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# Biosynthesis of Iridoid Monoterpenes in Insects: Defensive Secretions from Larvae of Leaf Beetles (Coleoptera: Chrysomelidae).

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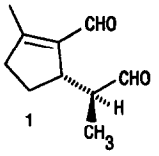
**Abstract:** Larvae of the leaf beetles *Phaedon amoraciae, Phaedon cochleariae, Gastrophysa viridula,* and *Plagiodera versicolora* biosynthesize the two iridoids chrysomelidial **1** and/or plagiodial **3** from geraniol **7** by an oxidative sequence related to that known from plants *en route* to the iridane skeleton. Following ω-oxidation of geraniol **7** the resulting 8-hydroxygeraniol **8** is oxidized further to 8-oxocitral **9**. The mode of the cyclization of 8-oxocitral **9** to chrysomelidial **1** depends on the chrysomelid species. In larvae of *Gastrophysa viridula* chrysomelidial **1** originates directly from the acyclic precursor **9** while in both *Phaedon spp.* chrysomelidial **1** is produced *via* plagiodial **3** followed by isomerization of the endocyclic double bond.

## INTRODUCTION

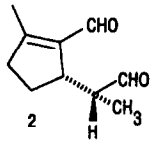
Methylcyclopentanoid monoterpenes possessing the iridane skeleton like **1** → **3** are widespread constituents of the defensive secretions of larvae of the phytophagous leaf beetles (Chrysomelidae).[1] The name "iridoid" originates from the fact that the first member of this class of compounds was isolated from the secretions of the ant *Iridomyrmex detectus*.[2] Iridoid dialdehydes are also used as chemical weapons by the stick insect *Anisomorpha buprestoides* (Phasmatidae). Anisomorphal **4** is sprayed from the prothoracic glands onto an enemy, when the insect is disturbed.[3] Other iridoids, in particular those possessing a lactone or hemiacetal functionality, act as pheromones during the sexual reproduction of aphids.[4]

The chrysomelid larvae (subfamily chrysomelinae) possess nine pairs of glands on the meso- and metathorax and on the first seven abdominal segments. The glands are eversible, and, when disturbed, a drop of secretion appears at the tip of the everted reservoirs. The low molecular fraction of the defensive secretions of, for example, larvae of *Phaedon amoraciae, Phaedon cochleariae,* and *Gastrophysa viridula* contains chrysomelidial **1** and epichrysomelidial **2** as the major products. Plagiodial **3** and plagiolactone **5** are typical for the larvae from *Plagiodera versicolora* which feed on willow trees.[1] Actinidine **6** is the major defensive compound of the phasmid *Megacrania tsudai* endemic to Taiwan[5] and certain staphylinid beetles.[1] At a

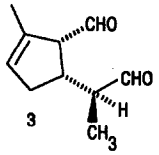
trace level **6** is found in the defensive secretions of all of the aforementioned chrysomelid larvae.



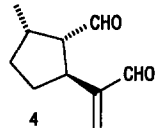

chrysomelidial



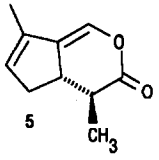

epichrysomelidial



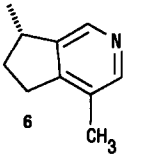

plagiodial




anisomorphal



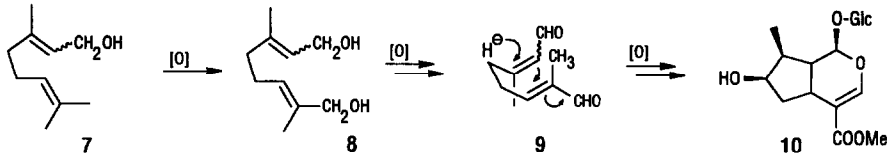

plagiolactone




actinidine

In plants the iridoids are most often present as water soluble glycosides. Via loganin **10** they are incorporated into thousands of indole- and isoquinoline alkaloids.[6,7] Due to their enormous number and their high pharmaceutical interest, the biosynthesis of the iridane skeleton in plants has been the subject of intensive research over the past thirty years.[8]

**Scheme 1**




loganin

The key steps of these plant biosyntheses are the ω-hydroxylation of geraniol **7** or the (2*Z*)-isomer nerol to 8-hydroxy(nerol)geraniol **8** and its further oxidation to the dialdehyde **9**. The latter can be cyclized and modified further to yield e.g. loganin **10** as the central intermediate towards the indole- and isoquinoline alkaloids.

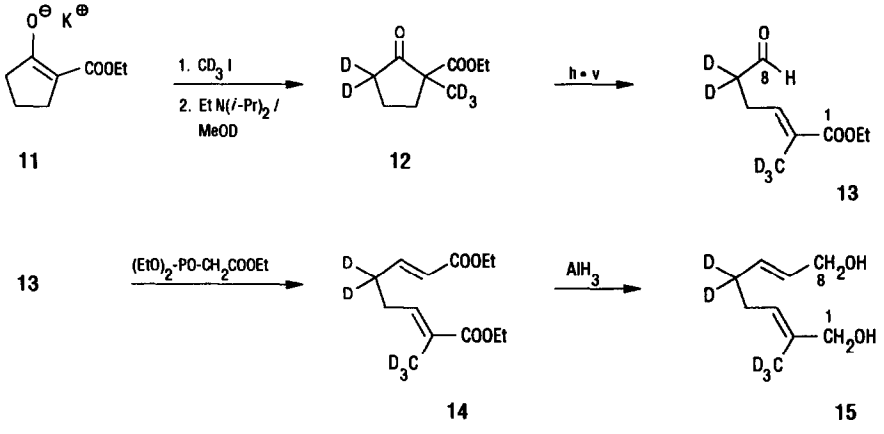
In contrast to the well studied plant metabolism there have been no detailed investigations[2] into the biosynthesis of iridodials in insects, *e.g.* **1** → **6**. In this and a previous communication[9] we report that the biosynthesis of **1**, **2**, **3** and **5** in the larvae of *P. amoraciae*, *P. cochleariae*, *G. viridula*, and *P. versicolora* is, in principle, similar to the known plant metabolism, but that the mode of the biosynthesis of chrysomelidial **1** from the acyclic precursor **9** reveals a surprising range of variations (Scheme 8 and 9). It is shown that larvae of the above leaf beetles can feed on leaves impregnated with artificial, deuterium labelled precursors, which are ingested, metabolized, and secreted as labelled metabolites together with the genuine defensive compounds. The compounds can be analyzed by mass spectrometry and, in conjunction with a specific labelling pattern of the administered precursors, details of the iridoid biosynthesis in insects become visible.

# RESULTS AND DISCUSSION

**I. Synthesis of deuterium labelled precursors.** As shown in Figure 1, chrysomelidial **1** and epichrysomelidial **2** are the dominant components of the larval defensive secretion of *G. viridula.* Taking into account that the rate of incorporation of labelled precursors into compounds of the lipid metabolism of insects or plants is usually very low (≤ 1%), labelled chrysomelidial **1** (by stable isotopes) would be buried under the huge signal of the natural product. In order to obtain usable mass spectra of metabolites of an administered precursor, we decided to use the deuterium labelled 3-norgeraniol **21** and related compounds like the diol **15** as the metabolic probe(s). Due to the lacking methyl group a resulting 1-norchrysomelidial **23** can be analyzed by mass spectroscopy without superposition by the corresponding natural product (Figure 1). The strategy is based on the assumption that the 3-norprecursors are transformed in an analogous manner to the iridoid dialdehydes as the natural substrates.

