CDCl_3$): δ [ppm] 1.54 (s, 1 H, -OH), 1.72 (s, 3 H, $-CH_3$), 2.2-2.32 (m, 2 H-C(5)), 2.38-2.5 (m, 2 H-C(4)), 4.08 (s,br. 1 H-C(8)), 5.62-5.72 (m, 1 H-C(6), 1H-C(7)), 6.42-6.52 (t, 1 H-C(3)). IR (neat): 3411, 2924, 2116, 2075, 1671, 1643, 1441, 1393, 1365, 1319, 1256, 1198, 1164, 1117, 1054, 1010, 974, 932, 806, 776, 662 cm^{-1}. MS (%): 138($M'^{\bullet}$-H_2O,15), 125(7), 124(6), 110(7), 109(5), 98 (5), 96(9), 86(16), 85(100), 84(12), 83(14), 82(9), 80(5), 72(6), 71(14), 70(5), 69(8), 68(9), 67(5), 58(6), 57(14), 56(25), 55(18), 54(9), 53(8), 44(26), 43(16), 42(20), 41(13), 40(9), 39(14). HR-MS: *m z* calcd. for $C_9H_{10}{}^2H_2O$($M'^{\bullet}$-18): 138.1014, found: 138.1038.

(2*E*,6*E*,1*S*,8*S*)-2-Methyl-[1,8-$^2H_2$]-octa-2,6-dien-1,8-diol {=(1*S*,8*S*)-[$^2H_2$]-3-nor-8-hydroxygeraniol} (13) EtOH (2.2 ml, 38 mmol), NAD^+ (10 mg, 0.015 mmol) and HLADH suspension (500 µl, 14U) were added to a shaken solution of hydroxyaldehyde **12** (0.08 g, 0.50 mmol) in phosphate buffer (10 ml, 0.1 M KH_2PO_4, pH=7.5). After 16 h a second portion of EtOH (2 ml, 35 mmol), NAD^+ (10.00 mg, 0.015 mmol) and HLADH suspension (200 µl, 6U) was added. After about 3 days ca. 50% of the hydroxyaldehyde **12** were reduced, and the soln. was extracted with ether (3 x 15ml). Chromatography on SiO_2 with ether/pentane (70:30, v:v) afforded the diol **13** as a viscous oil. Yield: 30.00 mg (38%). ^1H NMR (250 MHz, $CDCl_3$): δ [ppm] 1.55-1.62 (s, 2 H, -OH), 1.65 (s, 3 H, $-CH_3$), 2.07-2.15 (m, 2 H-C(4), 2 H-C(5)), 3.96 (s, 1 H-C(1)), 4.05 (s, 1 H-C(8)), 5,34-5.45 (m, 1 H-C(3)), 5,62-5,69 (m, 1 H-C(6), 1 H-C(7)). IR (neat): 3340, 2981, 2916, 2856, 2138, 1705, 1668, 1437, 1383, 1307, 1115, 1081, 1016, 972, 936 cm^{-1}. MS (%): (140 $M^{+\bullet}$-H_2O,8), 125(7), 122(16), 108(10), 97(6), 96(6), 95(7), 94(6), 92(7), 85(35), 84(25), 83(11), 82(10), 81(17), 80(13), 79(8), 75(8), 72(8), 71(11), 70(9), 69(28), 68(21), 67(10), 58(30), 57(13), 56(29), 55(81), 54(12), 53(11), 44(18), 43(100), 42(36), 41(28), 40(9), 39(18). HR-MS: *m/z* calcd. for $C_9H_{12}{}^2H_2O$ (M^+-H_2O): 140.1170, found: 140.1197.

