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Structure–Activity Relationships for Unsaturated Dialdehydes 10.† The Generation of Bioactive Products by Autoxidation of Isovelleral and Merulidial

Mikael Jonassohn,[a] Heidrun Anke,[b] Paloma Morales[a] and Olov Sterner[a]

[a]Division of Organic Chemistry 2, Chemical Center, University of Lund, P.O.B. 124, S-221 00 Lund, Sweden and [b]LB Biotechnologie der Universität Kaiserlautern, Paul-Ehrlich-Str. 23, D-67663 Kaiserslautern, FRG

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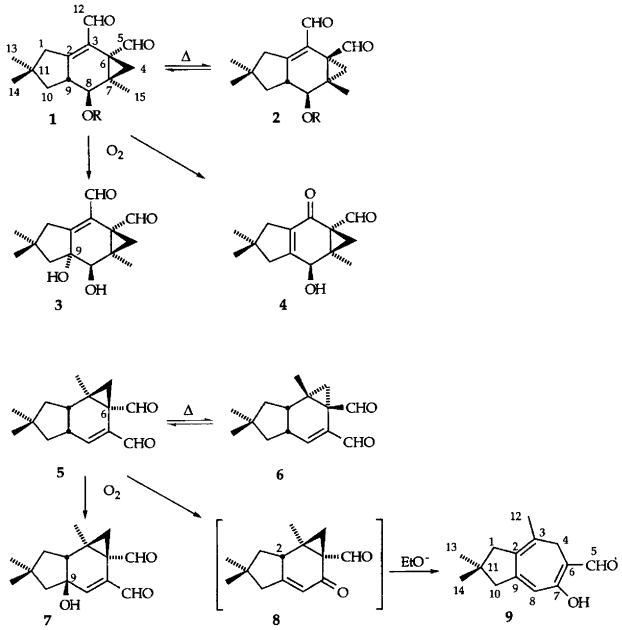
The autoxidation of the two mutagenic and antimicrobial sesquiterpenes merulidial (**1a**) and isovelleral (**5**) generates a number of bioactive products that are suggested to be partly responsible for the mutagenic and antimicrobial activities of the two compounds. The biological activities of the autoxidation products are, as far as has been possible to assay, of the same order as the parent compounds, and it is shown that they are formed in normal bioassay media. Merulidial (**1a**) is especially interesting, as its rate of autoxidation is considerably higher compared with non-mutagenic derivatives of **1a**. In addition, natural isovelleral [(+)-**5**] was found to be twice as mutagenic as synthetic isovelleral [(±)-**5**], indicating that (−)-isovelleral is only weakly active or inactive, and supporting the suggestion that the interaction of isovelleral (**5**) and its derivatives with DNA depends on the absolute stereochemistry of the unsaturated dialdehyde moiety.

Terpenoids containing an unsaturated dialdehyde functionality and possessing, for example, potent antibiotic and antifeedant activities have been isolated from a number of natural sources, and several studies have demonstrated the necessity of both aldehyde groups as well as the double bond for their biological activities.[2,3] In general, the unsaturated dialdehydes are believed to react as electrophiles with proteins and DNA, either as Michael acceptors[4] or by forming pyrrole derivatives via the reaction of both aldehyde groups with a primary amine.[5] However, QSAR studies have indicated that the reactivity of the unsaturated dialdehyde functionality is only one of several properties that are correlated with, for instance, the cytotoxicity[6] and the mutagenicity[7] of these compounds, and one cannot exclude that other molecular mechanisms might also contribute to their biological activities. Of special interest to us is the mutagenic activity in the Ames' Salmonella/microsome assay, as the mutagenicity of the dialdehydes is strongly enhanced by a cyclopropane ring in conjugation with the unsaturated dialdehyde functionality [as in merulidial (**1a**) and isovelleral (**5**), see Scheme 1]. In addition, small structural changes, such as inversion of the cyclopropane ring of isovelleral (**5**) (to form compound **6**), or acetylation of the hydroxy group of merulidial (**1a**), reduce the mutagenicity



Scheme 1. Compound **8** is a hypothetical intermediate. **a**: R=H; **b**: R=Ac; **c**: R=TBDMS.

