37. On the Stereochemistry of Natural Irones, Dihydroirones, and their Precursors

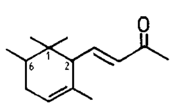
by **Wolfgang Krick, Franz-Josef Marner** and **Lothar Jaenicke***
Institut für Biochemie der Universität, An der Bottmühle 2, D-5000 Köln 1

(24.VIII.83)

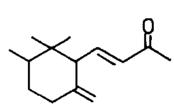
Summary

Natural irones from the essential oil of *Iris* rhizomes develop by oxidative degradation of C_{31}-triterpenoids produced by the plant. Two enantiomeric forms of irones are found in *Iris* oils of different origin. The optical properties and CD spectra of irones, dihydroirones and their C_{31}-precursors are reported and their absolute stereochemistry is determined.

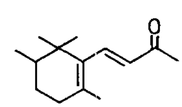
1. Introduction. – In following the biosynthesis of the irones **1a** to **1c** in the rhizomes of various *Iris* species we found their precursors to be a novel class of triterpenoids which, by oxidation, yield the irones or related compounds [1–3]. Thus, *Iris pallida* and *Iris florentina* contain iripallidal (**2**) and iriflorental (**3**), respectively, which already in contact with air release the typical violet-like scent of the ketones **1a** and **1b**.



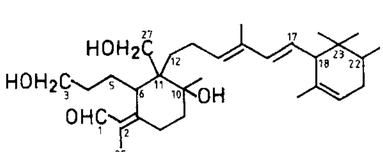
α-Irone **1a**



γ-Irone **1b**



β-Irone **1c**



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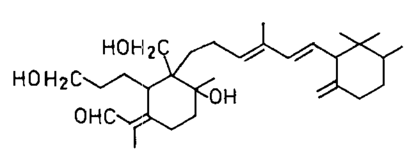

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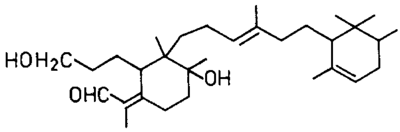
The oxidative degradation of α-irigermanal (**4**) and γ-irigermanal (**5**) isolated from *Iris germanica* L. needs somewhat more drastic conditions to yield the corresponding dihydroirones **6a** and **6b**.

The relative stereochemistry of γ-irigermanal (**5**) was well-established by X-ray crystallography [1], and the absolute configuration had to be either 6*R*,10*S*,11*S*,18*R*,22*S* or the opposite. We set out to answer this question by



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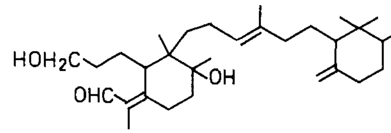

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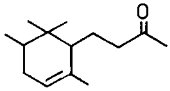
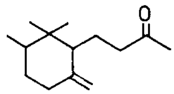
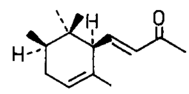


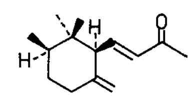
6a



6b



7a



7b

correlation of its oxidation product **6b** with (+)-*cis*-γ-irone (**7b**), the stereochemistry of which had been determined by *Rautenstrauch & Ohloff* [4] to be 2*R*,6*S*.

2. Absolute Configuration of the Irones from Various Sources. – The triterpenoids were isolated from the rhizomes as described earlier [1–3]. Upon oxidation of **2** and **3** with $KMnO_4$/crown-ether [5] **1a** or **1b** were obtained, respectively, in moderate yield. The optical rotations of these compounds are listed in *Table 1* together with the data of irones from other sources. Comparison of the spectroscopic properties to published values [4] established the ring-substituents as *cis*. Surprisingly, the chirality of the *cis*-α- and *cis*-γ-irone obtained in this way was the opposite to the dextrorotatory irones **7a** and **7b** examined by *Rautenstrauch et al.* [4]. The same was true for the *cis*-α-irone we isolated from a commercial *Iris* oil. *cis*-γ-Irone was not obtained pure enough in this separation to unequivocally decide upon its configuration.

