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Germacrenes from fresh costus roots

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Abstract

Four germacrenes, previously shown to be intermediates in sesquiterpene lactone biosynthesis, were isolated from fresh costus roots (*Saussurea lappa*). The structures of (+)-germacrene A, germacra-1(10),4,11(13)-trien-12-ol, germacra-1(10),4,11(13)-trien-12-al, and germacra-1(10),4,11(13)-trien-12-oic acid were deduced by a combination of spectral data and chemical transformations. Heating of these compounds yields (−)-β-elemene, (−)-elema-1,3,11(13)-trien-12-ol, (−)-elema-1,3,11(13)-trien-12-al, and elema-1,3,11(13)-trien-12-oic acid respectively, in addition to small amounts of their diastereomers. Acid induced cyclisation of the germacrenes yields selinene, costol, costal, and costic acid respectively. It is highly probable that the elemenes reported in literature for costus root oil are artefacts.

Keywords: *Saussurea lappa*; Asteraceae; Sesquiterpenes; Sesquiterpene lactone biosynthesis; Germacrenes; Elemenes; Eudesmanes

1. Introduction

Saussurea lappa Clarke, is a member of the Asteraceae indigenous to parts of India and Pakistan where it grows in the Himalayas at 2500–3500 m elevation. The dried 4–5 year old roots of this plant are known as costus roots and have a reputation for their medicinal properties as well as their fragrance. Attempts are being made to cultivate this plant, since it has become an endangered species due to ruthless collection from forest areas (Paul et al., 1960; Hatakeyama et al., 1989; Ayaz, 1996).

Various studies have shown that costus roots are extremely rich in sesquiterpene lactones (3% of fresh weight), of which the most important are dehydrocostus lactone and (+)-costunolide (**5**) (Paul et al., 1960; Somasekar Rao et al., 1960). The latter is the structurally simplest sesquiterpene lactone and is generally accepted as the common intermediate in the biosynthesis of most sesquiterpene lactones (Herz, 1977; Fischer, 1990). Costunolide (**5**) is presumably formed from farnesyl diphosphate (FPP) via (+)-germacrene A (**1**) (Fig. 1). Enzyme activities that introduce a carboxylic function in the isopropenyl side chain of (+)-germacrene A yielding germacra-1(10),4,11(13)-trien-12-oic acid (**4**) have recently been identified in chicory (*Cichorium intybus* L.), a vegetable rich in sesquiterpene lactones (de Kraker et al., 1998, 2001). Currently, investigations concerning the pathway for sesquiterpene lactones in chicory are hampered by the lack of a source of germacrene intermediates **1–4**, in particular germacrene acid (**4**). These germacrene compounds are themselves not present in chicory (Sannai et al., 1982), and to our knowledge only the isolation of (+)-germacrene A (**1**) from various plant species (e.g. Wichtman and Stahl-Biskup, 1987) and the partial purification of germacra-1(10),4,11(13)-trien-12-al (**3**) from *Vernonia glabra* (Steetz) (Bohlmann et al., 1983) have been described. A reason for the scarcity of reports on these germacrenes might be their instability. They are susceptible to proton-induced cyclisations and to heat induced (e.g. steam distillation, GC-analysis) Cope rearrangement yielding the eudesmane or elemene framework, respectively (Fig. 2) (Southwell, 1970; Takeda, 1974; Wichtman and Stahl-Biskup, 1987; Teisseire, 1994; de Kraker et al., 1998, 2001).

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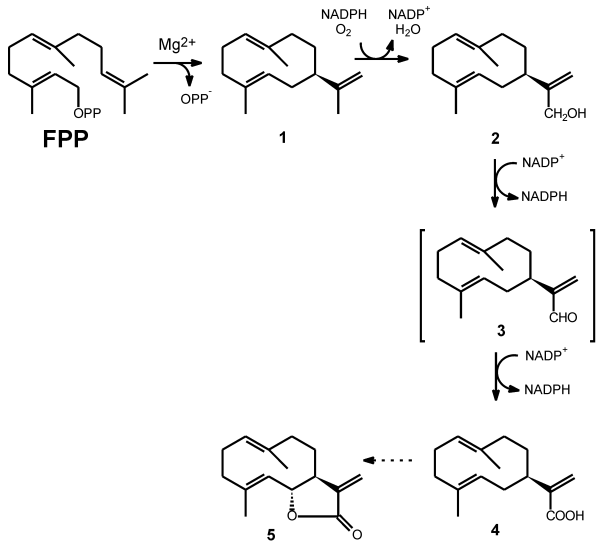

Fig. 1. Biosynthetic route from FPP to (+)-costunolide (**5**) via (+)-germacrene A (**1**), germacra-1(10),4,11(13)-trien-12-ol (**2**), germacra-1(10),4,11(13)-trien-12-al (**3**), and germacra-1(10),4,11(13)-trien-12-oic acid (**4**). Only the last step (dotted arrow) has not yet been demonstrated.

