Isolation and Structure Determination of the Precursors of α- and γ-Irone and Homologous Compounds from Iris pallida and Iris florentina

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Rhizomes of Iris pallida and Iris florentina contain — increasing on storage — violet-like smelling C_{14}-ketones (irones 1a–c) which develop by oxidative degradation of C_{3},triterpenoids. The structure of these precursors is reported as well as the structure of respective C_{30}-homologues. The unusual C_{13}-structures may derive biogenetically from the respective C_{30} compounds by a methylation which initiates the formation of the ring-closed irone-moiety.

Introduction

The characteristic violet-like smelling compounds of the essential oil of rhizomes of Iris florentina and Iris pallida have long been known to be the three isomeric irones (1a–c) [1].

It is well established that these ketones do not occur in freshly harvested plants but develop over years by a slow process — presumably an oxidative degradation of precursor-molecules [2]. In a previous study we showed that the dihydroirones (2a–b) are formed upon treatment of lipid-extracts of the rhizomes of Iris germanica L. with oxidative agents. The structure of their precursors was found to be α- and γ-irigermanal (3a and 3b), respectively [3].

Materials and Methods

Plant material

Rhizomes of Iris pallida were a kind gift of Dr. Steiner (Bonn) and were harvested in the garden of the Institut für Pharmakognosie, Universität Bonn, in the autumn of 1978 and 1979.

Rhizomes of Iris florentina were obtained by Bornträger & Schlemmer OHG, D-6521 Offstein, W.-Germany, in the fall of 1977 and 1981 as well as in the spring of 1981. If not used immediately the rhizomes were stored in a cold room (+ 4 °C).
Extraction and isolation procedure

Extraction of 1 kg of the rhizomes yielded between 10 and 30 g of crude extract [4] which was fractionated on silicagel using a petrolether/chloroform/acetone/methanol gradient. Final purification of the compounds was achieved by low-pressure liquid chromatography on a Merck Lobar Lichro-prep RP 8-column using methanol/water (80:20 or 90:10) as the eluent.

The compounds isolated amounted to 0.5% of the fresh weight of the rhizomes of \( I. \) florentina and to 1% in case of \( I. \) pallida.

Analytical Methods

The purity of the compounds was determined by HPLC using a Kontron model 200 HPLC-system equipped with a Kontron 720 LC uv-monitor and reversed phase (RP18)-columns.

Gas chromatographic separations were carried out on a Carlo Erba 2900 capillary column GC equipped with WCOT columns (50 m, 0.35 mm i.d.) coated with OV 61 and Ucon 75 H 90 000 respectively.

Mass spectra were recorded on a Finnigan MAT 4510 mass spectrometer. For the chemical ionisation (Cl) and negative chemical ionisation (NCI) experiments CH\(_4\) or NH\(_3\) were used as reactant gases.

Except for the 400 MHz-spectra \( ^1 \)H- and \( ^13 \)C-NMR-spectra were obtained on a Varian EM 390 and a Varian CFT 20, respectively. Chemical shifts are reported in \( \delta \)-units (ppm) relative to Me\(_4\)Si (\( \delta 0 \)).

UV-spectra were determined on a Varian Cary 14 spectrometer and optical rotations were measured on a Zeiss 0.005° precision polarimeter.

Spectral properties

**Iripallidal** (5) formed a glasslike solid: UV-spectrum (ethanol): \( \lambda_{\text{max}}(\epsilon) \) 238 (29 500). Mass-spectrum (El, 70 eV): 486 (M\(^+\)), 468, 450, 439, 428, 416, 398, 358, 343, 331, 313, 304. \([\text{M}+2\text{H}]^{2+} \) 7.4° (CH\(_2\)Cl\(_2\), \( c = 19.0 \)).