dramatically.[2,8] A QSAR study has suggested that the absolute configuration of the cyclopropane ring of isovelleral (**5**) is important for its interaction with the DNA,[7]

† For Part 9 see Ref. 1.

while the acetyl group of acetylmerulidial (**1b**) possibly blocks the cyclopropane ring (C-7) from nucleophilic attack.[8]

It has also been observed that merulidial (**1a**) is easily autoxidised, during, for instance, chromatography on alumina gel, yielding primarily hydroxymerulidial (**3**) and the deformylated derivative **4**.[9] Acetylmerulidial (**1b**) was found to be comparatively stable towards autoxidation on alumina, and as merulidial (**1a**) is mutagenic and acetylmerulidial (**1b**) is not,[8] the possibility that autoxidation products formed during the bioassay at least partially are responsible for the mutagenicity of merulidial (**1a**) should be considered. In order to study this possibility, the rates of autoxidation of merulidial (**1a**), isovelleral (**5**), and their isomers **2a** and **6** on alumina and in buffered water solutions have been investigated. In addition, the mutagenicity of the TBDMS derivatives **1c** and **2c** as well as of racemic isovelleral [(±)-**5**] has been assayed and is compared with that of merulidial (**1a**) and natural isovelleral [(+)-**5**].

Results and discussion

The autoxidation of merulidial (**1a**) on alumina gel has previously been shown to proceed rapidly and to be complete within minutes,[9] while isovelleral (**5**) is oxidised considerably slower. Interestingly, compared with merulidial (**1a**) its isomer **2a** is considerably more stable towards autoxidation (see the Experimental part for details), and 25% of compound **2a** could be recovered even after 2 h in contact with alumina. 20% of the TBDMS ether of merulidial (compound **1c**) was recovered after 2 h, confirming the difference in susceptibility between merulidial (**1a**) and its 8-*O*-protected derivatives discussed above. The rate of oxidation of isovelleral (**5**) and its isomer **6** is approximately equal, 25% of both compounds remained unchanged after 5 h contact with alumina. Both merulidial (**1a**) and isovelleral (**5**) are slowly autoxidised when left in organic solvents for several days at room temperature. However, bioassays are normally conducted in aqueous media, and the stability of compounds **1a**, **2a**, **5** and **6** in 0.1 M buffers (containing only inorganics) at pH 5.6, 7.4 and 9.0 was therefore monitored by HPLC. The results are shown in Table 1 as $t_{1/2}$, i.e., the half-life of the compounds at the respective pH.

All four compounds are reasonably stable at pH 5.6, but at pH 7.4 and particularly at pH 9.0 the compounds are degraded. The mutagenicity assay used in this investigation is performed on agar plates in a minimal medium at pH 7.4[10] and in order to investigate the degradation of merulidial (**1a**) and isovelleral (**5**) at this pH, plates containing 20 ml of growth medium and 200 μg of the dialdehydes (but no test organism) were incubated for 48 h at 37°C. At 6, 12, 24 and 48 h intervals, agar samples were extracted with ethyl acetate and the extracts were analysed by TLC. The major product of both dialdehydes after 6 h incubation is the 9-hydroxy derivative (compounds **3** and **7**, respectively), although several minor unidentified products were also formed, and this shows that autoxidation is responsible for at least a substantial part of the degradation. The unsaturated dialdehydes are degraded much more rapidly in bioassay medium complemented with amino acids or proteins,[11] and it has been assumed that this is due to the reaction of nucleophiles (e.g., cysteine) with the electrophilic dialdehydes. However, when isovelleral (**5**) was allowed to react with methylcysteine in phosphate buffer (0.1 M, pH 7.4)–ethanol 9:1, isovelleral rapidly disappeared without the formation of a major product. Instead a large number (>20 according to TLC) of trace products with very differing polarity were formed, and, owing to the limited amounts of isovelleral available, it has not been possible to characterise these (unpublished results). In contrast, the reaction between methylcysteine and muzigadial, a related sesquiterpenoid unsaturated dialdehyde isolated from the bark of the East African plant *Warburgia ugandensis*, in chloroform, gave a single product in 79% isolated yield.[12] Apparently, the reactions of isovelleral (**5**) with biomolecules are less straightforward, possibly due to competing autoxidation catalysed by components in the assay medium.