Table 1. *Optical Rotations of Various Irones* (purity by GC)

Compound	$[\alpha]^{20}_{578}$	Chemical	*c* (g/100 ml) purity (%)	Source
(−)-*cis*-α-irone (**8a**)	−111°	97	8.8	Oxidation of **2**
	−115°	94	0.65	Comm. oil No. 1
(+)-*cis*-α-irone (**7a**)	+109°			[4] (oil No. 5)
(−)-*cis*-γ-irone (**8b**)	−1°	99	0.5	Oxidation of **3**
(+)-*cis*-γ-irone (**7b**)	+2°			[4] (oil No. 5)

To check our results we recorded CD spectra of the compounds, since such data had been published [6] for the irones that were used in [4] [(+)-*cis*-α-irone (**7a**): λ_{max} ($\Delta\varepsilon$) 318 (−0.23), 247 (+12.18), 216 nm (−4.0); (+)-*cis*-γ-irone (**7b**): λ_{max} ($\Delta\varepsilon$) 372 (+0.05), 354 (+0.15), 340 (+0.22), 326 (+0.21), 315 (+0.15), 223 nm (+3.86)]. Our irones showed exactly opposite behaviour *(Fig.)* thus proving them to be (−)-(2*S*,6*R*)-*cis*-α-irone (**8a**) and (−)-(2*S*,6*R*)-*cis*-γ-irone (**8b**).

3. The Dihydroirones. – As in the case of the irones the oxidation of α-irigermanal (**4**) or γ-irigermanal (**5**) yielded the corresponding *cis*-dihydroirones **6a** and **6b**. Independently these compounds were synthesized by selective hydrogenation of **8a** and **8b** following the procedure of *Ojiwa & Kogure* [7]. *Table 2* lists the values found for the specific rotation of these ketones.

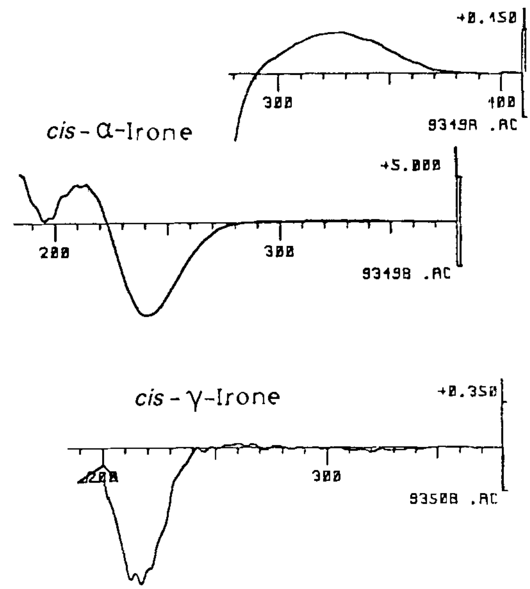


Figure. *CD spectra of (−)*-cis-α- (1.7 mM/l in CH_3CN) *and (−)*-cis-*γ-irone* (5.2 mM/l in CH_3CN)

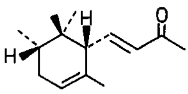
Table 2. *Optical Rotations of Dihydroirones* (purity by GC)

Compound	$[\alpha]_{578}^{20}$	Chemical purity (%)	*c* (g/100 ml)	Source
(−)-*cis*-α-dihydroirone (**9a**)	−10°	98	0.76	Oxidation of **4** from *I. germanica*
	−7°	91	0.64	Oxidation of **4** from *I. pallida*
	−12°	83	1.27	Reduction of **1a** from iripallidal
	−8°	85	3.5	Reduction of **1a** from comm. oil No. 1
(−)-*cis*-γ-dihydroirone (**9b**)	−53°	100	1.7	Oxidation of **5** from *I. germanica*
(+)-*cis*-γ-dihydroirone	+57°	90	1.33	Reduction of **1b** from *Iris* oil No. 5

Dr. *Rautenstrauch* kindly gave us a sample of his irone mixture, which contained (+)-(2*R*,6*S*)-*cis*-γ-irone (**7b**) [4]. After reduction of the oil we separated (+)-(2*R*,6*S*)-*cis*-γ-dihydroirone. This proves the (2*S*,6*R*)-nature of the (−)-*cis*-γ-dihydroirone (**9b**), obtained by oxidation of γ-irigermanal (**5**).

