Synthesis of Decaline Analogues of Isovelleral

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Decaline analogues of the bioactive fungal sesquiterpene (+)-isovelleral (1a), retaining the bicyclo[4,1,0]hept-2-en-1,2-dicarbaldehyde system, were prepared, and their cytotoxic and antimicrobial activities were compared with those of the natural product. While the two isomers (±)-2 and (±)-3 were as active as isovelleral (1a), the isomer (±)-4 was approximately 10 times less potent.

**Key words:** Bioactive Sesquiterpenoids, Unsaturated 1,4-Dialdehydes, Isovelleral

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### Introduction

Isovelleral (1a), an extremely pungent sesquiterpene isolated from the fruit bodies of *Lactarius vellereus*, possesses several potent biological activities [1]. It is formed enzymatically from an inactive precursor in seconds as a response to injury to the fruit body, apparently to protect it from parasites [2]. The unsaturated 1,4-dialdehyde moiety, which also is present in a number of other terpenoids claimed to be part of natural defence systems, is thought to be responsible for the biological activities of 1a [3]. However, the cyclopropane ring of 1a may also be involved in the biological activity. When 1a was allowed to react with a primary amine in ethanol the dialdehyde was transformed to a pyrrol ring and the cyclopropane was opened by the solvent as it is slightly activated towards nucleophilic attack [4]. When the activities of the two previously prepared bicyclic analogues 5 and 6 were compared, 5, with the methyl group attached to the cyclopropane ring, was approximately 10 times less potent than 6, lacking this methyl [4]. The structures are shown in Fig. 1.

This would be expected if a nucleophilic attack on the methylated cyclopropane carbon (C3) is part of the molecular mechanism by which isovelleral (1a) exerts its activity. The isovelleral analogue lacking the methyl groups, trimethylisovelleral (1b) was consequently prepared and found to be approximately 10 times as cytotoxic towards common tumour cell lines as isovelleral itself [5]. In addition, as 1a is considerably more potent that 5, the cyclopentane ring of isovelleral also appears to contribute to the biological activity. It has been shown for other terpenoid unsaturated dialdehydes that the dihedral angle between the two aldehyde groups in the unsaturated dialdehyde moiety is correlated with the antimicrobial activity [6]. The cyclopentane ring of 1a would certainly affect the positioning of the aldehyde groups in space by altering the conformation of the six-membered ring, however, it is not evident that the best effect is accomplished with a cyclopentane ring. The purpose of this study was therefore to prepare and assay decalin analogues of isovelleral (e.g. 2), with a cyclohexane ring instead of a cyclopentane and lacking methyl groups.

### Results and Discussion

The decalin analogues 2, 3, and 4 were synthesized according to the procedure described in Scheme 1, starting from commercially available hydroxytetralin 7.

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Fig. 1. Analogues of isovelleral.
Birch reduction of the methyl ether and acidic hydrolysis afforded the bicyclic enone 8 [7, 8], which was transformed into an epimeric mixture of the ester 9 after treatment with LDA followed by reaction with methylcyanoformate [9]. Hydrogenation of 9 resulted in a cis/trans mixture of the saturated β-keto ester from which the major cis isomers (10) could be obtained in 48% yield after chromatography on silica gel. The lack of selectivity, which is in contrast to the hydrogenation of indanes, can be explained by the reversed facial discrimination of the two epimers, and the lowest energy conformers of both epimers are shown in Fig. 2.

The oxidation of 10 to the α, β-unsaturated β-keto ester with PhSeCl and hydrogen peroxide, preceding the cyclopropanation, also generated isomeric mixtures [10, 11], as the allylic bridgehead proton was prone to epimerisation and the initially desired cis compound was to some degree transformed to the thermodynamically more stable trans compound (ΔE = 6 kJ/mol). However, this was not considered a problem as we desired several stereoisomers of the final products for determining structure-activity relations. By not isolating the α, β-unsaturated β-keto ester, but instead treating it immediately with the cyclopropanation reagent [12], the epimerisation gave approximately a 1:1 mixture of cis and trans decalines, and while the cyclopropanation of the cis compound was stereoselective, as expected, adding from the least hindered face.