A convenient route to the deuterium labelled diol [$^2H_5$]-**15**, corresponding to the first intermediate of the biosynthesis of the iridane skeleton in plants, is outlined in Scheme 2. Alkylation of the potassium salt of the ß-ketoester **11** with $^2H_3CI$ and base catalyzed exchange of the acidic α-protons of [$^2H_3$]-**12** yields [$^2H_5$]-**12**. Irradiation of the ß-ketoester induces a *Norrish I* cleavage, and the acyclic aldehyde [$^2H_5$]-**13** is obtained as a mixture of isomers (2*E*:2*Z*. = 2:1).[10] Their separation is achieved by chromatography on $SiO_2$. Subsequent alkylenidation of (2*E*)-[$^2H_5$]-**13** with the anion derived from triethylphosphono acetate and reduction of the resulting (2*E*,7*E*)-diester [$^2H_5$]-**14** with $AlH_3$ provides the diol [$^2H_5$]-**15**. If the reduction is carried out with $Al^2H_3$, four additional deuterium atoms can be introduced at C(1) and C(8), respectively. Analogous treatment of (2*Z*)-[$^2H_5$]-**13** gives (2*Z*,7*E*)-[$^2H_5$]-**15**. To avoid the concomitant reduction of the C(6)=C(7) double bond, the reduction of the ester moieties has to be carried out with $AlH_3$ or DIBAl-H.

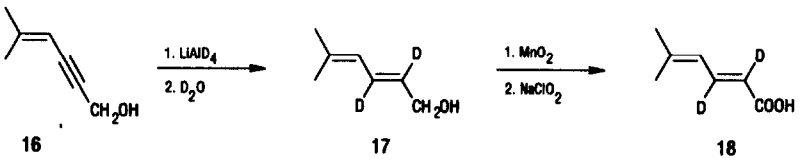
**Scheme 2**




The synthesis of the deuterium labelled 3-norgeraniol (4*R*,5*S*)-[$^2H_2$]-**21** and its enantiomer (4*S*,5*R*)-[$^2H_2$]-**21** is outlined in Scheme 4. The key step of this approach is the biocatalytic reduction of the dienoic acids [$^2H_2$]-**18** and [$^1H$]-**18**, using broken cells of *Clostridium tyrobutyricum* (strain: DSM 1460).[11] The enoate reductase of this bacterium catalyzes the enantiospecific transfer of two hydrogen atoms across the double

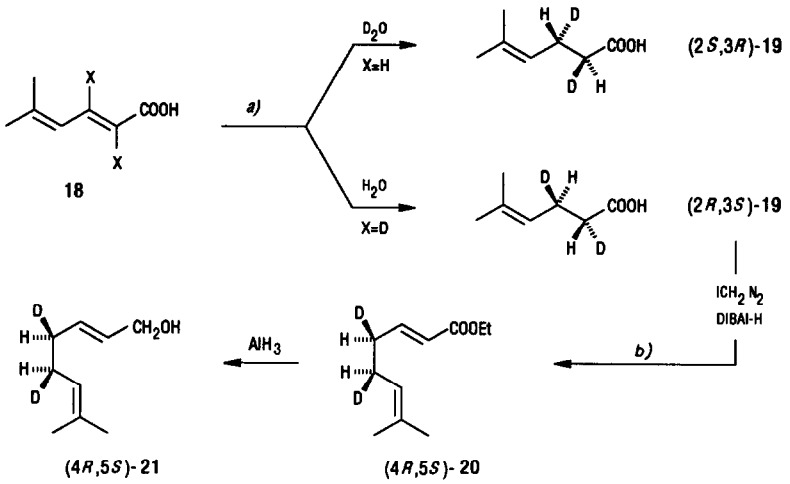
bond of a broad range of α,ß-unsaturated acids.[11] The process is well suited for preparative scale reactions. As outlined in Scheme 4, the reduction can be carried out in $^1H$-buffers as well as in buffered $^2H_2O$-solutions. The hydrogen or deuterium atoms are delivered to the two trigonal centres at C(2) and C(3) of the dienoic acid **18** in an exclusive *anti-Si-Si* fashion[13] and, hence, both enantiomers of the acids (2*S*,3*R*)-**19** and (2*R*,3*S*)-**19** are available by proper combination of the precursor **18** (X = H or $^2H$) with the suitable buffer system ($H_2O$ or $^2H_2O$). The conjugated double bond C(4)=C(5) is not affected. The deuteriated acid [$^2H_2$]-**18** is available from the alkynol **16** by reduction with $LiAl^2H_4$ and $^2H_2O/^2HCl$ for the hydrolysis of the organoaluminum intermediate (Scheme 3). Successive treatment of **17** with $MnO_2$ and a buffered solution of $NaClO_2$ as the oxidant gives [$^2H_2$]-**18** in 61% overall yield.[12]

**Scheme 3**




16 17 18

**Scheme 4**

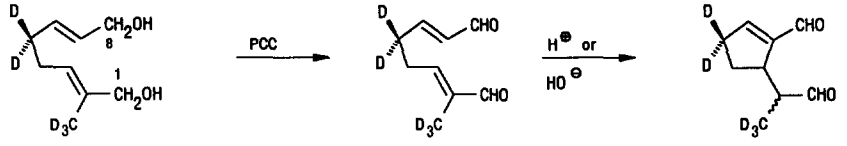



a) broken cells of *Clostridium tyrobutyricum*, phosphate buffer (p(D)H = 7.0), $H_2$ gas b) $(EtO)_2PO\text{-}CH_2COOEt$

As proved by their mandelate-diesters and $^1H$ NMR[14] the acids (2*S*,3*R*)-**19** and (2*R*,3*S*)-**19** are virtually optically pure (> 97% e.e.). Reductive alkylidenation[15] of the methyl ester of (2*R*,3*S*)-[$^2H_2$]-**19** with DIBAl-H and the anion of triethylphosphono acetate gives (4*R*,5*S*)-[$^2H_2$]-**20**. Reduction with $AlH_3$ provides the 3-norgeraniol (4*R*,5*S*)-[$^2H_2$]-**21**. Repetition of the sequence with (2*S*,3*R*)-[$^2H_2$]-**19** yields the enantiomer (4*S*,-5*R*)-[$^2H_2$]-**21**.

**II. Biomimetic cyclization of the deuterium labelled dialdehyde [$^2H_5$]-22.** The deuterium labelled 1-norchrysomelidial [$^2H_5$]-**23**, required as an authentic reference for the identification of the labelled metabolites, is available by a biomimetic cyclization of the corresponding acyclic dialdehyde precursor (Scheme 5). Thus, following oxidation of the diol [$^2H_5$]-**15** the resulting dialdehyde [$^2H_5$]-**22** can be cyclized with dilute base[16] (ca. 5 min, 0.01 N NaOH in MeOH/$H_2O$) or, preferably, by reflux in formic acid (50%, 1 h).[17] In both cases a mixture (ca. 1:1) of the 1-norchrysomelidial [$^2H_5$]-**23** and its diastereomers is obtained without significant loss of deuterium atoms from C(5) of the cyclopentene ring. Due to the formation of by-products, the base catalyzed cyclization[16] is less satisfactory. From a mechanistic view the base-induced cyclization of **22** can be rationalized as a *tandem-Michael* addition started by addition of a nucleophile ($H_2O$ or $CH_3OH$) to the C(6)=C(7) double bond, followed by cyclization and elimination of the conjugate base yielding the 3-norchrysomelidial [$^2H_5$]-**23**.

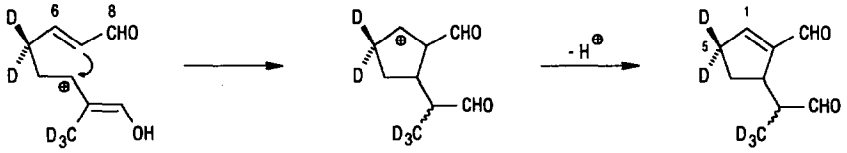
**Scheme 5**




**15** **22** **23**

As a matter of fact, the retention of both deuterium atoms at C(5) is in contradiction to a mechanistic proposal of *Uesato et al.*,[17] who considered plagiodial **3** as an intermediate of the acid catalyzed cyclization of 8-oxocitral **9** to chrysomelidial **1**. With respect to the retention of deuterium atoms at C(5) the acid catalyzed cyclization of the dialdehyde [$^2H_5$]-**22** should proceed as outlined in Scheme 6.