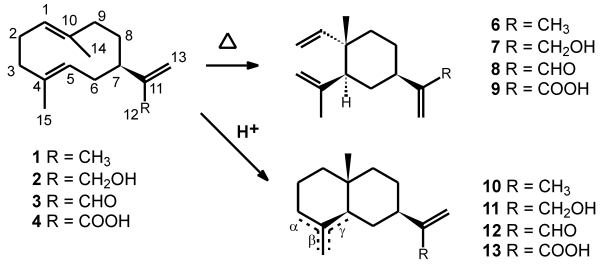

Fig. 2. Heat induced Cope-rearrangement of **1–4** yields (−)-β-elemene (**6**), (−)-elema-1,3,11(13)-trien-12-ol (**7**), (−)-elema-1,3,11(13)-trien-12-al (**8**), and elema-1,3,11(13)-trien-12-oic acid (**9**); acidic conditions are expected to give selinene (**10**), costol (**11**), costal (**12**), and costic acid (**13**).

The presence of β-elemene (**6**), (−)-elema-1,3,11(13)-trien-12-ol (**7**), and (−)-elema-1,3,11(13)-trien-12-al (**8**) in commercially available costus root oil has drawn our attention to these roots (Klein and Thömel, 1976; Maurer and Grieder, 1977). Since this oil is obtained by steam distillation and elemenes are generally considered to be artefacts, we assumed that costus roots originally contain the corresponding germacrenes **1–3**. The reported isolation of costic acid (**13**) from costus roots also suggests the presence of germacrene acid (**4**) (Bawdekar and Kelkar, 1965), since HCl was used in the isolation procedure which might have initiated the cyclisation of germacrene acid.

The aim of the present research was to investigate the presence of the germacrenes **1–4** in fresh costus roots, and to develop a mild isolation procedure for these compounds so that they can be used for further studies on sesquiterpene lactone biosynthesis.

2. Results and discussion

Et_2O extracts of commercially available dried costus roots, costus resinoid and costus root oil were screened on GC–MS for the presence of germacrene A (**1**). This germacrene can be measured as a well resolved peak at a GC-injection port temperature of 150 °C. The more polar germacrenes **2–4** cannot be measured as a real peak, because they will rearrange during migration through the GC-column to their faster migrating elemenes and will be observed as a broad "hump" in the baseline such as described for hedycaryol and 7-hydroxygermacrene (Stahl, 1984; de Kraker et al., 1998, 2001). Yet, in all of these extracts we would detect only the Cope-rearrangement product β-elemene (**6**). It was concluded that the germacrene A originally present in these materials had been exposed to heat and that the commercially available materials are not suitable for the isolation of germacrenes. Interestingly, in the resinoid and dried roots the previously unreported elema-1,3,11(13)-trien-12-oic acid (**9**) was detected in addition to (−)-elema-1,3,11(13)-trien-12-ol (**7**) and (−)-elema-1,3,11(13)-trien-12-al (**8**).

In contrast, the Et_2O extract of fresh costus roots did contain germacrene A (**1**), whereas no (−)-β-elemene (**6**) was observed. At the applied GC-injection port temperature of 150 °C, also the foreseen "broad humps" of the oxygenated germacrenes **2–4** were visible in the GC-chromatogram instead of sharp peaks of the oxygenated elemenes **7–9**.