Table 1. The half-lives in hours of compounds **1a**, **2a**, **5** and **6** in buffered water solutions at 37 °C (see the Experimental part for details).

Comp. No.	$t_{\frac{1}{2}}$/h pH 5.6	pH 7.4	pH 9.0
1a	170	23	10
2a	>5000	1000	63
5	2500	390	180
6	>5000	420	280

Basic conditions obviously accelerate the autoxidation of the dialdehydes (see Table 1). Treatment of isovelleral (**5**) in ethanol with sodium ethoxide resulted in the immediate formation of hydroxyisovelleral (**7**) together with some minor products (*vide infra*). This suggests that the autoxidation of the unsaturated dialdehydes proceeds via the formation of an allylic carbanion, which either can be oxidised to the corresponding radical that can react with O_2 to the peroxyl radical, or react directly with O_2 to the corresponding peroxide anion.[13] The rates of autoxidation on alumina can be compared with the calculated enthalpy change for abstracting the C-9 proton of compounds **1a**, **2a**, **5** and **6** to form their corresponding C-9 anions (see the Experimental part). AM1[14] calculations predict that the enthalpy change for this is 6.6 kcal mol^{-1} less for merulidial (**1a**) than for compound **2a**, while the difference between isovelleral (**5**) and compound **6** is only 1.2 kcal mol^{-1} (less for the latter). Consequently, the C-9 anion of merulidial (**1a**) should be formed considerably more easily than that of compound **2a**, while one cannot expect a big difference between isovelleral (**5**) and compound **6**, and this is in agreement with the observed rates of autoxidation of the compounds.

The 9-hydroxy derivatives **3** and **7** would be formed by decomposition of the corresponding hydroperoxides initially formed. Compound **4** is presumably formed via C-3 oxidation of the allylic radical of merulidial (**1a**) (to the corresponding β,γ-unsaturated α-peroxy-aldehyde) and oxidative deformylation (see Ref. 15 for an example of a similar deformylation). In addition, 9-hydroxyisovelleral (**7**), a minor product of the autoxidation was obtained from the preparative autoxidation of isovelleral (**5**) in EtOH–EtO (see the Experimental part). Structure determination showed that it is the norvellerane **9**, presumably formed via the base-sensitive hypothetical intermediate **8**, which would be the deformylated equivalent of compound **4**. Interestingly, in a recent investigation of the fruit bodies of *Lactarius vellereus*, the natural source of isovelleral (**5**), in which the fruit bodies were extracted by soaking them for 45 days in ethanol, two normarasmanes with the same carbon skeleton as compound **8** were isolated and reported as natural products.[16] As these are similar to compound **8**, and as they could not be detected in another investigation of the same mushroom,[17] it is at present uncertain whether they should be considered to be true metabolites.

