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Acid- and Enzyme-Catalysed Cyclisation Reactions of (*Z*,*E*)-1(10),4-Cyclodecadiene Derivatives as Model Systems for Melampolides

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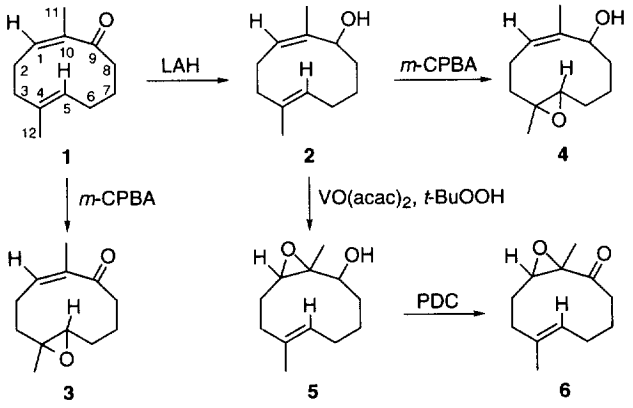
Abstract: The acid- and enzyme-catalysed cyclisations of a number of (*Z*,*E*)-1(10),4-cyclodecadiene derivatives were investigated. In contrast to the regular transannular C–C bond formation as observed for (*E*,*E*)-1(10),4-cyclodecadiene systems, these melampolide-like systems cyclised via a number of different reaction pathways in which the oxygen function at C_9 plays a crucial role. It was also found that these melampolide-like systems preferably react from the anti conformation. This finding is not in agreement with the postulate that melampolides are intermediates in the biosynthesis of trans-fused guaianes. Molecular mechanics and dynamics calculations indicated that the anti conformation is also the conformation in which these compounds preferably exist. © 1997 Elsevier Science Ltd.

Introduction

It has long been recognised that (*E*,*E*)-germacrane sesquiterpenes are the biosynthetic precursors of eudesmanes, guaianes, elemanes and other types of sesquiterpenes[1,2]. The isolation of configurationally isomeric germacranes, i.e. (*Z*,*E*)- (melampolides)[3,4], (*E*,*Z*)- (heliangolides)[5] and (*Z*,*Z*)-germacranes[6], has led to a number of considerations about their possible role in sesquiterpene biosynthesis[7]. Especially melampolides have been the subject of these biosynthetic considerations and it has been postulated that the biosynthesis of trans-fused guaianes proceeds via melampolides. However, due to the scarcity of experimental data, this postulate is rather speculative. Therefore, in order to gather more detailed information on this hypothetic route toward trans-fused guaianes, we decided to investigate the acid- and enzyme-induced cyclisation reactions of some melampolide-like systems, i.e. (*Z*,*E*)-cyclodeca-1(10),4-dien-9-one (**1**) and the structurally related compounds **2**–**6** (Scheme 1). Since these compounds all possess an oxygen function at C_9, just as most naturally occurring melampolides, they can be considered as ideal model systems for melampolides, all the more so because they can easily be prepared. Additionally, molecular mechanics and dynamics calculations were performed on these compounds to determine their lowest energy conformation[8]. To the best of our knowledge, this work represents the first detailed study on biomimetic cyclisations of functionalised (*Z*,*E*)-1(10),4-cyclodecadienes[9].

Results and discussion

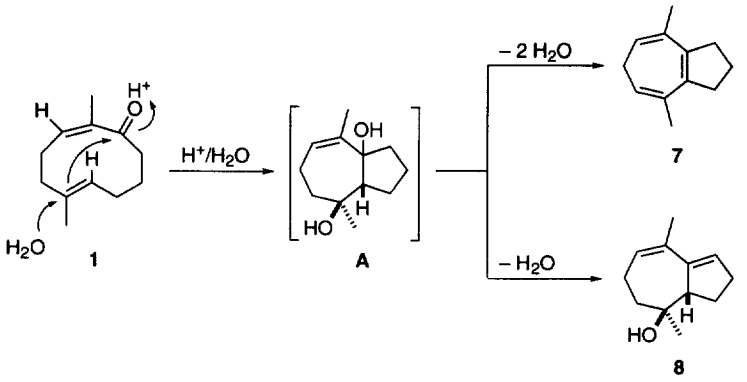
Synthesis of the (Z,E)-cyclodecadienes 1–6 - (*Z,E*)-cyclodeca-1(10),4-dien-9-one (**1**)[10] and the corresponding alcohol **2** were prepared as described[9]. Treatment of **1** and **2** with *m*-CPBA gave the epoxides **3** and **4**, respectively, in fair yields (Scheme 1). Selective epoxidation of the α,β-unsaturated double bond in **1** with H_2O_2 in the presence of NaOH to prepare directly compound **6** could not be achieved. The failure of this epoxidation reaction can be rationalised by a distorted conjugation between the C_9 carbonyl and the C_1–C_{10} double bond in **1**[11]. Normally, the β olefinic proton of an α,β-unsaturated carbonyl system resonates at δ values > 6.5 ppm. In the ^{1}H NMR spectrum of **1**, however, H_1 appears at δ 5.38 which points to strong disturbance of the conjugation. Such a distorted conjugation was also found in **3** as is indicated by the appearance of H_1 at δ 5.54 in its ^{1}H-NMR spectrum. A more successful synthetic route toward **6** started from the alcohol **2**. After Sharpless epoxidation[12] of **2**, PDC oxidation of the resulting epoxide **5** provided **6** in good yield. The way in which the structures **1–6** are depicted in Scheme 1 indicates that, at this stage of the research, the stereochemical and conformational features of these compounds could not be determined with certainty.