In order to isolate germacrenes **1–4**, 300 g of fresh costus roots was powdered in liquid N_2 and extracted with Et_2O. The organic extract was shaken with aq Na_2CO_3 to remove germacrene acid (**4**) and other acidic/polar compounds; the lactones (mainly costunolide and dehydrocostus lactone, 1.5 g) were partially removed by crystallisation from pentane (Somasekar Rao et al., 1960). Germacrenes **1–3** could be selectively extracted from pentane with aq $AgNO_3$ (Southwell, 1970; Minnaard, 1997), because of the complex-formation between the Ag^+-ions and the diene-ring system present in the germacrenes. Subsesquent flash column chromatography of the obtained crude germacrene-mixture in combination with a second $AgNO_3$ extraction yielded: 1.5 mg germacrene A (**1**), 1.5 mg germacrene aldehyde (**3**), and a mixture of germacrene alcohol (**2**) with β-costol (**11**), hedycaryol, costunolide (**5**), and dehydrocostus lactone. The isolated germacrene A contained a small impurity of 4% (determined by GC–MS) humulene that is extracted with aq $AgNO_3$ as well due to the presence of a cylic diene moiety. Purification of the germacrene alcohol (**2**) was more elaborate because β-costol (**10**) with its two adjacent exocyclic bond is also extracted with aq $AgNO_3$, whereas α- and γ-costol are not. Furthermore germacrene alcohol, in our hands, could hardly be separated from costunolide on silica gel. Argentation chromatography yielded 3 mg of germacrene alcohol (**2**)

that was, however, unlike the isolated germacrenes **1**, **3** and **4**, contaminated with elematrien-12-ol (**7**) (approx. 30%).

The germacrene acid (**4**) containing alkaline (Na_2CO_3) extracts were carefully brought to pH 6 and extracted with Et_2O. As expected from the protocol of Bawdekar and Kelkar (1965), the sesquiterpenoid acids did not dissolve in aq $NaHCO_3$ and acidification to a lower pH was not necessary, thus preventing the undesired cyclisation of germacrene acid to costic acid (**13**). Extraction with aq $AgNO_3$ and column chromatography yielded 1.5 mg of germacrene acid (**4**).

GC-analyses of germacrene aldehyde (**3**) at a GC-injection port temperature of 150 °C results in a "broad hump" is (Fig. 3 A) that displays the mass spectrum of elematrien-12-al (**8**) over its entire range. At a GC-injection port temperature of 250 °C a sharp peak of elematrien-12-al is visible instead (Fig. 3B), because the germacrene aldehyde is immediately rearranged in the GC-injection port and not during its migration through the GC-column (de Kraker et al., 2001). In the presence of *p*-toluenesulfonic acid the germacrene aldehyde is completely cyclised to costal (**12**) (Fig. 3C). This indicates that the sample is free of elemene aldehyde, because the elemene aldehyde does not cyclise to costal. ^{1}H NMR of the germacrene aldehyde confirmed that it did not contain any of its Cope rearrangement product, since a double doublet at δ 5.85 of $C_2{=}C_1H{-}C_{10}$ typical for the elemene aldehyde was clearly missing. Unfortunately, in solution many germacrenes show broadened NMR signals or even multiple NMR signals, due to the existence of different conformations of the ten-membered ring (Fig. 4) (Takeda, 1974; Minnaard, 1997). This hinders a complete interpretation of the NMR. Most striking is the presence of two aldehyde proton signals of similar height at δ 9.37 and δ 9.35 that cannot be due to coupling with a proton at C_{13}, accompanied by two very small signals at δ 9.47 and δ 9.45 (ratio 1:1:0.01:0.01).

The existence of different germacrene conformations also explains the formation of a small amount of diastereomeric elematrien-12-al during the Cope rearrangement of germacrene aldehyde (Fig. 3B). Cope rearrangement is a stereospecific reaction that preferably proceeds via a chair-like transition state (March, 1992), via either the UU or DD conformation of the germacrene (Fig. 4). However, the UU conformation with the isopropenal group in the equatorial position predominates and consequently the diastereomer of **8** will be formed only in low yield (Takeda, 1974; Piet et al., 1995; Minnaard, 1997). Small amounts of diastereomeric elemenes were also detected for the GC-injection port induced Cope rearrangement of germacrene A, germacratrien-12-ol