The lowest energy conformer of each compound was determined by a conformational search. Calculations were performed using MacroModel v8.6 (Force field: MMFFs; Solvent: Octanol (using the analytical Generalized Born/Surface-Area (GB/SA) model); Minimization method: TNCG; Conformational search: MonteCarlo (MCMM); Steps: 2000). The energy was then determined by equilibrium geometry *ab initio* calculation (6-31G**) using MacSpartan-Pro.

Scheme 1. Reaction conditions: (a) i) MeI, K₂CO₃, DMF, 55 °C; ii) Li, NH₃, EtOH, THF, −78 °C; iii) 10% HCl, MeOH, 66% (3 steps); (b) DIPA, n-BuLi, MeO₂CCN, THF, −78 °C, 69%; (c) HCO₂NH₄, 10% Pd/C, MeOH, reflux; (d) i) PhSeCl, pyridine, 30% H₂O₂, CH₂Cl₂; ii) Me₂SOCH₂, THF, 20 °C, 54%; (e) DIPA, n-BuLi, PhNTf₂, THF, 77%; (f) Pd(OAc)₂, PPh₃, CO, MeOH, Et₃N, DMF, 20 °C, 90%; (g) DIBAL-H, THF, 75%; (h) oxalyl chloride, DMSO, Et₃N, CH₂Cl₂, −78 °C, 78%.

![Scheme 1](image_url)
Table 1. Dihedral angles and distances of relevant conformers of 1a–4:  

<table>
<thead>
<tr>
<th>Compound (conformer)</th>
<th>ΔE</th>
<th>Dihedral angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a(1)</td>
<td>0</td>
<td>39.2</td>
</tr>
<tr>
<td>1a(2)</td>
<td>4.21</td>
<td>34.2</td>
</tr>
<tr>
<td>2(1)</td>
<td>0</td>
<td>54.5</td>
</tr>
<tr>
<td>2(2)</td>
<td>11.68</td>
<td>30.7</td>
</tr>
<tr>
<td>3(1)</td>
<td>0</td>
<td>30.8</td>
</tr>
<tr>
<td>3(2)</td>
<td>15.49</td>
<td>31.9</td>
</tr>
<tr>
<td>4(1)</td>
<td>0</td>
<td>56.3</td>
</tr>
<tr>
<td>4(2)</td>
<td>23.93</td>
<td>53.9</td>
</tr>
</tbody>
</table>

a Calculations were performed using MacroModel v8.6 (Force field: MMFFs; Solvent: Water (using the analytical Generalized Born/Surface-Area (GB/SA) model); Minimization method: TNCG; Conformational search: MonteCarlo (MCM/MM); Steps: 2000); b the two lowest conformers, no rotamers, were taken into consideration; c energy is given in kJ/mol; d the dihedral angle between the two carbonyl carbons.

On the other side, the cyclopropanation of the trans compound gave a 1:1 mixture of cyclopropane stereoisomers. This resulted in a 2:1:1 mixture of cyclopropanes (as determined by NMR), and the remaining steps were carried out without separating the isomers. Introduction of the second ester group [13] and the oxidation/reduction sequence to transform the ester groups to aldehyde groups [14, 15] followed essentially a previously described procedure developed for the total synthesis of (+)-isovelleral [16], affording a mixture of the three decalin-type dialdehydes 2, 3 and 4 as racemates. Final purification by HPLC gave the pure compounds for characterisation and biological assays. The structure and relative stereochemistry of each dialdehyde was determined by NMR spectroscopy.

