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Biotransformation of (±)-4,8-dimethylcyclodeca-3(E),7(E)-dien-1β-ol and (+)-Hedycaryol by *Cichorium intybus*.

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Abstract – The biotransformation of the synthetic (E,E)-1,5-cyclodecadienol **5** and (+)-hedycaryol (**11**) by a root suspension of fresh chicory has been investigated. Incubation of **5** with a root suspension gave a 2 : 1 mixture of the epimeric eudesmanediols **7a** and **7b** whereas **11** was selectively converted into cryptomeridiol (**12**). An explanation for the obtained results is proposed.

INTRODUCTION

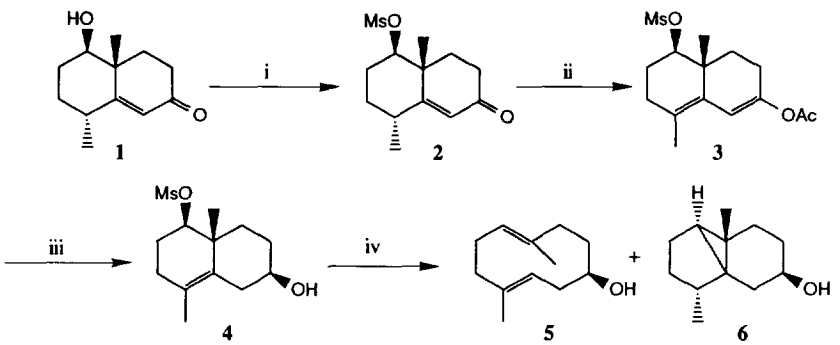
Recently, *in vivo* transformations have provided direct evidence that germacrane sesquiterpenes are important intermediates in the biosynthetic pathways towards guaiane, eudesmane and other types of sesquiterpenes[1-3]. The co-occurrence of germacranolides, eudesmanolides and guaianolides in chicory (*Cichorium intybus*)[4,5] has initiated our biosynthetic studies on sesquiterpene lactones in *C. intybus*. In our previous paper we reported the biotransformation of a synthetic (E,E)-1,5-cyclodecadienol (**9**), a model compound for germacranes, into two epimeric *trans*-eudesmanediols by a root suspension of fresh chicory[6]. This cyclodecadienol, however, lacks the methyl group at C-4 which is characteristic for germacranes and possesses a relatively small hydroxyl group at C-7. In this paper the role of the methyl group at C-4 as well as the influence of the substituent at C-7 on the transannular cyclisation reaction of germacranes by *C. intybus* is described. For this purpose, the synthesis of (±)-4,8-dimethylcyclodeca-3(E),7(E)-dien-1β-ol (**5**) was performed and the biotransformation of compound **5** and the natural sesquiterpene (+)-hedycaryol (**11**) by *C. intybus* was studied.

RESULTS AND DISCUSSION

Over the years, several approaches towards the construction of the chemically and thermally labile germacrane ring system have been reported[7]. The base induced fragmentation of an appropriately functionalised

hydroborated naphthalene system, utilised by Marshall[8], can elegantly be used in a synthetic route which leads to the formation of the (E,E)-1,5-cyclodecadiene ring system. This method has succesfully been applied in the synthesis of both (±)-and (+)-hedycaryol (**11**)[9,10].

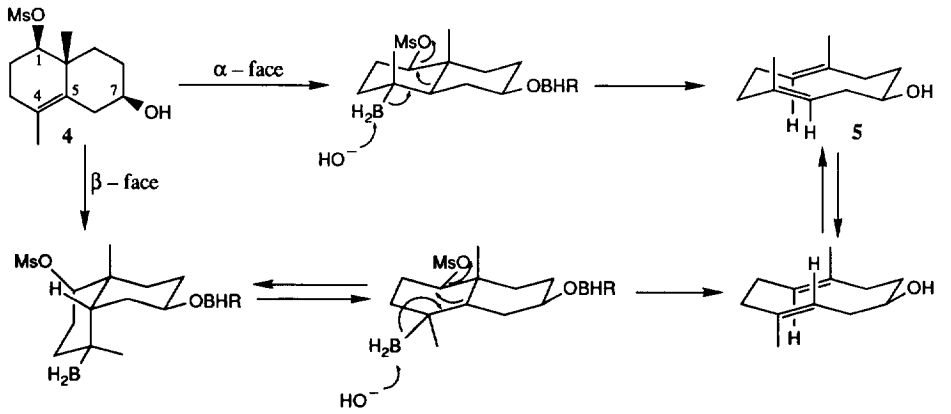
Octalol **1** was obtained from the readily available Wieland-Miescher ketone in a 6 step reaction sequence[11,12]. Exposure of **1** to mesylchloride and subsequent enolisation of the enone followed by reduction of the dienol acetate **3** gave the mesylate **4** (Scheme 1). Hydroboration-fragmentation of **4** proceeded almost quantitatively into a 9 : 1 mixture of (±)-4,8-dimethylcyclodeca-3(E),7(E)-dien-1β-ol (**5**) and 5β-hydroxy-tricyclo[5.3.$0^{1,7}$.$0^{2,7}$]decane (**6**). Alcohol **5** was purified by aqueous $AgNO_3$ extraction[13].



