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# Biotransformation of allylically activated (E,E)-cyclodeca-1,6-dienols by *Cichorium intybus*

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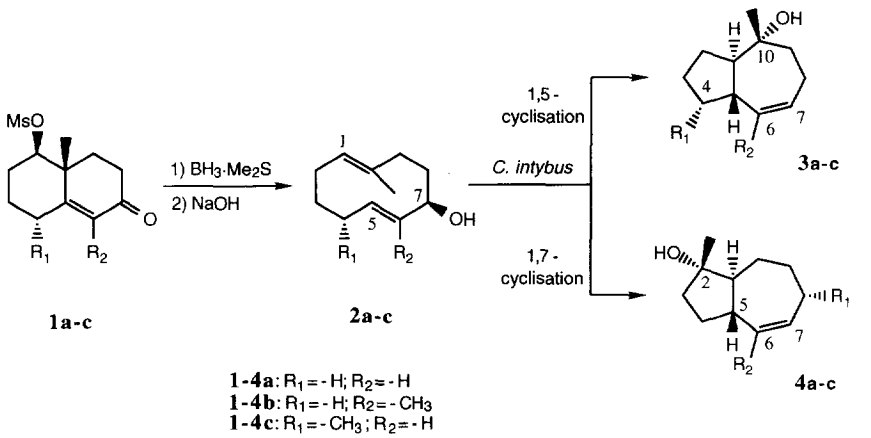
**Abstract** — The biotransformation of (E,E)-cyclodeca-1,6-dienols **2a-c** by a root suspension of fresh chicory (*Cichorium intybus*) is described. Incubation of **2a-c** gave mixtures of products arising from 1,5- or 1,7-cyclisation (**3a-c** and **4a-c**, respectively). An explanation for the obtained results is proposed together with a new biosynthetic route for the bitter principles in chicory. Copyright © 1996 Elsevier Science Ltd

## Introduction

It is widely recognised that germacrane sesquiterpenes are important intermediates in the biosynthesis of guaiane-, eudesmane- and other types of sesquiterpenes. Chicory (*Cichorium intybus*) is a well-known source of guaiane sesquiterpene lactones which give the edible chicory sprouts its distinctive bitter taste[1,2]. The roots of the chicory, extremely bitter-tasting to humans, are currently a waste product and mainly serve as cattle feed. In our previous papers we have demonstrated the applicability of a root suspension of fresh chicory as a cheap biocatalyst in the cyclisation of synthetic and natural (E,E)-cyclodeca-1,5-dienes and their epoxides[3-5]. Enzyme-mediated cyclisation of these substrates were assumed to start with protonation of either the double bond or the epoxide. We have now focused our attention on (E,E)-cyclodeca-1,6-dienes in which one of the double bonds is flanked by an alcohol group. The group of Marshall has demonstrated that solvolysis of the *p*-nitrobenzoate derivatives of these activated ten-membered rings resulted in a stereo- and regioselective cyclisation to yield hydroazulene compounds with a trans-fused framework (**3**)[6-8]. These findings indicate that an allylic leaving group is also able to initiate cyclisation of suitably functionalised cyclodecadienes. In this paper the biotransformation of the allylically substituted (E,E)-cyclodeca-1,6-dienes **2a-c** by a root suspension of fresh chicory is described. Based on these results, a new tentative biosynthetic route for the bitter principles in chicory is proposed.

## Results and discussion

The substrates for our biotransformation studies were prepared from the readily available mesylates **1a-c**[9-11]. Hydroboration followed by base-induced fragmentation gave the (E,E)-cyclodeca-1,6-dienols **2a-c** in excellent yields. Cyclisation of **2a-c** was mediated by an enzymatic activation of the allylic alcohol followed by transannular cyclisation to give mixtures of 1,7- and 1,5-cyclisation products (**3a-c** and **4a-c**, respectively), as outlined in scheme 1[12]. Incubation reactions with inactivated enzyme did not show any conversion. The formation of the 1,7-cyclisation products (**4**) was predicted in the literature but these hydroazulenes were never actually found in the solvolysis of the *p*-nitrobenzoate derivatives of **2a** and **2c**[6-8].