\( ^1 \)H-NMR (CDCl\(_3\), 400 MHz): \( \delta 10.25 \) (s, 1 H), 5.96 (d, 15.5 Hz, 1 H), 5.43 (m, 1 H), 5.35 (dd, 10.3 Hz, 15.5 Hz, 1 H), 5.16 (t, 6.8 Hz, 1 H), 4.08 and 3.93 (AB-system, 11.2 Hz, 2 H), 3.66 (m, 1 H), 3.57 (m, 2 H), 2.8–1.0 (19 H), 1.85 (s, 3 H), 1.64 (s, 3 H), 1.50 (s, 3 H), 1.32 (s, 3 H), 0.85 (d, 6.8 Hz, 3 H), 0.83 (s, 3 H), 0.64 (s, 3 H).

Decoupling experiments proved the following signals to be coupled to each other: 5.35 and 5.96, 5.35 and 2.35, 4.08 and 3.93.

Upon addition of Eu(fod)\(_3\) the following shifts could be observed (CDCl\(_3\), 90 MHz): 10.25 to 10.5, 4.08 to 5.23, 3.93 to 5.08, 3.66 to 4.14, 1.85 to 2.07 and 1.32 to 1.59.

\( ^{13} \)C-NMR (CDCl\(_3\), 400 MHz): \( \delta 190.2 \) (d), 162.8 (s), 137.3 (d), 134.4 (s), 134.1 (s), 133.1 (s), 129.2 (d), 128.4 (d), 121.6 (d), 76.3 (s), 68.1 (t), 62.4 (t), 56.4 (d), 46.8 (s), 42.9 (d), 38.2 (d), 37.1 (t), 35.7 (s), 35.7 (t), 32.2 (t), 32.0 (t), 27.3 (t), 26.6 (q), 26.4 (q), 24.0 (t), 23.1 (q), 21.7 (t), 15.7 (q), 14.8 (q), 12.6 (q), 11.0 (q).

From the results of NMR- and mass-spectra the molecular formula C\(_{23}\)H\(_{30}\)O\(_4\) was assigned.

Oxidative degradation (see below) gave a 30% yield of \( \alpha \)-irone (1a).

**Iriflorental** (6) (a glasslike solid): UV-spectrum (ethanol): \( \lambda_{\text{max}}(\epsilon) \) 237.5 (26 900). Mass-spectrum (El, 70 eV): 486 (M\(^+\)), 468, 450, 428, 413, 331, 304. Mass-spectrum (Cl, CH\(_4\)): 487 (M + 1).

High resolution mass measurement: 486.3706. C\(_{23}\)H\(_{30}\)O\(_4\) requires: 486.3709 \([\alpha]_{\text{D}}^{20} \) + 47.5° (CH\(_2\)Cl\(_2\), \( c = 13.5 \)).

\( ^1 \)H-NMR (CDCl\(_3\), 400 MHz): \( \delta 10.25 \) (s, 1 H), 5.93 (d, 15.5 Hz, 1 H), 5.64 (dd, 15.5 Hz, 9.7 Hz, 1 H), 5.15 (m, 1 H), 4.72 (s, 1 H), 4.48 (s, 1 H), 4.07 and 3.92 (AB-system, 11.1 Hz, 2 H), 3.64 (m, 1 H), 3.57 (m, 2 H), 2.8–1.0 (22 H), 1.84 (s, 3 H), 1.68 (s, 3 H), 1.31 (s, 3 H), 0.85 (s, 3 H), 0.84 (d, 6.5 Hz, 3 H), 0.64 (s, 3 H).

\( ^{13} \)C-NMR (CDCl\(_3\), 400 MHz): \( \delta 190.2 \) (d), 162.9 (s), 150.6 (s), 136.8 (d), 134.1 (s), 133.1 (s), 129.6 (d), 126.6 (d), 107.8 (t), 76.3 (s), 68.1 (t), 62.3 (t), 58.3 (d), 46.8 (s), 42.9 (d), 42.3 (d), 38.7 (s), 37.1 (t), 36.5 (t), 35.6 (t), 32.2 (t), 32.2 (t), 27.7 (q), 27.3 (t), 26.3 (q), 24.0 (t), 21.7 (t), 16.1 (q), 14.3 (q), 12.6 (q), 11.0 (q).

