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Biotransformation of germacrane epoxides by *Cichorium intybus*

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Abstract: The biotransformation of germacrone-4,5-epoxide (**2**), germacrone-1,10-epoxide (**3**), isogermacrone-4,5-epoxide (**5**), germacrene B-4,5-epoxide (**6**) and germacrene B-1,10-epoxide (**7**) by a suspension of fresh chicory root (*Cichorium intybus*) was investigated. Enzyme catalysed cyclisations towards substituted guaianes and eudesmanes were observed.

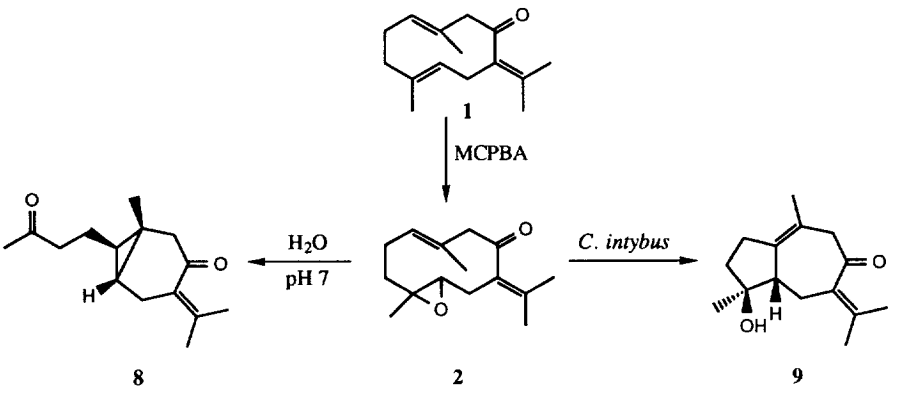
Introduction

Germacrane sesquiterpenes are believed to be important intermediates in the biosynthetic pathway towards guaiane-, eudesmane- and other types of sesquiterpenes. In our studies towards the partial elucidation of the biosynthesis of sesquiterpene bitter compounds in chicory (*Cichorium intybus*) we have reported the biotransformation of several synthetic and natural germacranes by a root suspension of fresh chicory [1,2].
Recently, several groups have reported the biotransformation of germacrone (**1**) by suspension cultured plant cells into highly functionalised metabolites with varying skeletal features[3-5]. Germacrone (**1**) can be isolated by simple crystallisation from the essential oil of *Geranium macrorrhizum* [6] and it can be transformed into a number of compounds suitable for biosynthetic studies. In this paper, the biotransformation of germacrone-4,5-epoxide (**2**), germacrone-1,10-epoxide (**3**), isogermacrone-4,5-epoxide (**5**), germacrene B-4,5-epoxide (**6**) and germacrene B-1,10-epoxide (**7**) by a root suspension of fresh chicory is described.

Results and discussion

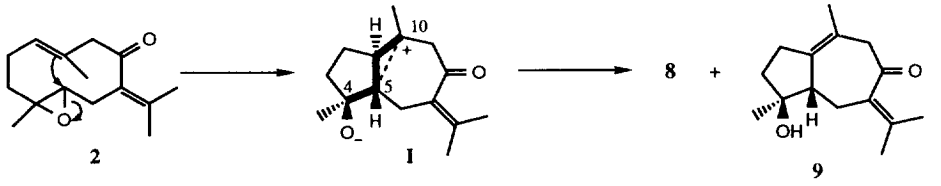
Treatment of germacrone (**1**) with an equimolar amount of MCPBA results in a mixture of the two mono-epoxides germacrone-4,5-epoxide (**2**) and germacrone-1,10-epoxide (**3**) of which **2** is the major component. When **2** was administered to a suspension of mortared chicory roots and incubated for 8 days, a 4 : 1 mixture of curcumenone (**8**)[7] and neoprocurcumenol (**9**)[8] was obtained. Their ^{1}H- and ^{13}C-NMR spectra are identical to those reported in the literature[7,9,10]. The stability of **2** towards the incubation medium and an inactivated chicory root sample was investigated as a control to test the possibility of non-enzymatic reactions. The conversion of **2** into **8** appeared to be an uncatalysed process. After 8 days, **2** was completely converted into **8**; no **9** was

detected in the controls. Thus, the formation of **9** appeared to be fully enzyme-mediated. The sp^2-bridgehead carbon in the guaiane system of **9** is characteristic for the bitter compounds in *C. intybus* [11-14]. Analytical HPLC-experiments using a chiral column revealed the racemic nature of **9**.



Scheme 1: Spontaneous and biotransformation of **2**.

The spontaneous formation of **8** *via* a homofragmentation reaction can only proceed through the *trans* guaiane intermediate **I** as depicted in scheme 2. Homofragmentations are fast and favoured reactions when orbital interaction through the three intervening C-C single bonds (through-bond interaction) is accompanied by 1,3-bridged through-space interaction[15]. Both conditions are met in a W-like conformation[16] as is present in **I**. A bridging between the cationic centre at C-10 and the back lobe of the O^- – C-4 – C-5 orbital is believed to be involved in this reaction.



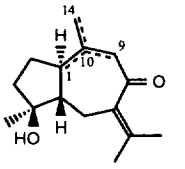
Scheme 2: Biotransformation of **2** into **9** through enzyme stabilised guaiane cation intermediates.

The enzymatically formed product **9** is the most stable of all *trans* guaiane double bond isomers as is determined from the calculation of their Heat-of-Formation (ΔH_f) using semi-empirical methods[17]. The ΔH_f values of **8** -**11** are given in table 1. Although the ΔH_f-value of **8** is 14.4 kcal/mol higher than **9**, spontaneous homo-fragmentation predominates the enzyme-mediated deprotonation.

In the Johnson model for oxidosqualene cyclising enzymes[18], cyclisation is initiated by proton donation from a specific amino acid residue which acts as a general acid catalyst. The ring fusion and the stereochemistry of the product(s) are directed by electron-rich, aromatic amino acid residues (e.g. tryptophan or tyrosine) which stabilise carbon centers that become cationic in transition states and/or high energy intermediates.