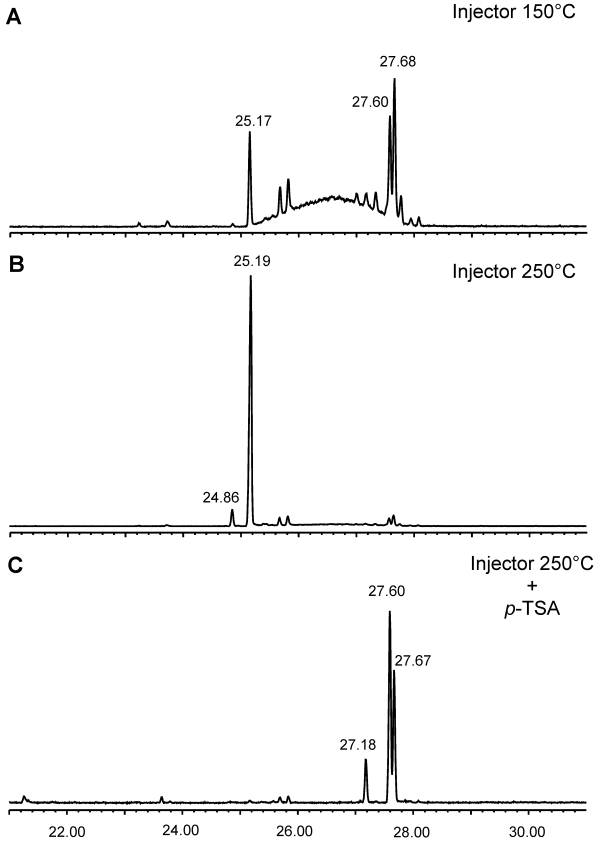

Fig. 3. GC–MS analyses of the germacrene aldehyde (**3**) at an injection port temperature of 150 °C (A) yields a broad hump that starts at the position of elematrien-12-al (**12**) (25.17 min). Injection at an temperature of 250 °C (B) gives almost complete rearrangement of **3** to elematrien-al (**12**) (25.19), whereas a small amount of diastereomeric elemene aldehyde can also be detected (24.86). In the presence of *p*-toluenesulfonic acid (C) the germacrene aldehyde (**3**) is completely converted into costal (**8**): γ-costal (27.18 min), β-costal (27.60 min), and α-costal (27.67 min).

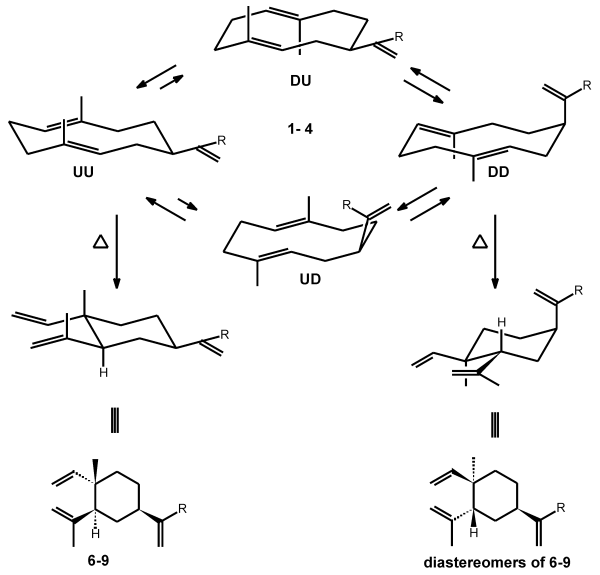

Fig. 4. Conformations of germacrenes **1–4** denoted as UU, UD, DU, and DD. U (up) and D (down) refer to the orientation of the C-14 and C-15 methyl groups. Both conformations UU and DD will easily undergo Cope rearrangement, however, the UU conformation is predominant and will yield elemenes **6–9**, whereas the diastereomeric elemenes that origin from the DD conformation will hardly be formed.

and germacratrien-12-oic acid. These diastereomers have a mass spectrum very similar to their true elemene and typically constitute only 2–7% of the total amount of Cope rearrangement product formed. Although we failed to detect the β-elemene diastereomer before (de Kraker et al., 1998), small amounts of it are not unusual in samples containing β-elemene/germacrene A (Dr. M. A. Posthumus, personal communication). The structure of the elematrien-12-ol diastereomer has tentatively been assigned by Maurer and Grieder (1977).

When the injection port temperature of the GC was varied between 150 and 250 °C, germacrene alcohol (**2**) and germacrene acid (**4**) showed chromatogram patterns similar to that of germacrene aldehyde (**3**). Both germacrene A (**1**) and germacrene acid (**4**) were completely converted into their eudesmane analogues (**10** and **13**, respectively) in the presence of *p*-toluenesulfonic acid. However, the GC-chromatogram of the germacrene alcohol (**2**) still showed a peak of elematrien-12-ol (**7**) after acid treatment, indicating that isolated sample of germacrene alcohol is actually a mixture of germacrene- and elemene alcohol. This was confirmed by ^{1}H NMR that showed a double doublet ($J = 18$ and 10 Hz) at δ 5.81 typical for elematrien-12-ol (Maurer and Grieder, 1977). It is not known whether the presence of elematrien-12-ol is caused by an intrinsic higher instability of the germacrene alcohol and/or by the more difficult and time-consuming purification of this compound. Although less likely, we cannot rule out that the elematrien-12-ol was already present in the crude plant material. In this respect it is remarkable that the germacratrien-12-ol produced in an enzyme assay from germacrene A (de Kraker et al., 2001) was free of any elematrien-12-ol and could be fully cyclised into costol (**11**), notwithstanding the fact that the germacratrien-12-ol had been exposed to 30 °C, a temperature unlikely to occur in costus roots that are underground.