Oxidation with KMnO\(_4\)/crown-ether (see below) yielded 12% of \( \gamma \)-irone (1c).

**Desoxy-iripallidal** (7) was found in trace amounts in the extracts of \( I. \) pallida and always came together with \( \alpha \)-irigermalin (3a). We were not able to separate the two compounds. The mixture showed the following properties: Mass-spectrum (Cl, CH\(_4\)): \( m/e \) 471 (M + 1, compound 7), \( m/e \) 473 (M + 1, compound 3a). \( ^1 \)H-NMR-spectrum (CDCl\(_3\),
90 MHz): (see Fig. 3, top). Except for the known signals for (3a) [3] the additional double bond showed up at 6.05 (d, 15 Hz, 1H) and 5.4 (m, 1H).

Upon oxidation with KMnO$_4$/crown ether $\alpha$-dimethylhydroirone (2a) and $\alpha$-irone (1a) were found in a 9:1 ratio.

Iso-iridogermanal (8a) came as a glasslike solid: UV-spectrum (ethanol): $\lambda_{\text{max}}(\epsilon)$: 256 nm (17 400). Mass-spectrum (EL, 70 eV): $m/e$ 456 (M-H$_2$O), 446, 387, 369, 351, 337, 319, 301. (NCI, CH$_4$): $m/e$ 474 (M$^+$). $[\alpha$$]_{D}^{20}$: + 34.4° (CH$_2$Cl$_2$, c = 0.9).

$\text{H}$-NMR-spectrum (CDCl$_3$, 400 MHz): $\delta$10.3 (s, 1H), 5.23 (t, 7.1 Hz, 1H), 5.06 (q, 7 Hz, 1H), 3.91 (t, 6.6 Hz, 1H), 3.56 (t, 6.3 Hz, 2H), 3.30 (d, 10.6 Hz, 1H), 2.6 (m, 1H), 2.05 to 2.17, 1.82 to 1.67, 1.61 (d, 1.2 Hz, 3H), 1.59 (d, 1.2 Hz, 3H), 1.53 (d, 1.2 Hz, 3H), 1.14 (s, 3H), 1.09 (s, 3H).

Decoupling experiments proved the following signals to be coupled to each other: 5.23 and 1.53, 5.06 and 1.67/1.57, 1.57 to 1.95, 1.14 to 1.35 and 1.09 to 1.40.

Upon oxidation of Eu(fod)$_3$ the following shifts could be observed (CDCl$_3$, 90 MHz): 10.3 to 11.0, 5.23 to 5.90, 5.06 to 5.45, 5.06 to 5.15, 3.91 to 4.72, 3.56 to 5.45, 3.30 to 3.95, 2.57 to 3.25, 2.21 to 2.80, 2.05 to 2.17, 1.82 to 1.74, 1.61/1.59 to 1.82/1.65, 1.53 to 1.95, 1.14 to 1.35 and 1.09 to 1.40.

$\text{C}$-NMR-spectrum (CDCl$_3$, 400 MHz): $\delta$190.2 (d), 163.4 (s), 138.1 (s), 137.1 (s), 133.0 (s), 131.4 (s), 125.5 (d), 124.2 (d), 120.1 (d), 76.8 (d), 74.8 (s), 62.7 (t), 44.8 (s), 43.6 (d), 39.8 (t), 37.0 (t), 34.2 (t), 32.6 (t), 26.8 (t), 26.6 (t), 26.1 (q), 25.6 (q), 23.1 (t), 21.8 (t), 17.9 (q), 17.7 (q), 16.3 (q), 11.8 (q), 10.9 (q). Upon oxidation with KMnO$_4$/crown ether 6-methyl-5-heptene-2-one (9) and 6,10-dimethylundeca-5,9-diene-2-one-3-ol (10) are found (see below).

From the spectral properties the molecular formula C$_{25}$H$_{30}$O$_4$ could be assigned.