Scheme 1: i: MsCl; ii: NaI, $(CH_3)_3SiCl$, Ac_2O; iii: $NaBH_4$; iv: 1) $BH_3{\cdot}Me_2S$ 2) NaOH.

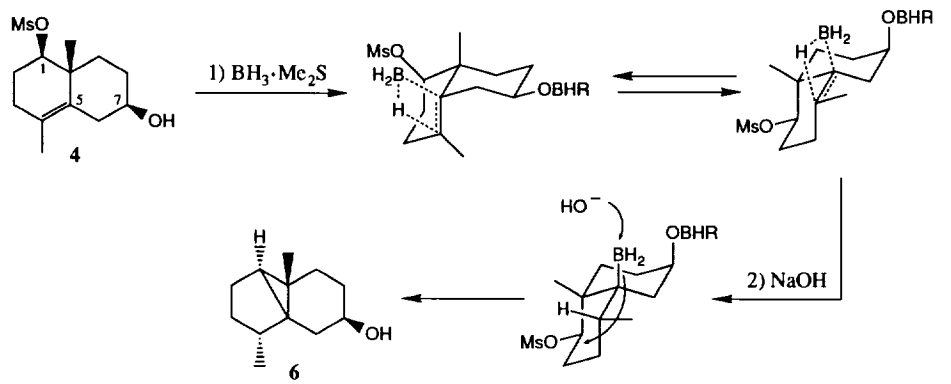
Hydroboration generally places the boron at the less substituted carbon of the double bond. In the case of the tetra-substituted unsaturated mesylalcohol **4**, discrimination of the two positions of the internal olefin is determined by both steric and electronic influences[14]. A four-centre transition state is suggested in the reversible hydroboration addition step[15]. α-Face hydroboration of **4** at C-4 will result in an *all*-chair conformation with the C-7 (di)alkoxyborane substituent in an equatorial position[16]. This conformation possesses the necessary geometry for a concerted fragmentation (Scheme 2). In the case of α-face hydroboration at C-5, γ-elimination can not occur because this conformation does not possess the necessary geometry for backside displacement of the mesylate[18,19]. Probably, the formation of (di)alkoxyboranes during the hydroboration of **4** is responsible for the predominating α-face attack of the $BH_3{\cdot}Me_2S$ complex. In a study towards the hydroboration-fragmentation reaction of functionalised decalin systems in which the C-7 hydroxyl group was replaced by a ether function which can not react with $BH_3{\cdot}Me_2S$, the formation of the tricyclodecane ring system was found to predominate[20].

β-Face hydroboration at C-4 followed by base induced fragmentation can also lead to the germacrane ring system. The intermediate borane then has to react through the energetically unfavourable boat-boat conformation to give **5** with the methyl groups *anti*-oriented towards each other. This mode of hydroboration, although unlikely, cannot be excluded but α-face hydroboration seems the more preferred pathway.



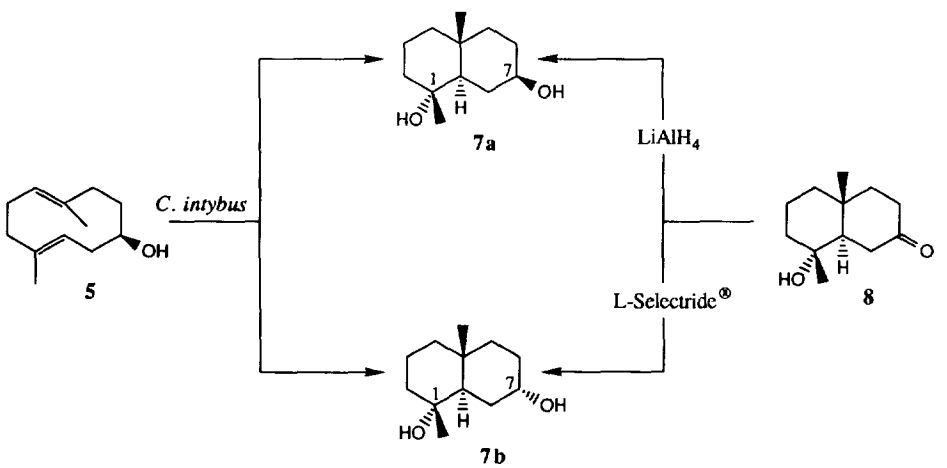
Scheme 2: Formation of the (E,E)-1,5-cyclodecadiene system due to α- and β-face hydroboration at C-4.

β-Face approach of the $BH_3 \cdot Me_2S$ complex towards C-5 will result in electrostatical repulsion between the boron and the axial (di)alkoxyborane at C-7. Only this conformation possesses the necessary geometry for backside displacement of the mesylate to yield **6** as outlined in scheme 3.



Scheme 3: Formation of the tricylodecane ring system due to β-face hydroboration of C-5.

When alcohol **5** was administered to a suspension of mortared chicory root and incubated for 10 days, two epimeric diols **7** were obtained in a 2 : 1 ratio. These diols could be separated using preparative capillary GC. The ^1H NMR spectrum of the minor compound (**7b**) exhibited a small multiplet at δ 4.20 ($W_{1/2}$ = 7.2 Hz) indicating H-1 to be in an equatorial position, while in the major compound (**7a**) a broad multiplet resonated at δ 3.64 ($W_{1/2}$ = 20.3 Hz) indicating the axial position of H-1. Both alcohols **7a** and **7b** were found to be identical upon comparison with authentical samples which were obtained through reduction of the known ketone **8**[21] (Scheme 4).



