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LOCALIZATION OF IRIDALS IN *IRIS GERMANICA* RHIZOMES

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Key Word Index—*Iris germanica*; Iridaceae; rhizomes; localization; triterpenoids; iridals; secondary plant products.

Abstract—Analysis of iridals in *Iris germanica* rhizomes of different ages indicated a higher level of these triterpenoids in young rhizomes. The analysis of small discs of rhizomes prepared from slices indicated an increasing dorso-ventral gradient of iridal amounts in 1- and 2-year-old rhizomes. Iridal composition was also found to differ, depending on the age of the rhizomes. Monocycloiridals are mostly represented in young rhizomes whereas cycloiridals appeared to be the main iridal form in the old ones. The study of the ratio free iridals (FI)/iridal esters (IE) revealed a higher ratio in the youngest rhizomes. Localization of iridals within rhizomes is correlated with previous results concerning their possible role and their biosynthesis in the plant.

INTRODUCTION

Since 1982, several unusual triterpenoids have been isolated from various *Iris* species [1–5]. These compounds may be distinguished as monocyclic iridals, bicyclic iridals and spiroiridals. Their common basic chemical structure is depicted in Fig. 1. In addition to their underivatized form, iridals may be esterified with fatty acids on the OH-group at C-3 (see Fig. 1 for carbon atom numeration).

Some of these compounds were also found in *Belamcanda* [7], a genus of Iridaceae closely related to *Iris* [8]. On oxidation some cycloiridals give rise to a fine violet-like fragrance (irones), used by perfumers.

It was recently suggested that these triterpenoids could be cell membrane constituents and could partially replace sterols in this cell compartment [9]. Dehydration experiments on *Iris* rhizome slices have also indicated the involvement of iridals in the maintenance of membrane integrity [10]. Their biosynthesis also seems to be located within the microsomal fraction. The iridal biosynthetic pathway is the same as that of sterols until 2,3-epoxysqualene [11,12]. After that, hydroxylations of iridals seem to be mediated by cytochromes P-450 [13] which are also membrane-located enzymes.

The rhizome is a key organ in the life of the *Iris*. It is both a storage organ which assures the perennity of the plant and constitutes the main way of species propagation. Iridals are present in rhizomes as well as in roots and leaves of studied species [14], but seeds seem to be free of them [15]. *Iris germanica* L. rhizomes contain *ca.* 70% free iridals whereas iridal esters are the major forms in roots and leaves [14].

Macroscopic localization of iridals and the study of FI/IE ratios in *I. germanica* rhizomes could contribute to our understanding the role of these compounds in this plant. This is the main aim of this work. For this study, slices were prepared from rhizomes of different ages and discs from slices as indicated in Fig. 2A (April harvest) and Fig. 2B (July harvest).

RESULTS AND DISCUSSION

Comparison between rhizomes

The results indicate that young rhizomes contain more iridals per g dry weight than older ones (Figs 3 and 4). Rhizomes I and II (Fig. 3) harvested in April as well as rhizomes I and II (Fig. 4) harvested in July, had the highest iridal content. However, the iridal amounts found

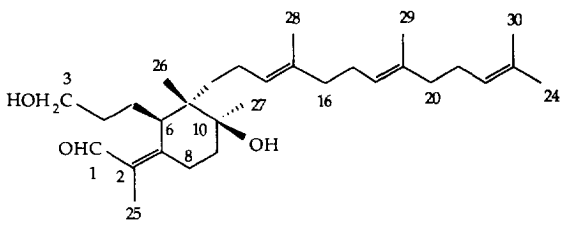

Fig. 1. Structure of iridal (21-desoxy-iridogermanal) according to [6].

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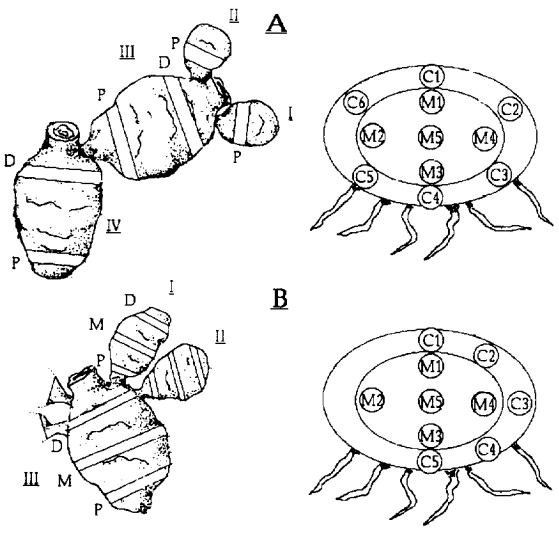