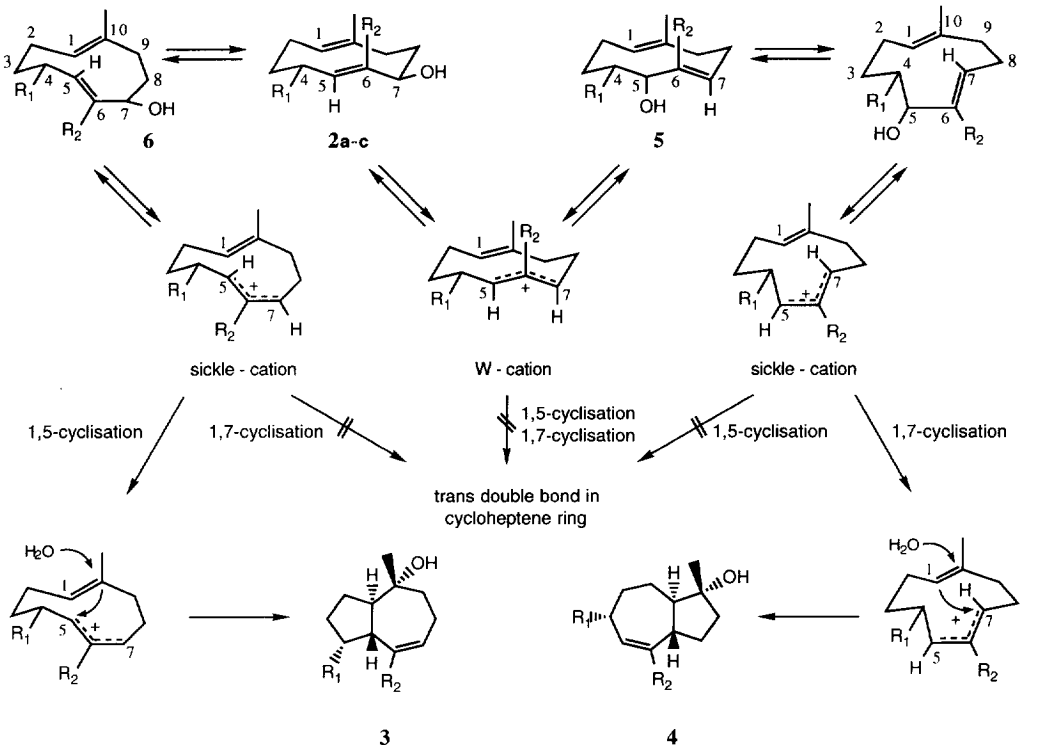



Scheme 1: Synthesis and biotransformation of **2a-c**.

Table 1.

| Precursor | Product ratio 1,5- vs 1,7-products | |
|---|---|---|
| **2a** | **3a** : **4a** | 4 : 1 |
| **2b** | **3b** : **4b** | 8 : 1 |
| **2c** | **3c** : **4a** | 3 : 1 |

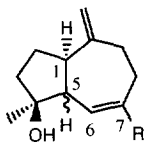
A rationalisation of the product formation is given in scheme 2. Ionisation of the allylic alcohol (**2**) would lead to the formation of an allylic cation, either sickle- or W-shaped, since the 10-membered ring system is flexible and can occur in multiple conformations through rotations around the $C_1$-$C_2$, $C_9$-$C_{10}$ and the $C_4$-$C_5$, $C_6$-$C_7$ bond. Both the 1,5- and the 1,7-cyclisation pathway through the W-cation would give a highly strained trans double bond in the cycloheptene ring. 1,7-Cyclisation through the sickle-cation would also lead to a highly strained trans double bond whereas 1,5-cyclisation would give an energetically more favourable cis double bond (**3**). The formation of the 1,7-cyclisation products can only be achieved via an enzyme mediated allylic isomerisation reaction of an allylic (E,E)-cyclodeca-1,6-dienol (**2**) into an allylic (E,E)-cyclodeca-1,5-dienol (**5**), as outlined in scheme 2. Rotation around the $C_5$-$C_6$, $C_7$-$C_8$ bond followed by ionisation of the intermediate allylic (E,E)-cyclodeca-1,5-dienol (**5**) generates a sickle-cation which will lead, after 1,7-cyclisation, to the energetically more favourable cis double bond in the cycloheptene ring (**4**).




