Hoogianal, a β-Irone Precursor from *Iris hoogiana* DYKES (Iridaceae)

by **Franz-Josef Marner*** and **Bernadette Hanisch**

Institut für Biochemie, Universität zu Köln, Zülpicher Str. 47, D-50674 Köln (phone: +49(0)221/4703206; fax: +49(0)221/4705067; e-mail: fj.marner@uni-koeln.de)

Fractionation of the lipid extract from rhizomes of *Iris hoogiana* DYKES resulted in the isolation of one new and several known iridals. The latter were identified by comparison with authentic standards as **1**–**5**. The structure of the new natural product, hoogianal (**11**), was elucidated by spectroscopic analysis. Oxidative degradation yielded β-irone (**10**), identified by GC- and LC-MS. The (–)-(2*S*)-configuration of this oxidation product was determined by enantioselective GC on a chiral cyclodextrin phase and by comparison with the corresponding ketones in laevo- and dextrorotatory commercial *Iris* oils.

Introduction. – Numerous Iridaceae have been studied within the last two decades, and more than thirty different iridals, mono- or bicyclic phytogenic triterpenoids (*e.g.*, **1**–**7**) and their fatty acid esters at C(3), were isolated from their essential oils [1] (*Fig. 1*). Among them are the bicyclic cycloiridals **6** and **7**, which are found in extracts of *I. pallida*, *I. germanica*, and *I. florentina* [2]. These compounds serve as precursors of *cis*-γ- (**8**) and *cis*-α-irone (**9**), respectively, which are constituents of the precious natural orris oil and responsible for its pleasant violet-like scent [1–3]. The origin of β-irone (**10**), present in varying amounts in commercial *Iris* oils, is not known. Since no triterpenoid precursor of this compound has been found to date, and since, in all cases studied, it showed the same configuration at C(2) as *cis*-γ-irone (**8**) [2–5], it is assumed to be derived from acid- or base-catalyzed isomerization of the latter during workup of the dried and powdered plant material [2][3]. We report here on the composition of the iridal fraction from extracts of *Iris hoogiana* DYKES and on the isolation and structure elucidation of a new β-irone precursor.

Results and Discussion. – *I. hoogiana* DYKES is reported to occur wild in the Pamir-Alai mountains [6]. Although not common as a garden plant, the species is sometimes available in the nursery trade. Upon HPLC[1]) analysis, lipid extracts of the rhizomes showed the presence of several iridals. Among them, the known triterpenoids 16-hydroxyiridal **1** (3.5%), iridal **2** (4%), 16-acetoxyiridal **3** (1%), 29-acetoxy-(28-ene)-spiroiridal **4** (1%), and the spirobicyclic (13*R*)-hemiacetal **5** (14%) were identified[2]). After isolation by MPLC, their nature was confirmed by comparison of their HPLC behavior and their UV and mass spectra with those of authentic standards [1].

---

[1]) Abbreviations: HPLC = high performance liquid chromatography, MPLC = medium pressure liquid chromatography; ESI = electrospray ionization; APCI = atmospheric-pressure chemical ionization; DAD = diode-array detector.

[2]) The C-skeleton of the iridals is numbered in analogy to squalene. For the systematic name of **11**, see *Exper. Part.*