The cytotoxic and antimicrobial activity of the new dialdehydes 2, 3 and 4 were compared with those of 1a. Previously, it has been shown that the antimicrobial and cytotoxic activities of the two enantiomers of isovelleral (1) are comparable [17], and it is reasonable to compare the potency of the racemic compounds 2, 3 and 4 with the natural product (+)-isovelleral (1a). The in vitro cytotoxicity towards the two cell lines L1210 (lymphocytic leukaemia mouse) and Colo 320 (human colon adenocarcinoma) showed that the cis isomer 2 and the trans isomer 3 are as potent as the natural product (IC50-values 0.1 μg/ml towards L1210 and 1 μg/ml towards Colo 320), while the trans isomer 5 was 10 times less potent. The antimicrobial activity towards the bacteria Bacillus subtilis, B. brevis, Micrococcus luteus and Enterobacter dissolvens, and the fungi Mucor miehei, Paecilomyces varioti, Penicillium notatum and Nematospora coryli was comparable for compounds 1a, 2 and 3, and in agreement with those already reported for isovelleral (1a) [4], while compound 4 was significantly less potent. The reason for this difference is not clear, but it has been shown that the dihedral angle between the aldehyde groups is important for the antimicrobial activity of other terpenoid unsaturated 1,4-dialdehydes [6]. The dihedral angles of the most stable conformers of compounds 1a–4 were calculated with MacroModel, and the results are shown in Table 1. Isovelleral (1a) has several low energy conformers below 12 kJ/mol, compound 2 has two while compounds 3 and 4 have only one (excluding rotamers). The more active compounds 1a–3 all have at least one low energy conformer in which the dihedral angle between the two aldehyde groups is approximately 30°, while this angle is almost doubled in the less active compound (4).

Conclusion

Decaline analogues of the fungal sesquiterpene isovelleral (1a) were synthesised and shown to be cytotoxic and antimicrobial agents, two of them as potent as isovelleral itself. However, the removal of the methyl group adjacent to the cyclopropane did not result in a 10-fold increase in the activity, as observed for 6 compared to 5 and 1b compared to 1a. Obviously, the six-six-three fused system of the compounds investigated here is less suitable for isovelleraloids with high activity, compared to the natural five-six-three fused variant.

Experimental Section

Materials were obtained from commercial suppliers and were used without further purification unless otherwise noted. THF was dried by refluxing over sodium/benzophenone ketyl immediately prior to use. CH2Cl2 and triethylamine were distilled from calcium hydride prior to use. DMF and DMSO were distilled under reduced pressure and kept over 4 Å MS. MeOH was dried by distilling from magnesium/iodine. All moisture and air-sensitive reactions were carried out under an atmosphere of dry nitrogen using oven-dried glassware. ESI-MS spectra (H3PO4 for calibration and as internal standard) were recorded with a Micromass Q-Tof Micro spectrometer, while EIMS (direct inlet, 70 eV) and CI spectra (direct inlet, methane) were recorded with a JEOL SX102 spectrometer. The NMR spectra (in CDCl3) were recorded with a Bruker DRX 400 spectrometer at 400 MHz (1H) and at 100 MHz (13C) and with a Bruker DRX 500 spectrometer at 500 MHz (1H) and at 125 MHz (13C), in CDCl3 or C6D6. Chemical shifts are given in ppm relative to TMS us-
ing the residual CHC\textsubscript{1} peak in CDCl\textsubscript{3} solution as internal standard (7.26 and 77.00 ppm, respectively relative to TMS) or using the residual C\textsubscript{6}H\textsubscript{5}D\textsubscript{2} peak in C\textsubscript{6}D\textsubscript{6} solution (7.16 and 128.06 ppm, respectively). Organic extracts were dried over MgSO\textsubscript{4}. All flash chromatography was performed on 60 Å 3 5–70 μm Matrex silica gel (Grace Amicon). TLC analyses were made on Silica Gel 60 F\textsubscript{254} (Merck) plates and visualised with anisaldehyde/sulphuric acid and heating.

HPLC separations were performed using a Dynamax SD-200 solvent delivery pump system with a Varian Microsorb HPLC separations were performed using a Dynamax SD- and visualised with anisaldehyde/sulphuric acid and heating.

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from 1H NMR (400 MHz, CDCl\textsubscript{3}): δ = 26.90, 27.30, 29.53, 34.80, 35.90, 36.87, 38.23, 124.61, 167.74, 200.36. – MS (El, 70 eV): m/z (%) = 150 (61) [M\textsuperscript{+}], 122 (60), 79 (34), 77 (13). – HRMS (El): C\textsubscript{10}H\textsubscript{12}O calcd. 150.1045 [M\textsuperscript{+}], found 150.1047.