Compound	ΔHf (kcal/mol)
8	– 80.49
9	– 94.86
10	– 90.81
11	– 87.03

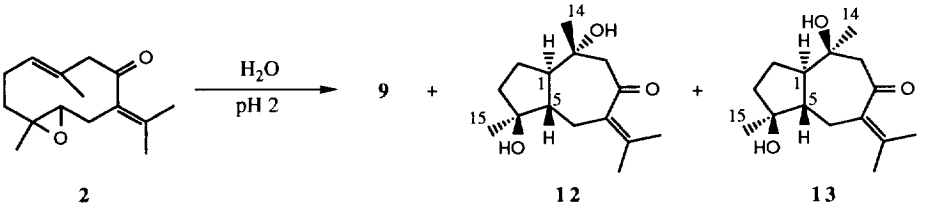
Table 1: ΔH_f values of **8** - **11**.



9 = Δ 1,10
10 = Δ 9,10
11 = Δ 10,14

This directing effect is known as 'the aromatic hypothesis' [19]. When we extrapolate this model for squalene cyclase to the germacrane cyclase from chicory roots, the π-systems of an aromatic side chain residue could interact with the positive charge at C-10 as present in **I**. This interaction might be responsible for the formation of neoprocurcumenol (**9**). There are two possible explanations for the product outcome. Firstly, the interaction can reduce the charge density at C-10 in intermediate **I** which will reduce the dipolar character of **I**. This will lead to reduced bridging between C-5 and C-10 necessary for homofragmentation reactions. Secondly, the interaction will distort the ideal W-like conformation. Even small deviations from this conformation influence the rate in which homofragmentation reactions occur[20]. This distortion gives way to other reactions of which selective deprotonation towards the thermodynamically most stable alkene is favoured.

Chemically induced cyclisation of **2** in an anhydrous medium gave mixtures of the three guaiane double bond isomers neoprocurcumenol (**9**), procurcumenol (**10**) and isoprocurcumenol (**11**). The stereochemistry of their ring fusion has recently been established as *trans* by means of NOE and X-ray crystallography[10]. Several groups have also reported the isolation of *cis*-fused guaianes originating from **2**, but no solid spectral or crystallographic evidence was presented[5,21,22]. Acid catalysed cyclisation of **2** in an aqueous medium gave **9** and two guaiane diols **12** and **13**. The ^{13}C-NMR spectra of **12** and **13** were identical to those of zedoarondiol and isozedoarondiol, respectively.



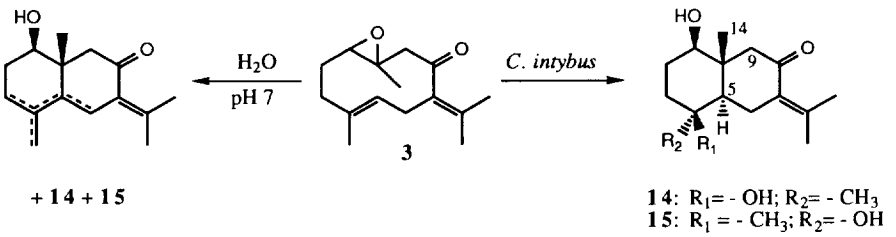
2 **12** **13**
C-14: δ 20.52 C-14: δ 32.15

Scheme 3: Aqueous cyclisation of **2** into **9**, **12** and **13**.

The ring fusion of **12** has unambiguously been established as *trans* by means of X-ray analysis[22]. According to Kuroyanagi *et al.*, isozedoarondiol (**13**) is a *cis* fused guaiane with Me-14 and Me-15 in an *anti* relationship towards the bridgehead protons, H-1 and H-5. In this relationship, the distance between the protons at Me-14 and Me-15 is calculated to be approximately 2.12 Å. This means that a NOE between Me-14 and Me-15 must be present. However when Me-14 (δ 1.04) was irradiated, no NOE with Me-15 (δ 1.23) was observed. In fact, a

clear NOE was present between Me-14 and H-1 (δ 2.64) indicating Me-14 to be in a *syn* relationship towards H-1. No NOE was observed between H-1 and H-5, revealing the *trans* fused ring structure. The *syn* relationship between H-1 and Me-14 was further ascertained from the ^{13}C-NMR chemical shift of C-14. Posner *et al.* reported a significant downfield shift for C-14 in guaianes possessing a *syn* relationship between H-1 and C-14 compared to their *anti* counterparts[23]. The C-14 of isozedoarondiol (**13**) in the ^{13}C-NMR spectrum resonates at δ 32.15. Compared to zedoarondiol (**12**), an 11.6 ppm downfield shift was observed for C-14 (δ 20.52). We therefore come to the conclusion that isozedoarondiol (**13**) possesses a *trans* fused guaiane skeleton and is, in fact, a C-10 epimer of zedoarondiol (**12**) as shown in scheme 3.

The minor epoxide formed upon treatment of germacrone (**1**) with one equivalent of MCPBA is germacrone-1,10-epoxide (**3**). When **3** was administered to a root suspension of mortared chicory and incubated for 8 days, a 1 : 2 mixture of epimeric diols **14** and **15** was obtained. Both controls gave a complex mixture of dehydrated products (total 52%) and both diols **14** (14%) and **15** (34%). The products could be separated using silica gel chromatography. Separation of the dehydrated products was unsuccessful but judging from the ^{1}H-NMR the mixture consisted of the α-, β- and γ-alkene as well as the conjugated isomer in a 1 : 1 : 2 : 1 ratio.