**Scheme 6**




**24** **25** **23**

Following protonation of the carbonyl oxygen at C(1) of [$^2H_5$]-**22**, the molecule will cyclize by interaction of the mesomeric oxo-carbenium ion [$^2H_5$]-**24** with the C(6)=C(7) double bond. The resulting carbenium ion [$^2H_5$]-**25** stabilizes by loss of a vicinal proton. Without assistance of a specifically positioned base (enzyme catalysis; *vide supra*) this will be the proton next to the aldehyde group, yielding the conjugated double bond of the 1-norchrysomelidial [$^2H_5$]-**23**.

**III. Biosynthetic studies with larvae of leaf beetles; the biosynthetic sequence.** When larvae of *G. viridula*, are reared on leaves of *Rumex patientia*, previously impregnated with the diol [$^2H_5$]-**15**, after about 2-

3 days significant amounts of the 1-norchrysomelidial [$^2H_5$]-**23** and its epimer can be detected in the defensive secretions by mass spectrometry (cf. Figure 1 and Figure 2).[9] As anticipated, the signal of the 1-norchrysomelidial [$^2H_5$]-**23** is not superimposed by a natural product, and the corresponding mass spectrum can be analyzed for structural details. This successful incorporation of a labelled precursor highlights two important aspects. *i)* the diol [$^2H_5$]-**15** is a suitable surrogate to follow the metabolism of natural 8-hydroxygeraniol **8** to the iridoid monoterpene dialdehydes in the defensive glands of chrysomelid larvae. *ii)* The occurrence of labelled 1-norchrysomelidial [$^2H_5$]-**23** and its epimer in the defensive secretion of *e.g. G. viridula* implies not only the ingestion of the precursors, but also their transport by the hemolymph to the defensive gland and their further metabolism to the iridane skeleton by specific enzymes within the gland cells or the gland reservoir, respectively. This may reflect a general ability of chrysomelid larvae to sequester plant derived acyclic monoterpene precursors like *e.g.* geraniol **7**, 8-hydroxygeraniol **8** or glycosidically bound derivatives[18] thereof as precursors for their own iridoid biosynthesis.[1]

If aq. solutions of the precursors [$^2H_5$]-**15** or [$^2H_2$]-**21** are injected into the hemolymph of 10-14-day old larvae (ca. 1 µl; 0.9% soln.), as soon as 4 h after the injection of the precursors [$^2H_5$]-**23** and its epimer are present in the secretion of surviving larvae (mortality ca. 30%). In the case of the 3-norgeraniol precursors like [$^2H_2$]-**21** this is the only successful incorporation technique, since the volatiles act as strong (feeding) deterrents.

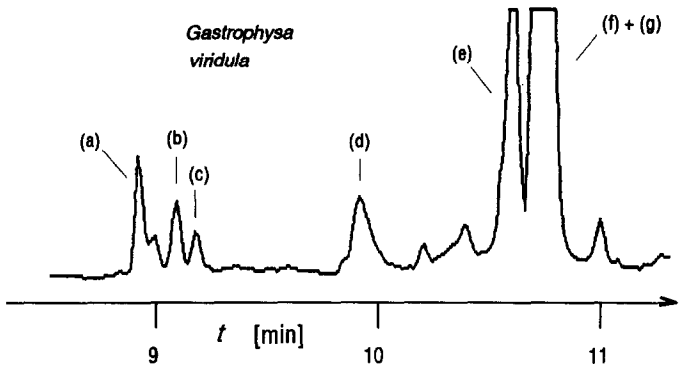


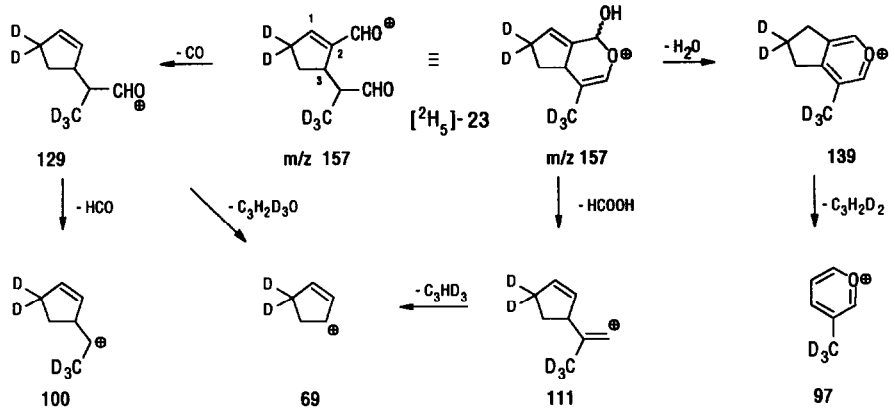

**Figure 1.** Section of the gas chromatogram of the volatiles from the defensive secretion of *Gastrophysa viridula* after feeding with [$^2H_5$]-**15**. Identification of compounds: **(a)** [$^2H_5$]-**23**, **(b)**/(c): plagiodial **3** and the epimer, **(d)**: actinidine **6**, **(e)**: [$^2H_5$]-**22**, **(f)**/**(g)**: chrysomelidial **1** and the epimer **2**. GC conditions: fused silica SE 30 (10 m x 0.32 mm) under programmed conditions (50°C for 2 min, then at 10°C $min^{-1}$ to 250°C). Detection and identification of compounds: *Finnigan* ion trap *ITD 800*.

In some experiments with larvae of *G. viridula* and [$^2H_5$]-**23** also the acyclic 3-nordialdehyde [$^2H_5$]-**22** is observed; an exceptionally large signal is shown in Figure 1. The compound is readily identified by its mass spectrum (see experimental). The isomeric diol (6*Z*)-3-nor[$^2H_5$]-**15** is not metabolized, indicating a high substrate specificity of the involved enzymes. Since the injection of the 3-norgeraniol [$^2H_2$]-**21** also leads to a labelled 1-norchrysomelidial [$^2H_2$]-**23**, the larvae must possess at least two different oxidases.[1] The first converts natural geraniol **7** into 8-hydroxygeraniol **8**, and the second type of enzyme(s) produces 8-

oxocitral **9** from the diol **8**. These findings perfectly match the biosynthesis of the iridane skeleton in plants which is outlined in Scheme 1 (cf. also Scheme 8 and 9).[8] A brief screening with larvae of other leaf beetles, like for example *Phaedon cochleariae, P. amoraciae, Plagiodera versicolora,* and *Prasocuris phellandrii* revealed that all of the above species are able to convert the 3-norprecursors, in particular the diol [$^2H_5$]-**15**, into the corresponding iridoid dialdehydes; the complete set of the required enzymes is, thus, widespread in the iridoid producing species of the subfamily Chrysomelinae.

**IV. Modes of the cyclization of the 3-nordialdehyde [$^2H_5$]-23 in the chrysomelid larvae.** The mass spectra (EI, 70 eV) of the 1-norchrysomelidial(s) [$^2H_n$]-**23** give valuable information about the number and position of the deuterium atoms. Using the biomimetic cyclization of deuterium labelled dialdehydes, like [$^2H_5$]-**23** and others, a set of specifically labelled iridoid dialdehydes could be synthesized and used to study the mass fragmentation pattern of these compounds. By analogy to the postulated fragmentation pattern of iridodial, presented by *Uesato et al.*,[19] the 1-norchrysomelidial [$^2H_5$]-**23** decays upon electron impact into the fragments outlined in Scheme 7.

