Isolation and Structural Determination of a New Methylated Triterpenoid from Rhizomes of *Iris versicolor* L.

Wolfgang Krick, Franz-Josef Marner, and Lothar Jaenicke
Institut für Biochemie der Universität zu Köln, An der Bottmühle 2, D-5000 Köln 1,
Bundesrepublik Deutschland

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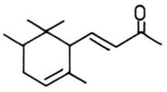
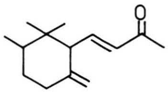
A monocyclic C_{31}-triterpenoid was isolated from rhizomes of *Iris versicolor* L. Its structure was elucidated as **12** by spectroscopy combined with oxidative degradation. The identity of the degradation-products was proven by comparison with synthetic references.

Introduction

The two isomeric irones **1 a – b** are constituents of the essential oil of *Iris florentina* and *Iris pallida* [1].

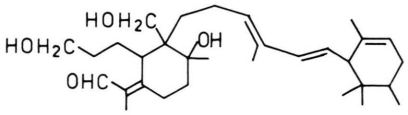


α-Irone(1a)

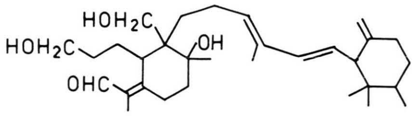


γ-Irone(1b)

In a previous communication we showed that these two irones develop on oxidative cleavage of the methylated triterpenoids iripallidal (**2a**) or its isomer iriflorental (**2b**) respectively [2].

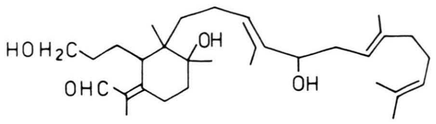


2a



2b

Their possible precursor on the biogenetic pathway from squalene is the C_{30}-compound isoiridogermanal (**3**) which was found in rhizomes of *Iris pallida* as well as *Iris florentina* [2].



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The cyclisation of the irone-moiety may be initiated by methylation of the isopropenyl-group of **3**. In this paper we will describe the isolation and structural determination of iriversical, the first monocyclic C_{31}-triterpenoid to be found in rhizomes of *Iris* species.

Materials and Methods

Plant material

Rhizomes of *Iris versicolor* L. were obtained by Bornträger & Schlemmer OHG, D-6521 Offstein, W.-Germany in May 1982.

Extraction and isolation procedure

Extraction of 950 g of the rhizomes with methanol and chloroform as described in [3] yielded 54 g of crude extract 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 Lichroprep RP-8 column using a methanol/water (80/20)-methanol gradient as the eluent [3].

Iriversical amounted to 0.2% of the fresh weight of the rhizomes. Isoiridogermanal (**3**) and 21-desoxy-iridogermanal [2] were found in trace amounts. The triterpenoids form colorless, lacquerlike solids.

Analytical methods

The purity of the compounds isolated was determined by HPLC on a Kontron Model 200 HPLC-system equipped with a Kontron model 720 uv-monitor and reversed phase (RP 18) columns.

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For the analytical gas-chromatography a Carlo Erba 2900 capillary gas-chromatograph equipped with WCOT glass-capillary columns (50 m × 0.35 mm) coated with OV 61 and Ucon 75 H 90000 respetively was used.

Mass spectra were recorded using a Finnigan MAT 4510 GC/MS-system.

Proton-NMR-spectra were obtained on a Varian EM 390. Chemical shifts were reported in δ-units (ppm) relative to Me_4Si ($\delta 0$).

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

Spectral properties of iriversical (**12**)

UV spectrum (ethanol): λ_{max} (ε): 256 nm (15200). Mass spectrum (EI, 70 eV): *m/e* 472 (M^+), 454, 439, 429, 409, 395, 383, 370, 357, 345, 332, 331, 329, 308, 303, 301.

Mass spectrum (CI, CH_4): 473 $(M+1)^+$. $[\alpha]^{20}_{578}$: +37° (CH_2Cl_2, c 5.2).

^1H-NMR spectrum ($CDCl_3$, 90 MHz): δ10.25 (s, 1H), 5.09 (m, 1H), 4.98 (t, 6.8 Hz, 1H), 4.71 (s, 1H), 4.66 (s, 1H), 3.60 (t, 6.3 Hz, 2H), 3.30 (dm, 11 Hz, 1H), 2.7–1.0 (21H), 1.84 (s, 3H), 1.60 (s, 3H), 1.52 (s, 3H), 1.18 (s, 3H), 1.09 (s, 3H), 1.02 (d, 7.2 Hz, 6H).