3-Oxo-1,3,5,6,7,8,9-octahydrooctahydronaphthalene-2-carboxylic acid methyl ester (9). To a solution of 10.8 ml (76.2 mmol) of diisopropylamine in 55 ml of THF, cooled to −78 °C, was added drop wise 7.8 ml (73.1 mmol, 9.4 M in hexanes) of n-BuLi. After 20 min a solution of 9.30 g (61.9 mmol) of the enone 8 in 40 ml of THF was added slowly. The solution was warmed to 0 °C after 20 min and stirred for 1 h at 0 °C. The solution was cooled to −78 °C and 7.95 ml (103.4 mmol) methylcyanofomate was added quickly. The solution was allowed to warm to room temperature and stirred for 40 min. 100 ml brine and 100 ml of ether were added and the layers separated.

The water layer was extracted with 100 ml ether and the combined ether layers were washed with 250 ml brine, dried and concentrated. The oil was purified with flash chromatography (H/E 10:1) to give 8.90 g (42.7 mmol, 69%) of a 3:1 epimeric mixture of the ester 9. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, major isomer): δ = 1.20 (m, 1H), 1.44 (m, 2H), 1.91 (m, 3H), 2.20 (m, 2H), 2.43 (m, 2H), 3.37 (m, 1H), 3.76 (s, 3H), 5.84 (s, 1H). – \textsuperscript{13}C\textsubscript{1}H\textsubscript{3} NMR (100 MHz, CDCl\textsubscript{3}, major isomer): δ = 25.73, 26.91, 32.50, 34.91, 35.51, 37.62, 52.57, 53.49, 167.78, 171.46, 194.50. – MS (El, 70 eV): m/z (%) = 208 (55) [M\textsuperscript{+}], 177 (18), 148 (42), 122 (100), 94 (26), 91 (23). – HRMS (El): C\textsubscript{12}H\textsubscript{16}O\textsubscript{3} calcd. 208.1099 [M\textsuperscript{+}], found 208.1102.

3-Oxodecahydronaphthalene-2-carboxylic acid methyl ester (10). 6.3 g (30.3 mmol) of the methyl ester 9 was dissolved in 580 ml of MeOH with 315 mg of 10% palla-
dium on carbon. 9.55 g (151.5 mmol) of ammonium formate was added and the reaction mixture was refluxed under N\textsubscript{2} for 30 min and then allowed to cool to room temperature. The reaction mixture was filtered through a bed of celite which was washed with MeOH. The solvent was removed under reduced pressure and the remains were dissolved in 500 ml ether and 500 ml water. The layers were separated and the aqueous layer was extracted with 500 ml of ether. The combined organic layers were dried and concentrated. Re-
crystallisation from heptane gave 3.08 g (14.5 mmol, 44%) of the trans isomers. The cis isomers were purified with flash chromatography to give 2.80 g (13.3 mmol, 40%): \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, major cis isomer): δ = 0.94 (m, 1H), 0.99 (m, 1H), 1.14 (m, 1H), 1.27 (m, 2H), 1.27 (m, 1H), 1.73 (m, 2H), 1.76 (m, 2H), 1.78 (m, 1H), 2.00 (dd, J = 16.9, 11.2 Hz, 1H), 2.09 (dd, J = 17.0, 5.1 Hz, 1H), 2.41 (dd, J = 17.0, 5.3 Hz, 1H), 3.68 (s, 3H), 12.1 (s, 1H). – \textsuperscript{13}C\textsubscript{1}H\textsubscript{3} NMR (100 MHz, CDCl\textsubscript{3}, major cis isomer): δ = 26.03, 26.22, 31.62, 33.37, 33.41, 37.50, 38.33, 38.86, 50.41, 91.59, 156.23, 170.52. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, major trans isomer): δ = 0.99 (m, 1H), 1.15 (m, 1H), 1.27
portions with 10 min intervals. 10 min after the last H2O2