The ^{1}H NMR of germacrene acid (**4**) was free of the double doublet at δ 5.85 from elematrien-12-oic acid (**9**) (de Kraker et al., 2001), but once again only few signals could be assigned. Similar observations were made by Zdero et al. (1991) for 3α- and 3β-acetoxygermacra-1(10)-,4,11(13)trien-12-oic acid whose structures could only be resolved after Cope-rearrangement to their corresponding elemene derivatives.

The isolation under mild conditions from fresh costus roots of (+)-germacrene A (**1**), germacra-1(10),4,11(13)-trien-12-al (**3**), and germacra-1(10),4,11(13)-trien-12-oic acid (**4**) clearly demonstrates that their reported elemene derivatives (**6–9**) are indeed heat-induced artefacts formed during drying of the roots or the manufacturing of costus resinoid/oil as literature on germacrenes suggests (e.g. Bohlmann et al., 1983; Wichtman and Stahl-Biskup, 1987; Teisseire, 1994). Very similar, has for example, been reported for the Australian shrub *Phebalium ozothamnoides* F. Muell whose main essential oil component is elemol, whereas the petroleum extract of macerated leaves yields hedycaryol instead (Southwell, 1970). Only in the case of germacra1(10),4,11(13)-trien-12-ol (**2**), it cannot completely be ruled out that a small quantity of its corresponding elemene is already present in fresh costus roots. The presence of elemenes instead of germacrenes was the only significant difference between an Et_2O-extract from fresh costus roots and the commercial costus root oil,[1] such as thoroughly described by Maurer and Grieder (1977). It is less certain whether the eudesmanes **10–13** have to be regarded as artefacts as well. Although they can be formed from germacrenes under acidic conditions, maybe during storage in the plant—an enzyme specifically producing β-selinene from FPP has been isolated from *Citronella mitis* fruits (Belingheri et al., 1992). It cannot be ruled out that costus root also contain such an enzyme and the eudesmanes present are genuine enzymatic products.

The presence of germacrenes **1–4** together with vast amounts of costunolide (**5**) in costus roots supports the pathway for costunolide as depicted in Fig. 1. The isolated germacrene acid (**4**) is currently being used to investigate the formation of costunolide (**5**) during sesquiterpene lactone biosynthesis in chicory.

3. Experimental

3.1. General experimental procedures

^{1}H NMR and ^{13}C NMR: recorded at 400 MHz with C_6D_6 as solvent and TMS as internal standard. GC–MS: HP 5890 series II gas chromatograph and HP 5972A mass selective detector (70 eV), equipped with a capillary HP5-MS column (30 m×0.25 mm, film thickness of 0.25 μm) at a helium flow rate of 0.969 ml min^{-1}, programmed at 55 °C for 4 min followed by a ramp of 5 °C min^{-1} to 280 °C, and a standard injection port temperature of 250 °C. CC: Silica gel flash (Janssen, 0.035–0.07 nm, pore 6 nm; column width 1.8 cm) (Still et al., 1978). 5% $AgNO_3$/Silica gel was prepared according to Maurer and Grieder (1977). TLC: Silica gel (Merck) 60 F254, eluent EtOAc–hexane (1:4); spray of 5 ml *p*-anisaldehyde in 5 ml H_2SO_4 and 95 ml EtOH, 1–2 min heating at 120 °C.

3.2. Plant material

Seeds of *Saussurea lappa* Clarke were introduced from Hortus Botanicus Vilr, Moscow (USSR) to the fields of Hokkaido Experimental Station for Medicinal Plants (Japan) in 1981 (Accession No. 9678-81) (Hatakeyama

[1] Less volatile compounds like fatty acids are not taken into account, because they are for obvious reasons not present in the costus oil that is produced by steam-distillation.

et al., 1989). Five-year old roots were harvested in August 2000 and transported by courier to The Netherlands. Upon arrival they were cut into pieces, powdered in liquid N_2 with a Waring blender and stored at −80 °C. Dried costus roots were obtained via internet from gaines.com (El Cajon CA, USA) and ginseng4less.com (Petaluma CA, USA).