^{13}C-NMR spectrum ($CDCl_3$, 400 MHz): δ190.1 (d), 163.3 (s), 155.9 (s), 135.3 (s), 134.9 (s), 132.9 (s), 124.0 (d), 123.4 (d), 106.1 (t), 74.9 (s), 62.7 (t), 44.7 (s), 43.3 (d), 39.5 (t), 38.4 (t), 37.2 (t), 36.8 (t), 33.7 (d), 33.0 (t), 32.5 (t), 26.6 (t), 26.4 (t), 26.0 (q), 23.8 (t), 22.0 (t), 21.8 (q), 21.8 (q), 17.8 (q), 16.0 (q), 15.9 (q), 10.8 (q).

Oxidation of iriversical (**12**) *with* $KMnO_4$*/crownether*

A solution of 9.5 mg (0.06 mmol) $KMnO_4$ and 29.6 mg (0.06 mmol) dicyclohexano-18-crown-6 in 3 ml benzene was added dropwise at room-temperature to a solution of 9.6 mg (0.02 mmol) iriversical (**12**) in 2 ml benzene [4]. The reaction mixture was stirred at room-temperature for 6 h. After filtration the products **4** and **5** were identified by gas-chromatographic and mass-spectrometric comparison with synthetic references (see below).

6-Methylheptane-2,5-dione (**4**)

Mass spectrum (EI, 70 eV): *m/e* 142 (M^+), 127, 99, 98, 71, 57, 55, 43, 41, 39.

Kováts-indices: Ucon 75 H 90000 (130 °C): 1459.8 ± 0.6, OV 61 (110 °C): 1084.8 ± 0.4.

5-Methylene-6-methylheptan-2-one (**5**)

Mass spectrum (EI, 70 eV): *m/e* 140 (M^+), 125, 122, 107, 97, 83, 82, 81, 79, 71, 69, 67, 58, 55, 54, 43, 41, 39.

Kováts-Indices: Ucon 75 H 90000 (110 °C): 1292.4 ± 0.4, OV 61 (110 °C): 1044.9 ± 0.3.

6,10-Dimethyl-9-methylene-5-undecen-2-one (**6**)

Mass spectrum (EI, 70 eV): *m/e* 208 (M^+), 190, 175, 165, 150, 135, 123, 107, 95, 83, 82, 81, 79, 67, 55, 53, 43, 41, 39.

Synthesis of 6-Methylheptane-2,5-dione (**4**) *and 5-methylene-6-methylheptan-2-one* (**5**)

5,6-Epoxy-6-methylheptan-2-one (**8**)

23.46 g 6-methyl-5-hepten-2-one (0.186 mol) was added to a solution of 0.193 mol perbenzoic acid in 275 ml ether at 0 °C. After standing at 0 °C for 16 h the solution successively was washed with 10% aqueous NaOH and water. The epoxide **8** was isolated in 63% yield (16.75 g).

Mass spectrum (EI, 70 eV): *m/e* 142 (M^+), 127, 114, 109, 100, 99, 85, 84, 83, 72, 71, 59, 58, 57, 55, 43, 41, 39.

^1H-NMR spectrum ($CDCl_3$, 90 MHz): δ2.73 (t, 7.2 Hz, 1H), 2.60 (t, 8.1 Hz, 2H), 2.18 (s, 3H), 1.80 (m, 2H), 1.30 (s, 3H), 1.27 (s, 3H).

6-Methylheptane-2,5-diol (**9**)

10.09 g $LiAlH_4$ (0.27 mol) were added at 0 °C to a solution of 11.81 g $AlCl_3$ (0.088 mmol) in 200 ml of dry ether. The solution was stirred for 30 min at room-temperature [5].

16.75 g epoxide **8** (0.12 mol) dissolved in 100 ml ether was added dropwise at such a rate that the temperature of the solution was maintained at 30 °C. After stirring for an hour at room-temperature the reaction mixture was hydrolyzed with wet ether and water at 0 °C.

Extraction with ether, drying over $MgSO_4$ and evaporation of the solvent yielded 16.3 g (94%) of a mixture of the 2,5- and the 2,6-diol (**9** and **9a**) in a

1:2 ratio. The crude mixture was used for the next step without further purification.

Mass spectrum (EI, 70 eV) **9**: *m/e* 128 (M-18)$^+$, 113, 103, 95, 86, 85, 73, 71, 69, 67, 58, 57, 56, 55, 45, 43, 41, 39.