**Scheme 7** Postulated fragmentation pattern for [$^2H_5$]-**23**




According to Scheme 7 the fragment at m/z = 69 (100%) is indicative for the number of deuterium atoms at the cyclopentene ring. It originates from the parent ion at m/z = 157 by a consecutive loss of carbon monoxide (→ m/z = 129) and $D_3CCH{=}COH$ (→ m/z 69). If a deuterium atom is present at the formyl group at C(2) of [$^2H_5$]-**23**, this is transferred to the ring, increasing the cyclopentenyl fragment by one mass unit. No transfer of deuterium atoms is observed from the deuteromethyl- or the formyl group of the $C_3$-side chain. The occurrence of a "cyclopentenyl cation" as the most prominent fragment is striking, and the structure of this peculiar ion is probably better represented by the corresponding isobaric but aromatic ethylcyclopropenylium cation. Owing to their rather high intensity, the fragments at m/z = 100 (14%) and m/z = 129 (12%) can be used to determine the total number of the deuterium atoms of the metabolites. In particular, at low concentrations the molecular ion at m/z = 157 is often not found.

As a matter of fact, the mass spectra of the metabolites $[^2H_n]$-**23** from the administration experiments with $[^2H_5]$-**15** and larvae of *Gastrophysa viridula* and *Phaedon amoraciae* or *P. cochleariae* exhibit a significant difference (Figure 2). Larvae of *G. viridula* cyclize $[^2H_5]$-**15** without loss of deuterium (m/z = 69; 100%). In contrast, the major fragment (m/z = 68; 100%) and the two signals at m/z = 99 and m/z = 128 of the same metabolites obtained from both *Phaedon* spp. are in agreement with the loss of one of the two enantiotopic deuterium atoms from C(5) of the precursor $[^2H_5]$-**15**. Obviously, these larvae produce at first the 1-norplagiodial $[^2H_4]$-**26** which is then isomerized to the more stable, conjugated 1-norchrysomelidial $[^2H_4]$-**23** (Scheme 8). Since small amounts of plagiodial **3** are present in the defensive secretion of these larvae, this biosynthetic sequence, involving the isomerization of plagiodial **3** as the final (enzyme catalyzed?) step, appears to be sufficiently evidenced.

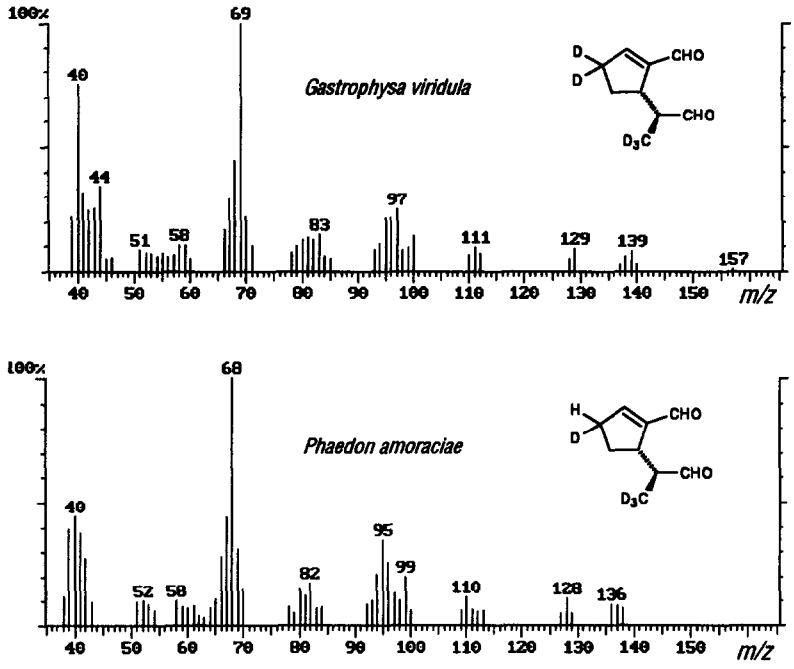
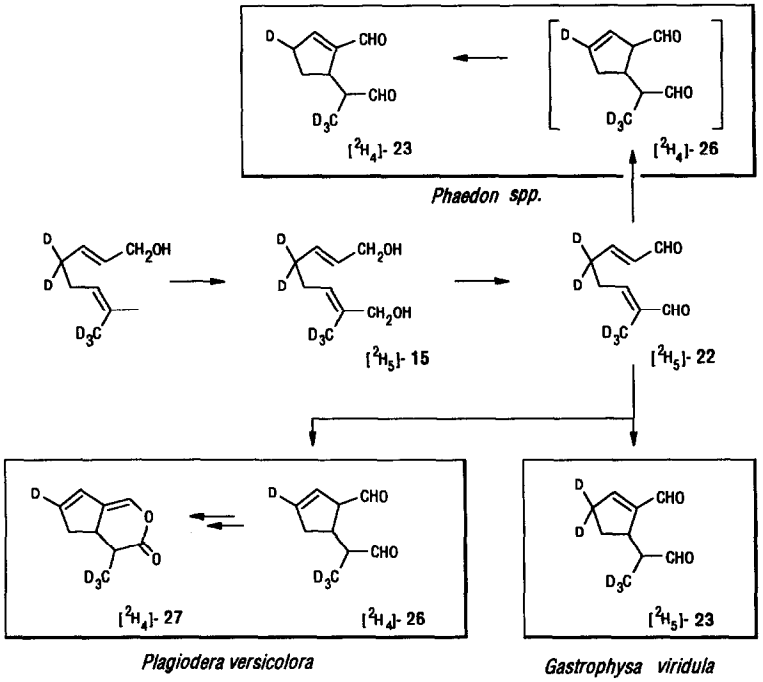



**Figure 2.** Mass spectra of $[^2H_5]$-**23** and $[^2H_4]$-**23** from the defensive secretions of *Gastrophysa viridula* (upper) and *Phaedon amoraciae* (lower) after feeding with $[^2H_5]$-**15**. *Finnigan* ion trap *ITD 800* (transfer line: 270°, scan range: 35-250 Dalton/sec).

Injection of the two chiral precursors (4*R*,5*S*)-$[^2H_2]$-**21** and (4*S*,5*R*)-$[^2H_2]$-**21** into the hemolymph of *P. amoraciae* proofs the removal the deuterium atom from C(5) of $[^2H_5]$-**15** to be specific for the C(4)-D*S* deuterium atom. The 3-norgeraniol (4*S*,5*R*)-$[^2H_2]$-**21** is transformed into the 1-norchrysomelidial $[^2H_1]$-**23**. The relevant fragments at m/z = 68, 125 and 135 *Da* are in agreement with the presence of one deuterium atom at the cyclopentene ring of the metabolite [$^2$H]-**23**. The control experiment with (4*R*,5*S*)-$[^2H_2]$-**21** corroborates the result and yields $[^2H_2]$-**23** with retention of both deuterium atoms (signals at m/z =69, 126 and

136 *Da*). Whether or not this stereochemistry is representative for the biosynthesis of plagiodial **3** in other chrysomelid larvae, remains to be established. Administration of [$^2H_5$]-**15** to larvae of *Plagiodera versicolora* leads to the 2-norplagiodial [$^2H_4$]-**26** and the 2-norplagiolactone [$^2H_4$]-**27**. Owing to the intense molecular ion of the lactone [$^2H_4$]-**27** at m/z = 154 (45%) the number of deuterium atoms is reliably determined. Mechanistic aspects of the additional steps between [$^2H_4$]-**26** and the lactone [$^2H_4$]-**27** remain to be clarified.