3.3. Chemicals and reference compounds

Costus resinoid and costus oil were obtained from Pierre Chauvet SA (Seillans, France). Costunolide (**5**) and dehydrocostus lactone were isolated from costus resinoid according to Fischer et al. (1990) making use of the described TLC-spot colour (Asakawa et al., 1981), and recrystallised in pentane; NMR date were identical to those reported in literature (Kuroda et al., 1987; Takahashi et al., 1987). Standards of (−)-elema-1,3,11(13)-trien-12-al (**8**) [NMR in C_6D_6 did not markedly differ from those recorded in $CDCl_3$ (Maurer and Grieder, 1977; Bohlmann et al., 1983)], elema-1,3,11(13)-trien-12-oic acid (**9**) and costal (**12**) were isolated/synthesised as previously described (de Kraker et al., 2001). A mixture of α- and γ-costic acid (**13**) was synthesised from a mixture of α- and γ-costal with $NaClO_3$ (de Kraker et al., 2001). (−)-Elema-1,3,11(13)-trien-12-ol (**7**) was a gift of Dr. B. Maurer (Firmenich SA, Geneva, Switzerland), standards of (+)-germacrene A (**1**) and (−)-β-elemene (**6**) were a gift of Professor W. A. König (Hamburg University, Germany).

3.4. Screening of crude materials for the presence of germacrene A (*1*)

Samples of dried costus roots were powdered in liquid N_2, and 0.2 g of powdered root was shaken in 3 ml Et_2O and centrifuged after 5 min. The Et_2O was passed through a Pasteur pipette with 0.4 g silica gel. A spatula tip of costus resinoid and a droplet of costus oil were also dissolved in 3 ml of Et_2O and passed over silica gel. The Et_2O extracts were screened at an injection port temperature of 150 °C for a germacrene A (**1**) peak that should be replaced by a peak of β-elemene (**6**) at an injection port temperature of 250 °C (de Kraker et al., 1998).

3.5. Isolation of germacrenes *1–3*

Powdered fresh costus root (300 g) was extracted twice with 500 ml of Et_2O at 6 °C; the Et_2O was filtered and the volume reduced to 75 ml by rotary evaporation at room temperature. The concentrate was extracted with 5% aq Na_2CO_3 (3×50 ml). The aq Na_2CO_3 layers were combined and stored at 4 °C. The Et_2O layer was washed with brine, dried with Na_2SO_4, and the solvent removed yielding 2.8 g of a yellow-brown solid. Lactones (1.5 g) were partially removed by dissolving the residue in pentane and cooling at −20 °C, after which the crystallised lactones were filtered off and the solvent removed in vacuo. This procedure was repeated twice. The obtained oil was dissolved in 15 ml pentane and extracted with 20% aq. $AgNO_3$ (4×10 ml). The Ag^+ ions were complexed by adding carefully 10 ml of aq. NH_3 (25%) on ice, after which the water layer was extracted with pentane (3×50 ml) (Southwell, 1970). The pentane layers were washed with brine and dried with Na_2SO_4 yielding 100 mg of a yellow oil. This oil was chromatographed with 350 ml pentane–CH_2Cl_2 (3:7) after which the column was eluted with CH_2Cl_2. Fractions of 10 ml were collected and monitored by GC–MS and TLC; costunolide and dehydrocostus lactone could easily be distinguished by their greenish-blue (R_f 0.30) and blue (R_f 0.35) spots respectively. After removal of aplotaxene [a major constituent of costus root oil (Klein and Thömel, 1976; Maurer and Grieder, 1977)] by a second silver-ion extraction (4×1.5 ml 20% aq. $AgNO_3$, etc.), fractions 2+3 yielded 4 mg of germacrene A (**1**) with an impurity of 4% of humulene. Fractions 19–33 yielded, after a second silver-ion extraction, 1.5 mg germacratrien-12-al (**3**). Fractions 40–64 contained a mixture of costunolide (**5**), dehydrocostus lactone, β-costol (**11**), hedycaryol and germacrene alcohol (**2**). Attempts to separate the latter from β-costol and costunolide by column chromatography on silica gel using pentane–CH_2Cl_2 (1:9) or Et_2O–pentane (1:4) failed. Column chromatography on 5% $AgNO_3$-silica gel (10 gram) was more successful: costol and subsesquently costunolide were eluted with Et_2O (400 ml), whereas germacratrien-12-ol (2 mg, of which ≈ 30% elematrien-12-ol) was eluted with 150 ml MeOH–Et_2O (1:9).