(2*E*,6*E*)-2,6-Dimethylocta-2,6-dien-1,8-dial (8)

Prepared from hydroxygeraniol **7** (0.90 g, 5.20 mmol) as described for **11**. Faint yellow liquid. Yield: 0.62 g (70%). ^1H NMR (250 MHz, $CDCl_3$): δ [ppm] 2.78 (s, 3H, $-CH_3$), 3.22 (s, 3H, $-CH_3$), 3.40-3.70 (m, 4 H-C(4,5)), 6.88-6.98 (d, J=8Hz, 1H-C(7)), 7.38-7.5 (t, 1H-C(3)), 10.4 (s, 1H-C(1)), 10.95 (33% *Z*) and 11.0 (66% *E*) (d, J=8 Hz, 1 H-C(8)). IR (neat): 3338, 2926, 2855, 2717, 1677, 1442, 1406, 1381, 1360, 1195, 1159, 1123, 1089, 1040, 1006, 823 cm^{-1}. MS (%):166 ($M^{+\bullet}$, 1), 151(6), 148(20), 138(10), 137(21), 133(6), 123(15), 121(22), 120(12), 119(11), 109(57), 108(29), 107(11), 105(18), 97(30), 96(10), 95(57), 94 (10), 93(13), 91(18), 84(96), 83(53), 82(56), 81(32), 80(18), 79(19), 77(15), 71(21), 69(18), 67(26), 65(7), 56(15), 55(100), 54(15), 53(35), 45(10), 43(89), 41(51), 39(48). HR-MS: *m/z* calcd. for $C_{10}H_{14}O_2$: 166.0993, found: 166.1007

Enzymatic reduction of the dialdehydes 8 and 11 with HLADH. General Procedure:

A soln. of the dialdehyde, *e.g.* **8** (0.20 g, 1.20 mmol) in phosphate buffer (30 ml, 0.05 M KH_2PO_4, pH=7.5) was shaken at rt. Ethanol-d_6 (1 ml, 17 mmol), NAD^+ (70.00 mg, 0,10 mmol), DCOONa (0.10 g, 1.40 mmol), HLADH suspension (700 µl, 19 U) in KH_2PO_4 buffer (20 mM) and formiate dehydrogenase (FDH, 700 µl, 53 U) were added. After 24 h a second portion of HLADH (400 µl , 10 U), NAD^+ (50.00 mg, 75.00 mmol), and ethanol-d_6 (200 µl, 3.40 mmol) were added. After three days the faint yellow soln. was extracted with ether (3 x 40 ml); the precipitated enzyme was filtered off. Chromatography on SiO_2 using ether/pentane (70:30, v:v) for elution afforded a mixture of the hydroxyaldehydes **14** (70%) and **15** (30%). Yield: 0.103 g (51%).

(2*E*,6*E*,8*R*)-2,6-Dimethyl-8-hydroxy-[8-^{2}H]-octa-2,6-dien-1-al {= (1*R*)-[1-^{2}H]-8-oxogeraniol} (14) and (2*E*,6*E*,1*R*)-3,7-Dimethyl-8-hydroxy-[8-^{2}H]-octa-2,6-dien-1-al {= (8*R*)-[8-^{2}H]-8-hydroxygeranial} (15)

^{1}H NMR (250 MHz, $CDCl_3$) of **14**: δ [ppm] 1.71 (s, 3 H,-CH_3), 1.78 (s, 3 H,-CH_3), 2.15- 2.6 (m, 2 H-C(4), 2 H-C(5)), 4.15 (d,br. J=8 Hz, 1 H-C(8)), 5.45 (d, J=8Hz, 1 H-C(7)), 6.45 (t, 1 H-C(3)), 9.38 (s, 1 H-C(1)); [8-^{2}H]-8-Hydroxygeranial **15**: 1.61 (s, 3 H, -CH_3), 1.63 (s, 3 H,-CH_3), 2.15-2.6 (m, 2 H-C(4), 2 H-C(5)), 3.98 (s,br. 1 H-C(8)), 5.39 (t, 1 H-C(6)), 5.89 (d, J=8Hz, 1 H-C(2)), 9.89 (30% 6*Z*) and 9.99 (70%, 6*E*) (d, 1 H-C(1)). IR (neat): 3411, 2922, 2858, 2717, 1683, 1444, 1405, 1382, 1360, 1256, 1195, 1158, 1124, 1078, 1032, 939, 824 cm^{-1}. MS (%):169 ($M^{+\bullet}$, 0.6), 151(33), 138(10), 137(10), 136(27), 135(6), 122(20), 121(12), 109(15), 108(13), 107(6), 98(6), 97(8), 96(6), 95(19), 94(10), 93(6), 86(7), 85(23), 84(100), 83(41), 82(22), 81(17), 80(9), 72(7), 71(6), 69(13), 68(8), 58(19), 56(17), 55(30), 54(7), 53(12), 43(46), 42(17), 41(33), 39(17). HR-MS: *m z* calcd. for $C_{10}H_{15}DO_2$: 169.1213, found: 169.1232.