Scheme 1

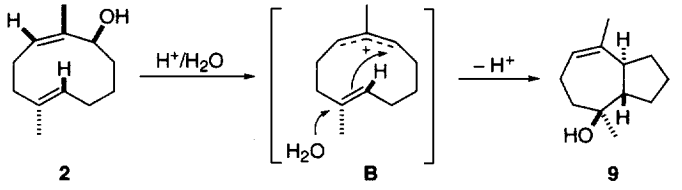
Acid-induced cyclisations of the (Z,E)-cyclodecadienes 1–6 - Acid-induced cyclisation of **1** afforded a ca 1:2 mixture of triene **7** and hydroazulene **8**, respectively (Scheme 2). The structure of the minor product **7** followed from the mass spectral data, [M$^+$] at *m/z* 160, and the NMR spectra. Both the ^{1}H and ^{13}C NMR spectra of **7** were surprisingly simple and indicated a symmetrical structure. The ^{13}C NMR spectrum of **8** showed a quaternary oxygen-bearing carbon together with two quaternary and two tertiary olefinic carbons. In the ^{1}H NMR of **8** two olefinic protons appeared at δ 5.58 and 5.70. The anti relationship between the Me_{12} group and the bridgehead proton at C_5 in **8** was ascertained by NOE difference experiments. Irradiation of H_5 (δ 3.02) did not exert a NOE on Me_{12} (δ 1.10) and *vice versa*. Together with the mass spectral data, [M$^+$] at *m/z* 178, these observations unequivocally establish the identity of **8**.

The formation of **7** and **8** assumedly proceeds via the intermediate hydroazulenic diol **A**. Loss of two molecules of water from **A** results in the formation of **7**, whereas loss of one molecule of water leading to the double bond in the five-membered ring accounts for the formation of **8**. Further information on the ring fusion in **A** and the conformational aspects of **1** could not be obtained from this experiment.



Scheme 2

Treatment of **2** with aqueous acid resulted in the formation of the previously reported hydroazulenic alcohol **9**[13] as the sole product (Scheme 3). A detailed NMR study of **9** was undertaken in order to establish the stereochemistry of this product unambiguously. Because of overlapped signals in its ^{1}H NMR spectrum taken in $CDCl_3$, the NMR measurements were recorded in C_6D_6 resulting in unobscured signals for the relevant protons, and with ^{1}H–^{1}H COSY and ^{1}H–^{13}C correlated NMR techniques the bridgehead protons H_5 and H_9 could now be assigned. The anti relationship between H_5 and Me_{12} and the trans ring junction in **9** was ascertained by NOE difference experiments. Irradiation of Me_{12} (δ 1.17) resulted in a clear NOE on H_9 (δ 2.19) but not on H_5 (δ 2.08).



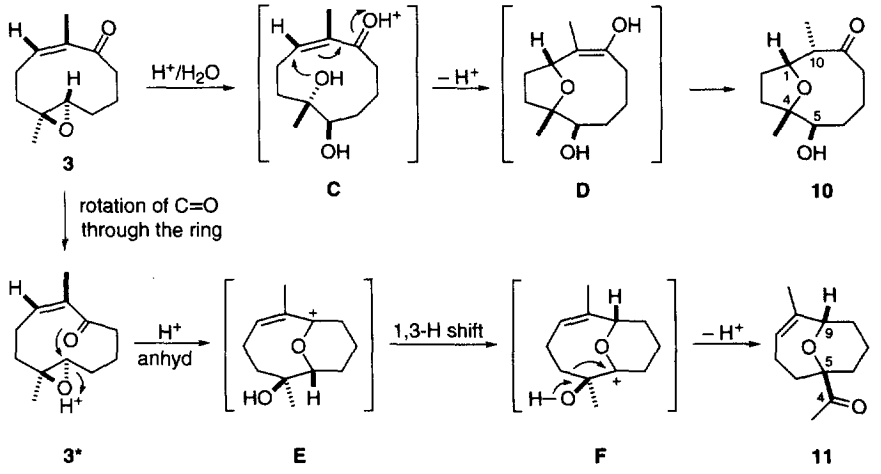
Scheme 3

The formation of **9** from **2** was thought to proceed via the intermediate allylic carbocation **B**[9]. The same allylic intermediate **B** has been proposed in the acid-induced cyclisation of (*E*,*E*)-6,10-dimethylcyclodeca-1(10),5-dien-7-ol also leading to alcohol **9**[13]. In this context, it is important to note that **2** presumably solely reacts from the anti conformation which is geometrically related to the trans product **9**[14]. This anti conformation seems to be typical for melampolides[15] as was deduced from *X*-ray studies on naturally occurring melampolides[3,4]. In the anti conformation, two-dimensionally represented by structure **2** in Scheme 3[16], the two double bonds are crossed and the hydroxyl group, which necessarily lies outside the ring, is favourably aligned for allylic ionisation probably intramolecularly assisted by the C_4–C_5 double bond. A similar rationalisation has been made for the solvolysis of esters of **2** in HOAc leading to the acetate of **9**[9].

Acid-induced cyclisation of **3** in aqueous medium gave the cyclic ether **10** as the main product and three other compounds which could not be identified. After chromatography, pure **10** was obtained in modest yield (Scheme 4). A detailed NMR study of **10** led to the unambiguous assignments of all the relevant signals. ^{1}H–^{1}H COSY

studies on **10** showed that H_{10} (dq, δ 3.51) was coupled to the Me_{11} protons (d, δ 0.90) and H_1 (ddd, δ 4.35). These data and the appearance of two tertiary oxygen-bearing carbons in the ^{13}C NMR spectrum indicated the presence of an ether bridge between C_1 and C_4 or C_5. Oxidation of **10** with PDC in DMF proved that the ether bridge is located between C_1 and C_4 as is indicated by the disappearance of the H_5 signal in the ^1H NMR spectrum of the oxidation product[17]. The stereochemistry at C_1, C_4, C_5 and C_{10} in **10** was ascertained by NOE difference experiments. Irradiation of H_1 (δ 4.35) and Me_{12} (δ 1.24) both resulted in a NOE on H_3 (δ 1.60), thereby supporting the syn spatial relationship between H_1 and Me_{12}. The downfield resonance of H_{10} (δ 3.51) and the upfield resonance of H_5 (δ 3.20) must be the result of magnetic anisotropy of the C_9 carbonyl group and are consistent with the assigned structure.

