198. Separation of Enantiomeric Irones by Gas-Liquid Chromatography on Modified Cyclodextrins

by **Franz-Josef Marner***

Institut für Biochemie der Universität zu Köln, Zülpicher Str. 47, D-5000 Köln 1

and **Torsten Runge** and **Wilfried A. König***

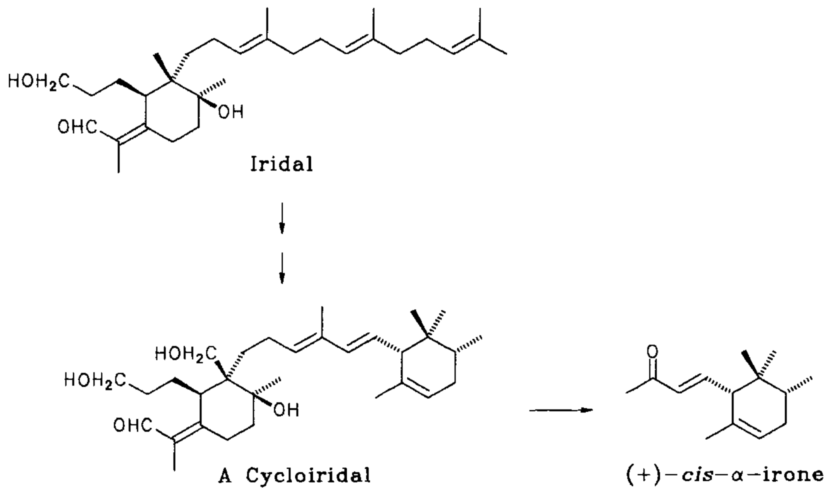
Institut für Organische Chemie der Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13

(10.X.90)

Natural irones found in the essential oil of *Iris* rhizomes often are mixtures of enantiomers. The separation of all optical isomers of the irones by GLC on modified cyclodextrins and the determination of their composition within different *Iris* oils is described. The significance for the biosynthesis of the cycloiridals, C_{31}-triterpenoids, which serve as precursors of the irones, is discussed.

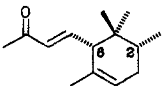
Introduction. – The irones are responsible for the violet-like scent of the precious *Iris* oil. These C_{14}-ketones are derived from oxidative degradation of methylated bicyclic triterpenoids, the cycloiridals, which are found together with the monocyclic iridals (*Scheme 1*) in roots and rhizomes of several *Iris* species [1] [2]. The irone moiety, biosynthetically, is formed by transfer of a Me group, probably *via* *S*-adenosylmethionine (SAM), to the terminal double bond of an open-chain iridal and cyclization as shown by incorporation experiments with radioactively labelled methionine [3].

Scheme 1. *Formation of Cycloiridals and Irones*

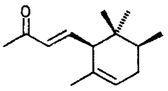


This reaction, however, usually does not lead to the specific formation of one product. Thus, most *Iris* oils contain the three α-, β-, and γ-double-bond isomers. The substituents at the two chiral centers C(2) and C(6) in the α- and γ-ketones predominantly are *cis* to each other, but also the diastereoisomeric *trans*-α-irone is found in minor amounts, and, on one occasion, the presence of traces of a *trans*-γ-isomer (with so far undetermined absolute configuration) has been reported [4]. In addition, from *Iris* oils of different origin, enantiomeric irones can be isolated. Thus, dextrorotatory irones are found in the oil of Italian *I. pallida* varieties [4–6] and the levorotatory enantiomers in a domestic *I. pallida* and in the oil from Moroccan *I. germanica* [1]. The corresponding cycloiridals with enantiomeric irone moiety were isolated from freshly harvested rhizomes of these *Iris* species [1] [7]. As indicated by the varying optical properties of the chromatographically pure ketones [1] [4–7], often mixtures of the two respective enantiomers are present.