Fig. 2. Drawings of rhizome (left) and plans of transverse slices (right) of *I. germanica* harvested in April (A) and July (B). A: I and II, yearly rhizomes; III, 1-year-old rhizome; IV, 2-year-old rhizome. B: I and II, yearly rhizomes; III, 1-year-old rhizome. Slices were prepared from proximal (P), medium (M) or distal (D) parts of rhizomes. C and M are discs sampled in the cortical or medullar zone, respectively.

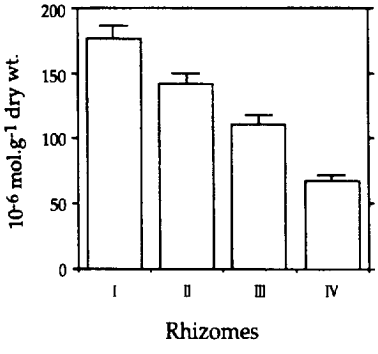


Fig. 3. Free iridal content in *I. germanica* rhizomes harvested in April. Rhizome numeration as indicated in Fig. 2(A). Results are means ± s.e. of 11, 11, 22 and 22 samples, respectively.

in young rhizomes in April (Fig. 3) are much higher than those found in July. This seems to indicate that there is a drop of iridal content during rhizome growth. This observation concurs with previous results obtained on leaves by Marner and Kerp [14]. These authors have indicated that leaf bud iridal contents decrease during leaf development.

Comparison between slices of rhizomes

Slices of 2-year-old rhizome IV (Fig. 2A), sampled in the proximal or distal parts of this organ, show little difference in their iridal content (Fig. 5). On the other hand, the iridal content of the 1-year-old rhizome III seems to be related to the location of the slice considered

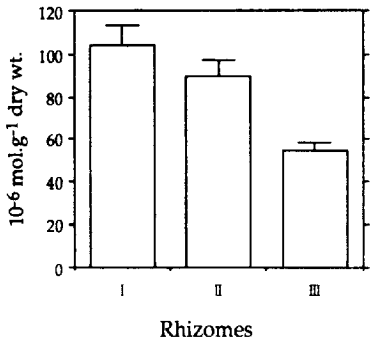


Fig. 4. Free iridal content in *I. germanica* rhizomes harvested in July. Rhizome numeration as indicated in Fig. 2(B). Results are means ± s.e. of 30 samples.

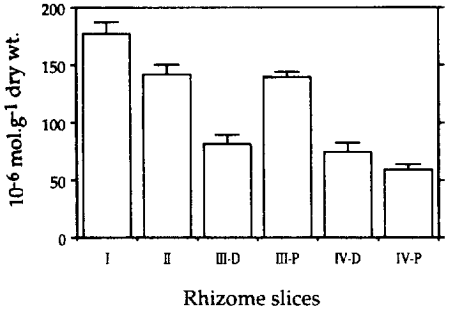


Fig. 5. Free iridal content in slices of *I. germanica* rhizomes harvested in April. Slice numeration as indicated in Fig. 2(A). Results are means ± s.e. of 11 samples.

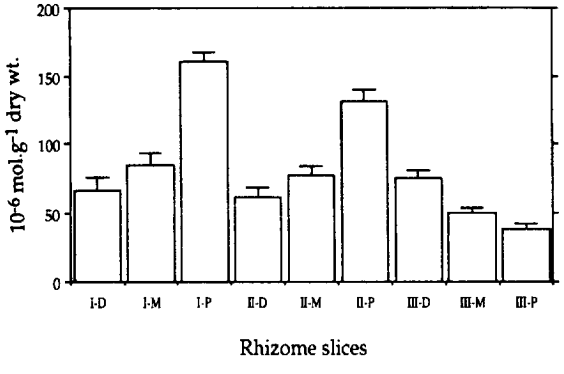


Fig. 6. Free iridal content in slices of *I. germanica* rhizomes harvested in July. Slice numeration as indicated in Fig. 2(B). Results are means ± s.e. of 10 samples.