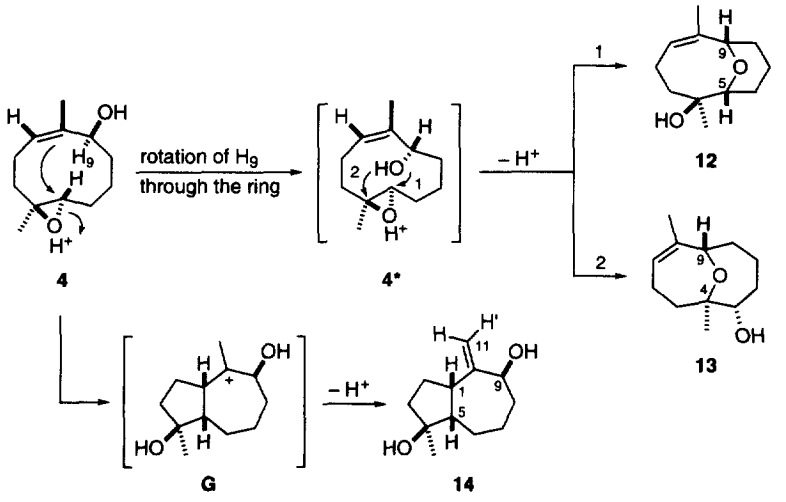
Under anhydrous conditions, acid treatment of **3** produced the cyclic ether **11** as the main product. The presence of an acetyl moiety in **11** was concluded from the base peak $[C_2H_3O]^+$ at *m/z* 43 and a prominent peak $[M - 43]^+$ at *m/z* 151 in its EI-mass spectrum, and the appearance of a three-proton singlet (Me_{12}) at δ 2.30 in its ^1H NMR spectrum. Chemical support for the presence of the acetyl group in **11** was obtained by reduction with $NaBH_4$ in EtOH. In the ^1H NMR spectrum of the reduction product the Me_{12} signal now appeared as a doublet at high field[18]. The structure of **11** was further elucidated by combination of ^1H and ^{13}C NMR, ^1H–^1H COSY and HETCOR spectra. NOE difference experiments revealed a weak but distinct NOE between H_9 and Me_{12} indicating their syn spatial relationship.



Scheme 4

In the presence of water, acid-catalysed opening of the epoxide ring in **3** probably produces the intermediate diol **C**. Protonation of the carbonyl group in **C** followed by 1,4-addition of the hydroxyl group to C_1 will then provide, via intermediate **D**, the cyclic ether **10**. Under anhydrous acid conditions, thus in the absence of external nucleophiles, rotation of the carbonyl function through the ring (**3** → **3***) and susequent intramolecular attack of this carbonyl at the C_5 position of the epoxide ring, activated by protonation, may result in the carbocationic intermediate **E**[19]. The formation of **11** from **E** can then be explained by a 1,3-H shift (**E** → **F**) and subsequent pinacol-like rearrangement[20]. The stereochemical features found for **10** and **11** suggest that **3** also reacts from the anti conformation, as is sketched in Scheme 4[16].

Acid-induced cyclisation of **4** in anhydrous environment afforded the cyclic ethers **12** and **13** and the diol **14** as main products, and several minor compounds which could not be identified (Scheme 5). It became clear from the MS and NMR data that both **12** and **13** contain a trisubstituted double bond, an ether bridge and an alcohol function. However, the location of the ether bridge (between C_4 and C_9 or C_5 and C_9) and the hydroxyl group (at C_4 or C_5) in **12** and **13** could not simply deduced from their NMR spectra. Therefore, both compounds were subjected to an oxidation reaction with PDC in DMF. Whereas **12** did not show any reaction, **13** was smoothly oxidised[21]. These findings are consistent with the structures assigned to **12** and **13**.

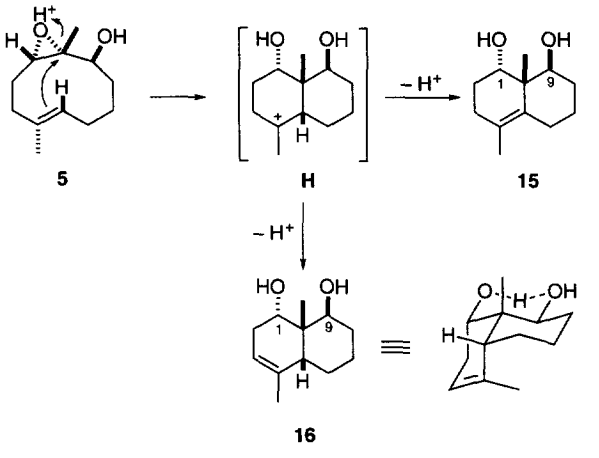


Scheme 5

The ^1H and ^{13}C NMR spectra of **14** showed signals only compatible with a hydroazulene skeleton possessing a 10(11)-exocyclic methylene (δ_H 5.04, br s, 1 H; δ_H 4.85, br s, 1 H; δ_C 154.14, s; δ_C 111.43, t), a tertiary hydroxyl group at C_4 (δ_C 81.82, s) and a secondary hydroxyl group at C_9 (δ_H 4.43, dd, J = 3.2, 6.9 Hz, 1 H; δ_C 75.74, d). The stereochemistry of **14** was deduced from NOE difference experiments. A clear NOE between H_1 (δ 3.33) and H_5 (δ 2.00) was consistent with the cis ring junction. Since no NOE was observed upon irradiation of Me_{12} (δ 1.12) on H_5, the anti relationship between Me_{12} and H_5 was ascertained. The β orientation of the hydroxyl group at C_9 followed from a very weak NOE between H_1 and H_{11} (δ 4.85) and a clear NOE between H_9 and $H_{11'}$ (δ 5.04) indicating the anti spatial relationship between H_1 and H_9. As a consequence, H_1 and the hydroxyl group at C_9 must have a syn spatial relationship. The chemical shift of H_1 at δ 3.33[22] and the coupling constant values (J = 3.2, 6.9 Hz) observed for H_9[23] were in agreement with the syn relationship.