$\text{21}$-Desoxy-iridogermanal (8b) (glasslike solid): UV-spectrum (ethanol): $\lambda_{\text{max}}(\epsilon)$: 255 nm (11 250). Mass-spectrum (EL, 70 eV): $m/e$ 440 (M-H$_2$O), 374, 356, 331, 317, 310. (NCI, CH$_4$): $m/e$ 458 (M$^+$). $[\alpha$$]_{D}^{20}$: + 34.4° (CH$_2$Cl$_2$, c = 0.9).

$\text{H}$-NMR-spectrum (CDCl$_3$, 400 MHz): $\delta$10.3 (s, 1H), 5.09 (t, 4.5 Hz, 1H), 5.07 (t, 4.5 Hz, 1H), 4.97 (t, 5 Hz, 1H), 3.6 (t, 5.4 Hz, 2H), 3.32 (m, 1H), 2.6 to 3.0 (2H), 1.83 (3H), 1.67 (3H), 1.59 (3H), 1.57 (3H), 1.52 (3H), 1.16 (3H), 1.09 (3H).

$\text{10}$-Desoxy-iridogermanal (8c) was isolated as a glass-like solid: UV-spectrum (ethanol): $\lambda_{\text{max}}(\epsilon)$: 256 nm (13 500). Mass-spectrum (EI, 70 eV): $m/e$ 440 (M-H$_2$O), 374, 356, 331, 317, 310. (NCI, CH$_4$): $m/e$ 458 (M$^+$). $[\alpha$$]_{D}^{20}$: + 33.4° (CH$_2$Cl$_2$, c = 4.2).

$\text{H}$-NMR-spectrum (CDCl$_3$, 90 MHz): $\delta$10.2 (s, 1H), 5.12 (m, 2H), 4.96 (t, 7.5 Hz, 1H), 4.38 (dt, 6.9 Hz, 7.5 Hz, 1H), 3.57 (t, 6.9 Hz, 1H), 3.38 (t, 8.4 Hz, 1H), 2.8 to 2.0 (20H), 1.80 (3H), 1.70 (3H), 1.67 (3H), 1.62 (3H), 1.50 (3H), 0.95 (3H), 0.82 (d, 6.3 Hz, 3H).

$\text{1C}$-NMR-spectrum (CDCl$_3$, 400 MHz): $\delta$190.0 (d), 163.6 (s), 134.7 (s), 134.4 (s), 133.1 (s), 131.4 (s), 128.3 (d), 127.5 (d), 124.7 (d), 65.7 (d), 62.5 (t), 48.1 (t), 43.2 (d), 40.0 (s), 39.4 (t), 35.6 (d), 31.7 (t), 31.3 (t), 30.4 (t), 27.3 (t), 26.3 (t), 25.7 (q), 24.1 (q), 23.9 (t), 21.0 (t), 18.1 (q), 16.1 (q), 15.7 (q), 15.2 (q), 10.7 (q).

Oxidative degradations

The oxidative cleavage of the compounds was carried out following the procedure of Sam and Simmons [5]: 0.7 mmol of the triterpenoid was dissolved in 40 ml of benzene and 36 mg (0.097 mmol) of dicyclohexano-18-crown-6 (EGA Chemie GmbH, D-7924 Steinheim, W.-Germany) was added. Within 8 h 460 mg (2.91 mmol) KMnO$_4$ was added in portions at room-temperature. The reaction mixture was stirred overnight, the benzene was distilled off, and after filtration the residue was chromatographed on silicagel using a pentane/pentane-ether (9:1) gradient as the eluent.

If possible, the compounds isolated were compared with commercially available substances by GC/MS and retention-indices:

Natural Iris-oil (Essence Iris Absolue) was obtained by P. Kaders, Hamburg, W.-Germany, and consisted of 60% $\alpha$-irone (1a) and 40% of the $\gamma$-isomer (1c), which were separated by preparative GLC (column: 2.5 m x 4 mm i.d., 20% PEG 4M on Chromosorb P, 60–80 mesh, 175 °C).