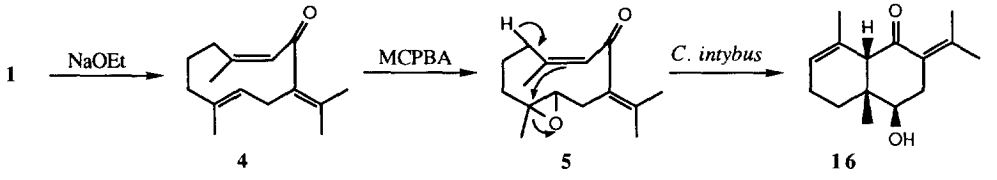
Scheme 4: Spontaneous and biotransformation of **3**.

Sakamoto *et al.*[5] reported the biotransformation of **3** by suspension cultured cells of *Curcuma zedoaria* into a *cis* and a *trans* fused eudesmane diol proposing that cyclisation had occurred through different germacrane conformers as postulated by Parker and Roberts[24]. However, NOE-experiments on both diols **14** and **15** clearly indicated a *trans* fusion of both decalin ring systems. Irradiation of **15** at Me-15 (δ 1.05) gave a positive NOE effect with Me-14 (δ 0.74). When Me-14 was irradiated a clear NOE was present between Me-14 and Me-15 and the equatorial H-9 proton (δ 2.46). A NOE between Me-14 and Me-15 in the spectrum of **14** was absent. In fact, a clear NOE was present between Me-15 (δ 1.25), H-6 (δ 2.70) and the H-5 bridgehead proton (δ 1.46) indicating the methyl group at C-4 to be in an equatorial position. Irradiation of Me-14 (δ 1.04) gave a positive NOE with the equatorial H-9 proton (δ 2.58). Our ^{1}H- and ^{13}C-NMR data of **14** are identical to those of the *cis* eudesmane as postulated by Sakamoto *et al.*[5]; hence, in our opinion their proposed structure of **14** has to be revised.

The 1,10-germacrone epoxide (**3**) appears to follow the same cyclisation pathway as the (E,E)-1,5-germacrenes. In our preceding paper[2] we have demonstrated that (E,E)-1,5-germacrenes cyclise through their *all-chair* conformation into eudesmanes with a *trans* fused ring structure. However, the cyclisation process of germacrone-1,10-epoxide (**3**) does not appear to be as concerted as that of the (E,E)-1,5-germacrenes. The

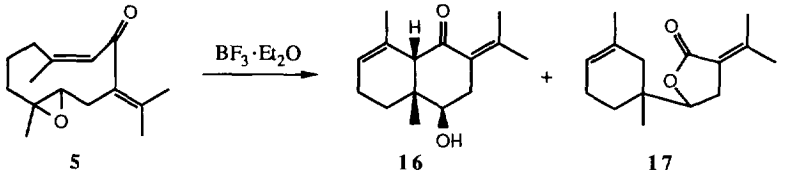
incorporation of a water molecule during cyclisation of (E,E)-1,5-germacrenes, initiated by chicory root enzymes, takes places from a *pseudo*-equatorial direction resulting in only one relative configuration at C-4. Cyclisation of **3**, on the other hand, does not show such a neat incorporation but a random uptake of a water molecule from the surrounding medium.

Isogermacrone (**4**), an (E,Z)-4,9-germacrene[25,26], was prepared by base-catalysed isomerisation of germacrone (**1**). Isogermacrone-4,5-epoxide (**5**) is the sole product formed upon treatment of **4** with one equivalent of MCPBA in the presence of solid Na_2CO_3. When **5** was administered to a suspension of freshly mortared chicory roots and incubated for 3 days, a *cis* fused eudesmane (**16**) was obtained. Both controls showed a 50% conversion into the same product. The ^1H- and ^{13}C-NMR data were identical to those reported in the literature[26].



Scheme 5: Synthesis and (bio)transformation of **5**.

The ring fusion of eudesmane **16** has unambiguously been established as *cis* by means of X-ray crystallography[26]. Chemically induced cyclisation of **5** using $BF_3{\cdot}Et_2O$ gave **16** and the lactone **17**[27] in 86% and 14% respectively. No lactone formation was observed in the enzymatic transformation and either controls.



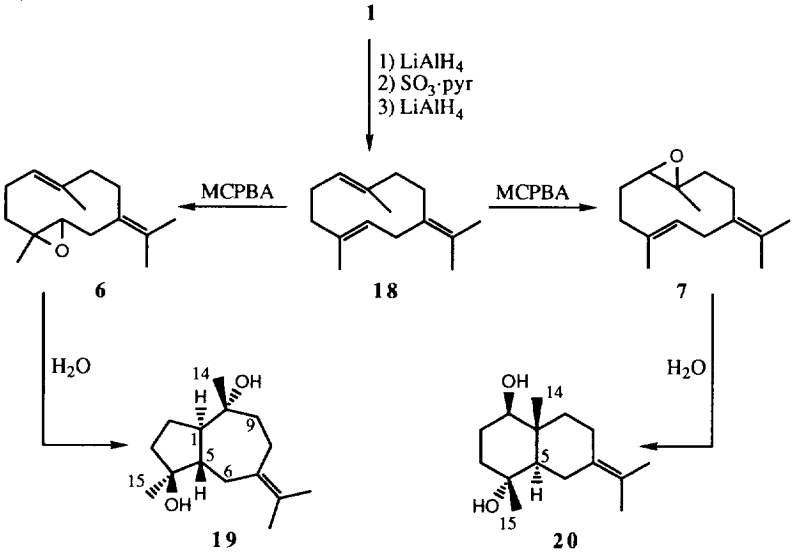
Scheme 6: Chemical cyclisation of **5** into **16** and **17**.

The non-enzymatic transformation of **5** into **16** reveals the instability of the epoxide towards the incubation medium. The catalytic role of the chicory root enzymes seems to be rather limited in this reaction, since they increase the rate of cyclisation only by a factor of 2. This very poor catalytic activity may suggest that epoxides derived from (E,Z)-4,9-cyclodecadienes are probably no intermediates in the biosynthesis of the bitter compounds in chicory. Also, no *cis*-fused eudesmanes have ever been reported as secondary metabolites in *C. intybus* or other members of the *Compositae* family.