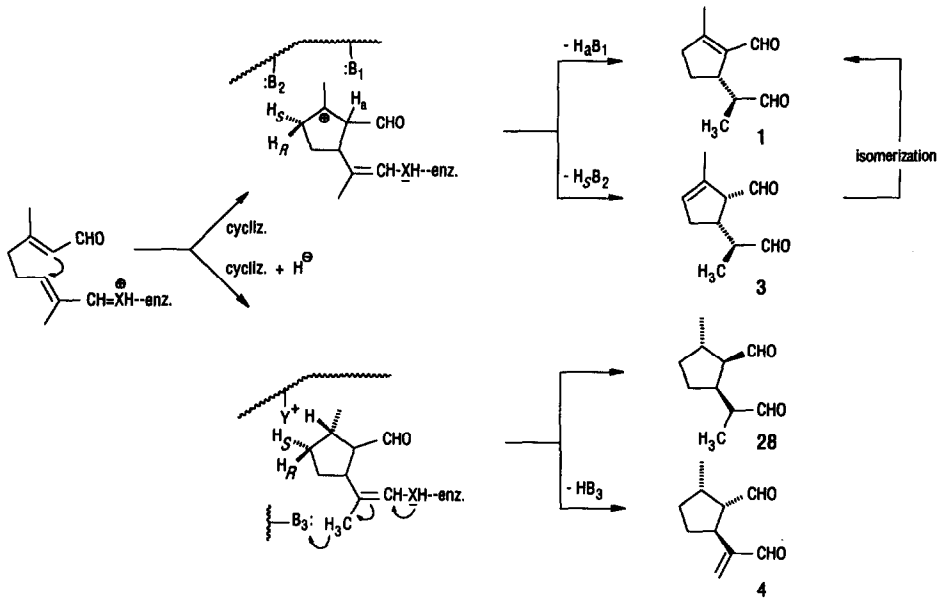
**Scheme 8** Biosynthetic pathways towards iridoid dialdehydes in chrysomelid larvae




The observation of two different modes of the biosynthesis of chrysomelidial **1** from 8-oxocitral **9** in larvae of different leaf beetles may reflect the existence of a common, transient intermediate within the diverging pathways. For example, an intermediate cation like **25** (Scheme 6) could stabilize by loss of a proton from one of the two adjacent carbon atoms yielding the cyclopentene double bond of either chrysomelidial **1** or plagiodial **3**, respectively. A tentative scheme which assumes a protonated intermediate (*e.g.* the oxygen of a carbonyl group or the corresponding iminium salt; X=N or O) in conjunction with specifically located basic groups, may account for the observed diversity (cf. Scheme 9). If a hydride (from NAD(P)H; = YH)[19] is delivered to the cation, the saturated cyclopentane iridodial **28** originates. The combination of hydride donation with the transfer of a proton from the methyl group C(10) to the base :$B_3$ may explain the formation of the exomethylene double bond of anisomorphal **4**, thus integrating all important iridoid dialdehydes from insects into a general mechanistic scheme. The isolation and characterization of individual enzymes of the biosynthetic sequence is the next and most important step to confirm and refine the postulated sequence. In this context is the recent isolation of glycosidically bound **8** from the defensive secretion of the chrysomelid larvae

of *P. versicolora* and *G. viridula* of particular importance;[18] the compound may act as a water soluble carrier of **8** between the larval digestive system and the defensive gland, indicating that even more enzymatic activities are required for the production of iridoid dialdehydes in chrysomelid larvae.

**Scheme 9** Mechanistic aspects of the cyclization of 8-oxocitral **9** to various iridoid dialdehydes in insects




A first approach towards the oxidase which converts 8-hydroxygeraniol **8** into the dialdehyde **9** was already successful and characterized this enzyme as an oxygen dependent oxidase [20]. Detailed results will be reported in due course.

## EXPERIMENTAL

General remarks. Reactions were performed under Ar. Solvents and reagents were purified and dried prior to use. Anh. $MgSO_4$ was used for drying. Boiling points are not corrected. The following spectroscopic and analytical instruments were used: $^1H$- and $^{13}C$ NMR: *Bruker Cryospec WM 250* and *Bruker WM 400*; $CDCl_3$, TMS as internal standard. IR: *Perkin-Elmer-882* IR spectrophotometer. MS: *Finnigan MAT 90* GLC/MS system and *Finnigan ITD 800* combined with a *Carlo-Erba* gas chromatograph, model *Vega*, equipped with a fused-silica capillary *SE 30*, (10m x 0.32 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, FRG) was used for liquid column chromatography.

**Ethyl[2-trideuteromethyl]cyclopentan-1-one-2-carboxylate [$^2H_3$]-12**

A soln. of $CD_3I$ (13,5 g, 93 mmol) and ß-ketoester **11** (14,19 g, 91,15 mmol) in *t*-butanol (90 ml) is added with stirring to a soln. of potassium *t*-butoxide (8,7 g, 91,15 mmol) in the same solvent (120 ml). Stirring is continued for 16 h, and the yellow precipitate is removed by suction. The solvent is evaporated to ca. 50 ml, and ether (100 ml) is added. The organic layer is washed with a sat. aq. soln of $NH_4Cl$ (70 ml) and dried. Removal of solvents and distillation affords [$^2H_3$]-**12** (10.25 g, 65%). B.p.: 95°C/7 torr). $^1H$ NMR (250 MHz, $CDCl_3$): δ 1.2 (t, $CH_3$) 1.8-2.6 (m, 2 H-C(3), 2 H-C(4), 2 H-C(5)), 4.15 (quart., -$OCH_2$); IR (neat): 2979, 2229, 1751, 1728, 1450, 1406, 1392, 1368, 1297, 1264, 1175, 1127, 1029, 916, 856, 837 $cm^{-1}$. MS (%): 173($M^{+\bullet}$,28), 156(9), 145(100), 128(52), 118(17), 116(86), 110(15), 100(34), 90(46), 72(47), 55(13), 44(13); HR-MS: m/z calcd. for $C_9H_{11}{}^2H_3O_3$: 173,1131 found: 173,1133.

**Ethyl[2-trideuteromethyl-5,5-$^2H_5$]cyclopentan-1-one-2-carboxylate [$^2H_5$]-12**

A soln. of [$^2H_3$]-**12** (10,2 g, 59,2 mmol) and ethyldiisopropylamine (3.1 ml, 18 mmol) in $CH_3OD$ (50 ml, 99,5% D) is stirred for 24 h at r.t. After removal of the solvent the crude residue is dissolved in ether (100 ml), and the organic layer is washed with dil. HCl (2 N, 30 ml), water and dried. Removal of solvents yields [$^2H_5$]-**12** (9.4 g, 90%). $^1H$ NMR (250 MHz, $CDCl_3$): δ 1,2 (t, $CH_3$), 1.8-2.6 (m, 2 H-C(3), 2 H-C(4)), 4.15 (quart., -$OCH_2$); IR (neat): 3458, 2979, 2880, 2228, 2124, 2070, 1751, 1728, 1451, 1391, 1367, 1264, 1178, 1111, 1093, 1070, 1034, 966, 914, 852, 778, 589, 488 $cm^{-1}$. MS (%): 175($M^{+\bullet}$,3), 147(100), 130 (40), 118(73), 102(40), 91(66), 74(38), 72(36); HR-MS: *m/z* calcd. for $C_9H_9{}^2H_5O_3$: 175,1256, found: 175,1242.

**(2*E*)-Ethyl-6-oxo[2-trideuteromethyl-5,5-$^2H_5$]hex-2-enoate [$^2H_5$]-13**

A soln. of the ß-ketoester [$^2H_5$]-**12** (9,37 g, 53,5 mmol) in benzene (190 ml) is irradiated under nitrogen with stirring and cooling (10 °C) for 30 h, using a low-pressure mercury lamp (Hanau, Q180, 180 W, 1.8 A). Following removal of the solvent, the mixture of isomers (starting material: 33%; (2*Z*)-[$^2H_5$]-**13**: 24%, (2*E*)-[$^2H_5$]-**13** 43%) is separated by chromatography ($SiO_2$, $Et_2O$/pentane; 30/70 v/v). (3*E*)-[$^2H_5$]-**13** is obtained as a colourless liquid (1,74 g, 27%). $^1H$ NMR (250 MHz, $CDCl_3$): δ 1.3 (t, -$CH_3$), 2.48 (d, 2 H-C(4)), 4.18 (quart., -$OCH_2$), 6.68 (t, 1 H-C(3)), 9.81 (s, 1 H-C(6)); IR(neat): 2982, 2939, 2906, 2825, 2725, 1710, 1644, 1465, 1446, 1391, 1372, 1266, 1137, 1114, 1096, 1059, 870, 841, 720 $cm^{-1}$. MS (%): 175($M^{+\bullet}$,30), 156(5), 146(20), 130(63), 118(56), 102(78), 100(76), 91(26), 85(41), 74(48), 72(43), 58(59), 43(100); HR-MS: *m/z* calcd. for $C_9H_9{}^2H_5O_3$: 175,1256, found: 175,1256.