Scheme 2: Proposed mechanism for the formation of **3a-c** and **4a-c** from **2a-c**.

The discrepancy between our findings and those reported in the literature may lie in the different reaction conditions. Solvolysis of the *p*-nitrobenzoate ester of **2a** and **2c** in a refluxing dioxane-water-$NaHCO_3$ mixture will result in a concerted cyclisation in which the $C_1$-$C_{10}$ double bond is actively involved the cyclisation process. The developing positive charge at $C_{10}$ is neutralised either by regioselective incorporation of a water molecule leading to **3** or deprotonated products[13] as reported by Marshall *et al.*[8]. In the biotransformation reaction of **2a-c**, initiated by *C. intybus*, the first step will be dissociation of the protonated allylic hydroxyl group into an intimate ion pair[14]. It is known from our earlier work that chicory root cyclising enzymes are able to stabilise a developing positive charge[5]. The prolonged lifespan of the allylic cation makes an *in situ* formation of an allylic (E,E)-cyclodeca-1,5-dienol (**5**, Scheme 2), possible in all three biotransformation reactions. The somewhat higher yield of the 1,5-cyclisation product obtained from **2b** is probably caused by steric factors. Due to steric hindrance between the methyl groups at $C_6$ and $C_{10}$ in **2b**, rotamer **6b** is preferred with respect to **5b**. This increased population of the sickle-cation leads to an increase of 1,5-cyclisation products at the expense of the 1,7-cyclisation products. The introduction of a methyl group at $C_4$ seems to have little influence on the product ratio. The stereochemistry of the 1,5-cyclisation product **3a** was established through an independent synthesis of its dihydro derivative[7]. Additional NOE-difference experiments confirmed these findings.

A detailed NMR study of the 1,7-cyclisation product **4a** was undertaken in order to establish the stereochemistry of this product. Since the 1,7-cyclisation products **4b** and **4c** are expected to be formed through




**7**: R = -CH(CH$_3$)$_2$; H$_5$ = β
**8**: R = -H; H$_5$ = α

Figure 1.

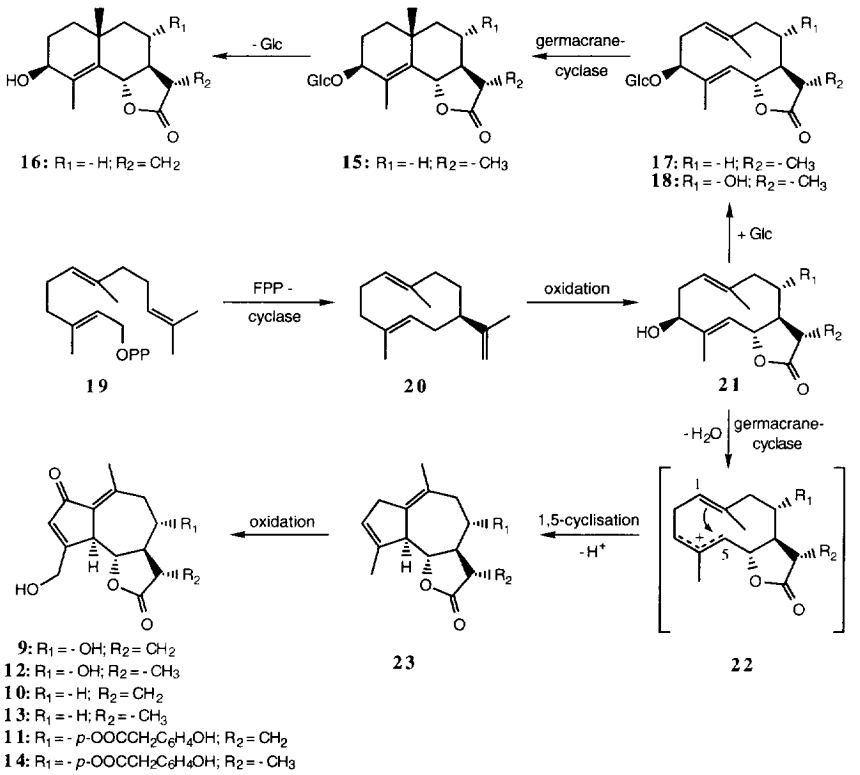
the same cyclisation pathway as that of **4a**, their stereochemistry must be identical to that of **4a**. It is known from NMR studies of alismol (**7**, Figure 1) and structurally related compounds, i.e. guaianes possessing a trans-fused ring structure and a double bond at $C_6$-$C_7$, that the *J*-value between $H_6$ and the bridgehead proton $H_5$ is relatively small: 2-3 Hz[15-17]. In a cis-fused hydroazulene ring system like that of 5-*epi*-dictamnol (**8**), $J_{6,5}$ was found to be 5.7 Hz[18]. This observation makes the coupling constant between the bridgehead proton and the olefinic proton adjacent to the bridgehead proton a useful tool in establishing the ring fusion in these hydroazulene ring systems. $J_{6,5}$ in **4a** was found to be 2.7 Hz, implying a trans-fusion of the hydroazulene ring. Additional NOE-difference experiments on $H_5$ in **4a** showed a small effect on the methyl group at $C_2$ and no effect on $H_1$, ascertaining the trans-fusion of the ring and establishing the anti-orientation between the methyl group at $C_2$ and the bridgehead proton, $H_1$.