6-Methyl-5-heptene-2-one (9) was obtained by EGA-Chemie GmbH, D-7924 Steinheim, W.-Germany.

For the synthesis of $\alpha$-dimethylhydroirone (2a) see [3]. 6,10-Dimethyl-undeca-5,9-diene-2-one-3-ol (10) was identified by its mass-spectrum (EI, 70 eV): $m/e$ 210 (M$^+$), 192, 177, 167, 165, 149, 141, 137, 123, 109, 95, 81, 74, 69, 55, 53, 45, 42, 40, 38.
Upon addition of N-methyl-N-trimethyl-silyl-trifluor-acetamide (MSTFA) the trimethylsilyl ether was formed. Mass-spectrum (EI, 70 eV): m/e 282 (M+), 264, 239, 192, 169, 157, 155, 149, 146, 130, 107, 93, 81, 73, 69, 45, 43, 41.

Oxidation of iso-iridogermanal (8a) with CrO3:2Pyr. Dipyridine-chromium(VI)-oxide oxidation [6] of 8a was carried out as follows: 0.45 ml pyridine were dissolved in 10 ml CH2Cl2. 279 mg (2.79 mmol) CrO3 was added, and the solution was stirred at room-temperature for 15 min. A solution of 132 mg (0.278 mmol) 8a was then added in one portion. After stirring at room-temperature for additional 30 min the CH2Cl2 was evaporated in vacuo. The residue was eluted with ether and the product purified by low-pressure liquid chromatography on a reversed phase column using methanol/water (80:20) as the eluent to yield 17.8 mg (13.6%).

The product showed the following spectral properties: UV-spectrum (ethanol): λmax(ε): 240 nm (11 000), 255 nm (sh). Mass-spectrum (EI, 70 eV): m/e 470 (M+), 455, 452, 397, 333, 315, 306, 301.

In the 1H-NMR-spectrum (CDCl3, 90 MHz) a new aldehyde signal appeared at δ 9.71 and one of the olefinic protons was shifted to δ 6.40.

Results and Discussion

Two compounds were isolated from Iris pallida as well as I. florentina and were named iripallidal (5) and iriflorental (6) after their main-occurrence in the respective species. Already on contact with air iripallidal releases α-irone (1a) and iriflorental γ-irone (1c) as opposed to the irigermanals (3a, 3b) which are quite stable under these conditions.

From their spectral properties it was evident that the irone-precursors isolated had to be similar in their overall structure to the substances from I. germanica L. However, in addition, in both iripallidal and iriflorental one double-bond and one extra hydroxy-group had to be present since 13C- and mass-spectra indicated a formula of C31H50O4. As mentioned above on oxidation the compounds yield α- or γ-irone (1a or 1c) respectively; thus the additional double-bond has to be in the irone-moiety between C-16 and C-17. This fits perfectly with the 1H-NMR signals for the protons of this double bond at δ 5.95 and 5.35 (Fig. 1, middle: iripallidal) or δ 5.93 and 5.64 (Fig. 1, bottom: iriflorental). The coupling of the two protons (J = 15.5 Hz) indicates an E-geometry.

Compared to the irigermanals the signal for one methyl-group is missing in the NMR-spectra of iripallidal and iriflorental which, instead, show an AB-system for two protons at δ 4.08 and 3.93 (4.07 and 3.92 respectively, see Fig. 1, middle and bottom). Clearly one methyl-group has been oxidized to a CH2OH-group.

Since the methyl-group in β-position to the aldehyde-function still is present, either C-26 or C-27 had to bear the hydroxy-function. As all attempts of a glycol-cleavage failed which should have resulted in the formation of a keto-group in C-10, in case C-26 was the CH2OH, we assigned it to C-27. Consequently iripallidal has structure 5 and iriflorental structure 6.
Extracts from *Iris pallida* contained in trace amounts a compound which was identical with iripallidal except for the missing hydroxy-group in C-27 (desoxy-iripallidal (7)).