(2*E*,6*E*,1*R*,8*R*)-2,6-Dimethyl-[1,8-$^2H_2$]-octa-2,6-dien-1,8-diol. {= (1*R*,8*R*)-[$^2H_2$]-8-hydroxygeraniol} (16)

A soln. of the hydroxyaldehydes **14** and **15** (0.10 g, 0.06 mmol) in phosphate buffer (15 ml, 0.05 M KH_2PO_4, pH=7.5) was shaken at rt. Then, ethanol-d_6 (0.60 ml, 10 mmol), NAD^+ (35.00 mg, 0.05 mmol), DCOONa (27.00 mg, 0.39 mmol), HLADH (400 µl, 10 U) in KH_2PO_4 buffer (20 mM) and formiate dehydrogenase (FDH, 300 µl, 23 U) in glycerine were added. A second portion of NAD^+ (0.05 g, 0.075 mol), ethanol-d_6 (300 µl, 5 mmol), DCOONa (25 mg, 0.36 mmol), HLADH suspension (200 µl , 5 U) and FDH-solution (200 µl, 5 U) was added after 24 h. To complete the reduction, further portions of the above reagents were added after 36 and 48 h. After 60 h the faint yellow soln. was extracted with ether (3 x 30 ml), the precipitated enzyme was filtered off, and the diol **16** was purified by chromatography on SiO_2 using ether/pentane (70:30, v:v) for elution. Yield: 0.07 g (68%). ^{1}H NMR (250 MHz, $CDCl_3$): δ [ppm] 1.64 (s, 3H, -CH_3), 1.68 (s, 3H, -CH_3), 2.00-2.25 (m, 4 H-C(4,5)), 3.99 (s, 1 H-C(1)), 4.10-4.18 (m, 1 H-C(8)), 5.31-5.43 (m, 2 H-C(3,7)). IR (neat): 3327, 2917, 2154, 1668, 1443, 1383, 1304, 1069, 1015, 939, 902, 839 cm^{-1}. MS (%): 154($M^{+\bullet}$-H_2O, 2), 139(5), 138(6), 135(5), 123(7), 122(15), 121(12), 110(7), 109(8), 108(7), 107 (6), 97(10), 96(10), 95(14), 94(16), 93(8), 91(5), 85(21), 84(44), 83(13), 82(7), 81(16), 75(6), 73(33), 72(7), 71(14), 70(10), 69(58), 68(47), 67(15), 61(22), 58(7), 57(10), 56(13), 55(16), 53(7), 45(48), 43(100), 41(26). HR-MS: *m z* calcd. for $C_{10}H_{14}D_2O$: 154.1326, found: 154. 1192 ($M^{+\bullet}$-H_2O).

Determination of the enantiomeric excess of the hyroxyaldehydes (14) and (15) via their α-methoxymandelate esters (18) and (19)

To a stirred solution (-10°C) of *S*-(+)-2-methoxy-2-phenylethanoic acid (37.0 mg, 0.22 mmol) and 4-dimethylaminopyridine (3.0 mg, 0.024 mmol) in CH_2Cl_2, a solution of the hydroxyaldehydes **14** or **15** (25.0 mg, 0.15 mmol) and N,N'-dicyclohexylcarbodiimide (45.0 mg, 0. 22 mmol) in CH_2Cl_2 (3 ml) was added dropwise. The solution was stirred at -10°C for 3 h prior to the addition of ether (10 ml). The precipitated urea was filtered off, and the solvent removed under reduced pressure. Purification and separation of the