This paper and our earlier work[3-5] shows that the germacrane cyclase from *C. intybus* possesses a broad substrate specificity since the enzyme accepts (derivatives of) (E,E)-1,5-, (E,Z)-1,6- and (E,E)-1,6-cyclodecadienes as substrates for biotransformation reactions. Its mode of action seems to consist of protonation of the most nucleophilic site of the substrate followed by stabilisation of the positively charged transition state. Depending on the conformation and configuration of the substrate, selective water incorporation or deprotonation leads to the final product. Acid-induced cyclisation studies on **2a-c** in an aqueous medium revealed an identical product formation in a ratio which was almost identical to that observed in the biotransformation studies. This means that the ring fusion of the cyclisation products appears to be dependent on the geometry and position of the double bond in the cyclodecadiene framework rather than being directed by the enzyme. Additional research towards the influence of the double bond geometry of the cyclodecadiene framework on the ring fusion of the cyclisation products, is currently being conducted at our department.

The chicory root-mediated cyclisation reactions described in this paper may be of biosynthetic importance. Chicory is a rich source of guaiane lactones i.e. lactucin (**9**), 8-deoxylactucin (**10**) and lactucopicrin (**11**), as well as their 11,13-dihydroderivatives (**12-14**)[2]. The group of Seto[19] reported the presence of two eudesmanolides sonchuside C (**15**) and cichoriolide A (**16**) together with the germacranolides sonchuside A (**17**) and cichoriolide C (**18**), as outlined in scheme 3. These germacranolides and eudesmanolides possess a hydroxyl function at $C_3$, which is sometimes glucosilated, while the guaianolides lack this function and have a carbon-carbon double bond instead. It is possible that this hydroxyl group is originally present in the biosynthetic precursors of the guaianes and is protonated by chicory root cyclases in the same way as described for **2a-c**. A new biosynthetic route for the sesquiterpene lactones from chicory, based on this hypothesis can be proposed and is presented in scheme 3.

Cyclisation of farnesyl pyrophosphate (**19**) by a specific cyclase of *C. intybus* yields germacrane A (**20**)[20] which is transformed, after several oxidation steps, into intermediate **21**. This intermediate might be the branching point in the biosynthesis of guaianolides, eudesmanolides and germacranolides. Enzyme-mediated cyclisation of **21** would start with the protonation of the $C_3$-hydroxyl group giving allylic cation **22**. This cation then might give **23** after a 1,5-cyclisation, followed by a selective deprotonation towards the bridgehead carbon

atom of the cyclised cationic intermediate. Compound **23**, which has not been detected in *C. intybus* thus far, has to be further oxidised in order to give guaianolides **9-14**. Glucosilation of the $C_3$-hydroxyl function of **21** gives sonchuside A (**17**) and cichorioside C (**18**) which may be cyclised by germacrane cyclasing enzymes into the corresponding eudesmanolides, e.g. **15**. Chicory root-mediated cyclisations of (E,E)-cyclodeca-1,5-dienes into decalins has been demonstrated before[3,4]. Presumably, glucosilation of the $C_3$-hydroxyl group prevents the 1,5-cyclisation process towards the guaianolides, although an unglucosilated eudesmanolide (**13**) has been isolated from *C. intybus*. However, **13** may be the result of glucosidase activity which has been reported in the chicory[21].