The first step in the formation of **12** and **13** from **4** is most likely rotation of H_9 through the ring (**4** → **4***). After protonation of the epoxide ring in **4***, nucleophilic attack of the hydroxyl group at C_4 (route 1) gives rise to the formation of **12**, whereas attack at C_5 (route 2) accounts for the formation of **13**. In the formation of the cis-fused hydroazulene **14**, the epoxide ring of **4**, activated by protonation, undergoes a Markovnikov-type transannular cyclisation to give the intermediate carbocation **G**. Loss of a proton from C_{11} in **G** accounts for the formation of **14**. The stereochemical features of the products formed in this reaction indicate that **4**, just as **2** and probably also **3**, preferably reacts from the anti conformation[16].

Acid-induced cyclisation of **5** gave the perhydronaphthalenediols **15** and **16** as main products, and several minor compounds which could not be identified (Scheme 6). The structure of **15** was deduced from its ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum of **15** exhibited two one-proton doublets of doublets at δ 3.76 (J = 4.7, 6.8 Hz) and 3.93 (J = 4.9, 11.0 Hz). Together with the resonances at δ 72.47 (d) and 74.97 (d) in the ^{13}C NMR spectrum, these data indicated the presence of both an axial and equatorial hydroxyl group in **15**. The axial hydroxyl group must be located at C_1 because H_1 and Me_{11} in **15** must have a syn relationship on mechanistic grounds. Consequently, the equatorial hydroxyl group is located at C_9. The absence of olefinic signals in the ^1H NMR spectrum and two singlets at δ 125.35 and 133.02 in the ^{13}C NMR spectrum pointed to the presence of a tetrasubstituted double bond in **15**. These considerations have led to the conclusion that **15** possesses the structure as depicted in Scheme 6.



Scheme 6

The structure and stereochemistry of **16** followed from its NMR spectra and NOE difference experiments. The two one-proton doublets of doublets at δ 3.64 (J = 6.3, 10.2 Hz) and 4.04 (J = 4.8, 11.1 Hz) in the ^1H NMR spectrum of **16** revealed the presence of two equatorial hydroxyl groups. The presence of two protons linked to oxygen-bearing carbons was further supported by the appearance of two doublets at δ 70.00 and 77.62 in the ^{13}C NMR spectrum. The appearance of a one-proton signal at δ 5.37 and a three-proton broad singlet at δ 1.58 was consistent with a trisubstituted double bond between C_3 and C_4. Similarly to **15**, H_1 and Me_{11} in **16** must have a syn relationship on mechanistic grounds. This syn relationship was confirmed by irradiation of Me_{11} resulting in a clear NOE on H_1. A NOE between H_9 and Me_{11} was not observed and this meant that H_9 and Me_{11} have a trans relationship. The occurrence of a clear NOE between Me_{11} and H_5 (δ 2.08) indicated that **16** possesses a cis-fused perhydronaphthalene skeleton. The sharp signals and couplings in the ^1H NMR spectrum further suggested that **16** exists in one distinct conformation. These findings all combined have led to the conclusion that **16** exclusively adopts the steroid conformation as depicted in Scheme 6. This conformation is strongly favoured by the possibility to form a stable intramolecular hydrogen bridge. The formation of **15** and **16**, which can easily be explained by proton-induced opening of the epoxide ring, transannular cyclisation leading to the intermediate carbocation **H** and proton loss, clearly shows that **5** predominantly reacts from the anti conformation[16].

Acid-induced cyclisation of **6** in both aqueous and anhydrous media gave no unambiguous product formation.

In both reactions a complex mixture was obtained and no further cyclisation studies on **6** were undertaken.

Molecular mechanics and dynamics calculations on **1–5** using the CHARMm force field[24] were performed to support the experimental findings described above. To determine the lowest energy conformations of **1–5**, the structure of each compound was geometry optimised in order to relax the conformation and to remove steric overlap. The resulting conformation was used as starting structure for molecular dynamics simulation[25]. In these simulations, Newton's equations of motions are integrated numerically for every atom in the molecule using the potential energy function[24]. All atoms were treated individually; bond lengths and angles containing hydrogen atoms were kept constant using the SHAKE[26] algorithm. The simulation was used to generate "random" structures of the compounds involved. In total 1000 random structures for each compound were obtained and each structure was geometry optimised individually using the steepest descents algorithm (500 steps), followed by the conjugate gradient algorithm until the energy gradient $\nabla E \leq 0.001$ kcal mol^{-1} Å^{-1}. From these calculations, it appeared that the lowest energy conformation of all five compounds is the anti conformation as is exemplified by the stereoscopic representations of the molecular structures of **1** and **3** (Figure 1).

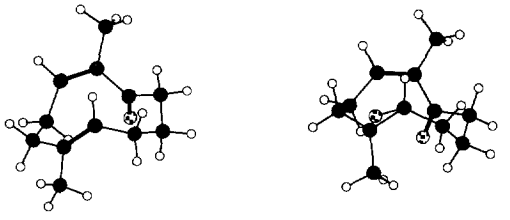


Figure 1. The lowest energy conformations of **1** and **3**

Enzyme-induced cyclisations of the (Z,E)-cyclodecadienes 1–5 - Next to the acid-catalysed cyclisation, also the enzyme-induced cyclisation of the compounds **1–5** was studied using a chicory (*Cichorium intybus*) root homogenate[13,27]. The substrate specificity of this homogenate was tested by comparing the transformations with a boiled root sample (enzyme blank) and the incubation medium (solvent blank). The products obtained from the acid-catalysed reactions described above were used as reference material for GC-MS analysis. Incubation of **1** with a root suspension of fresh chicory led to the formation of the same products (**7** and **8**) as the acid-catalysed reaction. Also the ratio in which **7** and **8** were formed was identical. Both blanks did not show any reaction. The azulenic alcohol **9** was the sole product obtained from incubation of **2**. Acid treatment of **2** showed a similar result. Also in this case, both blanks did not show any conversion. The compounds **3** and **5** were resistant toward enzymatic transformation. Compound **4** was partly converted into the products **12** and **14** by the enzyme-containing suspension, but the same reaction was also observed in the two blanks. The other product **13** formed upon acid treatment of **4** could not be detected.