MS (EI, 70 eV): (0.19 mmol, 43% from
purified by flash chromatography (H/E 4:1) to give 43 mg
with brine, dried and concentrated. The crude product was
with ether and the combined etheral phases were washed
phases were separated. The aqueous phase was extracted
The solution was diluted with both ether and water and the
was stirred for 40 min at
Θ
C. 510 mg (12.16 mmol) of NaF followed by 3 ml
of water were added to the cooled solution. The reaction
mixture was diluted with 30 ml of water and extracted twice
with 30 ml of ethyl acetate. The combined organic extracts
were dried and concentrated. Flash chromatography (H/E 4:1, 1% EtOH) afforded 59 mg (75%) of

2-Trifluoromethanesulfonyloxy-1,3a,4,5,6,7a,7b-octahydro-1aH-cyclopropa[a]naphthalene-1a-carboxylic acid methyl ester (11). 1.60 g (8.32 mmol) of PhSeCl was dissolved in 80 ml of CH2Cl2 under N2 and cooled to 0 °C. To the cooled solution was added 732 µl
(9.07 mmol) of pyridine and after 20 min of stirring 1.55 g
(7.56 mmol) of

in 16 ml of CH2Cl2 was added drop wise. After 30 min the reaction mixture was extracted with
2 × 40 ml of 10% aqueous HCl and cooled in an ice-bath. A solution of 30% aqueous H2O2 was added in 515
portions with 10 min intervals. 10 min after the last H2O2
addition 40 ml of water was added and the phases were
separated. The organic phase was washed with saturated aqueous NaHCO3, dried and concentrated giving 1.71 g of

crude product, which was used without further purification in the next step.

To 100 mg of the crude product was added 800 µl
(0.04 mmol, 0.5 M in THF) of dimethylsulfoxonium methylide. The cloudy white-yellowish solution was stirred at room temperature under nitrogen atmosphere for 40 min. The solution was diluted with both ether and water and the phases were separated. The aqueous phase was extracted with ether and the combined etheral phases were washed with brine, dried and concentrated. The crude product was purified by flash chromatography (H/E 4:1) to give 43 mg
(0.19 mmol, 43% from
of an isomeric mixture of

– MS (EI, 70 eV): m/z (%) = 222 (87) [M]+, 190 (64),

2-Trifluoromethanesulfonyloxy-1,3a,4,5,6,7a,7b-octahydro-1aH-cyclopropa[a]naphthalene-1a-carboxylic acid methyl ester (12). To an ice-cold solution of 237 µl
(1.68 mmol) of disobutylboron in 1.25 ml of THF was added drop wise 800 µl
(1.9 M in cyclohexane) of n-BuLi under a nitrogen atmosphere. After 10 min the solution was cooled to −78 °C and 308 mg (1.39 mmol) of

11 dissolved in 0.63 ml of THF was added drop wise. The reaction mixture was stirred for 40 min at
−78 °C when 536 mg (1.50 mmol) of

in 1.5 ml of THF was added. The resulting solution was allowed to reach room temperature and stirred for 1 h. The reaction mixture was then diluted with 10 ml saturated aqueous NaHCO3 and extracted with 10 ml of ether. The organic phase was washed with water, brine, dried and concentrated. The crude product was purified by flash chromatography (H/E 4:1, 1% EtOH) to give 379 mg (1.07 mmol, 77%) of

– MS (EI, 70 eV): m/z (%) = 534 (35) [M]+, 323 (19), 297 (8), 253 (6),
221 (52), 189 (100), 161 (48), 145 (22), 91 (38), 79 (22). – HRMS (EI): C14H17F3O5S calcd. 354.0749 [M]+, found 354.0758.