**(2*E*,4*E*)-Diethyl[2-trideuteromethyl-5,5-$^2H_5$]octa-2,6-dienoate [$^2H_5$]-14**

A chilled soln. of triethylphosphono acetate (2,2 g, 9,8 mmol) in THF (85 ml) is metallated with *n*-BuLi (4 ml of a 2,5 M soln. in hexane, 9.8 mmol). Stirring is continued for 20 min prior to the addition of a soln. of esteraldehyde [$^2H_5$]-**13** (1,2 g, 6,9 mmol) in THF (15 ml). Following usual work-up (partitioning between ether and water) the diester is purified by chromatography on $SiO_2$ with ether/pentane (20/80 v/v), yielding [$^2H_5$]-**14** as a colourless liquid (0.73 g, 43%). $^1H$ NMR (250 MHz, $CDCl_3$): δ 1.29 (m, 6H, -$CH_3$), 2.32 (d, J=8,5 Hz, 2 H-C(4)), 4.15 (quart. 4H, -$OCH_2$-), 5.90 (d, 1 H-C(7)), 6.70 (t, 1 H-C(3)), 6,95 (d, J=15Hz, 1H-C(6)); IR (neat): 3417, 2982, 2938, 2905, 2874, 2209, 1718, 1653, 1465, 1446, 1391, 1367, 1309, 1264, 1178, 1095, 1044, 993, 869, 840, 810, 721, 614, 494 $cm^{-1}$. MS (%): 245($M^{+\bullet}$,2), 200(17), 198(19),

183(30), 170(8), 158(65), 154(35), 130(83), 112(100), 102(84), 85(35), 74(57), 56(47), 41(20); HR-MS: *m/z* calcd. for $C_{13}H_{15}{}^2H_5O_4$: 245,1675; found: 245,1688.

**(2*E*,4*E*)-[2-Trideuteromethyl-5,5-$^2H_5$]octa-2, 6-diene-1,8-diol [$^2H_5$]-15**

A chilled suspension of $LiAlH_4$ ( 633 mg, 16,7 mmol) in ether (35 ml) is gradually treated with $AlCl_3$ (0.80 g, 6 mmol). After 1 h a soln. of the diester [$^2H_5$]-**14** (0.7 g, 2.9 mmol) in ether (10 ml) is added slowly. Stirring is continued for 3 h, the mixture is hydrolyzed by slow addition of dil. NaOH (10 ml, 2N), the precipitate is filtered off, and the aq. layer is carefully extracted with ether (3 x 50 ml). Chromatography on $SiO_2$, using ether/pentane for elution (30/70 v/v), yields [$^2H_5$]-**15** as a viscous oil (0.40 g, 86%). $^1H$ NMR (250 MHz, $CDCl_3$) δ 1.45 (s, -OH), 1.60 (s, -OH), 2.05 (d, 2 H-C(4)), 3.92 (s, 2 H-C(1)), 4.02 (d, J=4,2, 2 HC(8)), 5.32 (t, 1 H-C(3), 5.58 (m, 1 H-C(6), 1 H-C(7)); $^{13}C$ NMR (400 MHz, $CDCl_3$): δ 12,93 (C(9)), 26,8 (C(4)), 31,5 (C(5)), 63,6 (C(1)), 68,6 (C(8)), 125,2 (C(3)), 129,4 (C(6)), 132,3 (C(7)), 135,1 (C(2)); IR (neat): 3327, 2922, 2861, 2195, 2105, 1718, 1669, 1559, 1436, 1221, 1093, 1003, 972, 866, 701 $cm^{-1}$. MS (%): 160 ($M^{+\bullet}$,0.04), 143(8), 124(9), 112(7), 98(7), 87(79), 82(17), 71(27), 58(32), 56(71), 46(100); HR-MS: *m/z* calcd. for $C_9H_{11}{}^2H_5O_2$: 160,1401, found: 160,1393.

**(2*E*)-5-Methyl[2,3-$^2H_2$]hexa-2,4-dien-1-ol [$^2H_2$]-17**

The alkynol **16**[21] (4.54 g, 41 mmol), dissolved in abs. THF (50 ml), is added slowly to chilled and well stirred suspension of $LiAlD_4$ (1.73 g, 41 mmol) in the same solvent (200 ml). Following 4 h stirring at r.t. the mixture is hydrolyzed by slow addition of $D_2O$ (60 ml, 99% $^2H$) and dil. DCl (40 ml, 2 N, 99% $^2H$). After 30 min the product is extracted with ether (3 x 70 ml) and purified by chromatography on $SiO_2$ using ether/-pentane (40/60, v/v) for elution. Colourless liquid (3.3 g, 70%). $^1H$ NMR (250 MHz, $CDCl_3$): δ 1.77 (s, 3 H-C(6)), 1.79 (s, 3 H-C(7)), 4.19 (s, 2 H-C(1)), 5.86 (s, 1 H-C(4)); IR (neat): 3334, 2967, 2912, 2862, 2732, 2229, 1705, 1645, 1565, 1442, 1376, 1234,1207, 1152, 1095, 1046, 991, 861, 808, 731, 701, 421$cm^{-1}$. MS (%): 114($M^{+\bullet}$,100), 99(62), 96(38), 95(22), 93(7), 81(61), 80(32), 78(25), 71(22), 70(37), 69(41), 68(22), 67(10); HR-MS: *m/z* calcd. for $C_7H_{10}D_2O$: 114,1013, found: 114,0980.

**(2*E*)-5-Methyl[2,3-$^2H_2$]hexa-2,4-dienoic acid [$^2H_5$]-18**

The alcohol [$^2H_5$]-**17** (3.2 g, 28 mmol) is first oxidized to the aldehyde by stirring with $MnO_2$ (32 g) in $CH_2Cl_2$ (100 ml) at r.t. After 4 h the solvent is removed, and the aldehyde is dissolved in a mixture of *t*-butanol (1 l) and 2-methyl-2-butene (130 ml). Then a buffered soln. (150 ml) of the oxidant $NaClO_2$ (22.3 g) and $NaH_2PO_4$ (22.3 g) in water (150 ml)) is slowly added. When the yellow soln. decolourizes (ca. 1 h), most of the organic solvent is removed, and the aq. soln. is adjusted to pH 9 with dil. NaOH. Organic impurities are removed by extraction with pentane. The aq. layer is acidified (pH 3) with dil. HCl, and the acid is extracted with ether (3 x 50 ml). [$^2H_5$]-**18** is obtained by crystallization from hexane at -20 °C as a colourless solid (2.2 g, 61%). $^1H$ NMR (250 MHz, $CDCl_3$): δ 1.90 (s, 3 H-C(6)), 1.92 (s, 3 H-C(7)), 6.02 (s, 1 H-C(4)); IR (KBr): 3946, 2891, 3755, 3426, 2990, 2938, 286, 2650, 2540, 2341, 1686, 1624, 1576, 1411, 1382, 1345, 1281, 1166, 1011, 952, 866, 793, 746, 677, 498 $cm^{-1}$. MS (%, methyl ester): 142($M^{+\bullet}$,50), 127(63), 111(39), 84(8), 83(100), 82(37), 81(29), 80(10), 67(8); HR-MS (methyl ester): *m/z* calcd. for $C_8H_{10}{}^2H_2O_2$: 142,0962, found: 142,0947.