These enzyme-induced cyclisation studies on **1–5** show that only the conversions of **1** and **2** are accelerated by the root suspension of chicory. In contrast to the reaction outcome of enzyme- and acid-catalysed cyclisation reactions of (*E,E*)-germacranes[27b] and their epoxides[27c], the products and product ratio in the enzyme- and acid-catalysed cyclisation reaction of **1** and **2** are almost identical, which indicates that the catalytic activity of the chicory root enzymes is limited to enzyme-mediated protonation of the most nucleophilic site of the substrate, thereby initiating the cyclisation reactions observed for these compounds.

Conclusions

Although the accumulated results permit the generalisation that the cyclisation reactions of **1–5** preferentially proceed from the anti conformation, the variety of products formed in these reactions points to a number of different cyclisation pathways. It is therefore assumed that the anti conformation from which these melampolide-like compounds most likely react inhibits the transannular cyclisation reactions that are common in the (*E,E*)-germacrane series[15a]. Consequently, functional groups, in these specific cases the oxygen function at C_9, can interfere with the cyclisation process as the formation of the compounds **7–13** clearly shows.

The preference of the melampolide-like compounds studied by us, and possibly melampolides in general, to cyclise from the anti conformation is not in agreement with the postulate that the biosynthesis of trans-fused guaianes proceeds via melampolides[28]. Cyclisation to trans-fused guaianes should imply that melampolides react from the syn conformation! Also the observation that a cis-fused azulene is formed in the cyclisation reaction of **4** makes this postulate less probable. However, it is realised that additional ring substituents, such as annulated lactone rings present in the majority of naturally occurring sesquiterpenes, can have profound effect on the conformational behaviour of the ten-membered ring system in melampolides. Therefore, the possibility that melampolides may play a role in the biosynthesis of trans-fused guaiane sesquiterpenes can not completely be excluded.

Experimental

General. UV spectra were determined in MeCN on a Perkin Elmer Lambda 18 spectrometer. NMR experiments were conducted with Bruker AC-E 200 and DPX 400 instruments; signals are reported in parts per million (δ), referenced to the solvent used. MS data were determined at 70 eV on a Hewlett Packard 5890B series Mass Selective Detector, coupled with a DB-17 fused silica capillary column, 30 m × 0.25 mm i.d., film thickness 0.25 μm. Helium was used as the carrier gas. HRMS data were obtained with a Finnigan MAT 95 spectrometer. Column chromatography was performed on Merck silica gel 60 using mixtures of petroleum ether (bp. 40–60 °C) (PE) and EtOAc as the solvent system. Solvents were dried and freshly distilled by common practice. Product solutions were dried over $MgSO_4$ prior to evaporation of the solvent under reduced pressure by using a rotary evaporator. (*Z,E*)-cyclodeca-1(10),4-dien-9-one (**1**) and the corresponding alcohol **2** were prepared as described[9].

(*Z*)-4,10-Dimethyl-4,5-epoxycyclodec-1(10)-en-9-one (3). To a stirred solution of 42 mg of **1** in 3 ml of CH_2Cl_2 were successively added 3 ml of water, 0.5 g of $NaHCO_3$ and 60 mg of *m*-CPBA (70–75%) at 0 °C. After being stirred for 30 min, the two-phase mixture was separated and the aqueous layer was extracted with 2 × 5 ml of CH_2Cl_2. The combined organic layers were washed with brine, dried and evaporated. The remaining residue (50 mg) was chromatographed (25% EtOAc in PE) to give 34 mg of **3**: UV λ_{max} 242 nm; ^{1}H NMR ($CDCl_3$) δ 1.05–1.35 (m, 2 H), 1.19 (s, 3 H), 1.65–2.60 (m, 8 H), 1.95 (br s, 3 H), 2.63 (dd, J = 2.5, 10.6 Hz, 1 H), 5.54 (br t, J = 7.9 Hz, 1 H) ; ^{13}C NMR ($CDCl_3$) δ 17.87 (q), 19.87 (q), 21.15 (t), 23.17 (t), 28.24 (t), 37.16 (t), 41.91 (t), 60.56 (s), 62.21 (d), 132.79 (d), 139.75 (s), 207.95 (s); MS *m/z* (relative intensity) 194 (M^+, 3), 176 (2), 133 (44), 122 (39), 109 (59), 95 (96), 79 (43), 67 (35), 55 (39), 43 (100); HRMS calcd for $C_{12}H_{18}O_2$ (M^+) 194.1307, found 194.1304.

(*Z*)-4,10-Dimethyl-4,5-epoxycyclodec-1(10)-en-9-ol (4). The alcohol **2** (98 mg) was treated with *m*-CPBA as described above. Workup yielded 95 mg of an oil which was chromatographed (40% EtOAc in PE) to give 64 mg of **4**: ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 1 H), 1.25 (m, 1 H), 1.32 (s, 3 H), 1.58 (OH), 1.64 (br s, 3 H), 1.65–2.10 (m, 7 H),

2.25 (m, 1 H), 2.56 (dd, J = 1.3, 9.9 Hz, 1 H), 4.44 (t, J = 8.0 Hz, 1 H), 5.32 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 16.66 (q), 16.69 (q), 22.07 (t), 23.73 (t), 26.67 (t), 34.98 (t), 37.30 (t), 60.44 (s), 65.45 (d), 67.29 (d), 128.10 (d), 138.59 (s); MS *m/z* (relative intensity) 196 (M^+, 0.5), 181 (10), 163 (9), 149 (7), 145 (5), 135 (16), 123 (24), 109 (60), 97 (47), 79 (47), 67 (30), 55 (53), 43 (100); HRMS calcd for $C_{12}H_{20}O_2$ (M^+) 196.1463, found 196.1458.