The two 9-hydroxylated derivatives **3** and **7** formed by autoxidation are both more mutagenic than their parent dialdehydes.[2] The chemical structures of compounds **4** and **8**, both possessing a doubly activated cyclopropane ring, suggest that they are strongly electrophilic and that they may be mutagenic. The mutagenic activity in the Ames' Salmonella/microsome assay towards the tester strains TA98 (sensitive to frameshift mutations) and TA100 (sensitive to base-pair substitutions) of compound **4** was therefore compared with that of merulidial (**1a**) and compound **2a** (results given in Table 2). The two TBDMS ethers **1c** and **2c** were included in the assay in order to confirm the previous result that 8-*O*-protected derivatives of merulidial (**1a**) are non-mutagenic. In addition, the mutagenic activity of racemic isovelleral [(±)-**5**] was compared with that of natural isovelleral [(+)-**5**]. It is interesting to note that the deformylated derivative (**4**) possesses mutagenic activity and that its potency is comparable to that of merulidial (**1a**), and that the merulidial diastereomer **2a** is completely devoid of mutagenic activity. The latter is approximately 10 times less toxic towards the test organism (*Salmonella typhimurium*) than merulidial (**1a**), indicating that the antibiotic activity also decreases with the inversion of the cyclopropane ring (*vide infra*). Compound **9**, which was assayed in the absence of compound **8**, is, as expected, devoid of mutagenic activity. More interesting is the fact that the mutagenic activity of racemic isovelleral [(±)-**5**] is only approximately half of that of the natural enantiomer [(+)-**5**] (see Table 2), indicating that the (−)-enantiomer [(−)-**5**] is inactive or only weakly active. This supports the suggestion that the interaction of isovelleral (**5**) depends on the absolute configuration of its cyclopropanedialdehyde moiety,[7] and is in agreement with the earlier finding that compound **6** possesses only approximately 10% of the mutagenic activity of isovelleral (**5**).[2] The mutagenic activity of the 9-hydroxy derivative of compound **6** (not shown in Scheme 1) is also approximately 10% of that of compound **7**. However, it should be noted that the two compounds **5** and **6** possess similar antibiotic activity.[18] In contrast, no antimicrobial activity at all could be detected for compound **2a** in the plate diffusion assay towards the bacteria *Bacillus brevis, B. subtilis, Enterobacter dissolvens* and *Micrococcus luteus*, and the fungi *Mucor miehei, Paecilomyces varioti, Nematospora coryli* and *Penicillium notatum* (up to 100 μg/disc, see Ref. 19 for details about the assay).

We propose that bioactive products formed by autoxidation of natural unsaturated dialdehydes in bioassay media are partially responsible for the activities of the dialdehydes. Unfortunately the complexity of the reactions that the dialdehydes undergo in such media and the limited amounts of them available, hampers quantitative studies of the autoxidation and the isolation and characterisation of the many products formed. However, we have shown that autoxidation takes place in the medium used in the Ames' mutagenicity test and that the products

Table 2. The mutagenic activity of compounds **1a**, **1c**, **2a**, **2c**, **4**, (+)−**5**, (±)−**5** and **9** in the Salmonella/microsome assay in the absence of metabolic activation.

Comp. (No.)	μg/plate[a]	Mutagenic response[b]		Mutagenic activity[c]	
		TA98	TA100	TA98	TA100
1a	20	$67^{0.98}$	$77^{0.90}$	0.90	0.99
1c	200	0	0	0	0
2a	200	0	0	0	0
2c	200	0	0	0	0
4	60	$68^{0.92}$	$260^{0.97}$	0.26	0.96
(+)−**5**	0.5	$48^{0.98}$	$159^{0.90}$	25	76
(±)−**5**	1	$50^{0.98}$	$156^{0.94}$	12	36
9	75	0	0	0	0

[a]The highest non-toxic dose. Each plate contained 20 ml of medium. [b]The mutagenic response is recorded as the number of revertant colonies in excess of the solvent control at the given concentration. Superscripts are correlation coefficients, a correlation with a correlation coefficient below 0.60 was considered insignificant and is given as 0. [c]The mutagenic activity is given by the slope of the dose–response curve in number of excess revertants per nmol.

formed are bioactive, in some cases even more so than the parent compounds. In the case of merulidial (**1a**) and its derivatives, there is a striking correlation between bioactivity and susceptibility to autoxidation. This was not observed with isovelleral (**5**) and its isomer **6**, which are autoxidised at approximately the same rate but possess very different mutagenic activity. However, the antimicrobial and cytotoxic activities of compounds **5** and **6**, and indeed also of their 9-hydroxylated derivatives, are comparable,[18] and the difference in mutagenicity could at least in part be due to the fact that the major autoxidation product of isovelleral (**5**) (compound **7**) is a potent mutagen while that of compound **6** is not.[2] Our results also suggest that the mutagenic activity of isovelleral (**5**), but not the antimicrobial and cytotoxic activities, depend on the absolute stereochemistry of the compound, and for the future it would be highly interesting to prepare and assay the unnatural enantiomer of isovelleral [(−)-**5**]. In conclusion, in order fully to understand quantitative structure–activity relationships for this class of compound one should consider their autoxidation, not as an alternative but as a parallel mechanism of action.