1,3a,4,5,6,7a,7b-Octahydro-1aH-cyclopropa[a]naphthalene-1a,2-dicarboxylic acid dimethyl ester (13). 348 mg
(0.94 mmol) of

triethylamine, 1.59 ml (39.2 mmol) MeOH, 6.6 mg (0.03 mmol) of palladium acetate and 15.4 mg (0.06 mmol) of triphenylphosphine were dissolved in 4 ml of DMF. CO was bubbled through the solution and the reaction mixture was stirred for 2 h under a CO atmosphere at room temperature. The mixture was diluted with 40 ml of ether, washed with 40 ml of water and 40 ml brine, dried and concentrated. Flash chromatography (H/E 4:1, 1% EtOH) gave 282 mg
(quant) of


(2-Hydroxymethyl-1,3a,4,5,6,7a,7b-octahydro-1aH-cyclopropa[a]naphthalene-1a-yl)-methanol (14). 100 mg
(0.38 mmol) of

was dissolved in 1 ml of THF under a nitrogen atmosphere and cooled to −78 °C and 3.0 ml of (1.0 M in hexane) of DIBAL-H was added dropwise. The solution was stirred at room temperature for 30 min and then cooled to 0 °C. 510 mg (12.16 mmol) of NaF followed by 3 ml
of water were added to the cooled solution. The reaction mixture was diluted with 30 ml of water and extracted twice with 30 ml of ethyl acetate. The combined organic extracts were dried and concentrated. Flash chromatography (H/E 1:1, 0.1% MeOH) afforded 59 mg (75%) of

– MS (EI, 70 eV): m/z (%) = 208 (18) [M]+, 159 (100), 105 (100), 91 (100), 79 (62). – HRMS (EI): for C13H20O2 calcd. 208.1463 [M]+, found 208.1470.

1,3a,4,5,6,7a,7b-Octahydro-1aH-cyclopropa[a]naphthalene-1a-2-dicarbaldehyde (2, 3 and 4). To a solution of 54 µl
(0.76 mmol) of DMSO in 1 ml of CH2Cl2 at −78 °C was added 26 µl
(0.30 mmol) of oxalyl chloride. The solution was stirred for 15 min when 15.8 mg (0.08 mmol) of
in 1 ml of CH2Cl2 was added. After 30 min stirring at −78 °C, 254 µl
(1.82 mmol) of triethylamine was added. The stirring was continued for an additional 30 min before the reaction mixture was allowed to reach room temperature. After 20 min stirring at room temperature the mixture was diluted with 10 ml of water and 10 ml of ether. The phases were separated and the aqueous phase was extracted with 10 ml of ether. The combined ether extracts were dried and concentrated. Flash chromatography (H/E 8:1) afforded 12 mg (80%) of the isomeric mixture. The isomers were separated on normal phase HPLC in the same system, to afford 5 mg of
2, 2.5 mg of 3 and 2.5 mg of
4.
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(1H), 2.38 (m, 1H), 6.87 (d, J = 1.9 Hz, 1H), 9.58 (s, 1H), 9.80 (s, 1H). $^1$H NMR (125 MHz, CDCl$_3$): δ = 20.11, 23.34, 24.87, 26.12, 29.85, 30.12, 30.39, 33.45, 33.81, 142.04, 153.82, 192.48, 199.40. – HRMS (CI): C$_{13}$H$_{17}$O$_2$ [M+H]$^+$, calcd. 205.1229, found 205.1223.

3: $^1$H NMR (500 MHz, C$_6$D$_6$): δ = 0.53 (dd, J = 7.0, 4.4 Hz, 1H), 0.65 (m, 1H), 0.78 (m, 2H), 0.87 (m, 2H), 0.94 (m, 1H), 0.99 (m, 1H), 1.33 (m, 2H), 1.50 (m, 2H), 1.92 (dd, J = 8.8, 4.4 Hz, 1H), 6.50 (d, J = 2.0 Hz, 1H), 9.56 (s, 1H), 9.96 (s, 1H). $^{13}$C NMR (125 MHz, C$_6$D$_6$): δ = 18.28, 20.13, 26.41, 30.65, 31.73, 35.07, 36.11, 37.95, 45.53, 139.92, 153.16, 192.69, 199.60. – HRMS (CI): C$_{13}$H$_{17}$O$_2$ [M+H]$^+$, calcd. 205.1229, found 205.1235.

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