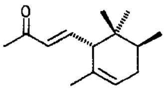
Irones



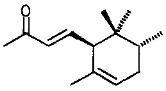
(+)-(2*R*,6*S*) (*cis*-α)



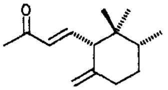
(–)-(2*S*,6*R*) (*cis*-α)



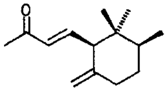
(+)-(2*S*,6*S*) (*trans*-α)



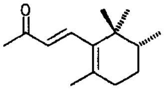
(–)-(2*R*,6*R*) (*trans*-α)



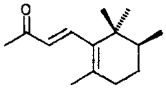
(+)-(2*R*,6*S*) (*cis*-γ)



(–)-(2*S*,6*R*) (*cis*-γ)

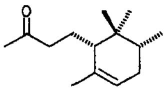


(+)-(2*R*) (β)

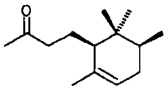


(–)-(2*S*) (β)

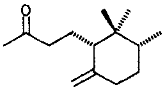
Dihydroirones



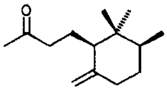
(+)-(2*R*,6*S*) (*cis*-α)



(–)-(2*S*,6*R*) (*cis*-α)



(+)-(2*R*,6*S*) (*cis*-γ)



(–)-(2*S*,6*R*) (*cis*-γ)

It was impossible, however, to determine exactly the enantiomeric purity of the compounds. The composition of the mixtures, therefore, could only be estimated by extrapolation of their optical rotations to the highest values measured [5]. Nothing is known to date about the optical purity of the dihydroirones. Their precursors, so far only found in extracts of *I. germanica*, are stable to autooxidation but readily cleaved by oxidizing agents [8]. Thus, they are only minor components in natural oils from this species [9].

Since a modified cyclodextrin as chiral stationary phase recently has successfully been employed for the GLC separation of the α-ionone and α-damascone enantiomers [10], we decided to study the irones and dihydroirones from several sources with this technique.

Results. – Separation of the enantiomeric irones was achieved either on octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-γ-cyclodextrin [11] or on octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin [12], the latter being more efficient and giving shorter retention times. The dihydroirones only were separated on the second stationary phase. Some of the chromatograms obtained for different irone or dihydroirone mixtures are shown in the *Figure*. The elution orders of the *cis*-α- and *cis*-γ-irones and of the dihydroirones were determined by injection of isolated compounds, the ee of which was ascertained by measuring their optical rotation. The knowledge that the *Firmenich Iris* oil obtained from *Ohloff*, the chromatogram of which is shown in *Fig.*, *b*, contains a (+)-*trans*-α-excess [5] [6] served for the assignments of the *trans*-α-enantiomers. The attribution of the β-irones is based on the following observations and considerations: *i*) no cycloiridals with β-irone substructure have been detected so far. *ii*) β-Irone only is found in samples that have been stored for some time or were prepared by oxidative degradation of the triterpenoid precursors with pyridinium chlorochromate (PCC). *iii*) The amount of this isomer in the samples obtained with PCC greatly depends on the reaction conditions and increases with the relative amount of oxidant used and the age (acidity) of the reagent. *iv*) β-Irones only are