Part of the 90 MHz-spectrum of 7 is shown in Fig. 3 (top).

From their $^{13}$C- and mass-spectra the two other compounds isolated from *I. pallida* as well as *I. florentina* extracts appear to be isomers of iridogermanal (4). As shown in Fig. 2, the difference between 4 (top) and iso-iridogermanal (8a) (middle) is in the position of the secondary hydroxy-group in the side-chain.

The signal at $\delta$ 3.91 indicates that the proton next to the OH is coupled only to a CH$_2$ and not to an olefinic proton as in iridogermanal. Thus five possible positions had to be considered for the hydroxy-group viz. C-8, C-9, C-12, C-16 or C-20. When recorded in presence of Eu(fod)$_3$, the proton-NMR of 8a results in a large shift of one olefinic proton from $\delta$ 5.23 to $\delta$ 5.9 and a somewhat smaller shift of another olefinic proton from $\delta$ 5.06 to 5.45. Therefore only C-16 or C-20 could bear the oxygen-function. This was confirmed by oxidation of 8a with CrO$_3$-bipyridyl-complex [6]. A dramatic shift of the NMR-signal at 5.23 to 6.40 proved the appropriate proton to be now in $\beta$-position of an $\alpha$, $\beta$ unsaturated carbonyl-group: the alcohol function had been oxidized to a ketone. Final confirmation that C-16 was the CHOH-group was achieved by oxidative degradation of 8a with KMnO$_4$, as 6-methyl-5-heptene-2-one (9) and 6,10-dimethyl-undeca-5,9-diene-2-one-3-ol (10) were formed.

Thus the structure of iso-iridogermanal has been established as 8a.

\[
\begin{align*}
&\text{Fig. 2. 400 MHz-$^1$H-NMR spectra of iridogermanal (4), iso-iridogermanal (8a) and 21-desoxy-iridogermanal (8b).} \\
&\text{Fig. 3. 90 MHz-$^1$H-NMR spectra of desoxy-iripallidal (7) and 10-desoxy-iridogermanal (8c).}
\end{align*}
\]
Table I. Occurrence of triterpenoids in different *Iris* species.

<table>
<thead>
<tr>
<th>Main-products (&gt;10%)</th>
<th>Side-products (1-10%)</th>
<th>Traces (&lt;1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. germanica</em> 3a, 3b, 4</td>
<td>8b, 8c</td>
<td>5, 6</td>
</tr>
<tr>
<td><em>I. pallida</em> 5</td>
<td>8a, 3a</td>
<td>7</td>
</tr>
<tr>
<td><em>I. florentina</em> 6</td>
<td>8a</td>
<td>5, 8b</td>
</tr>
</tbody>
</table>

The other isomer of 4 and 8a is the desoxy-compound 8b. The 'H-NMR (Fig. 2, bottom) shows the absence of any OH in the side chain. All other signals of 4 are still present. Furthermore the mass-spectrum proves the absence of oxygen by the molecular ion m/e 458, 16 mass units less than the M⁺ of 4 and 8a (m/e 474).

The same molecular weight and elemental composition has been found for an additional isomer of iridogermanal which was isolated in trace amounts from rhizomes of *Iris germanica* L. As can be taken from Fig. 3 by the signal at δ 4.38 (Fig. 3, bottom), this compound still possesses the OH-function in C-21. Since the terminal CH₂OH (δ 3.57) was still present too, the oxygen at C-10 had to be missing. Thus the structure of 10-desoxy-iridogermanal is 8c.

Table I shows the occurrence of all triterpenoids isolated from the three species. It is evident that the distribution is quite characteristic and may be used for chemotaxonomic purposes of *Iris* species. We found a seasonal dependence of isomer distribution only in the case of *I. germanica* as reported previously [3]. A precursor for β-irone (1b) was not found in any of the species. We therefore assume that β-irone derives by isomerization of the α- or γ-isomer.

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[4] For a detailed description of the extraction-procedure see [3].