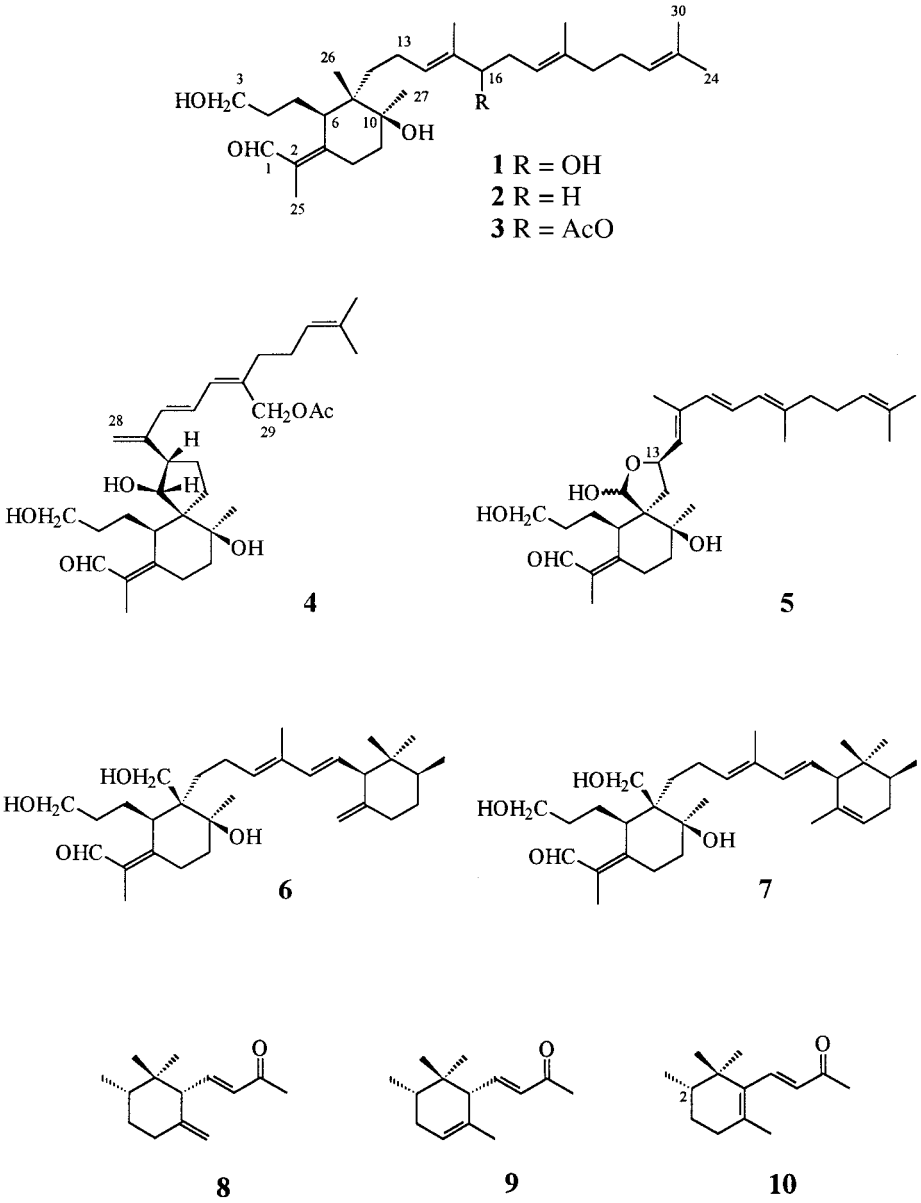
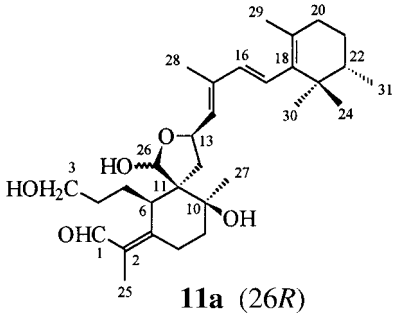


Fig. 1. *Iridals, spiroiridals, cycloiridals, and irones*

The main product of the iridal fraction (76.5%), hoogianal (**11**), proved to be a hitherto unknown compound. Its UV spectrum showed an absorption λ_{max} 254 nm, typical of the usual α,β-unsaturated aldehyde moiety of the iridals. Unusual, however, was a shoulder at 238 nm, which pointed to the presence of a second chromophore. In the ESI-MS[1]), a pseudomolecular ion $[M + Na^+]$ at *m/z* 523 was observed, whereas in

the APCI[1]) mode, only ions caused by the successive loss of H_2O, *i.e.* $[M - H_2O + H^+]$ at *m/z* 483 and $[M - 2\ H_2O + H^+]$ at *m/z* 465, were present. These results and the ^{1}H- and ^{13}C-NMR spectra led to a molecular composition of $C_{31}H_{48}O_5$. Four olefinic C=C bonds and one C=O group count for five of the eight of unsaturations. Therefore, the molecule contains three ring systems.



11a (26*R*)
b (26*S*)

HPLC[1]) Analysis of the isolated substance **11** showed that two isomeric compounds in a 3:1 ratio were present; these are in an equilibrium, since after separation, the same isomer composition was found again. This behavior is similar to that of hemiacetal **5**, which consists of two epimers that interconvert about C(26)[2]) [7]. Indeed, the presence of two epimeric spirobicyclic hemiacetal moieties was easily deduced from the appropriate signals in the ^{1}H-, ^{13}C-, and 2D-NMR spectra (H,H-COSY, HMQC, HMBC, ROESY), and the structure of hoogianal was established as the tricyclic 26-hydroxy-13-oxaspiro-22-methyl-β-cycloiridal **11**[2]).