Evidently, the (−)-*cis*-*a*-dihydroirone (**9a**) obtained by oxidation of *a*-irigermanal (**4**) possesses the (2*S*,6*R*)-configuration and this is also true for the reduction product of (−)-*cis*-*a*-irone (**8a**) from iripallidal (**2**) and from a commercial *Iris* oil.

4. The precursors. – From the absolute configuration of (−)-*cis*-γ-dihydroirone (**9b**) the stereochemistry of its precursor (+)-γ-irigermanal (**10**) was easily deduced as 6*R*,10*S*,11*S*,18*R*,22*S*.



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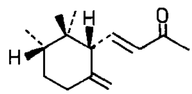

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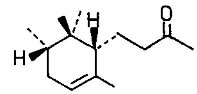

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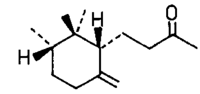

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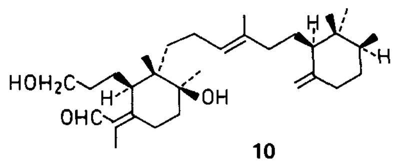

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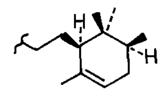


10a

Considering the number of chiral centres, the optical rotation of the various triterpenoids given in *Table 3* is of no use for the determination of their stereochemistry. The same applies to the CD spectra recorded for γ-irigermanal (**5**), iripallidal (**2**) and iriflorental (**3**). No reasonable correlation can be made to derive the absolute stereochemistry of **2** or **3**.

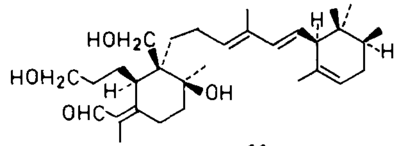
Table 3. *Optical Rotations of the Triterpenoids*

Compound	$[\alpha]^{20}_{578}$	*c* (g/100 ml)	Source
2	−7°	19.0	*I. pallida*
3	+48°	13.5	*I. germanica* and *I. florentina*
4	+36°	7.2	*I. germanica* and *I. pallida*
5	+15°	14.4	*I. germanica*

By the above-mentioned oxidations it is obvious, however, that the chiral centres in the irone or dihydroirone moiety all have the same chirality, namely 18*R*,22*S*. Since the ^{1}H- and ^{13}C-NMR data for the polyfunctional six-membered ring do not differ significantly for the irigermanals, for iripallidal, or for iriflorental [1–3] – except for C(27) which in **4** and **5** is a methyl group and in **2** and **3** a hydroxymethyl group – we assume the absolute stereochemistry of all triterpenoids mentioned to be 6*R*,10*S*,11*S*,18*R*,22*S* as shown in formulae **10** and **11**.

5. Discussion. – It is widely accepted that enantiomeric terpenes are found in nature and racemic mixtures may even occur within the same plant. Although we did not find stereoisomers among the triterpenoids we isolated from the same source, we can infer them to occur in different *Iris* rhizomes from the experiments reported here and earlier [1–3]. The optical rotation of *Iris* oil has been found to be both dextro- and laevorotatory [8]. The same holds true for four different *Iris* oils we examined. After separation of the irone mixture from impurities such as fatty esters, the optical rotation and composition were determined as in *Table 4*.