(*E*)-4,10-Dimethyl-1,10-epoxycyclodec-4-en-9-ol (5). To a solution of 484 mg of **2** in 15 ml of benzene was added 50 mg of $VO(acac)_2$ and 0.9 ml of *t*-BuOOH (5–6 M in decane). The reaction mixture was stirred for 1.5 h, and then washed with 2 × 25 ml of 10% aqueous $Na_2S_2O_3$. The combined aqueous layers were back-extracted with 2 × 25 ml of EtOAc. The combined organic layers were dried and evaporated to give 660 mg of a brownish oil. Purification of this oil by chromatography (20% EtOAc in PE) gave 346 mg of **5** as a colourless oil: ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 3 H), 1.25–1.90 (m, 6 H), 1.64 (br s, 3 H), 1.95–2.25 (m, 4 H), 2.35 (br d, J = 13.8 Hz, 1 H), 2.82 (dd, J = 3.7, 10.7 Hz, 1 H), 3.24 (m, $W_{1/2} \approx$ 10 Hz, 1 H), 5.14 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.71 (q), 19.81 (q), 27.45 (t), 28.48 (t), 30.50 (t), 31.88 (t), 32.76 (t), 64.46 (s), 66.31 (d), 74.36 (d), 125.30 (d), 134.46 (s); MS *m/z* (relative intensity) 178 (M^+ – 18, 1), 163 (2), 139 (26), 112 (38), 97 (27), 81 (30), 71 (60), 67 (38), 55 (77), 43 (100); HRMS calcd for $C_{12}H_{18}O$ (M^+ – 18) 178.1358, found 178.1358.

(*E*)-4,10-Dimethyl-1,10-epoxycyclodec-4-en-9-one (6). To a stirred solution of 2.0 g of PDC in 5 ml of DMF was added 346 mg of **5** in 3 ml of DMF. The reaction mixture was stirred at room temperature overnight, and then diluted with 80 ml of water. After extraction with 6 × 30 ml of ether, the combined organic layers were washed with brine, dried and evaporated. The remaining colourless oil (290 mg) was almost pure **6**: ^{1}H NMR ($CDCl_3$) δ 1.30 (m, 1 H), 1.57 (s, 3 H), 1.61 (br s, 3 H), 1.65–1.85 (m, 2 H), 1.95–2.50 (m, 7 H), 2.88 (dd, J = 3.1, 10.3 Hz, 1 H), 4.82 (br t, J = 8.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 19.26 (q), 19.73 (q), 25.25 (t), 27.81 (t), 27.90 (t), 31.82 (t), 39.05 (t), 63.50 (s), 64.77 (d), 126.58 (d), 134.44 (s), 205.55 (s); MS *m/z* (relative intensity) 194 (M^+, 2), 179 (5), 176 (7), 161 (14), 151 (19), 133 (54), 111 (60), 95 (64), 81 (68), 67 (59), 55 (59), 43 (100); HRMS calcd for $C_{12}H_{18}O_2$ (M^+) 194.1307, found 194.1302.

Acid-induced cyclisations of (*Z*,*E*)-cyclodecadienes 1–5. a. To a solution of 425 mg of **1** in 15 ml of acetone were added 10 ml of water and 25 drops of H_2SO_4. The reaction mixture was stirred at room temperature overnight, and then carefully neutralised with solid $NaHCO_3$. After removal of acetone under reduced pressure, the remaining aqueous layer was extracted with 2 × 20 ml of EtOAc. The combined organic layers were dried and evaporated to give 367 mg of an oil. Chromatography (15% EtOAc in PE) afforded 73 mg of **7** and 142 mg of **8**.

7: UV λ_{max} 255 nm; ^{1}H NMR ($CDCl_3$) δ 1.78 (t, J = 7.3 Hz, 2 H), 1.78 (br s, 6 H), 2.01 (t, J = 6.8 Hz, 2 H), 2.59 (t, J = 7.3 Hz, 4 H), 5.08 (br t, J = 6.8 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.29 (2 q), 22.18 (t), 27.15 (t), 35.55 (2 t), 116.63 (2 d), 133.54 (2 s), 142.28 (2 s); MS *m/z* (relative intensity) 160 (M^+, 26), 145 (100), 130 (20), 128 (19), 115 (26), 105 (14), 91 (26), 77 (21), 65 (18), 51 (23), 39 (51); HRMS calcd for $C_{12}H_{16}$ (M^+) 160.1252, found 160.1248.

8: UV λ_{max} 233 nm; ^{1}H NMR ($CDCl_3$) δ 1.10 (s, 3 H), 1.47 (OH), 1.70–1.80 (m, 2 H), 1.81 (br s, 3 H), 1.90–2.10 (m, 3 H), 2.25–2.50 (m, 3 H), 3.02 (br t, J = 5.7 Hz, 1 H), 5.58 (br t, J = 5.6 Hz, 1 H), 5.70 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 22.48 (q), 23.35 (q), 24.06 (t), 26.31 (t), 31.27 (t), 44.27 (t), 56.64 (d), 75.63 (s), 128.77 (d), 128.84 (d), 132.63 (s), 143.78 (s); MS *m/z* (relative intensity) 178 (M^+, 4), 160 (11), 145 (13), 135 (28), 120 (17), 105 (50), 91 (43), 79 (37), 65 (18), 43 (100); HRMS calcd for $C_{12}H_{18}O$ (M^+) 178.1358, found 178.1358.