The exocyclic α,β-unsaturated aldehyde moiety of the major isomer **11a** gives rise to NMR signals at δ(H) 10.27 and δ(C) 190.9 (for signals of the minor epimer **11b**, see *Tables 1* and *2*). The quaternary C-atoms of the acrylaldehyde unit of **11a** appear at 132.6 (C(2)) and 162.0 (C(7))[2]) and those of the methine group CH(6) at δ(H) 3.65–3.75 and δ(C) 42.9. A NOE cross-peak between H–C(1) and H–C(6) (*Fig. 2*) in the ROESY spectrum establishes the usual configuration of the exocyclic C=C bond. C(10) gives rise to a signal at δ(C) 73.9, C(11) appears at δ(C) 59.9 and the CH_2OH group of the 3-hydroxypropyl side chain at δ(H) 3.5–3.75 and δ(C) 62.1. The presence of the hemiacetal ring is recognized by resonances at δ(C) 99.3 (δ(H) 5.71; H–C(26)), δ(C) 42.7 (δ(H) 1.25–1.4 and 1.7–1.8; 2 H–C(12)) and δ(C) 73.3 (δ(H) 5.14–5.22; H–C(13)). H–C(13) is coupled to its olefinic neighbor H–C(14), which appears as a br. *d* at δ(H) 5.41 and 5.43. Extension of the olefinic system up to C(19) is established by appropriate long-range couplings (H–C(14)/C(15), H–C(16)/C(14) and H–C(17), H–C(17)/C(18) and C(19) and direct couplings (*J* (H–C(16),H–C(17) = 16 Hz). The latter and a strong allylic coupling of H–C(14) to H–C(28) are typical for the (*E*)-configuration of the C(14)=C(15) and the C(16)=C(17) bonds. The fully substituted C(18)=C(19) bond is part of a β-irone ring system, and the ring closure between C(18) and C(23) is suggested by a cross-peak H–C(17)/C(23) in the HMBC spectrum. C(22) is substituted by the additional Me group, giving a *d* at δ(H) 0.87 in the ^{1}H-NMR.

Comparison of the spectral data with the values found for other iridals and biosynthetic considerations [1] suggest a (6*R*,10*S*,11*R*)-configuration for the three chiral centers of the iridal ring system. In the ROESY spectrum, both epimers show cross-peaks for Me(27) and H–C(14), thus establishing the (13*R*) configuration. The proton at the epimeric center C(26) correlates with Me(27) in the main and with H–C(6) in the minor isomer (*Fig. 2*). Therefore, the main component **11a** is (26*R*)- and the minor component **11b** (26*S*)-configured.

Table 1. *^{1}H-NMR Chemical Shifts δ of* **11a** *and* **11b** *in $CDCl_3$. δ* in ppm rel. to $SiMe_4$, *J* in Hz.

	11a	**11b**	**11a**		**11b**
H–C(1)	10.27 (*s*)	10.2 (*s*)	2 H–C(20)	1.8–2.05 (*m*)	1.8–2.05 (*m*)
2 H–C(3)	3.5–3.75 (*m*)	3.5–3.75 (*m*)	2 H–C(21)	1.4–1.6 (*m*)	1.4–1.6 (*m*)
2 H–C(4)	1.15–1.25, 1.4–1.5 (2*m*)	1.15–1.25, 1.4–1.5 (2*m*)	H–C(22)	1.3–1.5 (*m*)	1.2–1.5 (*m*)
2 H–C(5)	1.8–2.2 (*m*)	1.8–2.2 (*m*)	Me(24)[a])	0.81 (*s*)	0.81 (*s*)
H–C(6)	3.65–3.75 (*m*)	3.27 (br. *d*, *J* = 9.4)	Me(25)	1.8 (*s*)	1.81 (*s*)
2 H–C(8)	2.4–2.55, 2.67–2.77 (2*m*)	2.4–2.55, 2.67–2.77 (2*m*)	H–C(26)	5.71 (br. *s*)	5.18 (br. *s*)
2 H–C(9)	1.35–1.45, 1.65–1.77 (2*m*)	1.35–1.45, 1.65–1.77 (2*m*)	Me(27)	1.21 (*s*)	1.45 (*s*)
2 H–C(12)	1.25–1.4, 1.7–1.8 (2*m*)	1.5–1.7 (*m*)	Me(28)	1.8 (*s*)	1.81 (*s*)
H–C(13)	5.18 (*q*, *J* = 8)	4.59 (*q*, *J* = 8)	Me(29)	1.62 (*s*)	1.62 (*s*)
H–C(14)	5.42 (br. *d*, *J* = 8)	5.42 (br. *d*, *J* = 8)	Me(30)[a])	0.96 (*s*)	0.96 (*s*)
H–C(16)	5.91 (*d*, *J* = 16)	5.93 (*d*, *J* = 16)	Me(31)	0.87 (*d*, *J* = 6.5)	0.87 (*d*, *J* = 6.5)
H–C(17)	6.08 (br. *d*, *J* = 16)	6.08 (br. *d*, *J* = 16)			