mandelate esters **18** (from **14**) and **19** (from **15**) was achieved by chromatography on SiO_2 using ether/pentane (50/50, v/v) for elution. Yield: 30.0 mg (62%) each. ^{1}H NMR (250 MHz, $CDCl_3$) **18**: δ [ppm] 1.67 (s, $-CH_3$), 1.72 (s, $-CH_3$), 2.11-2.5 (m, 4 H-C(4,5)), 3.4 (s, $-OCH_3$), 4.65 (d, ^{3}J=7 Hz, 1 H_s-C(8)), 4,75 (s, 1H-C2'), 5.3 (d, J=7 Hz, 1 H-C(7)), 6.39 (t, ^{3}J=7 Hz, 1 H-C(3)), 7.3-7.48 (m, 5 H, aromat.-H), 9.35 (s, 1 H-C(1)). ^{1}H-NMR (250 MHz, $CDCl_3$) **19**: δ [ppm] 1.5 (s, $-CH_3$), 1.92 (s, $-CH_3$), 2.08-2.3 (m, 4 H-C(4,5)), 3.4 (s, $-OCH_3$), 4.42 (s, 1H, ethanoic acid), 4.76 (s, 1 H_s-C(8)), 5.20-5.31 (m, 1 H-C(6)), 5.85 (t, 1 H-C(2)), 7.3-7.5 (m, 5 H, aromat.-H), 9.9 (2 d, ^{3}J=10 Hz, 1 H-C(1)).

2,6-Dimethyl-octa-2,6-dienyl-1,8-di-(*S*)-(+)-methoxy-phenylethanoate (17)

Prepared from commercial 8-hydroxygeraniol **7** (40.0 mg, 0.24 mmol) as described before. Yield: 60.0 mg (50%). ^{1}H NMR: (250 MHz, $CDCl_3$): δ[ppm] 1.47 (s, $-CH_3$), 1.59 (s, $-CH_3$), 1.9-2.1 (m, 4 H-C(4,5)), 3.39 (s,$-OCH_3$), 3.41 (s, $-OCH_3$), 4.47 (s, 2 H-C(2) ethanoic acid), 4.5-4.7 (2 dd, J=13 and. 20Hz, 2 H-C(8)), 4.76 (d, J=6Hz, 2 H-C(1)), 5.18-5.3 (m, 1 H-C(3,7)), 7.28-7.47 (m, 10 H, aromat.-H). IR (neat): 3063, 3031, 2931, 2827, 1749, 1672, 1495, 1455, 1349, 1256, 1199, 1174, 1115, 1075, 1029, 1001, 924, 849, 732, 698, 619 cm^{-1}. MS (%): 466($M^{+\bullet}$, 0.07), 390(0.05), 372(0.2), 301(1.5), 135(3), 134(2), 122(8), 121(100), 118(1), 77(3). HR-MS: *m z* calcd. for $C_{28}H_{34}O_6$: 466.2355, found: 466,2339.

Rearing of insects and* in vitro *oxidations

Larvae of the leaf beetles *Phaedon armoraciae* were reared on Chinese cabbage from the local market at 16°C using a light regime of 12 h. Leaves were replaced every second day. Larvae of all other insects (cf. Table 1) were collected from their natural habitat near Bonn and Bayreuth. *In vivo* transformations: cabbage leaves were painted with 0.1% aq. solutions of the diols **7**, **13** and **20**. After drying up of the solutions, the larvae were allowed to feed for 2-3 days on the pre-treated leaves. Treatment of the larvae with tweezers led to discharge of the defensive glands. The metabolites were withdrawn as a small droplet using a pressure-lok mini injector from *Alltech*, Munich. The sample was analysed by GC-MS without preceding purification. *In vitro* oxidations: The defensive secretion from ten larvae was collected with small glass capillaries from the everted glands and pooled in a phosphate buffer (1.20 ml, NaH_2PO_4, 0.1 M, pH 7.5). 0.6 ml of this stock solution were degassed with helium. To start the reaction, 100 µl of a degassed solution of the diols **13**, **16** and **20** in the same buffer was added. The cuvette was briefly shaken to allow free access of air. The progress of the oxidations was then followed at 240 nm against the stock solution without added diol. For GC-MS 100 µl aliquots of the stock solutions were mixed with 100 µl of 0.1% aq. soln. of the diols. After standing for 24 h at rt. the products were extracted with ether (200 µl) and analysed by GC-MS.

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