b. The alcohol **2** (282 mg) was treated with diluted H_2SO_4 as described for **1**. Workup yielded 213 mg of an oil which was chromatographed (20% EtOAc in PE) to give 110 mg of **9**: ^{1}H NMR (C_6D_6) δ 1.17 (s, 3 H, Me_{12}), 1.28–1.49 (m, 2 H, $H_{8'}$ and $H_{7'}$), 1.58–1.68 (m, 2 H, $H_{3'}$ and H_7), 1.77 (br s, 3 H, Me_{11}), 1.78–1.85 (m, 4 H, H_3, H_6, $H_{6'}$ and H_8), 1.96 (quintet, J = 8.8 Hz, 1 H, $H_{2'}$), 2.08 (ddd, J = 6.6, 9.8, 9.8 Hz, 1 H, H_5), 2.19 (br q, J = 9.8 Hz, 1 H, H_9), 2.30 (m, 1 H, H_2), 5.58 (m, 1 H, H_1). The ^{13}C NMR and MS data of **9** were identical to those reported in the

literature[13].

c. A solution of 140 mg of **3** in 5 ml of acetone was added to 100 ml of water buffered at pH 2. The resulting suspension was stirred at room temperature for 72 h. The aqueous phase was extracted with 2 × 20 ml of CH_2Cl_2, and the combined organic layers were washed with brine, dried and evaporated. The remaining residue was purified by chromatography (30% EtOAc in PE) to give 32 mg of **10**: ^{1}H NMR δ 0.90 (d, J = 6.8 Hz, 3 H, Me_{11}), 1.24 (s, 3 H, Me_{12}), 1.50 (m, 1 H), 1.60 (ddd, J = 8.0, 12.5, 12.5 Hz, 1 H, H_3), 1.73 (OH), 1.80–2.12 (m, 6 H), 2.20 (ddd, J = 1.7, 8.2, 14.2 Hz, 1 H, $H_{8'}$), 2.84 (ddd, J = 2.1, 10.8, 14.2 Hz, 1 H, H_8), 3.20 (br t, J = 5.4 Hz, 1 H, H_5), 3.51 (dq, J = 6.7, 6.8 Hz, 1 H, H_{10}), 4.35 (ddd, J = 6.7, 6.7, 9.4 Hz, 1 H, H_1); ^{13}C NMR δ 11.79 (q, C_{11}), 19.98 (q, C_{12}), 25.00 (t), 25.47 (t), 35.60 (t), 36.32 (t), 46.10 (t), 50.00 (d, C_{10}), 74.34 (d, C_5), 83.11 (d, C_1), 85.74 (s, C_4), 218.68 (s, C_9); MS *m/z* (relative intensity) 212 (M^+, 4), 194 (7), 155 (12), 130 (17), 113 (21), 99 (29), 83 (13), 71 (15), 55 (33), 43 (100); HRMS calcd for $C_{12}H_{20}O_3$ (M^+) 212.1412, found 212.1411.

d. To a solution of 49 mg of **3** in 50 ml of toluene was added 5 mg of TsOH. The reaction mixture was stirred at room temperature for 20 h, and then washed with 2 × 20 ml of saturated aqueous $NaHCO_3$. The combined aqueous layers were back-extracted with 2 × 25 ml of ether. The combined organic layers were dried and evaporated to give 56 mg of an oil which was chromatographed (20% EtOAc in PE) to give 16 mg of **11**: ^{1}H NMR (C_6D_6) δ 1.35 (ddddd, J = 3.5, 3.5, 3.8, 3.8, 13.0 Hz, 1 H, H_7), 1.42 (br s, 3 H, Me_{11}), 1.43–1.62 (m, 4 H, H_6, $H_{6'}$, H_8, $H_{8'}$), 1.68 (dddd, J = 3.7, 3.7, 13.0, 13.0 Hz, 1 H, $H_{7'}$), 1.76 (dddd, J = 2.2, 6.0, 8.4, 15.1 Hz, 1 H, H_2), 2.03 (ddd, J = 2.2, 12.6, 14.4 Hz, 1 H, H_3), 2.30 (s, 3 H, Me_{12}), 2.37 (ddd, J = 2.3, 6.0, 14.4 Hz, 1 H, $H_{3'}$), 2.46 (dddddd, J = 2.3, 2.3, 2.3, 3.2, 12.6, 15.1 Hz, 1 H, $H_{2'}$), 4.45 (br d, J = 6.4 Hz, 1 H, H_9), 5.58 (br d, J = 8.4 Hz, 1 H, H_1); ^{13}C NMR (C_6D_6) δ 15.42 (t, C_7), 21.24 (q, C_{11}), 23.41 (t, C_2), 25.58 (q, C_{12}), 26.65 (t, C_8), 32.34 (t, C_6), 37.13 (t, C_3), 77.77 (d, C_9), 81.46 (s, C_5), 127.83 (d, C_1), 137.04 (s, C_{10}), 211.67 (s, C_4); MS *m/z* (relative intensity): 194 (M^+, 0.9), 176 (26), 151 (23), 133 (54), 105 (31), 93 (28), 81 (39), 67 (32), 55 (39), 43 (100); HRMS calcd for $C_{12}H_{18}O_2$ (M^+) 194.1307, found 194.1313.

e. A sample of **4** (55 mg) was treated with TsOH (6 mg) as described for **3**. Workup yielded 46 mg of an oil which was chromatographed (25% EtOAc in PE) to give 4 mg of **12**, 15 mg of **13** and 5 mg of **14**.

12: ^{1}H NMR ($CDCl_3$) δ 1.02 (s, 3 H), 1.15–1.50 (m, 2 H), 1.55–2.10 (m, 7 H), 1.65 (br s, 3 H), 2.80 (m, 1 H), 3.46 (OH), 3.75 (dd, J = 4.1, 13.1 Hz, 1 H), 4.35 (br s, $W_{1/2}$ ≈ 11 Hz, 1 H), 5.41 (br t, J = 7.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.73 (t), 20.66 (t), 21.74 (t), 22.01 (q), 23.99 (t), 24.90 (q), 36.92 (t), 72.91 (d), 77.67 (s), 81.07 (d), 125.08 (d), 140.60 (s); MS *m/z* (relative intensity) 178 (M^+ – 18, 4), 168 (2), 160 (3), 152 (7), 135 (14), 134 (12), 109 (27), 94 (84), 79 (100), 67 (20), 55 (19), 43 (38); HRMS calcd for $C_{12}H_{18}O$ (M^+ – 18) 178.1358, found 178.1353.