Three of the mixtures were dextro-, one was laevorotatory. We had sufficient amounts only of oil No. 1 in *Table 4* to carry out a GC separation of the isomers and only the *cis*-α-irone fraction was obtained pure enough to prove its chirality as 2*S*,6*R*. The same configuration, however, is possessed by all oxidation products of the triterpe-



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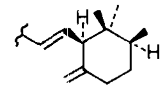

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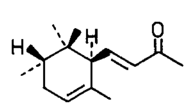
Table 4. *Optical Rotations and Composition of Various* Iris *Oils*

No.	Source	$[\alpha]_{578}^{20}$	Composition (%) *trans-α-*	*cis-α-*	*cis-γ-*	*β*-irone
1	*P. Kaders*, Hamburg (Morocco)	−23°	1	61	37	1
2	*F. Mülhens*, Cologne	+37°	16	36	38	10
3	*C. Georgie*, Böblingen	+27°	10	16	74	–
4	*Haarmann & Reimer*, Holzminden	+2°	26	34	40	trace
5	*Firmenich*, Geneva (Italy) [4]	+28°	16	39	43	2

noid precursors we isolated from fresh *Iris* rhizomes of different origin, namely (−)-*cis*-*α*- (**8a**), (−)-*cis*-*γ*-irone (**8b**) and (−)-*cis*-*α*- (**9a**) and (−)-*cis*-*γ*-dihydroirone (**9b**). The opposite chirality has been found for commercial *cis*-*α*- and *cis*-*γ*-irone by *Rautenstrauch & Ohloff* [4]. Reduction of an authentic sample of their *cis*-*γ*-irone to the corresponding *cis*-*γ*-dihydroirone and comparison of the latter with *cis*-*γ*-dihydroirone obtained by oxidation of *γ*-irigermanal (**5**) proved these contrasting results as did the recording of CD spectra from *cis*-*α*- and *cis*-*γ*-irone prepared by oxidation of the corresponding triterpenoids. *Table 4* shows that the dextrorotary *Iris* oils have other qualities in common, *e.g.* the high content in *trans*-*α*-irone which *Rautenstrauch et al.* [4] found to be (+)-(2*S*,6*S*)-*trans*-*α*-irone (**12**) in their oil. Since **12** differs from **7a** by the configuration at C(2), it seems unlikely that it is generated from **7a** by isomerization; it is more likely that there is a non-specific step in the biosynthesis of these compounds. (+)-(2*R*)-*β*-irone (**13**), on the other hand, is probably an isomerization product of the *α*- and/or *γ*-isomers.



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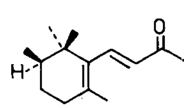

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Interestingly, *trans*-*α*- and *β*-irones are present only in traces in the laevorotatory commercial *Iris* oil. We could never detect these substances or their precursors either in extracts of *Iris* rhizomes or in their oxidation products. This allows the assumption that for the formation of the C_{31}-triterpenoids – which no doubt develop from squalene – different sets of enzymes with different stereospecifities must exist depending on the genetic or cultivational background of the *Iris* culture. So far we can only state that there may be a regional dependence of the nature of the irones. At least one of the dextrorotatory *Iris* oils was produced in Italy *(Table 4)*, whereas the laevorotatory oil was derived from *Iris* rhizomes in Morocco. Analogous results were published by the Crabalonas (see [8]) who found laevorotation for an oil from Moroccan *Iris germanica* and dextrorotation for an oil from *Iris pallida* from Tuscany. The rhizomes examined during the present study were exclusively grown in West Germany.

We thank Prof. Dr. *G. Snatzke*, Bochum, for the recording and extensive help in interpreting the CD spectra. Thanks are due to *Drs. Rautenstrauch* and *Ohloff, Firmenich SA*, Geneva, for discussion and for the supply of their *Iris* oil. Other essential oils were kindly donated by the following producers: *F. Mülhens*-4711, Cologne, *Haarmann & Reimer*, Holzminden, and *C. Georgie*, Böblingen. Prof. *M. Steiner*, Bonn, opened his garden for the cultivation of *Iris pallida*. Ms. *B. Spiolek* gave skilled technical assistance. Financial support by the *Deutsche Forschungsgemeinschaft*, Bad Godesberg, and the *Fonds der Chemischen Industrie*, Frankfurt, is gratefully acknowledged.