Scheme 3: Proposed biosynthesis of sesquiterpene lactones in *C. intybus*.

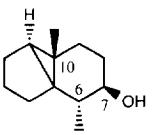
## Experimental

Melting points are uncorrected. $^1$H-NMR spectra were recorded in $CDCl_3$ (unless noted otherwise) at 200 and 400 MHz; $^{13}$C-NMR spectra were recorded at 25.3 MHz with TMS as the int standard. Analytical GC-MS was carried out as previously reported using a DB-17 column[4]. Column chromatography was performed on Merck silicagel 60 using petroleum ether (40-60) - EtOAc as the solvent system. The mesylates **1a** and **1c** were prepared according to the literature[9,11]. *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 **2a-c** 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. All substrates were incubated for 10 days after which the incubation medium was extracted with 1 ml of EtOAc. The crude mixtures were analysed by GC-MS.

**(E,E)-8-methylcyclodeca-2,7-dien-1-ol (2a).** To a solution of 7.85 g of mesylate **1a** in 300 ml of dry THF under a nitrogen atmosphere, cooled on ice, was added dropwise 40 ml of 2M $BH_3$·$Me_2S$ complex in THF. The reaction mixture was stirred overnight at room temperature after which 10 ml water was carefully added, followed by 150 ml of 4N NaOH. The resulting mixture was stirred for 1h, the organic phase was separated and the aqueous phase was extracted with 3 x 50 ml of ether. The combined organic layers were washed with 50 ml of brine, dried over $MgSO_4$ and evaporated to give 6.94 g of a yellow oil which was purified using colomn chromatography (PE-EtOAc 2:1) to yield 2.51 g of **2a** as a colourless oil. $^1$H-NMR of **2a** (200 MHz): δ 5.47 (ddd, 1H, $H_5$, $J_{5,6}$ = 15.5 Hz, $J_{5,4}$ = 10.7 Hz, $J_{5,4'}$ = 3.1 Hz), 5.19 (ddd, 1H, $H_6$, $J_{6,5}$ = 15.5 Hz, $J_{6,7}$ = 9.2 Hz, $J_{6,4}$ = 1.6 Hz), 4.88 (ddd, 1H, $H_1$, $J_{1,2}$ = 11.2 Hz, $J_{1,2'}$ = 1.3 Hz, $J_{1,C10\text{-}Me}$ = 1.3 Hz), 4.06 (ddd, 1H, $H_7$, $J_{7,8}$ = 10.7 Hz, $J_{7,6}$ = 9.1 Hz, $J_{7,8'}$ = 4.1 Hz), 2.35-1.93 (m, 6H), 1.70-1.40 (m, 5H), 1.50 (d, 3H, $C_{10}$-Me, $J_{C10\text{-}Me,1}$ = 1.3 Hz). $^{13}$C-NMR of **2a**: δ 137.21 (d), 130.82 (d), 130.70 (d), 130.15 (s), 75.72 (d), 37.44 (t), 33.43 (t), 29.18 (t), 29.00 (t), 27.33 (t), 16.69 (q). Mass spectrum of **2a** (m/e): [$M^+$] 166 (16), 148 (18), 133 (19), 124 (39), 109 (31), 107 (37), 105 (29), 97 (26), 95 (30), 93 (38), 91 (53), 83 (88), 81 (100), 80 (31), 79 (71), 77 (29), 67 (77), 55 (62), 53 (41), 43 (55), 41 (99), 39 (70).