Scheme 4: Synthesis and biosynthesis of **7a** and **7b**.

Both diols **7a** and **7b** originated from the two *syn* conformers of **5** with the double bonds in a crossed position as illustrated in figure 1. As the methyl groups invert, the position of the hydroxyl at C-1 changes from equatorial to axial. Cyclisation through conformer **I** leads to **7a**, while conformer **II** is responsible for the formation of **7b**. The *anti*-conformation of **5**, with the double bonds parallel towards each other, would lead to the formation of *cis*-eudesmanes. The fact that these compounds were not detected probably means that the activation barrier towards the formation of *cis*-fused eudesmanes is higher than for *trans*-eudesmanes.

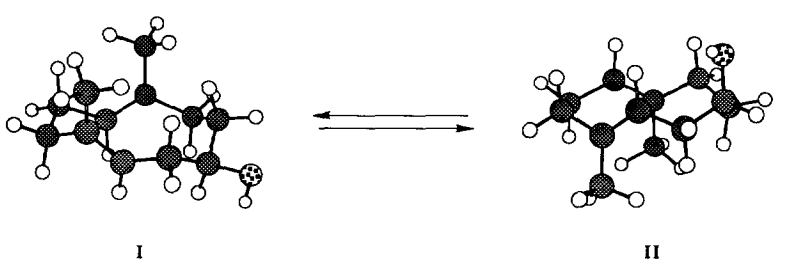
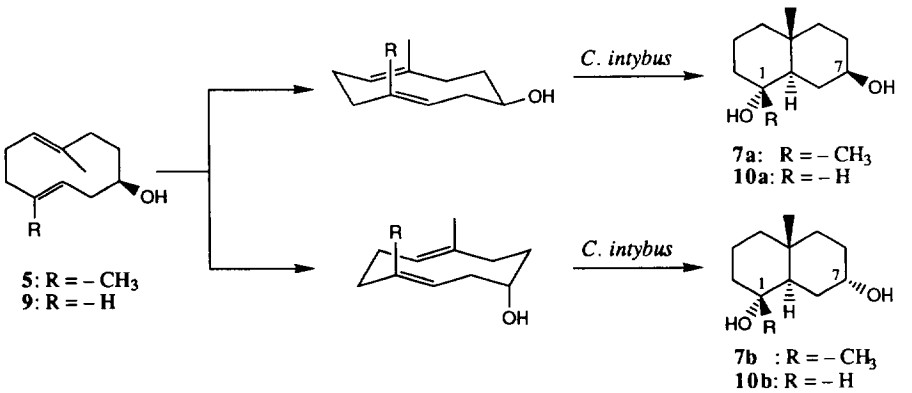


Figure 1: The two *syn* conformations leading to **7a** and **7b**.

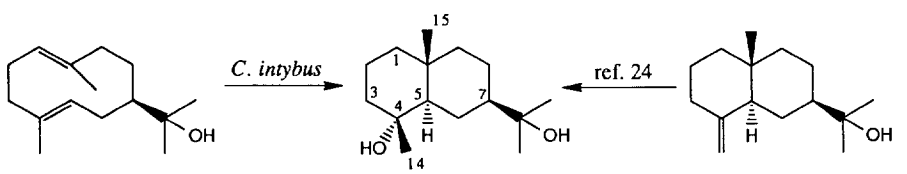
The unambiguously established outcome of the transannular cyclisation of **5** which resulted in the formation of the C-7 epimeric alcohol **7a** and **7b** instead of the expected C-1 epimeric alcohols led us to reinvestigate the cyclisation of **9**, the compound lacking methyl group at C-4. We had identified the major product, eudesmanediol **10a**, by direct comparison with an authentic sample. The minor product, eudesmanediol **10b** was assigned as the C-1 epimeric diol because it was assumed that cyclisation of **9** only proceeded through the *syn* conformation as postulated by Hendrickson[22], with the C-7 substituent in an equatorial position.



Scheme 5: Cyclisation of **5** and **9** through different conformers.

The ^{1}H NMR spectrum of **10a** showed two α-hydroxy protons at δ 3.58 (multiplet, $W_{1/2}$ = 21.5 Hz) and δ 3.40 (dt, J = 10.2 Hz, J = 4.5 Hz) while in the minor product, eudesmanediol **10b**, the two α-hydroxy protons resonated at δ 4.09 (multiplet, $W_{1/2}$ = 7.0 Hz) and δ 3.37 (dt, J = 10.6 Hz, J = 4.5 Hz). Based on the assumption that **9** would cyclise through only one conformer, we assigned the signal at δ 3.37 to H-7α. However, after separation of the epimeric mixture obtained by enzymatic cyclisation of **5**, the major product, eudesmanediol **7a**, displayed a multiplet at δ 3.64 ($W_{1/2}$ = 20.3 Hz). In the minor product, the α-hydroxy proton of the epimer **7b** resonated at δ 4.20 (multiplet, $W_{1/2}$ = 7.2 Hz). This indicates that the signal at δ 3.37 in the ^{1}H NMR spectrum of **10b** did not belong to H-7α but to H-1α. We therefore come to the conclusion that the identity of the minor product **10b** has to be revised from (1α,4aα,7α,8aβ)-decahydro-4a-methylnaphthalene-1,7-diol into (1α,4aβ,7α,8aα)-decahydro-4a-methylnaphthalene-1,7-diol. This means that the cyclisation of **9** proceeds in a similar way to that of **5**.