3.6. Isolation of germacra-1(10),4,11(13)-trien-12-oic acid (*4*)

The aq. $NaCO_3$ layers (150 ml) were carefully acidified on ice to pH 6 with 5 M HCl and extracted with Et_2O (4×60 ml). The Et_2O layers were washed with brine, dried with Na_2SO_4, concentrated to 15 ml, and extracted with 20% aq $AgNO_3$ (4×10 ml). The Ag^+ ions were precipitated with 35 ml 2 M NaCl, and the remaining solution was extracted with Et_2O (4×50 ml). After washing with brine and drying with Na_2SO_4, 20 mg of a yellow solid was obtained that mainly contained germacratrien-12-oic acid (**4**), costunolide (**5**) and dehydrocostus lactone. Column chromatography with 350 ml of EtOAc–pentane (1:4) removed the lactones after which the germacrene acid was eluted with 100 ml EtOAc. The last minor impurities were removed by column chromatography with Et_2O–pentane (1:4; 400 ml), and 1.5 mg of germacratrien-12-oic acid was obtained.

3.7. Acid induced cyclisation of germacrenes

A small amount (≈ 10 nmol) of germacrene (**1–4**) was dissolved in 2 ml of CH_2Cl_2 and a crystal of *p*-toluenesulphonic acid was added (Minnaard, 1997). After 7 min at room temperature, the solution was washed with 1 ml of $NaHCO_3$ (satd.) and passed through a Pasteur pipette filled with 0.25 g $MgSO_4$. The solvent was reduced to 100 μl under a stream of N_2 and the sample was analysed by GC–MS. In case of germacratrien-12-oic acid (**4**) $NaHCO_3$ was replaced by NaH_2PO_4. The experiments were repeated with elemenes **6–9** that did not cyclise.

3.8. Compound characterisation

3.8.1. Germacrene A (*1*), 4 mg

Colourless oil, purple TLC-spot R_f 0.75, R_I 1511, EIMS *m/z* (rel int) M^+ 204 (8), $[M–Me]^+$ 189 (37), 93 (100), 67 (99), 68 (94), 107 (79), 81 (77), 41 (68), 79 (67), 105 (59), 91 (57), 53 (54), 147 (47), 119 (43), 121 (41), 55 (41), 161 (36), 133 (36). Cope rearrangement to **6**, R_I 1396, EIMS *m/z* (rel int) M^+ 204 (1), $[M–Me]^+$ 189 (28), 93 (100), 81 (96), 67 (83), 68 (76), 41(67), 79 (64), 107 (63), 53 (51), 91 (48), 147 (45), 121 (42), 105 (42), 55 (41), 161 (31), 119 (31), 133 (29); and diastereomer of **6**, R_I 1388, EIMS *m/z* (rel int) M^+ 204 (1), $[M–Me]^+$ 189 (23), 93 (100), 81 (78), 67 (61), 68 (58), 41 (52), 79 (51), 107 (50), 91 (41), 121 (35), 161 (34), 55 (33), 53 (32), 105 (31), 119 (27), 133 (23), 147 (21). Cyclisation yields **10**, R_I 1479 (γ), 1491 (β) and 1500 (α) [MS-data are comparable with those of Maurer and Grieder (1977) and were used in combination with the R_I's reported by Adams (1995) to determine the elution order].

3.8.2. Germacra-1(10),4,11(13)-trien-12-ol (*2*), 2 mg, mixture with 7 (≈ 30%)

Colourless/slightly yellow oil, pink/purple TLC-spot R_f 0.29, R_I 1673 to ± 1806. Cope rearrangement to **7**, R_I 1673 EIMS *m/z* (rel int) M^+ 220 (1), $[M–Me]^+$ 205 (4), 79 (100), 81 (94), 41 (91), 91 (90), 67 (86), 93 (85), 55 (69), 105 (66), 53(60), 119 (58), 77 (55), 68 (53), 189 (42), 121 (37), 133 (35), 145 (33), 163 (25), 161 (25); and diastereomer of **7**, R_I 1657, EIMS *m/z* (rel int) M^+ 220 (1), $[M–Me]^+$ 205 (3), 81 (100), 41 (94), 79 (92), 93 (78), 67 (78), 91 (71), 69 (70), 55 (63), 105 (55), 119 (50), 77 (46), 53 (45), 161 (28), 133 (24), 145 (18), 189 (15). Cyclisation yields **11**, R_I 1755 (γ), 1775 (β) and 1781 (α) [MS-data are comparable with those of Maurer and Grieder (1977) and used to determine elution order].