13: ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H), 1.45 (br dd, J ≈ 3.0, 14.0 Hz, 1 H), 1.62 (br s, 3 H), 1.60–2.70 (m, 10 H), 3.59 (br d, J = 9.3 Hz, 1 H), 4.04 (dd, J ≈ 7.4, 9.3 Hz, 1 H), 5.24 (d, J = 6.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 18.52 (t), 23.10 (q), 24.40 (q), 24.63 (t), 33.57 (t), 34.34 (t), 38.19 (t), 75.24 (d), 79.50 (d), 81.41 (s), 122.26 (d), 141.53 (s); MS *m/z* (relative intensity) 196 (M^+, 0.5), 178 (4), 168 (3), 160 (4), 152 (10), 135 (21), 134 (18), 109 (37), 94 (100), 79 (98), 67 (28), 55 (26), 43 (63); HRMS calcd for $C_{12}H_{20}O_2$ (M^+) 196.1463, found 196.1460.

14: ^{1}H NMR ($CDCl_3$) δ 1.03 (m, 1 H), 1.12 (s, 3 H, Me_{12}), 1.45–1.90 (m, 11 H), 2.00 (ddd, J = 2.5, 10.2, 12.5 Hz, 1 H, H_5), 3.33 (br q, J ≈ 10 Hz, 1 H, H_1), 4.43 (dd, J = 3.2, 6.9 Hz, 1 H, H_9), 4.85 (br s, 1 H, H_{11}), 5.04 (br s, 1 H, $H_{11'}$); ^{13}C NMR ($CDCl_3$) δ 23.04 (t), 24.51 (q), 25.76 (t), 26.82 (t), 36.23 (t), 40.95 (t), 41.00 (d), 53.41 (d), 75.74 (d), 81.82 (s), 111.43 (t), 154.14 (s); MS *m/z* (relative intensity) 178 (M^+ – 18, 3), 163 (24), 160 (54), 145 (47), 135 (39), 120 (40), 105 (45), 93 (62), 91 (61), 79 (74), 67 (39), 55 (35), 43 (100); HRMS calcd for $C_{12}H_{18}O$ (M^+ – 18) 178.1358, found 178.1358.

f. A solution of 54 mg of **5** in 5 ml of acetone was added to 100 ml of water buffered at pH 1. The suspension

was stirred at room temperature for 20 h, and then extracted with 2 × 20 ml of CH_2Cl_2. The combined organic layers were washed with brine, dried and evaporated. The remaining residue (58 mg) was chromatographed (40% EtOAc in PE) to give 24 mg of **15** and 8 mg of **16**.

15: ^{1}H NMR ($CDCl_3$) δ 1.10 (s, 3 H), 1.18 (m, 1 H), 1.50–2.20 (m, 8 H), 1.59 (br s, 3 H), 2.40 (m, 1 H), 2.99 (OH), 3.36 (OH), 3.76 (dd, J = 4.7, 6.8 Hz, 1 H), 3.93 (dd, J = 4.9, 11.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 17.69 (q), 19.60 (q), 24.39 (t), 24.69 (t), 26.43 (t), 29.09 (t), 30.09 (t), 44.31 (s), 72.47 (d), 74.97 (d), 125.35 (s), 133.02 (s); MS *m/z* (relative intensity) 196 (M^+, 0.5), 178 (39), 145 (100), 121 (48), 119 (61), 107 (96), 91 (44), 79 (36), 67 (22), 55 (35), 43 (64); HRMS calcd for $C_{12}H_{20}O_2$ (M^+) 196.1463, found 196.1464.

16: ^{1}H NMR ($CDCl_3$) δ 1.10 (m, 1 H), 1.21 (s, 3 H), 1.35–1.90 (m, 5 H), 1.58 (br s, 3 H), 2.08 (m, $W_{1/2} \approx$ 11 Hz, 1 H), 2.15–2.50 (m, 2 H), 2.81 (OH), 2.94 (OH), 3.64 (dd, J = 6.3, 10.2 Hz, 1 H), 4.04 (dd, J = 4.8, 11.1 Hz, 1 H), 5.37 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.00 (q), 20.26 (t), 21.31 (q), 22.75 (t), 30.28 (t), 32.04 (t), 40.72 (s), 46.17 (d), 70.00 (d), 77.62 (d), 120.97 (d), 134.62 (s); MS *m/z* (relative intensity) 196 (M^+, 15), 178 (12), 163 (17), 145 (24), 135 (29), 107 (100), 91 (43), 79 (35), 55 (29), 41 (47); HRMS calcd for $C_{12}H_{20}O_2$ (M^+) 196.1463, found 196.1460.

Enzyme-induced cyclisations of (*Z*,*E*)-cyclodecadienes 1–5. A suspension of fresh chicory root (20% w/v) was produced by mortaring the root in a solution of 0.25 M sucrose, 3 mM Tris·HCl, 10 mM $MgCl_2$ and 0.2 % (w/v) Bovine Serum Albumin (BSA). The pH of this sucrose/Tris/$MgCl_2$/BSA-solution (STMB) was set at 6.5 using 2-morpholinoethanesulfonic acid (MES). The stability of the substrates towards the buffer and an inactivated chicory root sample (obtained by boiling the root suspension for 30 min) was investigated as a control to test the possibility of non-enzymatic reactions. Incubations were performed in sealed 4 ml vials at room temperature in a KS 500 shaker at 260 rpm containing 200 µl root suspension, 790 µl STMB-solution and 10 µl 0.1 M substrate in EtOH. The incubation medium was extracted with 0.5 ml of EtOAc and its contents were analysed by GC-MS.

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