(Fig. 5). The low iridal content of the III-D slice could be interpreted as a source/sink relationship between rhizome III and younger rhizomes I and II, respectively. It is interesting to see that in the July-harvested rhizomes there was no such relationship between rhizome III and younger rhizomes I and II (Fig. 6). Young rhizomes I and II (Fig. 6) behave in the same way. These results indicate again that there is a drop in the iridal content during elongation.

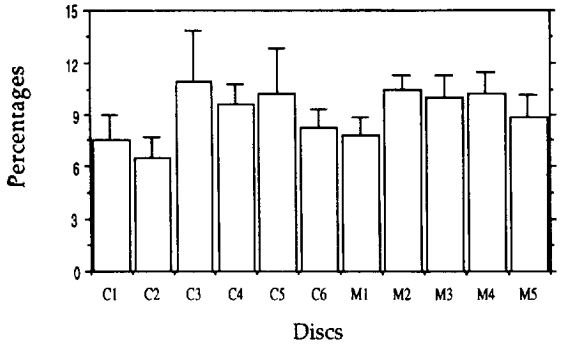


Fig. 7. Partition of free iridal percentages between discs sampled from cortical (C) and medullar (M) parts of *I. germanica* rhizomes harvested in April. Localization of discs as indicated in Fig. 2(A). Results are means ± s.d. of six rhizome slices.

Comparison between discs of slices

For this study, the iridal contents of each disc are replaced by their relative percentage within the slice. This is in order to avoid the important variability encountered between slice iridal contents (see above). On a transverse slice of *I. germanica* rhizome, two distinct zones are visible (Fig. 2): an outer zone constituted of cortical parenchyma (C) and an inner zone (M) of medullar parenchyma [16].

Visual analysis (Fig. 7) suggests the existence of disc groups representing nearly the same amounts of iridals in the cortex as well as in the medullar zone. Thus, the (C1, C2, C6) disc group is distinct from the (C3, C4, C5) group in the cortex. In the same manner, the (M1, M5) group is distinct from the (M2, M3, M4) group in the medullar zone. These qualitative observations are statistically analysed in Table 1.

Mann Whitney's U test [17] confirms significant differences between these disc groups. When these results are compared to the transverse slice schemes (Fig. 2), it is obvious that discs located in ventral parts contain significantly higher amounts of iridals than those of dorsal parts. Moreover, one may consider that there is an increasing dorso-ventral gradient of free iridals (FI) in the rhizome.

Table 2. Free iridals (FI)/iridal esters (IE) ratios, in cortical (C) and medullar zones (M) in *I. germanica* rhizomes of different ages

	Old rhizomes	*n*	Young rhizomes	*n*
C	3.22 ± 0.99a	24	3.67 ± 0.60a	12
M	3.50 ± 0.92a	20	5.01 ± 0.98b	10

Results are means ± s.d. of n discs analysed. Mann Whitney's U Test is used to compare the means obtained. Two different letters indicate significant differences between the means at 1% level. U test was carried out between lines and columns as well.

Ratio free iridals/iridal esters (FI/IE)

The results (Table 2) indicate that the FI/IE ratio is higher in young rhizomes and especially in their medullar parts. This seems to indicate that esterification could be enhanced in old rhizomes. Ageing induces cell membrane alteration [18] with free fatty acid (FFAs) liberation [19]. These FFAs could be sequestered by free iridals producing iridal esters. A similar behaviour was encountered when *I. germanica* rhizome slices were subjected to dehydration [10]. Nevertheless, iridal esters could be due to *de novo* synthesis and not necessarily to an esterification of free iridals [20].

Table 3 indicates the values of the FI/IE ratio within disc groups previously seen. Contrasting results are obtained when comparing old and young rhizomes. In old rhizomes, the dorsal part (C1, C2, C3 and M1, M5) presents a lower FI/IE ratio than the ventral part. Since the former part, in contact with air, is the one most exposed to dehydration, these results are in agreement with the increase of IE during water stress previously observed [10]. In young rhizomes the lower part and especially the cortical zone contains the highest IE proportion.