3.8.3. Germacra-1(10),4,11(13)-trien-12-al (*3*), 1.5 mg

Colourless/slightly yellow oil with strong mossy odour, pink spot R_f 0.58, R_I 1585 to ± 1715. ^1H NMR δ 9.35 (*s*) 9.37 (*s*), 9.45 (*s*) and 9.47 (*s*) (ratio 1:1:0.01:0.01, 1H, H-12); 5.77 (*br s*) and 5.72 (*s*) (ratio 1:1, 1H, H-13); 5.36 (*br s*) and 5.33 (*s*) (ratio 1:1, 1H, H′-13); 5.22 (*br s*,1H, H-1); 4.84 (*br d*, $J = 13.3$ Hz) and 4.62 (*br d*, $J = 10.3$ Hz) (ratio 1:1, 1H, H-5) 2.75–2.55 (*br m*, 1H, H-7). Cope rearrangement to **8**, R_I 1585 EIMS *m/z* (rel int) M^+ 218 (1), $[M–Me]^+$ 203 (12), 81 (100), 67 (70), 79 (68), 41 (63), 91 (56), 53 (50), 93 (46), 95 (38), 77 (38), 55 (37), 107 (37), 105 (35), 119 (27), 161 (25), 121 (24); and diastereomer of **8**, R_I 1570 EIMS *m/z* (rel int) M^+ 218 (ND), $[M–Me]^+$ 203 (8), 81 (100), 79 (64), 67 (60), 41 (57), 91 (48), 93 (43), 95 (41), 53 (40), 77 (36), 105 (36), 107 (35), 68 (34), 55 (34), 119 (25), 121 (22), 175 (22). Cyclisation yields **12**, R_I 1673 (γ), 1692 (β) and 1695 (α) [MS-data are comparable with those of Maurer and Grieder (1977) and used to determine elution order].

3.8.4. Germacra-1(10),4,11(13)-trien-12-oic acid (*4*), 1.5 mg

White crystals, blue TLC-spot (note: **9** gives pink spot) R_f 0.18, R_I 1771 to ± 1845. ^1H NMR δ 6.44-6.37 (*m*) and 6.36 (*s*) (ratio 1:1, 1H, H-13); 5.44 (*br m*) and 5.38 (*s*) (ratio 1:1, 1H, H′-13); 5.30–5.15 (*br s*, 1H, H-1); 4.85 (*br m*) and 4.63 (*br d*, $J = 10.6$ Hz) (ratio 1:1, 1H, H-5); 2.75-2.55 (*br m*, 1H, H-7). Cope rearrangement to **9**, R_I 1771 EIMS *m/z* (rel int) M^+ 234 (1), $[M–Me]^+$ 219 (7), 81 (100), 67 (50), 79 (47), 41 (43), 68 (40), 91 (38), 53 (34), 93 (29), 105 (29), 55 (26), 77 (26), 107 (26), 121 (23), 119 (19), 69 (17), 177 (15); and diastereomer of **9**, R_I 1750 EIMS *m/z* (rel int) M^+ 234 (ND), $[M–Me]^+$ 219 (4), 81 (100), 79 (67), 41 (58), 67 (56), 107 (55), 91 (49), 93 (49), 53 (41), 55 (40), 68 (39), 105 (38), 121 (35), 162 (33), 147 (26), 119 (25), 163 (25). Cyclisation yields **13**; R_I 1854 (γ), EIMS *m/z* (rel int) M^+ 234 (26), $[M–Me]^+$ 219 (100), 91 (57), 41 (35), 79 (35), 81 (34), 105 (34), 147 (34), 107 (32), 93 (31), 55 (30), 77 (30), 173 (25); R_I 1876 (β), EIMS *m/z* (rel int) M^+ 234 (25), $[M–Me]^+$ 219 (67), 91 (100), 79 (95), 93 (85), 41 (78), 121 (78), 77 (63), 81 (63), 55 (61), 67 (59), 105 (55), 107 (55), 53 (45), and R_I 1879 (α), EIMS *m/z* (rel int) M^+ 234 (27), $[M–Me]^+$ 219 (100), 91 (57), 79 (46), 41 (38), 81 (37), 107 (36), 105 (35), 93 (35), 55 (29), 205 (26) (elution order of isomers presumed to be the same as for **10–12**).

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