Experimental

The NMR spectra were recorded with a Varian XL300 and a Bruker ARX500 spectrometer in $CDCl_3$ and the chemical shifts are given in ppm with the solvent signal as the reference (7.26 ppm for ^1H NMR and 77.1 ppm for ^{13}C NMR). The coupling constants are given in Hz. The melting points (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin-Elmer 141 polarimeter at 22°C. EI mass spectra were run on a Jeol SX102 spectrometer (direct inlet, 70 eV). TLC analyses were made on Merck DC-Alufolien Kieselgel 60 F_{254} SiO_2 plates and were visualised by spraying with anisaldehyde–sulphuric acid and warming to 120°C. HPLC analyses were performed using an Altex 110A pump, a Merck 50943, LiChroCART 125-4, LiChrospher 100 RP-18 (particle size, 5 μm) column, a fixed wavelength absorbance detector LDC UV III Monitor 1203, a Hewlett Packard HP 3396A integrator, and MeOH–H_2O mixtures (50–70%) as elution solvents with a flow rate of 1 ml min^{-1}. The sesquiterpene dialdehydes were detected at 254 nm. Merulidial (**1a**) was isolated from submerged cultures of the fungus *Merulius tremellosus*,[20] isovelleral from fruit bodies of *Lactarius vellereus*,[17] while compounds **1c**, **2a**, **2c** and **9** were prepared as described below. Racemic (±)-isovelleral (**5**)[21] and compounds **6**[22] and **7**[2] were prepared as described previously. The Ames' Salmonella/microsome assay was made according to the standard procedure,[10] using plates containing a total of 20 ml of substrate. TA98 is a frameshift mutant strain and TA100 is a base-pair substitution mutant strain. All plates were triplicated, and at least five concentration levels (differentiated by a factor of 2) of each compound were tested. Acetone was used as the solvent throughout. The calculations of conformational energies were carried out as follows. The most stable conformers of the sesquiterpene dialdehydes were identified by molecular mechanics calculations [MacMimic (v. 2.9)/MM2(91) (v. 1.0) software package for Macintosh Quadra, obtained from InStar Software AB, IDEON Research Park, S-223 70 Lund, Sweden]. The data for these conformers were used as input to a semiemperical AM1 program [SPARTAN (v. 3.1) software package, obtained from Wavefunction Inc., 18401 Von Karman Avenue, Suite 370, Irvine, California 92715 USA] which calculated the AM1 energy-minimized conformers. This program was also used to energy minimize the corresponding allylic anions.

Merulidial TBDMS ether (**1c**). TBDMSOTf (373 μl, 1.23 mmol) was added dropwise to a solution of merulidial (**1a**) (101 mg, 0.41 mmol), 2,6-lutidine (213 μl, 1.83 mol) and dimethylaminopyridine (DMAP) (1 mg, 8 μmol) in CH_2Cl_2 (5 ml) at 0°C under an N_2 atmosphere. The reaction was complete in less than 30 min and was quenched with 2-propanol. The mixture was coevaporated with toluene under reduced pressure, and filtered through a silica pad to remove traces of lutidine. The crude product was mixed with a suspension of 0.5 g silica gel in 5 ml of CH_2Cl_2 to which 0.1 ml of a 10% solution of oxalic acid in H_2O had been added, and the hydrolysis was complete after approx. 20 min (according to TLC analysis). After filtration of the suspension through a Celite pad, the silica gel was washed with EtOAc and the combined filtrates were concentrated. The residue was purified by chromatography (SiO_2; heptane–EtOAc 2:1) to provide compound **1c** (129 mg, 91%). Recrystallisation (ligroin) gave white needles with m.p. 124–127°C; $[\alpha]_D$ −116.4° (*c* 1.00, $CHCl_3$). MS [*m/z* (% rel. int.)]: 362.2286 (M^+, 10%, calculated for $C_{21}H_{34}SiO_3$ 362.2277), 333 (13), 305 (54), 230 (83), 201 (45), 187 (29), 115 (39), 75 (100), 73 (79), 28 (55). ^1H NMR: δ 9.84 (s, 12-H), 9.61 (s, 4-H), 3.77 (d, J_{8-9} = 8.5, 8-H), 2.78 (dm, J_{1a-1b} = 18.4, 1-H_a), 2.65 (dd, J_{1a-1b} = 18.4, J_{1b-9} = 3.1, 1-H_b), 2.60 (m, 9-H), 1.99 (ddd, $J_{10a-10b}$ = 12.0, J_{9-10a} = 7.5, J_{1a-10a} = 1.5, 10-H_a), 1.74 (d, $J_{5en-5ex}$ = 5.2, 5-H_{ex}), 1.33 (dd, $J_{10a-10b}$ = 12.0, J_{9-10b} = 11, 10-H_b), 1.19 (d, $J_{5en-5ex}$ = 5.2, 5-H_{en}), 1.18 (s, 13-H_3), 1.12 (s, 14-H_3), 1.06 (s, 15-H_3), 0.93 [br s, SiC(CH_3)$_3$], 0.12 and 0.11 [2 s, Si(CH_3)$_2$]. ^{13}C NMR: δ 197.6 (C-4), 189.1 (C-12), 164.7 (C-2), 130.7 (C-3), 76.0 (C-8), 47.4 (C-9), 45.6 and 44.1 (C-1 and C-10), 38.5 and 38.0 (C-6 and C-11), 34.5 (C-7), 29.5 (C-14), 28.3 (C-15), 26.0 [SiC(CH_3)$_3$], 20.2 (C-5), 18.3 [SiC(CH_3)$_3$], 16.9 (C-13), −3.2 and −3.5 [Si(CH_3)$_2$].