Germacrene B (**18**), a naturally occurring and relatively stable germacrene, was obtained in two reductive steps from germacrone (**1**)[28-30]. Homogenous epoxidation using one equivalent of MCPBA led to over-epoxidised products, starting material and only minute amounts of mono-epoxides. Using a two-phase-system[31], the

desired mono-epoxides could be obtained in moderate yield. Column chromatography gave the 4,5-epoxide (**6**) and the 1,10-epoxide (**7**) as an inseparable mixture. Contrary to the work of Brown *et al.*, **7** did not disintegrate during chromatographic workup procedures[32]. Also, **6** could not be crystallised from the mono-epoxide mixture as reported. Consequently the mono-epoxide mixture was used for our biosynthetic studies. Unfortunately, **6** and **7** cyclise spontaneously under the incubation conditions. After 1 day, the chicory root suspension as well as both controls showed complete conversion. The 4,5-epoxide **6** was transformed into the guaiane-diol **19** and the 1,10-epoxide **7** into the eudesmane-diol **20**.

Both products have a *trans* fused ring system as was clearly established by means of 2D-NMR experiments. The position of the bridgehead protons H-1 and H-5 in **19** were ascertained by ^{1}H-^{13}C-coupled NMR to be δ 2.89 and δ 1.85 respectively (C_6D_6). Irradiation of **19** at Me-14 (δ 1.28) displayed a NOE with H-9 (δ 2.78) and H-5 (δ 1.85).



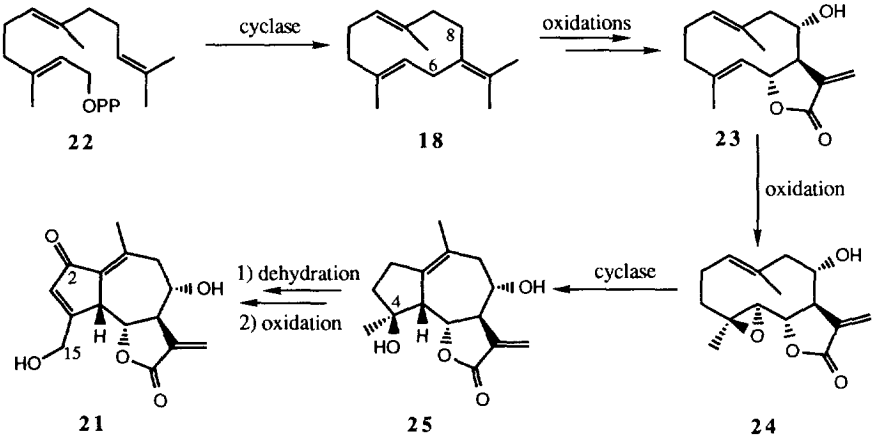
Scheme 7: Synthesis and cyclisation of germacrene B mono-epoxides **6** and **7**.

Irradiation at Me-15 (δ 1.33) gave a very clear NOE with H-6 (δ 2.37) but not with H-1. The lack of a NOE between Me-15 and H-1 does not implicate that the ring-fusion of **19** is *cis*, but it indicates that the distance between Me-15 and H-1 is to big to display a NOE. This was confirmed by semi-empirical calculations on **19** where the distance between the protons at Me-15 and H-1 was calculated to be 3.64 Å. No NOE was observed between H-1 and H-5. Irradiation of **20** at Me-14 (δ 0.96) gave a NOE with Me-15 (δ 1.12) and *vice versa*. Also, no NOE was observed between Me-15 and H-5 ascertaining the *trans* fusion of the decalin system of **20**.

The mode of the chemically induced cyclisation of the germacrone epoxides **2** and **3** is profoundly influenced by the carbonyl function at C-8 as compared to the cyclisation of the germacrene B epoxides **6** and **7**. This influence is best illustrated by the formation of the homofragmentation product **8** or a mixture of the epimeric guaiane diols **12** and **13** from **2**, whereas only one cyclisation product was obtained from **6**. The nature of the

directing effect of the carbonyl at C-8 on homofragmentation reactions in systems like **I** (Scheme 2) is not yet fully understood and is currently under investigation in our laboratory. The observation that **3** cyclises into two epimeric diols **14** and **15** whereas **7** gave only one product (**20**) cannot be ascribed to any electronic influence of the carbonyl function in **3**. Probably, the introduction of a sp^2 centre at C-8 influences the configuration of the cationic centre at C-4 in such an extend that water incorporation can occur from either side of the molecule.

In this paper and in our earlier work[1,2] we have shown that chicory root-mediated cyclisations of (E,E)-1,5-germacrenes and germacrane-1,10-epoxides, derived from (E,E)-1,5-germacrenes, give eudesmanes, whereas germacrane 4,5-epoxides derived from (E,E)-1,5-germacranes lead to guaianes. Since the large majority of the bitter principles in chicory possess the guaiane skeleton[14], it is quite likely that germacrane-4,5-epoxides play a crucial role in their biogenesis. A tentative route for the biosynthesis of lactucin (**21**), one of the major bitter principles in chicory, is shown in scheme 8.



Scheme 8. Proposed biosynthesis of lactucin in chicory roots.

Starting from farnesyl pyrophosphate (**22**), the action of a cyclase will first lead to germacrene B[33] (**18**). Oxidation of germacrene B in the side chain and the ring would lead to the lactone-alcohol balchanolide (**23**). Stereoselective epoxidation of the 4,5-double bond in **23** gives an intermediate epoxide **24** in which the essential groups are correctly placed for the cyclisation to guaiane **25**. Note that the product obtained after selective enzyme-mediated deprotonation of the guaiane intermediate **I** in scheme 2 has its double bond in the same position as lactucin (**21**). Elimination of the hydroxyl group at C-4 in **25** followed by hydroxylation on C-15 and oxidation on C-2 would give lactucin (**21**). The question whether formation of the lactone ring will occur before or after the hydroxylation on C-8 is unclear, although it is quite likely that hydroxylation will occur first because both C-6 and C-8 in germacrene B are allylic and, therefore, relatively reactive. Up to now, we have not observed any stereoselectivity in the cyclization reactions. Presumably, chirality is induced by stereoselective hydroxylating and epoxidizing enzymes. The role of the cyclases in chicory seems to be the initiation of the cyclization reaction and the selective deprotonation of the bicyclic intermediate.