Mass spectrum (EI, 70 eV) **9a**: *m/e* 131 (M-15)$^+$, 113, 103, 95, 84, 77, 73, 71, 69, 59, 58, 57, 56, 55, 45, 43, 42, 41, 39.

^{1}H-NMR-spectrum ($CDCl_3$, 90 MHz) **9** and **9a**: δ3.78 (m), 3.32 (m), 1.85–1.30 (m's), 1.50 (s), 1.24 (s), 1.20 (d, 7 Hz), 0.93 (d, 8 Hz).

6-Methylheptane-2,5-dione (4)

To a solution of CrO_3-pyridinum-complex prepared from 103.9 g pyridine (1.32 mol) and 66 g CrO_3 (0.66 mol) in 500 ml CH_2Cl_2 according to [6] a solution of 8 g of the diol-mixture **9** and **9a** (54.8 mmol) in 50 ml dichloromethane were added at once. The mixture was stirred for 30 min. The solution was decanted, and the brown residue was washed with 50 ml CH_2Cl_2. After evaporating the CH_2Cl_2 the residue was dissolved in ether. Insoluble material was filtered off and the filtrate was evaporated again.

The crude **4** was purified by chromatography on silicagel using a petrolether/ether-gradient as the eluent. Yield: 1.4 g (57.5% based on **9**. Mass spectrum (EI, 70 eV): see above.

Kováts-indices: Ucon 75 H 90000 (130 °C) 1459.8 ± 0.7, OV 61 (110 °C) 1084.0 ± 0.6.

^{1}H-NMR spectrum ($CDCl_3$, 90 MHz): δ2.70 (s, 1 H), 2.60 (m, 1 H), 2.18 (s, 4 H), 1.10 (d, 8.1 Hz, 6 H).

2-(1,3-Dioxolane)-6-methylheptan-5-one (10)

A solution of 810 mg of the diketone **4** (5.7 mmol), 0.39 g 1,2-dihydroxyethane (6.27 mmol) and 40 mg *p*-toluenesulfonic acid in 20 ml $CHCl_3$ was refluxed at the water separator for 2 h.

The solution was washed with 2 N NaOH and water, dried over $MgSO_4$, and the solvent was evaporated to give a 3:1 mixture of **10** and its isomeric 5-dioxolane **10a**. The mixture was used without further purification for the subsequent Wittig-reaction.

Mass-spectrum (EI, 70 eV) **10**: *m/e* 171 (M-15)$^+$, 143, 126, 111, 99, 87, 71, 55, 43.

Mass spectrum (EI, 70 eV) **10a**: *m/e* 143 (M-43)$^+$, 126, 115, 99, 71, 55, 43, 41.

5-Methylene-6-methylheptan-2-one (5)

A slurry of 8.9 g methyl-triphenylphosphonium-iodide (22 mmol) in 100 ml THF was treated at room-temperature with 42.3 ml of a 0.53 N solution of *n*-butyllithium in pentane (22.4 mmol). After 15 min at room-temperature a solution of 0.82 g of the ketal-mixture **10** and **10a** in 50 ml THF was added. After 3 h the reaction-mixture was hydrolyzed with 50 ml water and extracted with ether. The organic phase was dried over $MgSO_4$ and evaporated.

Mass spectrum (EI, 70 eV) **11**: *m/e* 169 (M-15)$^+$, 139, 133, 122, 107, 99, 87, 81, 67, 55, 43.

The crude product was dissolved in 50 ml acetone, 5 ml 1 N H_2SO_4 are added, and the solution was stirred at room-temperature for 3 h.

The acetone was evaporated and the residue extracted with ether. After drying over $MgSO_4$ and evaporation of the solvent the residue was chromatographed on silicagel using a petrolether/ether gradient. Final purification of **5** was achieved by preparative GC (2 m × 4 mm 15% DEGS on Chromosorb 60–80 mesh). A total of 9 mg of the ketone **5** was obtained. Mass-spectrum (EI, 70 eV): see above.

Kováts-indices: Ucon 75 H 90000 (110 °C) 1293.7 ± 0.9, OV 61 (110 °C) 1044.4 ± 0.3.

^{1}H-NMR spectrum ($CDCl_3$, 90 MHz): δ4.80 (s, 1 H), 4.62 (s, 1 H), 2.7–2.2 (m, 5 H), 2.17 (s, 3 H), 1.00 (d, 8 Hz, 6 H).