TBDMS ether **2c**. A solution of compound **1c** (148 mg, 0.41 mmol) in mesitylene (5 ml) was heated for 1 h at 180°C under an N_2 atmosphere in a high-pressure glass tube. After being cooled the solution was concentrated, and the residue was purified by chromatography (SiO_2; heptane–EtOAc 4:1 followed by CH_2Cl_2–EtOAc 50:1).

Besides starting material (25 mg, 17%, higher R_f), the isomer **2c** (80 mg, 54%, lower R_f) was obtained, and recrystallization in heptane gave white crystals with m.p. 146–149°C; $[\alpha]_D$ −36.2° (*c* 1.00, $CDCl_3$). MS [*m/z* (% rel. int.)]: 362.2293 (M^+, 22%, calculated for $C_{21}H_{34}SiO_3$ 362.2277), 333 (24), 305 (66), 277 (33), 230 (100), 201 (80), 187 (47), 115 (81), 75 (93), 73 (99). ^{1}H NMR: δ 9.86 (s, 12-H), 9.25 (s, 4-H), 3.05 (m, 9-H), 2.99 (d, J_{8-9} = 9.4, 8-H), 2.85 (dd, J_{1a-1b} = 18.4, J_{1a-10a} = 2.0, 1-H_a), 2.58 (dd, J_{1a-1b} = 18.4, J_{1b-9} = 2.7, 1-H_b), 2.06 (d, $J_{5en-5ex}$ = 4.8, 5-H_{ex}), 1.83 (ddd, $J_{10a-10b}$ = 12.5, J_{9-10a} = 7.6, J_{1a-10a} = 2.0, 10-H_a), 1.21 (s, 13-H_3), 1.16 (s,14-H_3), 1.15 (m 10-H_b), 1.08 (s, 5-H_3), 1.03 (d, $J_{5en-5ex}$ = 4.8, 5-H_{en}), 0.93 [br s, SiC(CH_3)$_3$], 0.10 and 0.05 [2 s, Si(CH_3)$_2$]. ^{13}C NMR: δ 199.2 (C-4), 189.3 (C-12), 172.9 (C-2), 130.5 (C-3), 81.3 (C-8), 48.8 (C-9), 44.2 (C-1), 43.4 (C-10), 39.6 and 38.5 (C-6 and C-11), 35.0 (C-7), 33.7 (C-5), 29.0 (C-14), 27.7 (C-15), 25.8 [SiC(CH_3)$_3$], 18.1 [SiC(CH_3)$_3$], 12.6 (C-13), −4.4 and −4.8 [Si(CH_3)$_2$].