**(E,E)-2,8-dimethylcyclodeca-2,7-dien-1-ol (2b).** To a solution of 3.85 g of mesylate **1b** in 150 ml of dry ether under a nitrogen atmosphere, cooled on ice, was added dropwise 25 ml of 2M $BH_3$·$Me_2S$ complex in THF. The reaction mixture was stirred overnight at room temperature after which 10 ml of water was carefully added followed by 50 ml of 4N NaOH. The resulting mixture was stirred for 1h, the organic phase was separated and the aqueous phase was extracted with 2 x 30 ml of ether. The combined organic layers were washed with 50 ml of brine, dried over $MgSO_4$ and evaporated to give 2.60 g of a yellow oil. GC analysis showed the presence of **2b** and the tricyclic compound **24** in a 7:3 ratio, respectively. Purification of **2b** from the reaction mixture was performed by $AgNO_3$-extraction as described[4] to yield 880 mg of **2b** and, after colomn chromatography of the residue (PE-EtOAc 2:1), 7 mg of **21**. m.p. of **2b**: 59.5-61°C. $^1$H-NMR of **2b** (200 MHz): δ 5.25 (dd, 1H, $H_5$, $J_{5,4}$ = 10.9 Hz, $J_{5,C6\text{-}Me}$ = 1.6 Hz), 4.83 (broad d, 1H, $H_1$, $J_{1,2}$ = 10.7 Hz), 4.22 (dd, 1H, $H_7$, $J_{7,8}$ = 11.0 Hz, $J_{7,8'}$ = 3.4 Hz), 2.30-2.05 (m, 6H), 1.80-1.45 (m, 5H), 1.57 (t, 3H, $C_6$-Me or $C_{10}$-Me, J = 1.4 Hz), 1.55 (t, 3H, $C_{10}$-Me or $C_6$-Me, J = 1.4 Hz). $^{13}$C-NMR of **2b**: δ 134.78 (s), 133.78 (d), 131.31 (d), 129.72 (s), 80.40 (d), 38.32 (t), 28.99 (t), 28.27 (t), 28.16 (t), 25.81 (t), 15.60 (q), 10.74 (q). Mass spectrum of **2b** (m/e): [$M^+$] 180 (9), 162 (23), 147 (29), 133 (28), 123 (19), 119 (20), 105 (35), 97 (100), 95 (40), 93 (45), 91 (34), 81 (37), 79 (46), 77 (24), 67 (66), 55 (59), 53 (40), 43 (64), 41 (99). $^1$H-NMR of **24** (200 MHz): δ 3.14 (m, 1H, $H_7$), 2.00-1.30 (m, 12 H), 0.98 (d, 3H, $C_6$-Me, $J_{C6\text{-}Me,6}$ = 6.8 Hz,); δ 0.96 (s, 3H, $C_{10}$-Me). $^{13}$C-NMR of **24**. δ 73.12 (d), 39.57 (s), 39.28 (d), 32.12 (t), 30.91 (t), 30.41 (d), 28.12 (t), 26.94 (t), 26.64 (t), 24.45 (s), 16.76 (q), 15.93 (q). Mass spectrum of **24** (m/e): [$M^+$] 180 (7), 162 (5), 147 (82), 134 (22), 133 (88), 123 (29), 121 (42), 120 (23), 119 (45), 111 (47), 108 (27), 107 (61), 106 (25), 105 (74), 95 (52), 93 (99), 90 (84), 81 (52), 79 (90), 77 (54), 67 (75), 65 (25), 55 (57), 53 (41), 43 (71), 41 (100), 39 (60).