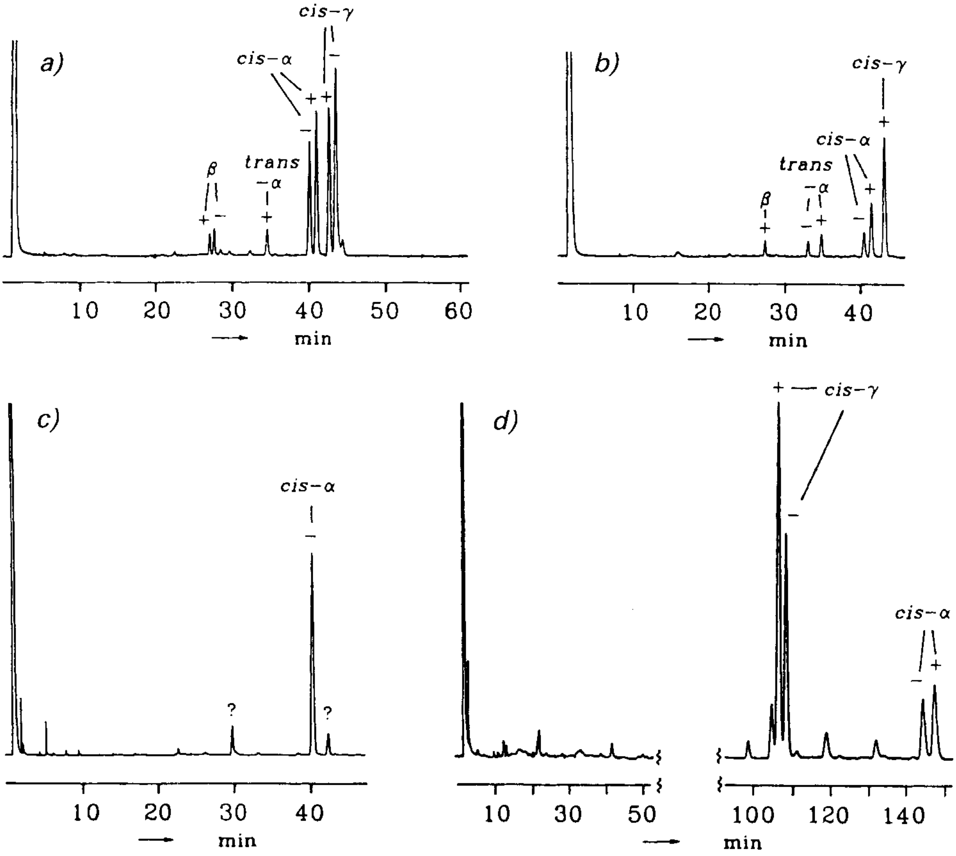


Figure. *GLC Separation of irones and dihydroirones on a 22-m* (0.25 mm i.d.) *open tubular* Pyrex *capillary column with octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin. a*) Irone mixture. *b*) *Firmenich Iris* oil. *c*) Oil from *I. pallida Bonn* (the peaks marked with question marks are no irones as was confirmed by GC/MS). *d*) Dihydroirone mixture. Column temperature: *a*)–*c*): 105°, *d*): 85°.

present in samples that contain the *cis*-γ-isomers and always show an enantiomeric pattern identical to the latter. It has been shown before that phosphoric acid induces the formation of (+)-β- from (+)-*cis*-γ-irone [5]. Therefore, the β-enantiomers exclusively are derived by (acid-catalyzed) rearrangement from their corresponding *cis*-γ-counterparts and the ee values of both have to be identical. The formation of *cis*-α-irone, which occurred during the isomerization of *cis*-γ-irone with phosphoric acid [5], could not be observed.

The separation of the compounds allows an exact calculation of the enantiomeric distribution. The results of the analyses are summarized in the *Table*. As can be seen, most of the samples originate from varieties of *I. pallida*. The finding that this species produces predominantly dextrorotatory irones coincides with the earlier results mentioned above.

Table. *Enantiomeric Excess* [%] *of Irones and Dihydroirones in Oils from Various Sources*

	(+)-*cis*-α	(−)-*cis*-α	(+)-*trans*-α	(+)-*cis*-γ	(−)-*cis*-γ	(+)-β	(−)-β
Irones:							
Iris oil (*Firmenich*)	38.3		20.3	100		100	
I. blanc	10.4		100	100		100	
I. pallida dalmatica	90.4		100	100		100	
I. pallida 'BS'	100		100	100		100	
I. pallida 'Cologne'	3.2		100	100		100	
I. pallida 'Bonn'		100					
Iris oil (*Kaders*)		81.9			37.2		39.5
I. germanica		67.9	100		81.2		81.9
I. pumila	100		100		12.8		30.7
Dihydroirones:							
I. germanica		100			100		