**Microbial Reduction of (2*E*)-5-Methylhexa-2,4-dienoic acids; General Procedure:** *Clostridium tyrobutyricum* (Strain: DSM 1460) was grown, stored, and manipulated as described.[11] For the experiment in $^2H_2O$ buffer, wet packed cells were freeze dried (under exclusion of oxygen) and re suspended in buffered $^2H_2O$. A total volume of 130 ml, containing the sodium salt of [$^1$H]-**18** or [$^2H_2$]-**18** (2.2 g, 10.6 mmol), 17.6 g of wet packed cells, methylviologen (40 mg, 0.15 mmol), and 0.1 M potassium-phosphate buffer (from $H_2O$ or $^2H_2O$, matching [$^1$H]- or [$^2H_2$]-**18**) at p(D)H 7.0 is shaken at 35° under an atmosphere of $H_2$ gas. The progress of the reaction is monitored by the consumption of the hydrogen gas, using a *Warburg* manometer or by GLC. The reduction is complete within ca. 12 h. The mixture is acidified (pH = 1.5) by addition of dil. $H_2SO_4$, and the product is extracted with ether (3 x 100 ml). Drying, evaporation of the solvents and rapid filtration over a small column of silica gel yields the acids (2*S*,3*R*)- or (2*R*,3*S*)-[$^2H_2$]-**19** (hexane/ether, 70:30), which are esterified with diazomethane and purified by chromatography on $SiO_2$.

**(2*R*,3*S*)-5-Methyl[2,3-$^2H_2$]hex-4-enoic acid (2*R*,3*S*)-[$^2H_2$]-19**

Prepared from [$^2H_2$]-**18** (2.2 g, 17 mmol) in an $H_2O$ buffer (0.87 g, 63%). $^1$H NMR (250 MHz, $CDCl_3$): δ 1.60 (s, 3 H-C(6)), 1.66 (s, 3 H-C(7)), 2.19-2.35 (m, 1 H-C(2), 1 H-C(3)), 5.08 (d, 1 H-C(4)); IR (neat): 3423, 2973, 2935, 2180, 1707, 1414, 1377, 1282, 1218, 1119, 1017, 934, 827, 419 cm$^{-1}$. MS (%, methyl ester): 144($M^{+\bullet}$,46), 143(5), 113(18), 112(17), 111(16), 86(18), 85(19), 84(53), 83(35), 75(58), 70(100), 69(38), 68(19); HR-MS (methyl ester): *m/z* calcd. for $C_8H_{12}{}^2H_2O_2$: 144,1119, found: 144,1131.

**(2*S*,3*R*)-5-Methyl[2,3-$^2H_2$]hex-4-enoic acid (2*S*,3*R*)-[$^2H_2$]-19**

From [$^1$H]-**18** (1.08 g, 8.6 mmol) in $^2H_2O$ buffer (0.64g, 57%). Spectroscopic data identical with (2*R*,3*S*)-[$^2H_2$]-**18**.

**Ethyl-(2*E*,4*R*,5*S*)-7-methyl[4,5-$^2H_2$]octa-2,6-dienoate (4*R*,5*S*)-[$^2H_2$]-20**

A soln. of the methyl ester of (2*R*,3*S*)-[$^2H_2$]-**19** (0.40 g, 2.76 mmol) in hexane (10 ml) is treated with stirring at -78°C with DIBAl-H (0.48 ml, 2.76 mmol). The progress of the reduction is followed by GLC. If the reduction is complete, the excess of the hydride is destroyed by addition of 0.1 ml abs. methanol. Then, a soln. of the anion of triethylphosphono acetate (5.5 mmol, cf. the preparation of [$^2H_5$]-**14**) in THF (20 ml) is added to the organoaluminum intermediate, and the mixture is allowed to come to r.t. Stirring is continued for 1 h, dil. HCl (10 ml, 2 N) is added, and the ester is extracted with ether. Chromatography on $SiO_2$, using ether/pentane (95/5) for elution yields a colourless liquid (0.44 g, 86%). $^1$H NMR (250 MHz, CDCl3): δ 1.28 (t, $-CH_3$), 1.60 (s, 3 H-C(9)), 1.69 (s, 3 H-C(8), 2.11-2.30 (m, 1 H-C(4), 1 H-C(5), 4.18 (q, $-OCH_2$), 5.09 (d, J=7Hz, 1 H-C(6)), 5.82 (d, J=16,2, 1 H-C(2)), 6.95 (dd, J=16.7, 1 H-C(3)); IR (neat): 3427, 2981, 2928, 2166, 1723, 1652, 1447, 1414, 1366, 1334, 1306, 1264, 1176, 1095, 1040, 985, 918, 818, 709, 418 cm$^{-1}$. MS (%): 184($M^{+\bullet}$,2.2), 169(1), 155(1), 139(10), 138(4), 137(2), 115(45), 110(8), 87(24), 70(100) ; HR-MS *m/z* calcd. for $C_{11}H_{16}{}^2H_2O_2$: 184.1432, found: 184.1472.

**Ethyl-(2*E*,4*S*,5*R*)-7-methyl[4,5-$^2H_2$]octa-2,6-dienoate (4*S*,5*R*)-[$^2H_5$]-20**

Prepared from the methyl ester of (2*S*,3*R*)-[$^2H_2$]-**19** (0.304 g, 1.65 mmol) as described before (0.18 g, 77%). Spectroscopic data identical with (4*R*,5*S*)-[$^2H_2$]-**20**.

**(2*E*,4*R*,5*S*)-7-Methyl[4,5-$^2H_2$]octa-2,6-dien-1-ol (4*R*,5*S*)-[$^2H_2$]-21**

The ester (4*R*,5*S*)-[$^2H_2$]-**20** (304 mg, 1.65 mmol) is reduced with $AlH_3$ (6.6 mmol) in ether as described for [$^2H_5$]-**15**. Chromatography affords the 3-norgeraniol (4R,5S)-[$^2H_2$]-**21** as a colourless liquid (180 mg, 77%). $^1H$ NMR (250 MHz, $CDCl_3$): δ 1.60 (s, 3 H-C(9)), 1.69 (s, 3 H-C(8)), 2.05 (s(br.), 1 H-C(4), 1 H-C(5)), 4.08 (d, J=4,8 Hz, 2 H-C(1)), 5.12 (s(br.), 1 H-C(6)), 5.57-5.75 (m, 1 H-C(2), 1 H-C(3)); IR (neat): 3350, 2968, 2927, 2731, 2145, 1726, 1665, 1636, 1447, 1374, 1297, 1199, 1092, 1002, 971, 902, 872, 824, 488, 457 $cm^{-1}$. MS (%): 142 ($M^{+\bullet}$,2), 124(2), 111(8), 109(2), 99(5), 98(3), 97(1), 95(1), 94(1), 73(8), 70(100), 69(10); HR-MS: *m/z* calcd. for $C_9H_{14}{}^2H_2O$: 142,1326, found: 142,1340.

**(2*E*,4*S*,5*R*)-7-Methyl[4,5-$^2H_2$]octa-2,6-dien-1-ol (4*S*,5*R*)-[$^2H_2$]-21**

Prepared from the ester of (4*S*,5*R*)-[$^2H_2$]-**20** (250 mg, 1.36 mmol) as described before. Yield: (173 mg, 89%). Spectroscopic data identical with (4*R*,5*S*)-[$^2H_2$]-**21**.