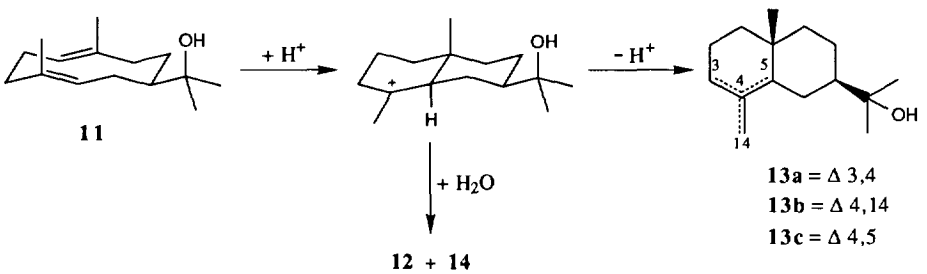
When (+)-hedycaryol (**11**)[10] was administered to a mortared root suspension of fresh chicory and incubated for four days, cryptomeridiol (**12**) was obtained as the sole product (scheme 6). The identity of **12** was confirmed by direct comparison with an authentic sample synthesised from natural β-eudesmol (**13c**)[23] by epoxidation and subsequent reduction of the 4,14-epoxide[24]. The ^{1}H- and ^{13}C NMR values of **12** are identical with those reported in the literature[25]. The strong NOE between C-14 and C-15 is also consistent with the assigned equatorial position of the hydroxyl function at C-4.



11 **12** **13c**

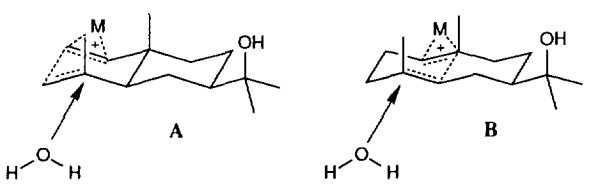
Scheme 6: Synthesis and biosynthesis of **12**.

In contrast to the selective biotransformation of **11**, its chemically induced cyclisation always gives rise to mixtures of eudesmanes. If water is present in the reaction medium, this mixture usually consists of **12** and α-, β- and γ-eudesmol (**13a, b** and **c** respectively). The ratio in which these products are formed depends on the reaction conditions. For instance, treatment of **11** with sulfuric acid in aqueous acetone gives a mixture of predominantly **12** and **13c** accompanied by small amounts of **13a**, **13b** and another eudesmane **14**, judging from its MS to be the C-4 epimeric alcohol of **12**. The ratio in which **12** and **13c** are formed appeared to be relatively constant when the water concentration exceeds 30% (v/v). These findings indicate that probably a carbocation intermediate is involved in the acid induced cyclisation of **11** (Scheme 7). Processes in which the capture of a nucleophile is accompanied by extensive elimination are believed to proceed through a step-wise formation of an intimate ion pair[26].



Scheme 7: Acid induced cyclisation of **11**.

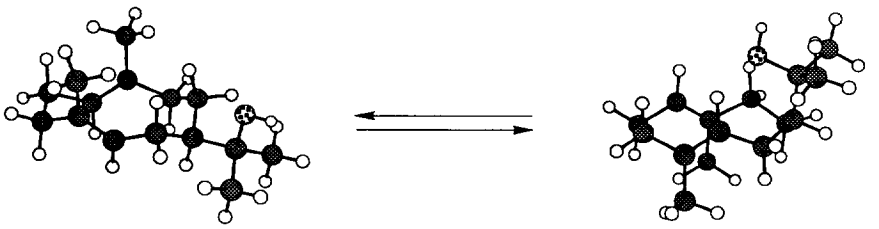
An olefinic cyclisation reaction which is thought to proceed more synchronously is the oxymetallation reaction. Renold *et al.* reported the oxymetallation of elemol using $Hg(OAc)_2$ in aqueous THF followed by reductive demercuration yielding 69% of **12** and 7% of a mixture of **13a–c**[27]. A product-like transition state followed by capture of the nucleophile from a *pseudo*-equatorial direction, as indicated in scheme 8, has been proposed to explain the product outcome. Treatment of hedycaryol (**11**) with $Hg(OAc)_2$ under identical reaction conditions gave almost the same ratio of **12** (60%) and **13a-c** (15%). This indicates that the oxymercuration-deoxymercuration reaction of elemol and hedycaryol probably proceeds via a similar type of transition state (**A** and **B** respectively). The more concerted nature of this reaction is reflected by the formation of a larger amount of **12**, as compared to the acid induced cyclisation of **11**.



M = Hg(OAc)

Scheme 8: Transition state intermediates of elemol (**A**) and hedycaryol (**B**) upon oxymetallation.