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**(E,E)-4β,8-dimethylcyclodeca-2,7-dien-1α-ol (2c).** To a solution of 2.00g of mesylate **1c** in 100 ml of dry THF under a nitrogen atmosphere, cooled on ice, was added dropwise 15 ml of 2M $BH_3$·$Me_2S$ complex in THF. The reaction mixture was stirred overnight at room temperature after which 10 ml of water was carefully added, followed by 25 ml of 4N NaOH. The resulting mixture was stirred for 1h, the organic phase was separated and the aqueous phase was extracted with 3 x 30 ml of ether. The combined organic layers were washed with 25 ml of brine, dried over $MgSO_4$ and evaporated to give 1.36 g of a yellow oil which was purified using colomn chromatography (PE-EtOAc 2:1) to yield 267 mg of **2c** as a colourless oil. $^1$H-NMR of **2c** (200 MHz, $C_6D_6$): δ 5.19 (dd, 1H, $H_6$, $J_{6,5}$ = 15.4 Hz, $J_{6,7}$ = 8.7 Hz), 5.01 (dd, 1H, $H_5$, $J_{5,6}$ = 15.4 Hz, $J_{5,4}$ = 9.2 Hz), 4.87 (broad d, 1H, $H_1$, $J_{1,2}$ = 9.5 Hz), 3.98 (ddd, 1H, $H_7$, $J_{7,8}$ = 10.6 Hz, $J_{7,6}$ = 8.8 Hz, $J_{7,8'}$ = 4.1 Hz), 2.25-1.90 (m, 4H), 1.80-1.35 (m, 3H), 1.42 (s, 3H, $C_2$-Me), 1.20-0.95 (m, 2H), 1.28 (s, 1H, -OH), 0.92 (d, 3H, $C_4$-Me, $J_{C4\text{-}Me,4}$ = 6.6 Hz). $^{13}$C-NMR of **2c** ($CDCl_3$). δ 142.49 (d), 130.40 (s), 130.33 (d), 129.42 (d), 75.75 (d), 39.51 (d), 37.39 (t), 36.37 (t), 29.87 (t), 28.17 (t), 22.18 (q), 16.68 (q). Mass spectrum of **2c** (m/e): [$M^+$] 180 (4), 162 (3), 147 (5), 138 (9), 123 (8), 121 (10), 95 (30), 93 (21), 91 (21), 83 (50), 81 (61), 79 (34), 67 (55), 55 (57), 53 (34), 43 (45), 41 (100), 39 (66).