Experimental

The same solvents for spectral measurements and column chromatography were used as described previously[2]. GC-MS conditions were identical to those previously reported[2]. A suspension of fresh chicory root (20% w/v) was produced by mortaring the peeled 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-soln (STMB) was set at 7.0 using 2-morpholino-ethanesulfonic acid (MES). The stability of the epoxides towards the buffer and an inactivated chicory root sample (obtained by boiling the suspension for 30 min) were investigated as a control to test the occurrence 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 of root suspension, 790 µl of STMB-solution and 10 µl of 0.1 M substrate in EtOH. Epoxide incubation times: **2**, **3**: 8 days; **5**: 3 days; **6**, **7**: 1 day. The incubation media were extracted with EtOAc and analysed on GC-MS. Chiral analytical HPLC was performed on a Varian 5000 HPLC equipped with an HPLC-chiral II ET 250/8/4 NUCLEOSIL® with 5% of *iso*-PrOH in hexane as the solvent system and a Spectraflow 773 absorbance detector set at 254 nm.

Germacrone (**1**): Isolated from the natural oil of *Geranium macrorrhizum* [34]. mp. 55-56 °C. ^{1}H- and ^{13}C-NMR data were identical to those reported in the literature[25]. Mass spectrum (m/e): 41 (55), 67 (70), 91 (40), 107 (100), 135 (64), 136 (55), 175 (23), 218 (14); Calc. for (M^+): 218.1670; Found 218.1671.

Epoxidation of Germacrone (**1**): To a stirred solution of 1.02 g of **1** (4.68 mmol) in 40 ml of CH_2Cl_2, cooled to 0 °C, 1.04 g of MCPBA (85-90%) was added and the reaction was stirred for 30 minutes. The reaction mixture was washed with 2 x 50 ml of sat. $NaHCO_3$ solution and the combined aqueous layers were extracted with 50 ml of CH_2Cl_2, the combined organic layers dried were on $MgSO_4$ and evaporated *in vacuo* to yield 1.08 g of a white solid. The epoxides were purified by column chromatography (Al_2O_3; 10% EtOAc in petroleum ether) to give 657 mg of **2** (mp 79-79.5 °C) and 116 mg of **3** (mp 63-64.5 °C). The ^{1}H- and ^{13}C-NMR were identical to those reported in the literature[9,28]. Mass: Calc. for **2** (M^+): 234.1620; Found: 234.1619; Calc. for **3** (M^+): 234.1620; Found: 234.1619.

Cyclisation reactions of **2**: A solution of 155 mg of **2** in 4 ml of EtOH was added to 75 ml of water buffered at pH 2.0 and shaken for 24h at room temperature. The reaction mixture was extracted with 2 x 25 ml of CH_2Cl_2 and 2 x 25 ml of EtOAc, the combined organic layers were dried on $MgSO_4$ and evaporated *in vacuo* to give 100 mg of a yellow oil. Column chromatography (Al_2O_3; EtOAc / petroleum ether 2 : 1) gave 30 mg of neoprocurcumenol (**9**), 32 mg of zedoarondiol (**12**) and 10 mg of isozedoarondiol (**13**). The ^{1}H- and ^{13}C-NMR of **9** and the ^{13}C-NMR of **12** and **13** were identical to those reported in the literature[9,10,22]. Mass spectrum of **9** (m/e): 43 (100), 67 (51), 68 (47), 77 (29), 79 (27), 91 (38), 93 (25), 105 (51), 121 (58), 163 (53), 173 (15), 176 (14), 177 (15), 201 (10), 219 (8), 234 (38).
^{1}H-NMR of **12** (500 MHz): δ 2.92 (d, 1H, H-9, J = 12.6 Hz); δ 2.78 (d, 1H, H-6, J = 14.7 Hz); δ 2.55 (d, 1H, H-9, J = 12.6 Hz); δ 1.98–1.92 (m, 2H); δ 1.89 (d, 3H, H-12, J = 1.5 Hz); δ 1.79 (d, 3H, H-13, J = 1.0 Hz); δ 1.75-1.63 (m, 4H); δ 1.37 (m, 1H); δ 1.17 (s, 3H, H-15); δ 1.14 (s, 3H, H-14). Mass spectrum of **12** (m/e): 43 (100), 81 (20), 109 (7), 149 (8), 173 (4), 191 (7), 234 (6), 252 (0).

^{1}H-NMR of **13**[35] (500 MHz): δ 3.06 (d, 1H, H-9, *J* = 16.1 Hz); δ 2.64 (dt, 1H, H-1, *J* = 10.0 Hz, *J* = 5.8 Hz); δ 2.34 (d, 1H, H-6, *J* = 14.2 Hz); δ 2.21 (dd, 1H, H-9, *J* = 16.1 Hz, *J* = 1.2 Hz); δ 1.84 (s, 3H, H-12); δ 1.82 (dd, 1H, H-5, *J* = 5.6 Hz, *J* = 1.9 Hz); δ 1.75 (m, 1H, H-2); δ 1.71 (s, 3H, H-13); δ 1.70-1.57 (m, 3H, H-3, H-6); δ 1.36 (m, 1H, H-2); δ 1.23 (s, 3H, H-15); δ 1.04 (s, 3H, H-14). Mass spectrum of **13** (m/e): 43 (100), 81 (9), 121 (9), 149 (8), 173 (5), 174 (5), 191 (10), 234 (5), 252 (0).