When **11** was incubated with a root suspension of fresh chicory, no eudesmols were detected after completion of the enzymatic reaction. Dehydration of **12** was not observed in both the reaction- and incubation media, even after prolonged exposure to the experimental conditions. Therefore, the exclusive formation of **12** in the biotransformation of **11** by *C. intybus* must have occurred through the *syn* conformer **III** as postulated in figure 2. Inversion of the methyl groups (**IV**) will place the large isopropanol group at C-7 in an unfavourable *pseudo*-axial position and cyclisation through this conformation is not likely to occur.



III IV

Figure 2: The two *syn* conformations of **11**.

The biotransformation of **5**, **9** and **11** is induced by enzymatic protonation of the 1,10-double bond followed by cyclisation and subsequent incorporation of a water molecule. The water molecule being stereoselectively incorporated from the α-side which indicates that the *syn* conformation is preserved during cyclisation and that the transannular cyclisation reaction initiated by *C. intybus* is of a concerted nature. Some decades ago, cyclisation through different conformations was postulated to be responsible for the wide variety of naturally occurring guaianes and eudesmanes[22,28,29]. More recently, Sakamoto *et al.* reported the formation of *cis*-fused eudesmanes and guaianes originating from germacrone epoxides, but no solid experimental or crystallographical data were presented[3]. Our findings confirm the generally accepted idea that 1,5-(E,E)-germacranes and germacranoids cyclise through the *all*-chair conformation. Since there are two *all*-chair conformations possible for the 10-membered ring system, additional ring substituents determine the ratio in which these conformers exist. The formation of two epimeric diols from **5** and **9** and only one from **11** indicates that the nature of the substituent at C-7 has a major influence on the conformation of the germacrane ring system. When we compare the transannular cyclisation of **5** and **9**, the C-4 methyl group has (surprisingly) little effect on the occurrence of both *syn* conformers. Upon cyclisation, both compounds yielded a C-7 epimeric mixture of eudesmanediols in a 2 : 1 ratio. Additional research towards the influence of substituents on the transannular cyclisation of germacrane sesquiterpenes and their epoxides induced by *C. intybus* is currently being conducted at our department.

EXPERIMENTAL

Melting points are uncorrected. The ^{1}H NMR spectra were recorded in $CDCl_3$ relative to TMS at 200 and 500 MHz; the ^{13}C NMR spectra were recorded at 25.3 and 125.7 MHz. Both α-, β- and γ-eudesmol (**13a-c**) and cryptomeridiol (**12**) in the crude reaction mixture were analysed by GC-MS and their Kovat's retention index.

Analytical GC-MS was carried out as previously reported using DB-17 and DB-WAX columns[6]. Preparative GC was performed with a Gerstel preparative DCS system using a Hewlett Packard methyl silicone pre-column (5 m x 0.53 cm; d_f = 2.65 μm) and a DB-WAX main column (60 m x 0.53 cm; d_f = 1.0 μm) in two separate Hewlett Packard 5890 II gas chromatographs using hydrogen as the carrier gas. GC analyses were carried out on a Fisons GC 8000 equipped with a FID and a DB-17 fused silica capillary column (30m x 0.25 mm, d_f 0.25 μm). Peak areas were integrated electronically with a Fisons integrator DP700. Column chromatography was performed on Merck silicagel 60 and deactivated Al_2O_3 (grade III) using petroleum ether (40-60) - EtOAc as the solvent system.

Plant material. 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 **5** and **11** towards the buffer and an inactivated chicory root sample (obtained by boiling the supernatant for 30 min) were investigated as a control to test the possibility of non-enzymatic reactions. Neither showed any reaction.

Incubations. 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 hedycaryol incubation medium was extracted after 4 days, the incubation medium containing **5** after 10 days with 0.5 ml 5% *iso*-PrOH in EtOAc. The crude mixtures were analysed by GC-MS.

(4aα,5α,8β)-4,4a,5,6,7,8-hexahydro-4a,8-dimethyl-2(3H)-naphthalene 5-(methanesulfonate) (**2**)

To a stirred solution of 1.44 g of **1** (7.42 mmol) in 20 ml of dry pyridine, cooled to 0°C, was added 0.8 ml of methanesulfonyl chloride. The reaction mixture was stirred at 0°C for 1h, after which cold saturated $NaHCO_3$ was added. The resulting mixture was taken up in 50 ml of CH_2Cl_2 and extracted with saturated $NaHCO_3$ (2 x 50 ml). The aqueous layers were extracted with 2 x 50 ml CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to yield 1.81 g (6.65 mmol, 90%) of **2** as a white solid. Mp: 118.5–120°C (dec.). ^{1}H NMR (200 MHz): δ 5.82 (d, 1H, H-1, J = 1.9 Hz); δ 4.44 (dd, 1H, H-5α, J = 11.2 Hz, J = 5.3 Hz); δ 3.02 (s, 3H, $-SO_2CH_3$); δ 2.43–2.38 (m, 3H); δ 2.15–2.04 (m, 3H); δ 2.00–1.88 (m, 3H); δ 1.24 (s, 3H, 5β–CH_3); δ 1.05 (d, 3H, 8α–CH_3). ^{13}C NMR: 198.71 (s), 168,25 (s), 123.96 (d), 87.05 (d), 40.91 (s), 38.94 (q), 34.35 (t), 33.12 (d), 33.03 (t), 31.42 (t), 28.19 (t), 17.46 (2 x q).