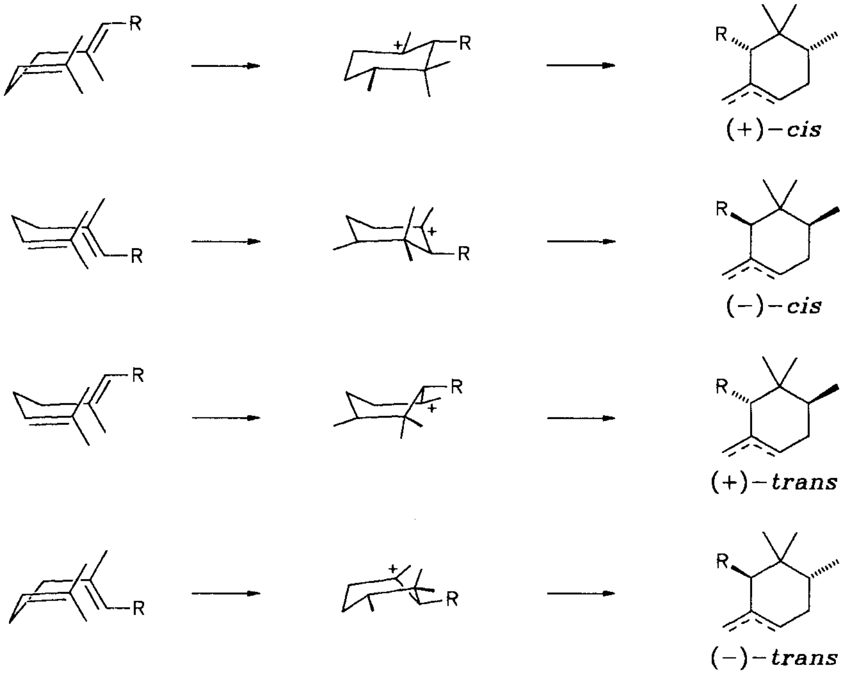
The ee values determined for the components of the *Firmenich* oil are identical with the calculations of *Rautenstrauch* and *Ohloff* [5], which were based on the optical rotation of isolated compounds. The various plants, which are identified as *I. pallida* by their outer appearance, apparently belong to different varieties of this species. This already is indicated by the fact, that they bloom at slightly different times, although growing next to each other. Except for one variety (*I. pallida Bonn*), which specifically – and only – synthesizes the (−)-*cis*-α-moiety, their irone mixtures contain pure (+)-*cis*-γ- and (+)-*trans*-α-irones but differ significantly in the enantiomeric purity of the *cis*-α-isomers. These differences may be useful for their identification.

The oil from *I. germanica* contains an excess of levorotatory *cis*-irones and in this respect – not, however, in their ee – is identical with the commercial oil (*Kaders*), which was obtained from a Moroccan variety of this species. Additionally, the latter is lacking the *trans*-α-irone, which is found enantiomerically pure in the domestic plant. The two *cis*-α- and *cis*-γ-dihydroirones obtained from this plant are optically pure (−)-enantiomers.

I. pumila, a dwarf iris belonging to the group of bearded irises [13], has not been reported as a source of irones before. The cycloiridals, however, can be detected in small amounts in lipid extracts of this plant. Again, the isomer distribution of the irones generated from the triterpenoids is significantly different from the other plants with optically pure (+)-*cis*-α- and (+)-*trans*-α-irones and an excess of the (−)-*cis*-γ-ketone. The reason for the extraordinary high ee value of (−)-β-irone has yet to be studied.

Discussion. – The varying optical purity of the irones certainly is due to a nonspecific course of the enzymatic methylation/cyclization of the open-chain iridals. As shown in *Scheme 2*, the formation of the four possible optical isomers depends solely on the way the homofarnesyl side chain is folded into the active site of the methylating enzyme. The two possible chair forms will yield the two *cis*- and the boat forms the *trans*-isomers. Thus, an enantiomer will be missing, if the appropriate precursor form does not fit into the enzyme pocket or, so it does, if the Me donor cannot reach the terminal double bond. The intermediate cyclohexyl-carbenium ion subsequently is deprotonated to give the α- or γ-irone moiety. Whether only one or both isomers are formed is determined by the position or flexibility of the base, which will abstract the proton. Molecular models of the boat and chair forms of iridals show that the differences are rather small, and the parts of the molecules involved in the single steps of the reaction are subjected only to slight shifts.

Scheme 2. *Formation of the Enantiomeric Irone Moieties*



Therefore, already very small distinctions in the conformation of the responsible enzyme can lead from a highly specific to an unspecific reaction. It will be interesting to find out, whether differences in the primary structure of the enzyme are the reason for the species- (or variety-)specific distribution of the irone isomers within the various plants, or whether this is due to different reaction conditions.

Financial support of the *Deutsche Forschungsgemeinschaft*, Bad Godesberg (Ma 1172/2-1 and Ko 608/9-1), and the *Fonds der chemischen Industrie*, Frankfurt, is gratefully acknowledged.

Experimental Part

Preparation of Capillary Columns with Chiral Stationary Phase. The syntheses of the cyclodextrin derivatives used in this study have been described in [11] [12]. *Pyrex* glass cap. columns (30 m and 22 m) were coated by the static procedure [14] after pretreatment of the inner glass surface with *Silanox* (*Cabot Corp.*, Boston, Mass., USA) according to the description in [15].