Merulidial isomer **2a**. An aqueous solution of HF (40%, 8 mg, 0.16 mmol) was added to a solution of the TBDMS ether **2c** (28 mg, 0.080 mmol) in CH_3CN (1 ml) at 0°C. The solution was stirred at r.t. until the reaction was complete (4 h) according to TLC analysis. Excess Na_2CO_3 solution (8%) was added, and the product was extracted with Et_2O. The Et_2O phase was washed with H_2O and dried. Concentration followed by chromatography (SiO_2; heptane–EtOAc 2:1) gave the isomer **2a** (16 mg, 81%) as a white solid. Recrystallisation (Et_2O) gave crystals with m.p. 141–144°C. Its ^{1}H NMR and MS data are identical in all respects with those previously published.[8] ^{13}C NMR: δ 199.2 (C-4), 189.3 (C-12), 172.1 (C-2), 130.8 (C-3), 80.3 (C-8), 48.3 (C-9), 44.1 (C-1), 43.1 (C-10), 39.8 and 38.2 (C-6 and C-11), 34.7 (C-7), 33.8 (C-5), 28.9 (C-14), 27.9 (C-15), 12.1 (C-13).

*3-Hydroxy-9,9,12-trimethylbicyclo[5.3.0]deca-1,3,6-triene-4-carbaldehyde (***9***).* To a solution of isovelleral (**5**) (100 mg) in EtOH (10 ml), a 1 M solution of sodium ethoxide in EtOH was added dropwise (1 drop per minute) until TLC revealed that the starting material had been consumed. The products were isolated by column chromatography (SiO_2; heptane–EtOAc 4:1) and besides 9-hydroxyisovelleral (**7**) (45 mg, 42%), 7 mg (7%) of compound **9** were obtained as a yellow oil. MS [*m/z* (% rel. int.)]: 218.1307 (M^+, 41, $C_{14}H_{18}O_2$ requires 218.1307), 203 (19), 189 (100), 175 (23), 115 (22), 91 (31) and 77 (26). ^{1}H NMR: δ 8.66 (s, 5-H), 6.14 (s, 8-H), 2.63 (s, 4-H_2), 2.39 (s, 10-H_2), 2.24 (s, 1-H_2), 1.91 (s, 12-H_3), 0.99 (s, 13-H_3 and 14-H_3). ^{13}C NMR: δ 186.5 (C-5), 175.0 (C-7), 159.3, 135.6 and 135.5 (C-2, C-3 and C-9), 119.6 (C-8), 104.8 (C-6), 51.2 and 45.7 (C-1 and C-10), 37.0 (C-11), 32.4 (C-4), 28.2 (C-13 and C-14), 21.8 (C-12).

Degradation of the dialdehydes in buffer. Two mmol of the dialdehydes were dissolved in CH_3CN (0.50 ml) and added to the buffer (10 ml) at 37°C. The following buffers were used: 0.1 M phosphate buffer (pH = 5.6), 0.1 M phosphate buffer (pH = 7.4) and 0.025 M borax buffer (pH = 9.0). The solution was then stirred at 37°C for a number of weeks, and samples (0.1 ml) of the reaction mixtures were taken at intervals, mixed with 0.1 ml MeOH and analysed by reversed-phase HPLC.

Autoxidation of the dialdehydes on alumina. The degradation on alumina (Merck Aluminiumoxid 90, standardized according to Brockmann, activity II-III) was performed by mixing each sesquiterpene dialdehyde (1.5–4.5 mg) with a tenfold excess of alumina in Et_2O (0.50 ml mg^{-1} dialdehyde). The suspension was then stirred at r.t. for 2 h (compounds **1a**, **1c** and **2a**) or 5 h (compounds **5** and **6**). After filtration of the suspension through a Celite pad, the alumina was washed with EtOAc and MeOH, and the combined filtrates were concentrated. The residue was dissolved in $CDCl_3$ (purity 99.95%, 1 ml mg^{-1} original substrate) and analysed by ^{1}H NMR spectroscopy. The integrals of the upfield aldehyde proton of each dialdehyde before and after degradation on alumina were compared, using the solvent signal (δ_H = 7.26) as an internal standard.

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