Experimental Part

General. Rhizomes of *Iris pallida Lam.* were cultivated. Rhizomes of *I. germanica* L. and *I. florentina* L. were obtained from *Bornträger & Schlemmer* oHG., Offstein. For an extensive description of the isolation procedure see [1]. A commercial *Iris* oil (No. 1) was obtained from *P. Kaders*, Hamburg. Analytical GC: *Carlo Erba* capillary gas chromatograph, Series *2900* equipped with *Duran* glass capillaries 50 m × 0.35 mm coated with *OV 101* or *Ucon 75 H 90000*. Prep. GC: *Hewlett-Packard* gas-chromatograph, Series *5720* with FID and outlet-splitter 1:100. Column: 20% *PEG 4M* on *Chromosorb P60* – 80 mesh. Temperature: 175°. Purification of the *Iris* oils was carried out by chromatography on silica gel *60* (70–230 mesh, *Merck*). Eluent: pentane ether (9:1). Optical rotations were recorded on a *Zeiss* 0.005° precision polarimeter in CH_2Cl_2 as the solvent.

Oxidative Degradation [5]. To a solution of the triterpenoid (1 mMol) in 50 ml of benzene, dicyclohexano-18-crown-6 (37 mg, 0.1 mMol) was added. Within 8 h $KMnO_4$ (500 mg, 3.2 mMol) was added in portions with stirring at r.t. The mixture was stirred overnight, the benzene was distilled off, and after filtration the residue was chromatographed on silica gel using a pentane ether (9:1) gradient. The irones or dihydroirones were obtained in 10–30% yield.

Selective Reduction of the Irones [7]. In a typical run, an irone-mixture (4.5 mMol) was mixed with tris(triphenylphosphine)chlororhodium(I) (21 mg) [9] and triethylsilane (0.9 ml, 5.5 mMol) and heated to 50–60°. The reaction was checked by GC. After 4 h a 1:1:1-mixture (15 ml) of MeOH, acetone and sat. K_2CO_3-solution was added for hydrolysis of the silylenol ether, and kept at 50–60° for 2 h. After addition of H_2O (20 ml) the product was extracted with Et_2O (50 ml), the ethereal layer dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by chromatography on silica gel with pentane/Et_2O (9:1) to yield 30–40% of the dihydroirones. Separation of the isomers was achieved by preparative GC.

CD Spectra. The CD spectra [λ_{max}[nm]($\Delta\varepsilon$)] were recorded in CH_3CN at r.t.

(+)-γ-Irigermanal (**10**) (c = 0.7 mMol/l): 336 (−0.5), 253 (+2.74), 246 (+1.98), 225 (−0.96), 205 (+4.89), 202 (+4.78).

(−)-Iripallidal (**11**) (c = 0.4 mMol/l): 334 (−0.15), 323 (−0.14), 250 (−12.51), 230 (+4.76), 213 (+3.87), 208 (+4.14), 198 (+7.05).

(+)-Iriflorental (**11a**) (c = 0.38 mMol/l): 336 (−0.09), 323 (−0.07), 288 (−0.09), 277 (−0.14), 250 (−2.99), 231 (+5.05), 213 (+1.83), 206 (+1.73), 198 (+1.37).

*(−)-*cis-*α-Irone* (**8a**), obtained in 97% purity by oxidation of **11** (c = 1.7 mMol/l): 327 (+0.14), 239 (−10.42), 214 (+4.31), 209 (+4.26).

*(−)-*cis-*γ-Irone* (**8b**), obtained in 99% purity by oxidation of **11a** (c = 5.2 mMol/l): 323 (−0.03), 318 (−0.03), 216 (−1.07), 211 (−1.04).

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