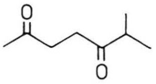
Results and Discussion

As seen from NMR- and mass-spectra, iriversical has the formula $C_{31}H_{52}O_3$. Comparison of the NMR-data of this triterpenoid with the compounds previously isolated from *Iris* rhizomes proved the irone-moiety to be missing but the substituted cyclohexane-system at the other end of the molecule to be present. In contrast to isoiridogermanal **3**, the open chain end of the molecule did not bear any hydroxy-group but, instead, one additional carbon. Three double-bonds had to be present in that part of the molecule. Two of these double-bonds are trisubstituted, one substituent being a methyl-group (δ1.52 and δ1.60 respectively). The third double-bond apparently was an olefinic methylene-group, the protons of which showed up at δ4.66 and δ4.71 in the ^{1}H-NMR.

The appropriate ^{13}C-NMR-signals are found at δ106.1 (t) and δ155.9 (s).

The isopropylidene-group of isoiridogermanal **3** is saturated in this compound because the methyl-groups at δ1.02 are coupled to a methine-group at δ2.1.

Oxidative degradation of iriversical with $KMnO_4$/crown-ether resulted in the formation of three compounds. Two of them were identified as 6-methylheptane-2,5-dione **4** and 5-methylene-6-methylheptan-2-one **5** by GC/MS and comparison with synthetic material.



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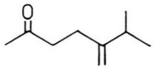

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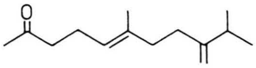
The third oxidation product presumably is 6,10-dimethyl-9-methylen-5-undecen-2-one **6** since the mass-spectrum shows a molecular ion at *m/e* 208. No further attempts have been made to verify this structure.



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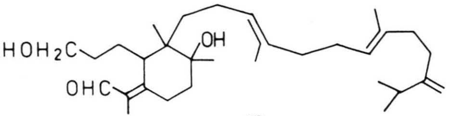
The synthesis of the diketone **4** and the ketone **5** followed the route outlined in scheme 1.

Epoxidation of 6-methyl-5-hepten-2-one **7** followed by reduction of the epoxide **8** with AlH_3 gave a mixture of the two diols **9** and **9a**. Oxidation of the diol mixture yielded the diketone **4** which was easily separated from unwanted products and proved identical with the corresponding oxidation-product from iriversical.

Upon condensation of the diketone **4** with dihydroxyethane the desired dioxolane **10** was formed in favour of the product **10a**. Wittig-reaction of **10** and **10a** with triphenylphosphonium-methylene and subsequent hydrolysis of the dioxolanes **11** and **11a** yielded a mixture of the two ketones **5** and **5a** which were separated by preparative GLC.



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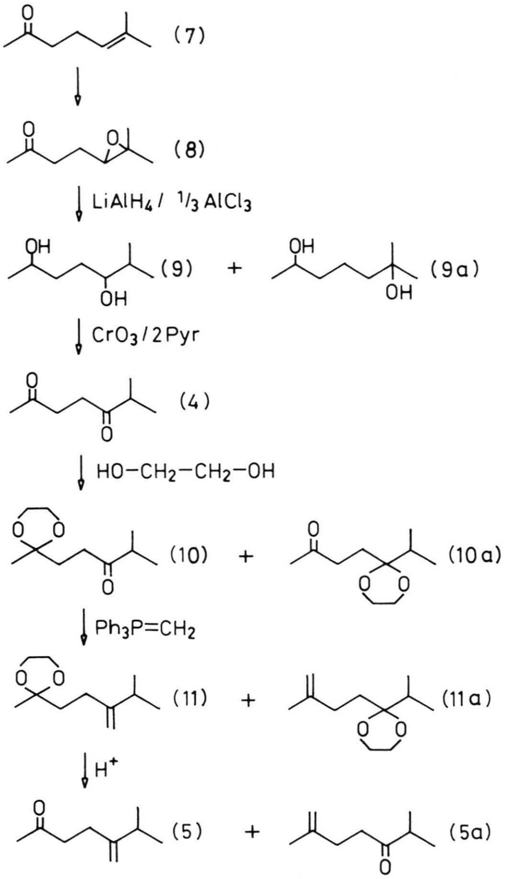

Scheme 1

Product **5** proved identical with the corresponding oxidation-product of iriversical.

Thus, from the unequivocally identified fragments **4** and **5**, the structure of iriversical is **12**.

Acknowledgements

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