[a]) Assignments may be interchanged.

A sample of hoogianal was oxidatively cleaved with $KMnO_4$/dicyclohexano[18]-crown-6 [2] to give β-irone (**10**), which was identified by comparison of its chromatographic and spectroscopic properties with a synthetic reference [8]. To solve the question of the configuration at C(22) of hoogianal, its oxidation product was analyzed by enantioselective GC in comparison to two samples of β-irone from commercial *Iris* oils, which were derived from Moroccan *I. germanica* and Italian *I. pallida*, respectively. It is well-established that laevorotatory irones are formed by *I. germanica*, whereas *I. pallida* produces predominantly the dextrorotatory isomers [1][4–5]. Thus, the (−)-(2*S*)-configuration of the β-irone (> 96% ee) obtained from **11** was determined. Therefore, hoogianal is a mixture of the (6*R*,10*S*,11*R*,22*S*,26*R*)- and (6*R*,10*S*,11*R*,22*S*,26*S*)-epimers (**11a** and **11b**, resp.).

Table 2. *^{13}C-NMR Chemical Shifts δ of* **11a** *and* **11b** *in $CDCl_3$. δ* in ppm rel. to $SiMe_4$

	11a	**11b**		**11a**	**11b**
C(1)	190.9	190.2	C(17)	127.9	128.2
C(2)	132.6	133.0	C(18)	137.3	137.3
C(3)	62.1	62.3	C(19)	128.3	128.3
C(4)	31.0	32.0	C(20)	31.7	31.7
C(5)	27.0	28.7	C(21)	27.0	27.0
C(6)	42.9	46.4	C(22)	38.9	38.9
C(7)	162.0	161.5	C(23)	37.3	37.3
C(8)	23.9	23.9	C(24)[a])	22.0	22.0
C(9)	38.3	38.1	C(25)	11.1	11.1
C(10)	73.9	74.8	C(26)	99.3	105.2
C(11)	59.9	54.4	C(27)	27.9	27.7
C(12)	42.7	43.8	C(28)	13.0	13.1
C(13)	73.3	69.2	C(29)	21.7	21.7
C(14)	129.0	128.5	C(30)[a])	27.5	27.5
C(15)	137.8	137.5	C(31)	16.4	16.4
C(16)	137.1	137.0			

[a]) Assignments may be interchanged.

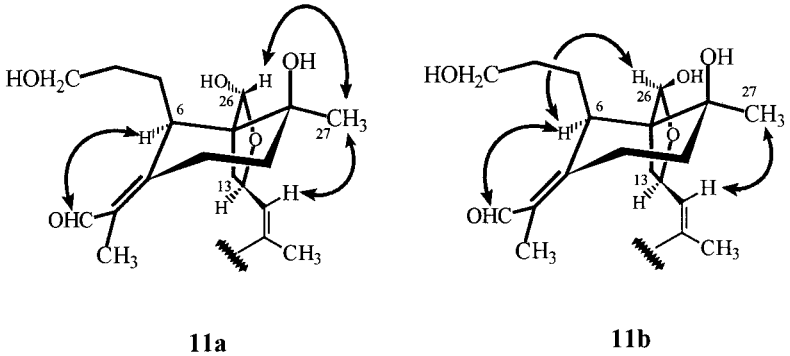


Fig. 2. *Conformation and selected NOE correlations of the hoogianal epimers* **11a** *and* **11b**

It has frequently been shown that the unmodified iridals often are accompanied by their fatty-acid esters at C(3) [1]. This also applies to the extract of *I. hoogiana* rhizomes, which contains a considerable quantity of esters (46% of the iridal fraction). The compounds were identified as the esters of hoogianal **11** with octanoic (31%), decanoic (33%), dodecanoic (8%), and tetradecanoic acid (18%) and the octanoic acid ester of the hemiacetal **5** (10%) by combined LC/UV (DAD) and LC/MS (APCI) analyses[1]). Additionally, the nature of the iridal moiety of the compounds was secured by HPLC analysis after enzymatic hydrolysis of the esters [9], and the fatty acids were analyzed by GC after transesterification with MeOH/HCl.

I. hoogiana, a member of the section *Regelia* [6], is the first example of an *Iris* species not belonging to the bearded irises (section *Iris*) to be found to contain an irone precursor. It should be interesting to find out whether further triterpenoids with irone substructures are to be found in other members of this subgenus. Although, from its olfactory properties, β-irone is not considered the most valuable of the irone isomers [5], *I. hoogiana* might prove an important source for this natural product. The biosynthesis of hoogianal presumably follows the same course as that shown for the bicyclic γ- and α-irone precursors **6** and **7** [1]. It may be inferred that in *I. hoogiana*, the hemiacetal **5** serves as the precursor, and the irone ring is formed by methylation of the terminal C=C bond of the terpenoid side chain and subsequent cyclization to the six membered ring.

The authors are indebted to Prof. Dr. *W. A. König*, Hamburg, for carrying out the enantioselective gas chromatography of the β-irone. We wish to thank Dr. *H. Schmickler*, Köln, for recording the NMR spectra and Dr. *C. Chapuis, Firmenich SA*, Genève, for a sample of racemic, synthetic β-irone.

Experimental Part

General. MPLC: *Büchi* chromatography pump, model *681*; 240 × 20 mm *RP-18* (14–40 μm) columns. Anal. HPLC: *Kontron* instrument, model *200*; *LiChrocart RP-18* column (125 mm, *Merck*); solvent: MeOH/H_2O 3:7 (5 min), linear gradient to 100% MeOH (15 min), 100% MeOH (20 min); flow 1 ml/min; *Hewlett-Packard 1040A* diode-array detector. UV Spectra: recorded during the HPLC run; λ_{max} in nm. HPLC/MS: *Finnigan MAT LCQ* equipped with a *Hewlett-Packard 1100* HPLC and APCI or ESI ion source; in *m/z*. GLC: *Fisons GC-8000* instrument; cap. column *DB225* (30 m × 0.25 mm); H_2 2 ml/min; temp. progr.: 80° (1 min) → 200° (10°/min). GC/MS: *Finnigan-MAT 4510* GC/MS, EI: 70 eV; in *m/z*. NMR: *Bruker AM-300*; ^{1}H 300 MHz; ^{13}C 75 MHz.

Plant Material. Rhizomes of *I. hoogiana* were obtained commercially from Friesland Staudengarten, Jever, Germany, in fall 1997 and 2000.

Extraction and Separation. The rhizomes (4 g) were cut into pieces and extracted 3 × with MeOH/$CHCl_3$ 2:1 (*v*/*v*). After evaporation, the residue was partitioned between Et_2O and H_2O. The org. phase was dried ($MgSO_4$) and evaporated to give the crude oil (310 mg). The extract was fractionated by MPLC (MeOH/H_2O gradient 60:40 (*v*/*v*) → 100% MeOH) to give successively 3.5 mg of 16-hydroxyiridal **1**, 2.1 mg of 16-acetoxyiridal **3**, 1.9 mg of 29-actoxy-(28en)-spiroiridal **4**, 3.8 mg of hemiacetal **5**, 18.0 mg of hoogianal (**11**), and 3.3 mg of iridal **2**. The known iridals **1**–**5** were identified by LC/UV and LC/MS in comparison to authentic standards. Subsequently, the fatty acid esters were eluted with 100% MeOH to give 2.4 mg of octanoic acid ester of **5** (UV: 260 (sh), 270, 281, 292 (sh); LC/MS (APCI): 595 ($[M - H_2O + H^+]$), 577 ($[M - 2\ H_2O + H^+]$)) and the esters of **11** (UV: 238 (sh), 254) with octanoic acid (8.1 mg) (LC/MS (APCI): 609 ($[M - H_2O + H^+]$), 591 ($[M - 2\ H_2O + H^+]$)), decanoic acid (12.9 mg) (LC/MS: 637 ($[M - H_2O + H^+]$), 619 ($[M - 2\ H_2O + H^+]$)), dodecanoic acid (6.9 mg) (LC/MS: 665 ($[M - H_2O + H^+]$), 647 ($[M - 2\ H_2O + H^+]$)), and tetradecanoic acid (3.7 mg) (LC/MS: 693 ($[M - H_2O + H^+]$), 675 ($[M - 2\ H_2O + H^+]$)).

A small sample of each ester was submitted to enzymatic hydrolysis according to [9], to give the iridals **5** and **11**, and a second part was reacted with 2N MeOH/HCl, yielding the methyl esters of the acids. HPLC analysis of the former and GC of the latter confirmed the nature of the iridal esters.

Hoogianal (=*(2Z)-2-{(1*R,*5*R,*6*R,*10*S*)- *and (1*S,*5*R,*6*R,*10*S*)-1,10-Dihydroxy-6-(3-hydroxypropyl)-10-methyl-3-{(1*E,*3*E*)-2-methyl-4-[(*5*S*)-2,5,6,6-tetramethylcyclohex-1-en-1-yl]buta-1,3-dienyl}-2-oxaspiro[4.5]dec-7-ylidene}propanal*; **11a** and **11b**, resp.) UV: 238 (sh), 254. ^{1}H-NMR ($CDCl_3$, 300 MHz): *Table 1*. ^{13}C-NMR ($CDCl_3$, 75 MHz): *Table 2*. ESI-MS (MeOH/H_2O 1:1 (*v*/*v*), 1 mM NaOAc): 523 ($[M + Na^+]$). LC/MS (APCI): 483 ($[M - H_2O + H^+]$), 465 ($[M - 2\ H_2O + H^+]$).

*(−)-(2*S*)-β-Irone* (=*(3*E*)-4-[(5*S*)-2,5,6,6-Tetramethylcyclohex-1-en-1-yl)but-3-en-2-one*; **10**). Hoogianal (**11**; 5 mg) was treated with $KMnO_4$/*cis*-dicyclohexano[18]crown-6 as described in [2], to give, after purification by chromatography (silica gel), **10**, identical in its chromatographic and spectroscopic properties with a synthetic standard. UV: 223, 296. GC/MS: 206 (1, M^+), 191 (61), 121 (10), 91 (9), 43 (100). LC/MS (APCI) 207 ($[M + H^+]$).

The configuration of **10** was determined by isothermal GC (120°; 25-m fused silica cap. column, coated with heptakis{6-*O*-[(*t*-butyl)dimethylsilyl]-2,3-di-*O*-methyl}-β-cyclodextrin (50% in polysiloxane *OV 1701* (*w*/*w*) [10]). As standards served: *i*) a dextrorotatory *Iris* oil with 10% (+)-β-irone (48% ee), distilled from Italian *I. pallida*, a gift of *4711 Eau de Cologne*, Cologne; *ii*) a laevorotatory *Iris* oil with 25% (−)-β-irone (34% ee), distilled from Moroccan *I. germanica*, a gift of *P. Kaders GmbH*, Hamburg.

REFERENCES

[1] F.-J. Marner, *Curr. Org. Chem.* **1997**, *1*, 153.
[2] W. Krick, F.-J. Marner, L. Jaenicke, *Z. Naturforsch., C* **1983**, *38*, 179.
[3] V. Rautenstrauch, G. Ohloff, *Helv. Chim. Acta* **1984**, *54*, 1776.
[4] F.-J. Marner, T. Runge, W. A. König, *Helv. Chim. Acta* **1990**, *73*, 2165.
[5] A. Galfré, P. Martin, M. Petrzilka, *J. Essent. Oil Res.* **1993**, *5*, 265.
[6] B. Mathew, 'The Iris', B. T. Batford Ltd., London, 1981.
[7] F.-J. Marner, A. Littek, R. Arold, K. Seferiadis, L. Jaenicke, *Liebigs Ann. Chem.* **1990**, 563.
[8] C. Chapuis, R. Brauchli, *Helv. Chim. Acta* **1993**, *76*, 2070.
[9] F.-J. Marner, I. Ritzdorf, G. Johnen, *Phytochemistry* **1993**, *33*, 573.
[10] W. A. König, N. Bülow, Y. Saritas, *Flavour Fragr. J.* **1999**, *14*, 367.