**Acid induced cyclisation of 2a-c.** Typical reaction: To a solution of 75 mg of **2a** in 10 ml of acetone-water (1:1) was added 5 drops of conc $H_2SO_4$. The reaction mixture was stirred overnight at room temperature and extracted with 2 x 10 ml of EtOAc. The combined organic layers were washed with 10 ml of saturated $NaHCO_3$ and 10 ml of brine, dried over $MgSO_4$, evaporated and subjected to column chromatography (PE-EtOAc 4:1) to give 15 mg of **3a** and 3 mg of **4a**. In a similar way, 50 mg of **3b** and 10 mg of **4b** was obtained from 110 mg of **2b** and 7 mg of **3c** from **2c**. The small amount of **4c** formed in the acid induced cyclisation could not be separated from **3c** due to identical $r_f$-values. $^1$H-NMR of **3a** (200 MHz, $C_6D_6$): δ 5.69 (m, 2H, $H_6$, $H_7$), 2.35-1.40 (m, 13H), 1.05 (s, 3H, $C_{10}$-Me). $^{13}$C-NMR of **3a** ($C_6D_6$): δ 136.04 (d), 129.94 (d), 74.40 (s), 54.73 (d), 42.82 (t), 41.09 (d), 34.24 (t), 27.18 (t), 24.06, (t), 23.76 (t), 21.72 (q). Mass spectrum of **3a** (m/e): [$M^+$] 166 (0.3), 148 (26), 133 (26), 119 (17), 108 (31), 93 (46), 91 (33), 81 (26), 80 (18), 79 (46), 77 (18), 67 (46), 43 (100), 41 (41), 39 (31). $^1$H-NMR of **4a** (400 MHz): δ 5.68 (dddd, 1H, $H_7$, $J_{7,6}$ = 11.6 Hz, $J_{7,8}$ = 7.4 Hz, $J_{7,8'}$ = 4.3 Hz, $J_{7,5}$ = 2.9 Hz), 5.33 (dt, 1H, $H_6$, $J_{6,7}$ = 11.6 Hz, $J_{6,5}$ = 2.7 Hz, $J_{6,8}$ = 2.7 Hz), 2.85 (dddd, 1H, $H_5$, $J_{5,1}$ = 6.1 Hz, $J_{5,4}$ = 5.8 Hz, $J_{5,7}$ = 3.0 Hz, $J_{5,6}$ = 2.5 Hz), 2.41 (m, 1H, $H_8$), 2.08-1.98 (m, 2H), 1.85 (ddd, 1H, $H_{10}$, $J_{10,10'}$ = 14.3 Hz, $J_{10,9}$ = 11.1 Hz, $J_{10,9'}$ = 3.3 Hz), 1.75-1.50 (m, 7H), 1.28 (s, 1H, -OH), 1.23 (s, 3H, $C_2$-Me). $^{13}$C-NMR of **4a**: δ 134.84 (d), 130.29 (d), 74.53 (s), 54.46 (d), 39.72 (d), 35.59 (t), 35.10 (t), 31.20 (q), 25.80 (t), 23.25 (t), 22.04 (t). Mass spectrum of **4a** (m/e): [$M^+$] 166 (0.7), 148 (26), 133 (36), 119 (25), 108 (31), 106 (15), 105 (27), 93 (52), 92 (19), 91 (48), 81 (25), 80 (26), 79 (52), 76 (23), 67 (48), 55 (17), 53 (17), 43 (100), 41 (48), 39 (36). $^1$H-NMR of **3b** (200 MHz): δ 5.48 (m, 1H, $H_7$), 2.20 (m, 2H), 2.00-1.30 (m, 11H), 1.66 (broad s, 3H, $C_6$-Me), 1.16 (s, 3H, $C_{10}$-Me). $^{13}$C-NMR of **3b**: δ 141.09 (s), 125.30 (d), 75.33 (s), 52.50 (d), 44.79 (d), 42.55 (t), 31.94 (t), 26.35 (t), 25.06 (t), 22.96 (t), 22.57 (q), 21.81 (q). Mass spectrum of **3b** (m/e): [$M^+$] 180 (0.3), 162 (30), 147 (51), 133 (60), 120 (19), 119 (22), 107 (27), 106 (18), 105 (38), 95 (22), 93 (31), 81 (22), 79 (45), 77 (23), 67 (36), 55 (24), 53 (19), 43 (100), 41 (47), 39 (30). $^1$H-NMR of **4b** (200 MHz): δ 5.54 (ddd, 1H, $H_7$, $J_{7,8}$ = 7.5 Hz, $J_{7,8'}$ = 5.8 Hz, $J_{7,5}$ = 1.6 Hz), 2.52 (m, 1H), 2.28 (m, 2H), 2.05 (m, 2H), 1.95-1.40 (m, 8H), 1.78 (s, 3H, $C_6$-Me), 1.12 (s, 3H, $C_2$-Me). $^{13}$C-NMR of **4b**: δ 143.35 (s), 123.03 (d), 75.00 (s), 51.83 (d), 44.72 (d), 35.63 (t), 33.24 (t), 30.31 (q), 29.93 (t), 26.54 (q), 26.05 (t), 22.60 (t). Mass spectrum of **4b** (m/e): [$M^+$] 180 (0.2), 162 (31), 147 (42), 133 (44), 122 (28), 120 (17), 119 (30), 107 (38), 105 (51), 94 (50), 93 (38), 91 (38), 79 (52), 76 (26), 67 (38), 55 (28), 43 (100), 41 (53), 39 (33). $^1$H-NMR of **3c** (200 MHz): δ 5.71 (m, 2H, $H_6$, $H_7$), 2.35-1.30 (m, 12H), 1.20 (s, 3H, $C_{10}$-Me), 0.85 (d, 3H, $C_4$-Me, $J_{C4\text{-}Me,4}$ = 6.9 Hz). $^{13}$C-NMR: δ 132.87 (d), 130.50 (d), 75.25 (s), 51.01 (d), 44.51 (d), 42.63 (t), 37.31 (d), 32.86 (t), 24.14 (t), 23.61 (t), 21.72 (q), 15.45 (q). Mass spectrum of **3c** (m/e): [$M^+$] 180 (0.1), 162 (8), 147 (10), 122 (11), 107 (15), 81 (27), 79 (29), 67 (19), 55 (16), 53 (15), 43 (100), 41 (38), 39 (28). Mass spectrum of **4c** (m/e): [$M^+$] 180 (4), 165 (8), 163 (9), 149 (15), 123 (26), 109 (50), 95 (42), 91 (18), 81 (57), 67 (54), 55 (54), 53 (28), 43 (24), 41 (100), 39 (56).

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