A solution of 153 mg of **2** in 4 ml of EtOH was added to 75 ml of water buffered at pH 7 and shaken for 7 days at room temperature. The reaction mixture was extracted with 25 ml of EtOAc and 50 ml of CH_2Cl_2. The combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated *in vacuo* to give 85 mg of a yellow oil. Column chromatography (Al_2O_3; 10% EtOAc in petroleum ether) gave 58 mg of curcumenone (**8**). The ^{1}H- and ^{13}C-NMR of **8** were identical to those reported in the literature[7]. Mass spectrum (m/e): 43 (100), 67 (44), 68 (54), 77 (18), 79 (21), 91 (22), 105 (16), 107 (20), 133 (18), 149 (28), 161 (30), 163 (18), 176 (47), 191 (7), 219 (5), 234 (9).

To a stirred solution of 329 mg of **2** in 10 ml of a 1 : 1 benzene / toluene mixture, cooled to 0 °C, was added 24 mg of pTSA. The reaction mixture was stirred for 4h, washed with saturated $NaHCO_3$ solution, the aqueous layer was extracted with 25 ml of EtOAc, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated *in vacuo* to give 330 mg of a dark yellow oil. Silica gel chromatograpy (EtOAc in petroleum ether 1 : 1) gave 163 mg of a 3 : 1 mixture of **9** and isoprocurcumenol (**11**) and 30 mg of procurcumenol (**10**). The ^{1}H- and ^{13}C-NMR of **10** were identical to those reported in the literature[9,10]. Mass spectrum of **10** (m/e): 43 (100), 55 (18), 57 (20), 67 (21), 69 (20), 77 (21), 79 (22), 91 (32), 105 (34), 123 (41), 133 (27), 145 (25), 147 (34), 165 (18), 173 (24), 191 (9), 201 (16), 216 (44), 234 (8). Mass spectrum of **11** (m/e): 43 (100), 67 (55), 77 (30), 79 (39), 105 (72), 107 (44), 119 (23), 121 (57), 131 (16) 133 (27), 145 (29), 158 (30), 173 (19), 191 (22), 234 (9).

Cyclisation reaction of 3: To a solution of 99 mg of **3** in 7.5 ml of acetone and 5 ml of water was added 5 drops of conc. H_2SO_4. After stirring for 75 min at room temperature, solid $NaHCO_3$ solution and 25 ml of CH_2Cl_2 was added. The mixture was extracted with 3 x 25 ml of CH_2Cl_2, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated *in vacuo* to give 97 mg of a colourless oil. Silica gel chromatography (EtOAc) yielded 35 mg of eudesmane double bond isomers, judging from the ^{1}H-NMR to be the α-, β- and γ-alkene as well as the isopropylidene conjugated isomer in a 1 : 1 : 2 : 1 ratio, 10 mg of **14** as a colourless oil and 25 mg of **15** (mp 197-198.5 °C) as a crystalline solid.

^{1}H–NMR of **14**: δ 3.32 (dd, 1H, H-1, *J* = 11.4 Hz, *J* = 4.0 Hz); δ 2.70 (dd, 1H, H-6, *J* = 16.0 Hz, *J* = 5.3 Hz); δ 2.58 (d, 1H, H-9, *J* = 16.0 Hz); δ 2.53 (m, 1H, H-6); δ 2.06 (dd, 1H, H-9, *J* = 15.0 Hz, *J* = 1.1 Hz); δ 2.06 (dd, 3H, H-12, *J* = 2.1 Hz, *J* = 1.3 Hz); δ 1.90-1.55 (m, 5H); δ 1.82 (d, 3H, H-13, *J* = 0.6 Hz); δ 1.46 (dd, 1H, H-5, *J* = 12.8 Hz, *J* = 5.5 Hz); δ 1.30-1.18 (m, 1H); δ 1.23 (d, 3H, H-15, *J* = 1.4 Hz); δ 1.03 (d, 3H, H-14, *J* = 1.0 Hz). ^{13}C-NMR: δ 202.12 (s), δ 146.49 (s), δ 129.80 (s), δ 78.60 (d), δ 70.88 (s), δ 55.29 (s), δ 47.49 (d), δ 40.26 (t), δ 39.59 (t), δ 29.94 (q), δ 26.68 (t), δ 25.80 (t), δ 23.81 (q), δ 23.07 (q), δ 12.61 (q). Mass spectrum (m/e): 39 (34), 41 (63), 43 (47), 55 (32), 67 (47), 79 (35), 83 (31), 91 (26), 105 (21), 115 (15), 119 (27), 133 (25), 147 (31), 148 (27), 163 (17), 173 (31), 201 (7), 219 (10), 234 (100), 252 (0); Calcd for [M^+]: 252.1725; Found: 252.1728. ^{1}H–NMR of **15**: δ 3.32 (m, 1H, H-1, *J* = 11.1 Hz, *J* = 4.0 Hz); δ 2.86 (dd, 1H, H-6, *J* = 15.4 Hz, *J* = 4.3 Hz); δ 2.56 (bs, 2H, –OH); δ 2.47 (d, 1H, H-9, *J* = 15.1 Hz); δ 2.10 (m, 1H, H-6); δ 1.97 (dd, 1H, H-9, *J* = 15.1 Hz, *J* = 1.0 Hz); δ 1.85 (d, 3H, H-12, *J* = 2.0 Hz); δ 1.69 (d, 3H, H-

13, J = 1.1 Hz); δ 1.65–1.35 (m, 5H); δ 1.04 (s, 3H, H-15); δ 0.75 (s, 3H, H-14). ^{13}C-NMR: δ 202.72 (s), δ 143.47 (s), δ 130.70 (s), δ 77.62 (d), δ 70.91 (s), δ 56.73 (s), δ 50.34 (d), δ 40.99 (t), δ 40.72 (t), δ 28.56 (t), δ 25.76 (t), δ 23.05 (q), δ 24.39 (q), δ 24.25 (q), δ 12.49 (q). Mass spectrum (m/e): 39 (47), 41 (100), 43 (58), 53 (40), 55 (36), 67 (42), 69 (31), 77 (40), 79 (35), 81 (22), 83 (19), 91 (36), 93 (26), 95 (20), 105 (26), 106 (18), 107 (33), 109 (27), 119 (29), 121 (56), 136 (34), 163 (35), 201 (8), 219 (5), 234 (98), 252 (0). Calcd for [M^+]: 252.1725; Found: 252.1728.