Table 1. Means of free iridal percentages per disc, in different disc groups from *I. germanica* rhizomes in cortical (C_1 to C_6) and medullar zone (M_1 to M_5) in function of the harvesting period

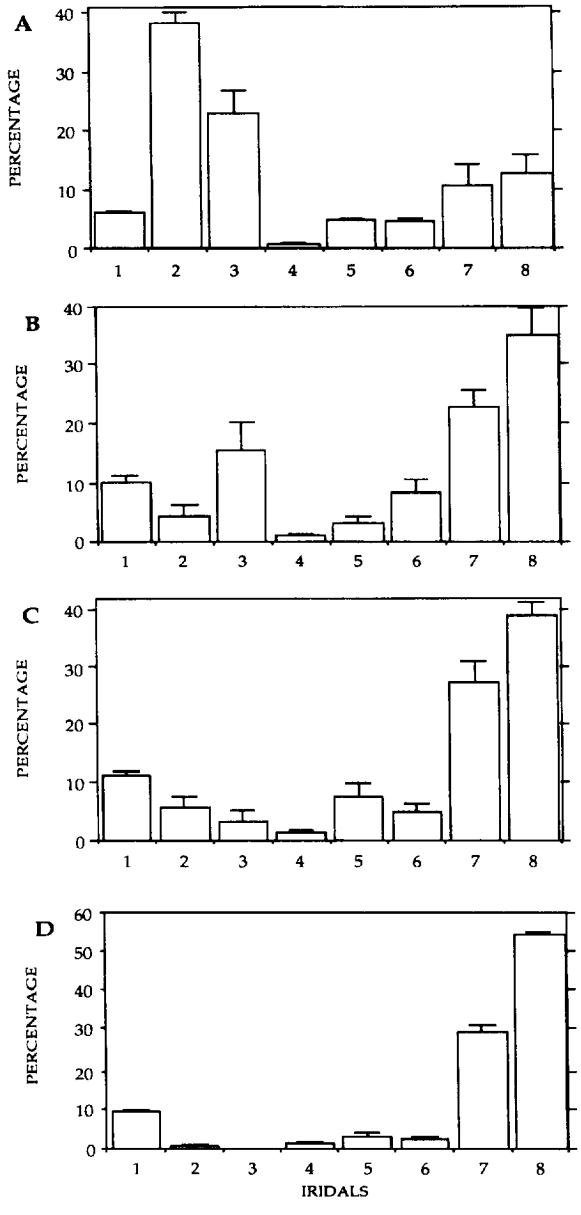
	Cortical zone				Medullar zone			
	C1, C2, C6	*n*	C3, C4, C5	*n*	M1, M5	*n*	M2, M3, M4	*n*
April (spring harvest)	7.4 ± 0.3a	18	10.2 ± 0.5b	18	8.3 ± 0.4a	12	10.2 ± 0.3b	18
	C1, C2, C3	n	C4, C5	n	M2, M4	n	M1, M3, M5	n
July (summer harvest)	8.1 ± 0.4a	27	10.8 ± 0.7b	18	12.1 ± 0.7c	18	10.0 ± 0.4b	27

Results are means ± s.e. of *n* discs analysed. Mann Whitney's U Test is used to compare the means obtained. Two different letters indicate significant differences between means at 1% level.

Table 3. FI/IE ratios in different disc groups from cortical (C_1 to C_6) and medullar zones (M_1 to M_5) of rhizomes of different ages

	Cortical zone				Medullar zone			
	C1, C2, C6	*n*	C3, C4, C5	*n*	M1, M5	*n*	M2, M3, M4	*n*
Old rhizomes	2.7 ± 0.6a	12	3.7 ± 1.1b	12	3.0 ± 0.4a	8	3.9 ± 0.9b	12
Young rhizomes	4.0 ± 0.6c	6	3.3 ± 0.4d	6	4.7 ± 0.9c	4	5.3 ± 1.1d	6

Results are means ± s.d. of *n* discs analysed. Mann Whitney's U Test is used to compare the means obtained. Two different letters indicate significant differences between means at 5% level.



Iridal composition in rhizomes of different ages

We have analysed the evolution of eight free iridals in rhizomes of four different ages. Our results are reported in Fig. 8. Young rhizomes (Fig. 8A) contain a high level of iridals **2** and **3**. Their level decreases with rhizome development (Fig. 8B) and iridals **7** and **8** become predominent. Iridal **6** reaches its highest level at this stage. In one- and two-year-old rhizomes (Fig. 8C and D) iridals **7** and **8** are always the major products. These latter compounds are precursors of γ-and α- irones, respectively [6]. It is well known by perfumers that only 2- or 3-year-old rhizomes should be used to give an optimal yield of irones. Figure 9 indicates the proportions of the three classes of iridals in the above rhizomes belonging to four age classes. It is significant that monocyclic iridals are the major form of iridals in young rhizomes. The decrease of these compounds gives rise to a concomittant increase of cycloiridals which represent about 80% of the iridals in the 2-year-old rhizomes. Spiroiridals always remain at a low level in *I. germanica* rhizomes. These results are in agreement with previous data concerning iridal biosynthesis in *I. pallida* cell suspension culture [20]. Some of these monocycloiridals could later be transformed to give spiro- and cycloiridals.