(4aα,5α)-2-acetoxy-3,4,4a,5,6,7-hexahydro-4a,8-dimethyl-naphthalene 5-(methanesulfonate) (**3**)

To a stirred mixture of 1.76 g of **2** (6.47 mmol) and 3.50 g of NaI in 40 ml of acetic anhydride, cooled to 0°C, was added dropwise 2.15 ml of $(CH_3)_3SiCl$. The reaction mixture was stirred for 1h at 0°C after which the solvents were evaporated *in vacuo*. The residu was taken up in 50 ml of CH_2Cl_2 and washed with saturated $NaHCO_3$ (2 x 25 ml) and saturated $Na_2S_2O_3$ (2 x 25 ml). The aqueous layers were extracted with 2 x 50 ml of CH_2Cl_2, washed with brine, dried over $MgSO_4$ and evaporated to dryness. The oily residue was chromatographed to yield 2.01 g (6.40 mmol, 99%) of **3** as a colourless oil.

^{1}H NMR (200 MHz): δ 6.01 (d, 1H, H-1, J = 2.0 Hz); δ 4.55 (dd, 1H, H-5α, J = 10.0 Hz, J = 6.5 Hz); δ 3.02 (s, 3H, $-SO_2CH_3$); δ 2.58–2.46 (m, 1H); δ 2.40–2.10 (m, 5H); δ 2.13 (s, 3H, $-OCCH_3$); δ 1.94 (ddd, 1H, J = 12.7 Hz, J = 5.8 Hz, J = 1.6 Hz); δ 1.62 (s, 3H, 8–CH_3); δ 1.55–1.42 (m, 1H); δ 1.06 (s, 3H, 4aβ–

CH_3). ^{13}C NMR: 169.43 (s), 147.73 (s), 129.12 (s), 127.98 (s), 112.54 (d), 87.04 (d), 38.71 (q), 36.57 (s), 32.81 (t), 31.47 (t), 24.69 (t), 23.95 (t), 21.02 (q), 18.27 (q), 17.35 (q).

(2α,4aα,5α)-1,2,3,4,4a,5,6,7-octahydro-4a,8-dimethyl-naphthalen-2-ol 5-(methanesulfonate) (**4**)

To a stirred solution of 1.97 g of **3** (6.27 mmol) in 40 ml of EtOH, cooled to 0°C, 1.98 g of $NaBH_4$ was added and the reaction was stirred overnight at room temperature. After carefully adding 3 ml of acetic acid, the reaction mixture was evaporated to dryness. The residue was taken up in in 50 ml of CH_2Cl_2 and washed with 2 x 50 ml saturated $NaHCO_3$. The combined aqueous layers were extracted with CH_2Cl_2 (2 x 25 ml), dried over $MgSO_4$ and evaporated *in vacuo* under exclusion of light to yield 1.62 g (5.91 mmol, 94%) of **4** as a colourless oil.

^1H NMR (200 MHz); δ 4.49 (dd, 1H, H-5α, J = 8.6 Hz, J = 7.0 Hz); δ 3.53–3.37 (m, 1H, H-2α); δ 3.00 (s, 3H, $-SO_2CH_3$); δ 2.74 (ddd, 1H, J = 13.6 Hz, J = 4.7 Hz, J = 2.1 Hz); δ 2.20–1.80 (m, 8H); δ 1.61–1.40 (m, 1H); δ 1.59 (s, 3H, 8–CH_3); δ 1.28–1.10 (m, 1H); d 1.09 (s,3H, 4β–CH_3). ^{13}C NMR: 130.81 (s), 126.01 (s), 89.02 (d), 70.67 (d), 38.76 (q), 38.36 (s), 36.24 (t), 35.15 (t), 31.42 (t), 30.82 (t), 25.15 (t), 18.99 (q), 18.11 (q).

4,8-dimethylcyclodeca-3(E),7(E)-dien-1β-ol (**5**)

To a stirred solution of 1.54 g of **4** (5.62 mmol) in 40 ml of dry ether under a nitrogen atmosphere, cooled to 0°C, was added dropwise 15.5 ml of 2M BH_3·Me_2S complex in THF which reacted vigorously as indicated by the evolution of hydrogen gas. The reaction was stirred for 8h at room temperature after which 3 ml of water followed by 25 ml of 4M NaOH was carefully added. The resulting two-phase mixture was stirred overnight after which the organic phase was separated and washed with 2M NaOH (2 x 50 ml). The combined aqueous layers were extracted with ether (2 x 50 ml) and the combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated to dryness to yield 950 mg (5.28 mmol, 94%) of an oil containing **5** and **6** in a 9 : 1 ratio according to GC. The oil was taken up in 50 ml of *t*-butylmethylether (*t*-BME) and extracted with 4 x 25 ml of 20% $AgNO_3$. The combined $AgNO_3$-layers were extracted with 25 ml of *t*-BME and then cooled to 0°C. The combined organic phase was washed with brine, dried over $MgSO_4$, concentrated and purified by column chromatography to yield 16 mg (0.15 mmol, 3%) of **6** as an oil. After addition of 70 ml of 25% ammonia, the $AgNO_3$-layers were extracted with 4 x 25 ml of *t*-BME. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated to dryness to yield 479 mg (2.66 mmol, 47%) of **5** as a solid. An analytical sample was recrystallised from diisopropylether. Mp: 71–72.5°C;