Isogermacrone (**4**): To a solution of 2.30 g of sodium in 100 ml of EtOH, cooled to 0 °C, was added dropwise 4.00 g of **1** in 30 ml of EtOH. After stirring for 3 days at room temperature, 2 ml of water was added and the solvent was evaporated. The residue was taken up in 50 ml of ether, washed with 50 ml of water, the combined aqueous layers were extracted with 2 x 50 ml of ether, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated *in vacuo* to give 3.65 g of **4** (mp 52-53.5 °C). The ^{1}H- and ^{13}C-NMR of **4** were identical to that reported in the literature[26]. Mass spectrum of **4** (m/e): 41 (86), 53 (41), 67 (66), 68 (100), 77 (33), 79 (37), 81 (28), 91 (42), 93 (34), 96 (34), 109 (32), 121 (25), 147 (16), 161 (11), 175 (9), 203 (15), 218 (17).

Isogermacrone-4,5-epoxide (**5**): To a solution of 3.65 g of **4** and 5 g of solid Na_2CO_3 in 30 ml of CH_2Cl_2 was carefully added 3.80 g of MCPBA. The mixture was stirred for 45 min at room temperature, 50 ml of water was added, the organic layer was separated, the aqueous layer was extracted with 2 x 50 ml of CH_2Cl_2, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated *in vacuo* to yield 3.84 g of **5** (mp 67-69.5 °C). The ^{1}H-NMR of **5** was identical to that reported in the literature[25]. ^{13}C–NMR: δ 202.72 (s), δ 152.74 (s), δ 133.23 (s), δ 130.50 (d), δ 130.46 (s), δ 64.14 (d), δ 60.07 (s), δ 36.76 (t), δ 28.83 (t), δ 28.35 (t), δ 23.53 (q), δ 21.94 (t), δ 21.47 (q), δ 19.81 (q), δ 15.92 (q). Mass spectrum (m/e): 39 (55), 41 (88), 43 (100), 53 (33), 55 (33), 67 (53), 79 (37), 95 (34), 105 (21), 107 (19), 109 (22), 121 (26), 149 (23), 161 (9), 163 (10), 191 (6), 219 (5), 234 (7).

Cyclisation of **5**: To a solution of 304 mg of **5** in 10 ml of dry ether, cooled to 0 °C, was added 0.8 ml of BF_3·Et_2O. The reddish mixture was stirred for 1h at 0 °C, 10 ml of water was added, the organic layer was separated, the aqueous layer was extracted with 20 ml of ether, the combined organic layers were washed with brine, dried on $MgSO_4$ and evaporated *in vacuo* to give 265 mg of a red oil. Silica gel chromatography (EtOAc / petroleum ether 1 : 2) gave 12 mg of **17** and 135 mg of **16** (mp 97-99 °C). The ^{1}H- and ^{13}C-NMR of **16** and **17** were identical to those reported in the literature[25]. Mass spectrum of **16** (m/e): 41 (55), 67 (48), 91 (35), 93 (100), 109 (56), 121 (91), 125 (24), 173 (5), 234 (4); Mass spectrum of **17** (m/e): 41 (45), 67 (43), 79 (48), 93 (76), 109 (35), 121 (100), 219 (5), 234 (6).

Germacrene B (**18**): To a solution of 5.08 g of **1** in 60 ml of dry ether, cooled to 0 °C, was carefully added 1.5 g of solid $LiAlH_4$. The grey suspension was stirred for 3h, Glauber's salt was added in small portions and the resulting mixture was stirred for 30 minutes. After addition of $MgSO_4$ the mixture was stirred for an additional 30 minutes, the solids were filtered off and the solvent was evaporated *in vacuo* to give 5.01 g of a colourless oil. Due to severe coalescence, both ^{1}H- and ^{13}C-NMR of germacrol were uninterpretable.

To a solution of 1.99 g of germacrol in 50 ml of dry ether was added 2.14 g of SO_3·pyridine complex and the resulting white suspension was stirred for 4h at room temperature. After cooling the suspension to –15 °C, 1.0 g of solid $LiAlH_4$ was carefully added in small portions. The reaction mixture was stirred for 2h and Glauber's salt was added in small portions. After an additional 30 minutes of stirring $MgSO_4$ was added and the solids were filtered off. The solvent was evaporated *in vacuo* to give 1.65 g of a yellow oil. Silica gel chromatography (petroleum ether) yielded 875 mg of **18** as a colourless oil. ^{1}H-NMR: δ 4.71 (bd, 1H, H-1, J = 12.4 Hz); δ 4.55 (bd, 1H, H-5, J = 10.5 Hz); δ 2.91 (bd, 1H, H-6, J = 13.1 Hz); δ 2.49 (m, 2H); δ 2.35–1.85 (m, 7H); δ 1.70, 1.68 (2s, 2 x 3H, H-12, H-13); δ 1.53, 1.50 (2s, 2 x 3H, H-14, H-15). ^{13}C-NMR: δ 137.07 (s), δ 133.19 (s), δ 131.63 (s), δ 128.15 (d), δ 126.31 (d), δ 124.47 (s), δ 40.42 (t), δ 38.83 (t), δ 33.53 (t), δ 32.46 (t), δ 25.74 (t), δ 20.71 (q), δ 20.40 (q), δ 17.02 (q), δ 16.02 (q). Mass spectrum (m/e): 41 (100), 53 (57), 55 (46), 67 (73), 79 (51), 81 (50), 91 (65), 93 (85), 121 (94), 133 (30), 147 (18), 161 (27), 189 (13), 204 (16).