Gas-Liquid Chromatography (GLC). For GLC investigations a *Carlo Erba Model 4160* gas chromatograph with split injection and flame-ionization detector was used. H_2 (0.6 bar) served as carrier gas. For electronic peak integration, a *Merck-Hitachi* integrator *Model 2000* was employed.

Sources for Irones and Dihydroirones. 1. Iris *Oils.* A sample of the *Iris* oil that had been used for the elucidation of the absolute configuration of the irones [5] was a friendly gift of *G. Ohloff* (*Firmenich SA*, Geneve). It presumably was derived from Italian *I. pallida* [4]. A commercial oil was obtained from *P. Kaders GmbH* (Hamburg), which had been distilled from Moroccan *I. germanica.*

2. Iris blanc. The irones of *Iris blanc*, dried and powdered rhizomes of *I. pallida* from Italy (a gift of *J. Garnero, P. Robertet & Cie.*, Grasse), were obtained by extraction and purification on silica gel.

3. *Irone Mixtures from Plants.* The rhizomes of various *Iris* species were extracted with $CHCl_3$/MeOH, the cycloiridal fraction was isolated by column chromatography on silica gel, and the irones were obtained by oxidative degradation of the triterpenoids with pyridinium chlorochromate (PCC) as described in [1] [8]. The following plants, all grown in the garden of the institute (Cologne), were used: *i*) three varieties of *I. pallida* identified by their appearance according to [13]. In this paper, they are named after their origin: *Cologne* (for years cultivated in the garden of the author), *BS* (originally obtained from *Bornträger* and *Schlemmer*, Offstein), and *Bonn* (harvested in the garden of the Institute of Pharmacognosy, University of Bonn). *ii*) *I. pallida dalmatica* (courtesy of the *Comitato per il concorso internazionale dell'iris*, Florence). *iii*) *I. germanica* and *I. pumila* were obtained from a garden supply.

4. *Isolated Irones.* Irone mixtures were separated by GLC [1] or by argentation chromatography [7]. Alternatively, the precursors were isolated by reversed-phase chromatography, and oxidatively cleaved as described above. Measurement of the optical rotation (*Zeiss 0.05°* precision polarimeter) of the respective compound indicated the predominant enantiomer.

5. *Dihydroirones.* Natural (levorotatory) dihydroirones were obtained by oxidation of the corresponding precursors from *I. germanica*, and the dextrorotatory enantiomers were synthesized by specific hydrogenation of the corresponding irones as described in [8].

REFERENCES

[1] W. Krick, F.-J. Marner, L. Jaenicke, *Z. Naturforsch., C* **1983,** *38,* 179.
[2] L. Jaenicke, F.-J. Marner, *Progr. Chem. Org. Nat. Prod.* **1986,** *50,* 1.
[3] F.-J. Marner, D. Gladtke, L. Jaenicke, *Helv. Chim. Acta* **1988,** *71,* 1331.
[4] V. B. Rautenstrauch, G. Ohloff, *Helv. Chim. Acta* **1984,** *67,* 325.
[5] V. B. Rautenstrauch, G. Ohloff, *Helv. Chim. Acta* **1971,** *54,* 1776.
[6] W. Krick, F.-J. Marner, L. Jaenicke, *Helv. Chim. Acta* **1984,** *67,* 318.
[7] F.-J. Marner, L. Jaenicke, *Helv. Chim. Acta* **1989,** *72,* 287.
[8] F.-J. Marner, W. Krick, B. Gellrich, L. Jaenicke, W. Winter, *J. Org. Chem.* **1981,** *47,* 2531.
[9] B. Maurer, A. Hauser, J.-C. Froideveaux, *Helv. Chim. Acta* **1989,** *72,* 1400.
[10] W. A. König, P. Evers, R. Krebber, S. Schulz, G. Fehr, G. Ohloff, *Tetrahedron* **1989,** *45,* 7003.
[11] W. A. König, R. Krebber, P. Mischnick, *J. High Res. Chromatogr.* **1989,** *12,* 732.
[12] W. A. König, D. Icheln, T. Runge, I. Pforr, A. Krebs, *J. High Res. Chromatogr.* **1990,** *13,* in press.
[13] B. Mathew, 'The Iris', B. T. Batford Ltd., London, 1981.
[14] J. Bouche, M. Verzele, *J. Gas Chromatogr.* **1968,** *6,* 501.
[15] W. A. König, K. Ernst, *J. Chromatogr.* **1983,** *280,* 135.