^1H NMR (200 MHz): Due to extensive coalescence of the ^1H NMR-spectrum of **5**, no additional information could be obtained other than **5** existing in multiple conformations in a $CDCl_3$ solution at room temperature. ^{13}C NMR: 137.76 (s), 131.01 (s), 126.70 (d), 126.48 (d), 76.15 (d), 38.68 (2 x t), 38.49 (t), 37.29 (t), 26.21 (t), 16.81 (q), 15.99 (q). Mass spectrum (*m/e*): [M^+] 180 (4), 162 (28), 147 (27), 123 (12), 121 (18), 120 (20), 119 (20), 105 (49), 93 (35), 91 (31), 81 (50), 79 (50), 68 (60), 67 (100), 55 (55), 53 (44), 43 (38), 41 (85), 39 (49); Calc. for: 180.1514, Found: 180.1512; Anal. calc. for $C_{12}H_{20}O$: C 79.94%, H 11.18%; Found: C 79.20%, H 11.33%. ^1H NMR (200 MHz) of **6**: δ 3.52–3.41 (m, 1H, H-1); δ 2.12 (dd, 1H, J = 12.9 Hz, J = 4.9 Hz); δ 2.00–0.80 (m, 12 H); δ 0.97 (d, 3H, 8α–CH_3); δ 0.93 (s, 3H, 4aβ–CH_3). ^{13}C NMR: 68.47 (d), 37.39 (d), 36.70 (t), 34.74 (d), 34.46 (t), 33.51 (t), 31.24 (t), 24.06 (t), 20.05 (q), 17.06 (q)[a].

[a] Quaternary carbons could not be assigned because the compound was not entirely pure.

(1α,4aβ,7β,8aα)-decahydro-1,4a-dimethyl-naphthalene-1,7-diol (**7a**)

To a stirred solution of 50 mg of **8** (0.26 mmol) in 5 ml of ether, cooled to 0°C, was added 100 mg of $LiAlH_4$. The resulting mixture was stirred for 2h after which 300 mg of Glauber's salt was added. Stirring was continued for 30 minutes followed by addition of $MgSO_4$, 15 minutes of stirring, filtering off the solids and evaporation of the solvent to yield 46 mg (0.23 mmol, 91%) of **7a** as a solid. Mp: 159–161°C;
^{1}H NMR (200 MHz): δ 3.64 (multiplet, 1H, H-7α, $W_{1/2}$ = 20.3 Hz); δ 2.11–2.04 (m, 1H); δ 1.81–1.72 (m, 2H); δ 1.52 (s, 2H, 2 x –OH); δ 1.59–1.27 (m, 7H); δ 1.22–0.95 (m, 3H); δ 1.01 (s, 3H, 8β–CH_3); δ 0.89 (s, 3H, 4aβ–CH_3). ^{13}C NMR: 72.01 (d), 71.82 (s), 52.41 (d), 42.99 (t), 42.52 (t), 40.53 (t), 34.10 (s), 31.09 (t), 30.49 (t), 22.74 (q), 20.10 (t), 18.57 (q). Mass spectrum (*m/e*): [M^+] 198 (6), 183 (2), 180 (4), 165 (5), 137 (12), 110 (26), 109 (16), 95 (41), 81 (25), 79 (17), 71 (38), 67 (28), 55 (28), 43 (100), 41 (39), 39 (16).

(1α,4aβ,7α,8aα)-decahydro-1,4a-dimethyl-naphthalene-1,7-diol (**7b**)

To a stirred solution of 2.39 g of **8** (12.2 mmol) in 100 ml of THF, cooled to –78°C under a nitrogen atmosphere, was added dropwise 22 ml of L-Selectride®. The resulting mixture was stirred for 30 minutes at –78°C after which the temperature was raised to room temperature over a 30 minute time period. A solution of 80% of EtOH was added followed by an additional 2h of stirring. The resulting solution was cooled to 0°C and 200 ml of 35% of H_2O_2 was added. After 3h, the organic solvents were evaporated and the aqueous phase extracted with CH_2Cl_2 (10 x 80 ml). The combined organic layers were washed with water (3 x 80 ml), brine, dried over $MgSO_4$ and evaporated *in vacuo* to yield 2.06 g (10.4 mmol, 85%) of **7b** as a solid. Mp: 117–119°C; ^{1}H NMR (200 MHz); δ 4.20 (multiplet, 1H, H-7β, $W_{1/2}$ = 7.2 Hz); δ 1.92–1.30 (m, 12 H); δ 1.56 (s, 2H, 2 x –OH); δ 1.21–1.14 (m, 1H); δ 1.07 (s, 3H, 8β–CH_3); δ 0.87 (s, 3H, 4aβ–CH_3). ^{13}C NMR: 71.71 (s), 66.84 (d), 47.33 (d), 43.72 (t), 41.01 (t), 38.42 (t), 34.95 (s), 28.48 (t), 28.21 (t), 22.19 (q), 20.12 (t), 17.59 (q). Mass spectrum (*m/e*): [M^+] 198 (0), 183 (17), 165 (18), 162 (9), 148 (10), 138 (11), 122 (26), 95 (60), 93 (20), 81 (25), 79 (17), 71 (25), 67 (27), 55 (26), 43 (100), 41 (38), 39 (15). Calc. for [M^+–H_2O]: 183.1384, Found: 183.1385.