Epoxidation of Germacrene B (**18**): To a solution of 2.29 g of **18** in 40 ml of CH_2Cl_2, 40 ml of water and 15 g of solid $NaHCO_3$, cooled to –10 °C, was added in small portions 1.95 g of MCPBA. The resulting suspension was stirred for 30 minutes at –10 °C. The organic layer was separated, washed with 4 x 25 ml of saturated $NaHCO_3$ solution, the combined aqueous layers were extracted with 2 x 50 ml of CH_2Cl_2, the combined organic layers were dried on $MgSO_4$ and evaporated *in vacuo* to give 2.32 g of a yellow viscous oil. Column chromatography (Al_2O_3, 5% of EtOAc in petroleum ether) yielded 735 mg of a mixture of **6** and **7** as a white solid. Extensive chromatography gave an analytical sample of **6**. ^{1}H-NMR: δ 4.99 (dd, 1H, H-1, J = 10.5 Hz, J = 5.7 Hz); δ 2.69 (bd, 1H, H-6, J = 14.1 Hz); δ 2.53 (dd, 1H, H-5, J = 9.3 Hz, J = 1.3 Hz); δ 2.51 (m, 1H); δ 2.30–1.85 (m, 8H); δ 1.71, 1.70, 1.67 (3s, 3 x 3H, H-12, H-13, H-14); δ 1.17 (s, 3H, H-15). ^{13}C-NMR: δ 135.33 (s), δ 129.83 (s), δ 129.24 (s), δ 124.27 (d), δ 66.35 (d), δ 61.19 (s), δ 40.28 (t), δ 37.49 (t), δ 36.24 (t), δ 36.12 (t), δ 23.93 (t), δ 20.77 (q), δ 20.69 (q), δ 17.18 (q), δ 16.56 (q).

Cyclisation of **6** *and* **7**: A solution of 100 mg of the mono-epoxide mixture in 1.5 ml of EtOH was added to 75 ml of water buffered at pH 7.0 and shaken for 24h at room temperature. The reaction mixture was extracted with 2 x 50 ml of CH_2Cl_2, the combined organic layers were dried on $MgSO_4$ and evaporated *in vacuo* to give 89 mg of a colourless oil. Column chromatography (Al_2O_3; EtOAc / petroleum ether 2 : 1) gave 13 mg of eudesmanediol **20** (mp 155-157 °C) and 26 mg of guaianediol **19** (mp 93-94.5 °C).
^{1}H-NMR of **19** (500 MHz; C_6D_6): δ 2.89 (dt, 1H, H-1, J = 10.0 Hz, J = 6.2 Hz); δ 2.78 (m, 1H, H-9, $W_{1/2}$ = 45.6 Hz); δ 2.41 (bs, 1H, –OH); δ 2.37 (dd, 1H, H-6, J = 13.2 Hz, J = 2.4 Hz); δ 2.28 (bs, 1H, –OH); δ 1.96-1.90 (m, 2H, H-8, H-9); δ 1.86-1.82 (m, 2H, H-3, H-5); δ 1.74 (m, 1H, H-3); δ 1.71 (s, 3H, H-12 or H-13); δ 1.69 (s, 3H, H-13 or H-12); δ 1.58 (ddd, 1H, H-8, J = 14.1 Hz, J = 12.6 Hz, J = 5.5 Hz); δ 1.51 (m, 1H, H-2); δ 1.38 (m, 1H, H-2); δ 1.37 (bd, 1H, H-6, J = 12.3 Hz); δ 1.33 (s, 3H, H-15); δ 1.28 (s, 3H, H-14). ^{13}C-NMR ($CDCl_3$): δ 131.22 (s), δ 126.02 (s), δ 82.36 (s), δ 74.69 (s), δ 53.34 (d), δ 52.56 (d), δ 37.21 (t), δ 30.82 (q), δ 30.50 (t), δ 28.87 (t), δ 26.77 (t), δ 25.09 (t), δ 24.91 (q), δ 20.16 (q), δ 20.04 (q). Mass spectrum (m/e): 41 (35), 43 (100), 55 (21), 67 (19), 79 (7), 81 (15), 91 (17), 107 (31), 119 (17), 122 (37), 159 (10), 162 (10), 187 (8), 220 (12), 238 (0.3). Calcd for [M^+ –H_2O]: 220.1827; Found: 220.1828.
^{1}H-NMR of **20**: δ 3.26 (d, 1H, H-1, J = 10.5 Hz, J = 4.6 Hz); δ 2.76 (dt, 1H, H-6, J = 13.5 Hz, J = 2.5 Hz); δ 2.53 (ddt, 1H, H-9, J = 13.7 Hz, J = 6.3 Hz, J = 2.1 Hz); δ 1.90–1.55 (m, 7H); δ 1.66 (d, 3H, H-12 or H-13, J = 0.9 Hz); δ 1.63 (s 3H, H-13 or H-12); δ 1.45 (dd, 1H, J = 11.5 Hz, J = 4.1 Hz); δ 1.12 (s, 3H, H-15);

δ 1.08 (m, 1H); δ 0.94 (d, 3H, H-14, J = 0.6 Hz). ^{13}C-NMR: δ 130.34 (s), δ 121.30 (s), δ 79.38 (d), δ 71.65 (s), δ 53.72 (d), δ 41.19 (t), δ 41.03 (t), δ 39.14 (s), δ 28.52 (t), δ 24.88 (t), δ 24.33 (t), δ 22.10 (q), δ 20.00 (q), δ 19.94 (q), δ 12.40 (q). Mass spectrum (m/e): 41 (45), 43 (100), 55 (34), 67 (24), 93 (29), 107 (27), 119 (22), 121 (19), 135 (21), 159 (20), 163 (17), 176 (21), 187 (16), 203 (14), 220 (9), 238 (24). Calcd for [M^+]; 238.1933; Found 238.1934.

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35. DMSO-d_6 and CD_3OD were added to enhance solubility.

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