In conclusion, our results indicate that iridals are not uniformly distributed in rhizomes. Young ones have higher iridal levels than old ones. Moreover, in the same rhizome, this level increases from old parts to young

Fig. 8. Changes in percentages of eight iridals in *I. germanica* rhizomes of increasing age. A: yearly rhizomes harvested in April; B: yearly rhizomes harvested in July; C: one-year-old rhizomes; D: two-year-old rhizomes. Results are means ± s.e. of 22, 40, 10 and 20 samples for the rhizomes A, B, C and D, respectively. Iridals investigated are: monocycloiridals: **1**, 16-hydroxyiridal; **2**, 16,17-didehydro-26-hydroxyiridal; **3**, 16,17-didehydroiridal; spiroiridals: **4**, 29-hydroxy-spiroiridal; **5**, 29-acetoxy-spiroiridal; **6**, spiroiridal; Cycloiridals: **7**, iriflorental; **8**, iripallidal.

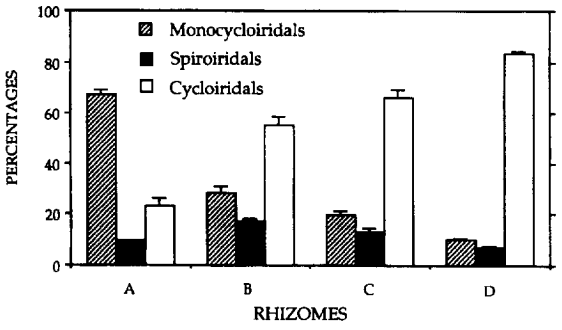


Fig. 9. Changes in percentages of mono-, spiro- and cycloiridals in *I. germanica* rhizomes of increasing age. A: yearly rhizomes harvested in April; B: yearly rhizomes harvested in July; C: one-year-old rhizomes; D: two-year-old rhizomes. Results are means ± s.e. of 22, 40, 10 and 20 samples for the rhizomes A, B, C and D, respectively.

parts in old rhizomes, whereas the opposite is found within young rhizomes. In slices, an increasing dorso-ventral gradient is present. We have also hypothesized sink/source relationships between young and old rhizomes. Marner and co-workers have reached the same conclusion after the incorporation of radiolabelled iridals into rhizomes (personal communication).

Under normal culture conditions, free iridals constitute the main iridal form. Nevertheless, the study of the FI/IE ratio indicates a heterogeneity of repartition. Old rhizomes as well as dorsal parts contain a higher proportion of esterified iridals. Senescence in old rhizomes and dehydration in dorsal parts in contact with air are known to induce membrane alteration. The increase of IE proportions in such tissues can be related to the role of iridals within cell membranes [9, 10]. The differing iridal composition of rhizomes of different ages indicates clearly that monocycloiridals are mainly located within young rhizomes. These results support the hypothesis identifying monocycloiridals as the first step in iridal biosynthesis as previously seen [20].

EXPERIMENTAL

Plant material. Rhizomes of *I. germanica* L. were obtained from Dr E. Zaid (Laboratory of Botany and Plant Physiology, University Mohamed V, Rabat, Marocco) in 1989 and cultivated until now in the field of the University of Montpellier II.

Sampling. Slices of rhizomes (*ca* 4 mm thick) and discs (4 × 4 mm) from these slices are prepd (see Fig. 2 for their localization).

Extraction. The discs (*ca* 50 mg fr. wt) were ground and extracted (× 3) with 1.5 ml of $CHCl_3$–MeOH (2:1). The three crude extracts were combined, filtered through glass wool and dried over Na_2SO_4. The solvent was evapd in a N_2 stream and the extract redissolved in a known vol. of $CHCl_3$–MeOH (2:1).

Analysis. Samples were analysed by HPLC using a MeOH–H_2O gradient as indicated previously in ref. [10]. Free iridals and iridal esters were determined by comparing their R_fs and UV with those of authentic standards obtained from Dr F.-J. Marner.

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