β-Eudesmol-4,14-epoxide

To a solution of 425 mg (1.91 mmol) of β-eudesmol[23] in 20 ml of MeOH was added magnesium monoperoxyphthalate (1.2 equivalents) and the mixture was stirred overnight at room temperature. After addition of 10 ml of saturated $Na_2S_2O_3$ and 10 ml of saturated $NaHCO_3$ the mixture was extracted with CH_2Cl_2 (6 x 20 ml), washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to yield a colourless oil. The crude product was purified by column chromatography to give 335 mg (1.41 mmol, 74%) of eudesmol-4,14-epoxide (α/β : 9/1) as an oil which solidified upon standing. The crude epoxide was recrystallised from diisopropylether to give the pure β-epoxide. Mp 58 – 59.5°; ^{1}H NMR: δ 2.65 (dd, 1H, *J* = 4.7 Hz, *J* = 1.8 Hz, H–14a); δ 2.46 (d, 1H, *J* = 4.7 Hz, H-14b); δ 1.98 (s, 1H, –OH); δ 1.65–1.13 (m, 14 H); δ 1.09 (s, 3H, Me–12)[b]; δ 1.08 (s, 3H, Me-13)[b]; δ 0.78 (s, 3H, Me-15); ^{13}C NMR: 72.55 (s), 59.38 (s), 50.88 (t), 48.95 (d), 47.23 (d), 41.47 (t), 40.99 (t), 35.76 (s), 35.50 (t), 27.39 (q), 26.90 (q), 22.33 (t), 21.04 (t), 20.56 (t), 16.99 (q); Mass spectrum (*m/e*) : [M^+] 238 (0.1), 220 (6), 205 (15), 165 (26), 149 (37), 147 (34), 93 (31), 91 (30), 81 (27), 79 (32), 59 (100). [b] Assignments may be interchanged

Cryptomeridiol (**12**)

To a cold suspension (–10°C) of 120 mg of $LiAlH_4$ in 10 ml dry of THF was added dropwise a solution of 300 mg of β-eudesmol-4,14-epoxide (1.35 mmol) in dry THF. The mixture was stirred overnight at room temperature, cooled to 0° and the excess $LiAlH_4$ was destroyed with Glauber's salt. After stirring for 30 minutes at room temperature, $MgSO_4$ was added and the solids were filtered off. Evaporation of the solvent yielded 292 mg (1.21 mmol, 90%) of **2** which was recrystallised from diisopropylether. Mp 135.5 – 137°; $[\alpha]_D$: –24.8° ($CHCl_3$; c 0.83); ^1H NMR: δ 1.92 (ddd, 1H, H-6, J = 12.7 Hz, J = 5.5, Hz, J = 2.5 Hz); δ 1.79 (ddt, 1H, H-3, J = 12.5 Hz, J = 3.2 Hz, J = 1.6 Hz); δ 1.20 (s, 6H, Me-12,13); δ 1.11 (d, 3H, J = 0.7 Hz, Me-14); δ 0.86 (s, 3H, Me-15); ^{13}C NMR, IR and MS data correspond with those reported in the literature[24,25,30].

Acid induced cyclisation of **11**

Typical reaction: A 10 µl solution of 0.1 M of **11** in EtOH was added to a stirred solution of 390 µl acetone and 600 µl of 1M H_2SO_4, stirred overnight at room temperature, extracted with 1 ml of water / EtOAc (1 : 1) and analysed by GC. The resulting solution contained cryptomeridiol (**12**, 31%), α-eudesmol (**13a**, 14%), β-eudesmol (**13b**, 11%), γ-eudesmol (**13c**, 42%) and probably *epi*-cryptomeridiol (**14**, 4%). Mass spectrum **14** (*m/e*): [M^+] 240 (0), 225 (1.3), 207 (8), 189 (10), 164 (40), 149 (92), 123 (31), 109 (37),108 (33), 81 (37), 71 (31), 67 (27), 59 (92), 43 (100), 41 (44).

Oxymercuration-deoxymercuration of **11**

To a stirred solution of 19 mg of **11** in 1 ml of THF was added dropwise a suspension of 30 mg of $Hg(OAc)_2$ in 1 ml of water and 1 ml of THF. After stirring for 5 min at room temperature, 1 ml of 3 M NaOH was added immediately followed by a solution of 10 mg of $NaBH_4$ in 1 ml of 3M NaOH. Mercury settled and the mixture was extracted with 4 x 10 ml of EtOAc. The organic layers were washed with 20 ml of saturated NH_4Cl and brine, dried over Na_2SO_4 and evaporated *in vacuo* to give 26 mg of a colourless oil. The mixture was analysed by GC to determine its composition. It contained cryptomeridiol (**12**, 60%), α-eudesmol (**13a**, 2%), β-eudesmol (**13b**, 9%), γ-eudesmol (**13c**, 4%) and two unidentified compounds (7% of a compound with M^+ = 220, 6% of a compound with M^+ = 204).

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