**(2*E*,6*E*)-2-Methylocta-2,6-diene-1,8-dial (= 3-nor-8-oxocitral) [$^2H_5$]-22**

A soln. of the diol [$^2H_5$]-**15** (0.44 g, 2.73 mmol) in $CH_2Cl_2$ (30 ml) is stirred with pyridiniumchloro chromate (1.76 g, 8.2 mmol) for 3 h. Then pentane is added to precipitate the chromium salts, and after filtration the solvents are removed. The crude product is re dissolved in pentane, and $MgSO_4$ is added to absorb last traces of the chromium salts. Filtration, removal of the solvent, and chromatography on $SiO_2$ with ether/pentane (70/30) affords [$^2H_5$]-**22** (0.304 g, 71%) $^1H$-NMR (250 MHz, $CDCl_3$): δ 2.58 (d, 2 H-C(5)), 6.18 (dd, J=15.7/7.8, 1 H-C(2)), 6.46 (t, J=7 Hz, 1 H-C(3)), 6.85 (d, J=15,7 Hz, 1 HC(6)), 9.42 (s, 1 H-C(8)), 9.54 (d, J=7,8 Hz, 1 H-C(1)); $^{13}C$ NMR: 194.8, 193.5, 155.6, 151.2, 140.4, 133.8, 30.7, 26.9, 8.6. IR (neat): 3354, 2930, 2822, 2725, 2214, 1866, 1638, 1405, 1386, 1304, 1245, 1207, 1133, 1074, 1013, 986, 909, 854, 735, 506. MS (%): 157($M^{+\bullet}$,8), 144(5), 128(8), 120(5), 109(18), 108(17), 107(11), 100(10), 96(7), 85(35), 81(35), 79(20), 73(38), 72(20), 70(11), 64(8), 58(100), 55(19), 53(12), 43(21), 42(21), 41(85), 40(65); HR-MS *m/z* calcd. for $C_9H_7{}^2H_5O_2$: 157.1151, found: 157.1117.

**Biomimetic Cyclization of the Dialdehyde [$^2H_5$]-22; General Procedure.** A soln. of the dialdehyde [$^2H_5$]-**22** (0.15 g, 0.95 mmol) in HCOOH/$H_2O$ (6/4, v/v) is refluxed for ca. 1 h. The products are extracted with ether. Acid is removed by washing with a sat. soln of $NaHCO_3$ and water. Chromatography on $SiO_2$, using pentane/ether (7:3, v/v) for elution, yields [$^2H_5$]-**23** as a 1:1 mixture of the 1-norchrysomelidial and 1-norepichrysomelidial which were not separated (63 mg. 42%). $^1H$ NMR (250 MHz, $CDCl_3$): 1-norchrysomelidial: δ 1.62 (dd, J=13.7,4.8, 1 H-C(4)), 2.09 (dd, J=13.7,8.8, 1 H-C(4)), 3.15 (s, 1 H-C-CHO, 3.61-3.66 (m, 1 H-C(3)), 6.97 (s, 1 H-C(1)), 9.70 (s, 1 -CHO, 9.78 (s, 1 H-C(7)); $^{13}C$ NMR: δ 204, 189.7, 155.5, 147.2, 47.5, 41.2, 32.8, 24.9; 1-norepichrysomelidial: δ 1,75 (dd, J=13.7/4.8 Hz, 1 H-C(4)), 2,23 (dd, J=13.7/8.8, 1 H-C(4)), 2,81 (s(br.), 1 H-C(2)), 3.36-3.41 (m, 1 H-C(3)), 6,97 (s, 1 H-C(1), 9.67 (s, 1 H-C(7)), 9,78 (s, 1 H-C(9)); $^{13}C$ NMR: δ 204.4, 189.7, 155.5, 147.2, 47.6, 43.6, 32.8, 27.5; IR (neat): 3405, 2949, 2839, 2718, 2254, 2228, 2073, 1705, 1672, 1600, 1455, 1428, 1379, 1334, 1258, 1170, 1104, 1051, 914, 836, 793, 733, 647, 467. MS (%): 157($M^{+\bullet}$,1.4), 139(13), 138(8), 129(60), 128(23), 114(10), 113(4), 112(10), 111(20), 110(11), 109(6), 100(35), 99(20), 98(20), 97(60), 96(61), 95(58), 94(20), 93(8), 83(18), 82(17), 81(18), 80(18), 79(17), 78(10), 70(18), 69(100), 68(61), 67(22), 66(10), 60(10); HR-MS m/z calcd. for $C_9H_7{}^2H_5O_2$: 157.1151, found: 157.1182.

**Administration of Labelled Precursors to the Chrysomelid Larvae.** Larvae of the leaf beetles *Phaedon armoraciae* (L.) and *Phaedon cochleariae* (F.) are continuously reared at 18 °C and a light-dark interval of 12 h on leaves of Chinese cabbage from the local market. Leaves were replaced every two days. Larvae of *Plagiodera versicolora* (Laich.) and *Gastrophysa viridula* (Deg.) were collected near Karlsruhe from willow trees and leaves of *Rumex patientia*, respectively. Aq. solns. of the labelled precursors are prepared by sonication (2 min, 225-400 watt, 35kHz) of [$^2H_5$]-**15** (ca. 10 mg) in tap water (1 ml). In the case of the 3-norgeraniol [$^2H_2$]-**21** (10 mg) a stable emulsion is obtained from DMSO (10 mg) and water (1 ml). For the feeding experiments the leaves were impregnated on both sides with the aq. solns. After drying up of solutions, larvae of a leaf beetle species were allowed to feed for ca. 2-3 days on the pre-treated leaves (ca. 7 larvae per leaf). The solns. of the 3-norgeraniol derivatives [$^2H_2$]-**21** were directly injected into the hemolymph of 10-14 day-old larvae, using a thin, handmade glass capillary tube. The soln. is injected by applying a slight positive pressure. The metabolites were collected from the defensive glands using a *pressure-lok mini injector* from *Alltech*, Munich.

**Mass Spectroscopic Analysis of the Metabolites.** Treatment of the larvae with tweezers leads to discharge of the defensive glands. The pale yellow, viscous liquid is taken up with a syringe possessing a whole in the movable plunger (cf. general). The sample is analyzed by GC-MS without preceding purification.

**Mass Spectra of Labelled Metabolites**

**[$^2H_4$]-23:** 138($M^{+\bullet}$-$H_2O$, 7), 137(8), 136(9), 128(12), 110(13), 99(21), 97(12), 96(27), 95(37), 94(22), 84(9), 83(9), 82(17), 81(12), 80(15), 70(28), 69(32), 68(100), 67(42), 66(30), 61(10), 58(12), 52(12),

**[$^2H_2$]-23:** 136($M^{+\bullet}$,-$H_2O$, 12), 126(5), 108(17), 107(17), 97(29), 96(22), 95(31), 93(27), 92(21), 83(17), 81(29), 80(17), 79(21), 78(18), 69(100), 68(50), 67(48), 66(22), 57(11), 55(25).

**[$^2H_1$]-23:** 135($M^{+\bullet}$, -$H_2O$,12), 125(5), 110(6), 108(3), 107(10), 96(28), 95(19), 93(21), 92(12), 82(12), 81(9), 80(20), 79(16), 78(15), 77(11), 68(100), 67(31), 66(28), 56(13), 55(12).

**[$^2H_4$]-26**: 138($M^{+\bullet}$, -$H_2O$), 127(4), 120(4), 110(5), 109(11), 100(12), 99(47), 98(13), 97(18), 96(45), 95(65), 94(22), 83(16), 82(18), 81(18), 80(23), 79(20), 78(30), 77(10), 71(16), 70(45), 69(58), 68(83), 67 (100), 66(39), 65(10), 61(30), 59(15), 58(18), 57(14), 56(15), 55(15), 54(13), 53(18), 52(21), 51(21), 46(73), 43(18), 42(31), 41(41), 40(6*2).

**[$^2H_4$]-27:** 154($M^{+\bullet}$,48), 126(18), 125(5), 108(42), 107(10), 98(9), 97(32), 96(14), 95(15), 94(29), 93(19), 82(10), 81(18), 80(55), 79(24), 78(25), 77(12), 69(12), 68(21), 67(100), 66(49), 65(12), 64(10), 59(55), 54(8), 53(17), 52(20), 51(22), 50(11), 43(11), 42(9), 41(21), 40(51), 39(40).

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