Composition of Iridals, Unusual Triterpenoids from Sword-Lilies, and the Seasonal Dependence of Their Content in Various Parts of Different *Iris* Species

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Iridals, Triterpenoids, *Iris pallida* Lam., *Iris germanica* Linn., *Iris pseudacorus* Linn.

The composition of iridals, new triterpenoids from sword-lilies, and their fatty acid esters in roots, rhizomes and leaves of *I. germanica* Linn., *I. pseudacorus* Linn. and two different varieties of *I. pallida* Lam. was determined. Quantitative analysis of their total content in the various parts of the plants in the course of the year showed specific seasonal changes in rhizomes and leaves. