PERGAMON

Phytochemistry 52 (1999) 1597–1600

PHYTOCHEMISTRY

Isolation and structure determination of three epoxidized iridals from *Iris cristata*

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Received 8 June 1998; accepted 13 March 1999

Abstract

Three new iridals were isolated from rhizomes of *Iris cristata*. After spectral analysis their structures were respectively established as 22,23-epoxy-21-hydroxyiridal **5**, 22,23-epoxyiridal **6** and 22,23-epoxy-10-deoxy-21-hydroxyiridal **7**.

Keywords: Iris cristata; Iridaceae; Epoxid; Iridals; Triterpenoids

1. Introduction

Iridals are triterpenoids, which can be isolated from various *Iris* species and were first described in 1982 (Marner, Krick, Gellrich, Jaenicke & Winter, 1982). Some of the compounds were shown to be the precursors of the irones, which are used by perfumers for their pleasant violet-like scent. Since then, more than 30 different iridal structures have been elucidated. The natural products are divided into three classes: monocycloiridals, bicycloiridals and spirioiridals (Marner, 1997). Recently, we showed for some iridals that they can play a role as constituents of cell membranes comparable to that of sterols (Bonfils et al., 1995; Bonfils, Sauvaire & Maurin, 1996; Leconte, Bonfils & Sauvaire, 1997). In a previous paper we described the first epoxidized monocycloiridal **1** from *I. germanica* (Bonfils, Marner & Sauvaire, 1998). The present work deals with the isolation and structure elucidation of three new epoxidized monocyclic iridals found in *I. cristata*.

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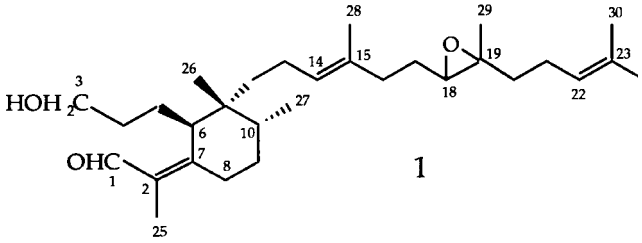
2. Results and discussions

The organic extract of fresh rhizomes of *I. cristata* showed on HPLC analysis six main peaks. Besides the two known iridals 16-hydroxyiridal **2** and 29-acetoxyspiroiridal **3** four unidentified compounds were present. Three of them were isolated by TLC and RP-HPLC and their structure was elucidated by spectroscopic means. The fourth unknown compound could not be isolated because of its low amount within the extract and its instability under our chromatographic conditions.

The first isolated compound shows an UV spectrum with a λ_{max} at 258 nm, indicating the presence of the typical α, β unsaturated carbonyl group. The FAB mass spectrum gave ions at m/z 491 in the positive and 489 in the negative mode, respectively, indicating a molecular weight of 490 g mol^{-1}. The structure elucidation was carried out by ^{1}H and ^{13}C NMR spectra and ^{1}H,^{1}H-COSY, ^{13}C,^{1}H-correlation and long-range experiments. The ^{13}C spectra along with the results of mass spectrometry led us to establish a molecular composition of $C_{30}H_{50}O_5$. The NMR spectra showed the typical signals of the iridal cyclohexane ring system and its substituents, as obtained previously for iridal **4**

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00186-7



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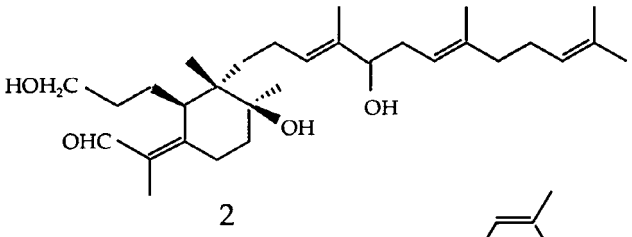

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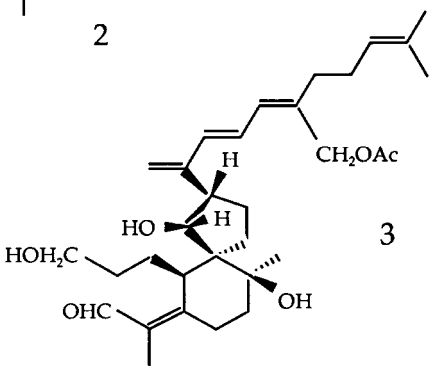

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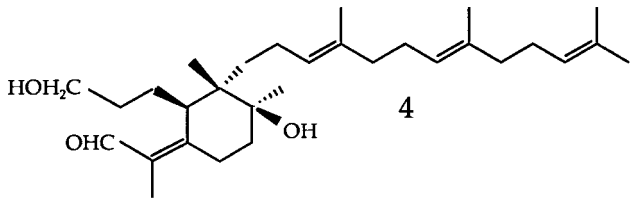

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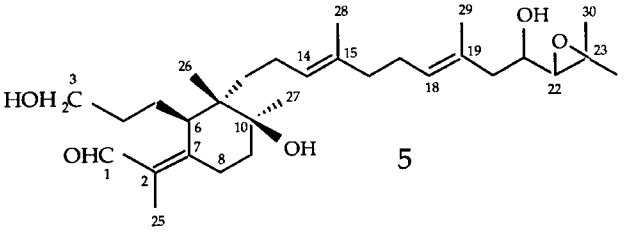

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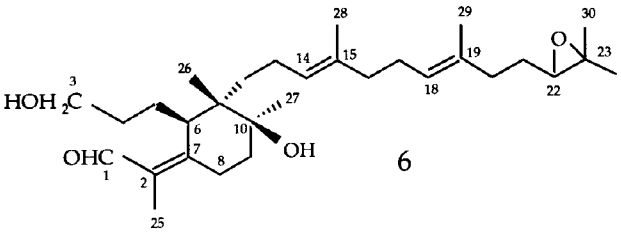

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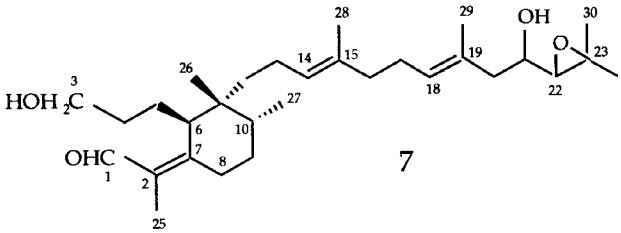

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(Krick, Marner & Jaenicke, 1983). Thus, besides the α, β unsaturated C-1 carbonyl group at δ 190.08, the corresponding double bond shows signals at δ 162.96 (C-7) and 133.14 (C-2). The resonance of C-10 appears at δ 75.06 as a singlet, indicating that it bears an OH group, and is a quaternary carbon. This is confirmed by the ^{1}H signal of the C-27 methyl group, which shows up as a singlet. A third oxygen atom is present, as usual, at C-3 in the propanol side chain, which is seen at δ 63.03 in the ^{13}C and at δ 3.58 in the ^{1}H NMR spectrum. In contrast to iridal **4** the homofarnesyl side chain of the new compound bears only two olefinic double bonds. Instead, three C-atoms are bound to oxygen, as seen by their resonances at $\delta C = 59.04$, 66.02 and 66.73, respectively. The first one is a quaternary carbon and the others carry one proton each ($\delta H = 2.59$ and 3.41, respectively). The presence of only one OH group is confirmed by comparison of ^{1}H spectra obtained in DMSO and in a mixture of DMSO and D_2O. In the first spectrum three OH resonances can be seen, which disappear in the second. Thus, two carbon atoms have to be bound to one oxygen and therefore one of the terpenopid double bond has to be epoxidized. Since the signal of the proton at the epoxide carbon ($\delta H = 2.59$, $\delta C = 66.02$) is a doublet, it is obvious that it is connected to the secondary CHOH group giving resonances at δH 3.41 and δC 66.73, respectively. The 2D NMR experiments helped in locating the epoxide moiety. Since both olefinic proton at 5.18 and 4.93 ppm show allylic coupling with only one methyl group each (at 1.61 and 1.49, respectively) there cannot be a double bond at the end of the homofarnesyl side chain. Also, in the long range spectrum the first olefinic proton ($\delta H = 5.18$) shows cross peaks with the ^{13}C resonances of the allylic methylene groups C-17 and C-20 and the C-29 methyl group. Accordingly, the second olefinic proton ($\delta H = 4.93$) shows cross peaks with C-13, C-28 and C-16. This confirms that the epoxide moiety has to be located between C-22 and C-23. Therefore, the compound is the 22,23-epoxy-21-hydroxyiridal **5**.

The second isolated compound shows an UV spectrum with a λ_{max} at 258 nm. The FAB MS indicated ions at m/z 475 (pos.) and 473 (neg.) indicating a molecular weight of 474 g mol^{-1}. Along with the ^{13}C

NMR data we established a molecular composition of $C_{30}H_{50}O_4$. The ^1H and ^{13}C NMR signals showed in general the same chemical shifts and coupling patterns as observed for compound **5**, except for the signals due to the hydroxy group at C-21 which is missing here. Comparison of ^1H spectra obtained in DMSO and DMSO + D_2O showed that only two OH groups are present in the molecule, which are located at C-3 and C-10. Since the DEPT spectrum indicates one CH_2 group more and one CH group less than for compound **5**, the OH group at C-21 is missing and the compound is the 22,23-epoxyiridal **6**. Again, this was confirmed by 2D NMR experiments, which showed that the CH group of the epoxide moiety (C-22) is coupled to a triplet of a CH_2 at C-21.

The UV spectrum of the third compound again showed a λ_{max} at 258 nm. As before, the FAB MS indicated a molecular weight of 474 g mol^{-1} by molecular ions at *m/z* 475 in the positive and 473 in the negative mode and a molecular composition of $C_{30}H_{50}O_4$ was established by connecting the ^{13}C NMR and MS data. The ^1H and ^{13}C NMR signals compared well with those obtained for compound **5**, except for the signals at C-10, which is not hydroxylated in this compound. Only two OH groups are present here as seen by comparison of ^1H spectra obtained in DMSO and DMSO/D_2O. Since C-10 gives rise to a signal at δ 35.73, it does not bear an OH, but instead is substituted by a proton. This is confirmed by the appearance of the C-27 proton signal as a doublet. Therefore, this compound is the 22,23-epoxy-10-deoxy-21-hydroxyiridal **7**. From biosynthetic considerations and from the identical NMR data we have assigned to the iridals **5** and **6** the same 6R,10 S,11 S-configuration as found for other monocyclic iridals before (Marner, 1997). Accordingly, the 10-deoxyiridal **7** has a 6 S,10R,11R-geometry (Marner, 1997). The sterochemistry at the epoxy rings still has to be determined.

3. Experimental

General. HPLC: Gilson apparatus consisting of 2 Gilson 305 and 302 pumps (25 SC heads), a Gilson 112 UV-Vis detector. Columns: semi-preparative Hibar Lichrospher RP-18, 100 Å, 10 μm, 250 × 25 mm, (Merck, Germany) and analytical Kromasil, C18, 5 μm, 100 Å, 250 × 4.6 mm (Touzart & Matignon, France). UV detection at 254 nm. MS: Finnigan-MAT 4510 GC/MS, solid probe (EI: 70 eV), FAB: Jeol DX-300; NMR: Bruker AM-300 (^1H: 300 MHz, ^{13}C: 75 MHz) (Cologne, Germany), Bruker AM-400 (^1H: 400 MHZ, ^{13}C: 75 MHz) (Montpellier, France).

Plant material. *I. cristata* Solander was obtained from Dr R. Nicholson (Jessie Smith Institute, USA) via the Botanical Garden of Montpellier.

Extraction and isolation. Rhizomes of *I. cristata* were cleaned under tap water and cut into pieces before they were ground in a grinder (Janke & Kunkel, model A10). The mash (FW ≈ 40 g) was deposited into cellulose extraction thimbles (Whatman, 41 × 123 mm) and extracted threefold with EtOH/H_2O (70:30, v/v) at room temperature in a Tecator apparatus. For the purification procedure see Bonfils et al. (1998), note that no CC on silica gel was carried out here.

(6R,10 S,11 S)-22,23-Epoxy-21-hydroxyiridal **5**. UV λ_{max} (methanol) 258 nm; EIMS *m/z* (rel. int) 490 $[M]^+$ (0.15), 472 $[M\text{-}H_2O]^+$ (0.18), 55 (76), 43 (100), 41 (50); FABMS *m/z* 491 $[M+H]^+$ (pos.), 489 $[M-H]^-$ (neg.), ^1H-NMR ($CDCl_3$: δ 10.15 (1H, s, H-1), 5.18 (1H, *t*, *J* = 6.6 Hz, H-18), 4.93 (1H, *t*, *J* = 6.5 H-14), 3.58 (2H, *t*, *J* = 6.4, H-3), 3,41 (1H, m, H-21), 3.29 (1H, *dd*, H-6), 2.59 (1H, *d*, *J* = 8, H-22), 2.59/2.53 (2H, *m*, H-8), 2.43/2.12 (2H, *m*, H-20), 2.11 (2H, *m*, H-17), 2.03/1.76 (2H, *m*, H-5), 1.97 (2 H, *m*, H-16), 1,86/1.66 (2H, *m*, H-9), 1.84 (2H, *m*, H-13), 1.81 (3H, *s*, H-25), 1.61 (3H, *s*, H-29), 1.49 (3H, *s*, H-28), 1.36 (3H, *s*, H-30), 1.34/1.27 (2H, *m*, H-4), 1.33 (3H, *s*, H-24), 1.23/1.13 (2H, *m*, H-12), 1.14 (3H, *s*, H-27), 1.06 (3H, *s*, H-26); ^{13}C-NMR ($CDCl_3$): δ 190.08 (*d*, C-1), 162.96 (*s*, C-7), 135.05 (*s*, C-15), 133.14 (*s*, C-2), 130.9 (*s*, C-19), 129.18 (*d*, C-18), 124.59 (*d*, C-14), 75.06 (*s*, C-10), 66.73 (*d*, C-21), 66.02 (*d*, C-22), 63.03 (*t*, C-3), 59.04 (*s*, C-23), 45.61 (*t*, C-20), 44.72 (*s*, C-11), 43.36 (*d*, C-6), 39.37 (*t*, C-16), 37.06 (*t*, C-12), 36.96 (*t*, C-9), 32.69 (*t*, C-4), 26.59 (*t*, C-5), 26.29 (*q*, C-27), 26.22 (*t*, C-17), 24.77 (*q*, C-24), 23.85 (*t*, C-8), 22.1 (*t*, C-13), 18.81 (*q*, C-30), 17.88 (*q*, C-26), 15.95 (*q*, C-29), 15.71 (*q*, C-28), 10.94 (*q*, C-25).

(6R, 10 S, 11 S)-22, 23-Epoxy-iridal **6**. UV λ_{max} (methanol) 258 nm; FABMS *m/z* 475 $[M+H]^+$ (pos.), 473 $[M-H]^-$ (neg.); ^1H-NMR ($CDCl_3$): δ 10.12 (1H, *s*, H-1), 5.05 (1H, *t*, *J* = 7 Hz, H-18), 4.88 (1H, *t*, *J* = 6.6 H-14), 3.54 (2H, *t*, *J* = 6, H-3), 3.24 (1H, *dd*, *J* = 9 Hz, H-6), 2.63 (1H, *t*, *J* = 6.3, H-22), 2.52/2.47 (2H, *m*, H-8), 2.05/1.87 (2H, *m*, H-5), 1.97 (2 H, *m*, H-16), 1.87/1.75 (2H, *m*, H-17), 1.87 (2H, *m*, H-21) 2.1/1.90 (2H, *m*, H-9), 1.80 (2H, *m*, H-13), 1.79 (3H, *s*, H-25), 1.60 (3H, *s*, H-29), 1.55 (2H, *m*, H-20), 1.51 (3H, *s*, H-28), 1.26 (3H, *s*, H-30), 1.23/1.13 (2H, *m*, H-12), 1.19/1.15 (2H, *m*, H-4), 1.23 (3H, *s*, H-24), 1.07 (3H, *s*, H-27), 1.05 (3H, *s*, H-26); ^{13}C-NMR ($CDCl_3$): δ 190.01 (*d*, C-1), 163.28 (*s*, C-7), 135.38 (*s*, C-15), 133.57 (*s*, C-2), 134.06 (*s*, C-19), 124.75 (*d*, C-18), 123.94 (*d*, C-14), 75.05 (*s*, C-10), 39.58 (*t*, C-21), 64.18 (*d*, C-22), 63.06 (*t*, C-3), 58.33 (*s*, C-23), 27.43 (*t*, C-20), 44.70 (*s*, C-11), 43.33 (*d*, C-6), 37.18 (*t*, C-16), 36.97 (*t*, C-12), 36.27 (*t*, C-9), 32.70 (*t*, C-4), 26.60 (*t*, C-5), 26.32 (*q*, C-27), 26.51 (*t*, C-17), 24.90 (*q*, C-24),

23.83 (*t*, C-8), 22.1 (*t*, C-13), 18.74 (*q*, C-30), 17.86 (*q*, C-26), 15.99 (*q*, C-29), 15.92 (*q*, C-28), 10.94 (*q*, C-25).

(6 S, 10 R, 11R)-22, 23-Epoxy-10-deoxy-21-hydroxyiridal **7**. UV λ_{max} (methanol) 258 nm; EIMS *m/z* (rel. int) 474 $[M]^+$ (0.1), 456 $[M\text{-}H_2O]^+$ (0.5), 109 (49), 55 (89), 43 (100), 41 (55); FABMS *m/z* 475 $[M+H]^+$ (pos.), 473 $[M-H]^-$ (neg); ^{1}H-NMR ($CDCl_3$): δ 10.18 (1H, *s*, H-1), 5.20 (1H, *t, J* = 6.8 Hz, H-18), 4.96 (1H, *t, J* = 6.6 H-14), 3.60 (2H, *t, J* = 6.4, H-3), 3,42 (1H, *m*, H-21), 3.38 (1H, *dd*, H-6), 2.63/2.15 (2H, *m*, H-8), 2.60, 1H, *d, J* = 8 Hz, H-22), 2.43/2.11 (2H, *m*, H-20), 2.12 (2H, *m*, H-17), 1.98 (2H, *m*, H-16), 1.88 (1H, *m*, H-10), 1.78 (3H, *s*, H-25), 1.77 (2H, *m*, H-13), 1.63 (2H, *m*, H-5), 1.60/1.38 (2H, *m*, H-9), 1.61 (3H, *s*, H-29), 1.49 (3H, *s*, H-28), 1.37 (3H, *s*, H-30), 1.33 (3H, *s*, H-24), 1.31 (2H, *m*, H-4), 1.17/1.10 (2H, *m*, H-12), 0.96 (3H, *s*, H-26), 0.81 (3H, *d, J* = 6.8 Hz, H-27); ^{13}C-NMR ($CDCl_3$): δ 190.13 (*d*, C-1), 163.39 (*s*, C-7), 134.71 (*s*, C-15), 133.26 (*s*, C-2), 130.83 (*s*, C-19), 129.28 (*d*, C-18), 125.02 (*d*, C-14), 35.73 (*d*, C-10), 66.69 (*d*, C-21), 66.03 (*d*, C-22), 62.96 (*t*, C-3), 59.07 (*s*, C-23), 45.62 (*t*, C-20), 40.12 (*s*, C-11), 43.25 (*d*, C-6), 39.39 (*t*, C-16), 31.67 (*t*, C-12), 30.50 (*t*, C-9), 31.46 (*t*, C-4), 23.94 (*t*, C-5), 15.20 (*q*, C-27), 26.27 (*t*, C-17), 24.78 (*q*, C-24), 27.44 (*t*, C-8), 21.08 (*t*, C-13), 18.81 (*q*, C-30), 24.18 (*q*, C-26), 15.94 (*q*, C-29), 15.70 (*q*, C-28), 10.83 (*q*, C-25).

Acknowledgements

The financial support of the Région Languedoc Roussillon and Pôle Européen is gratefully acknowledged.

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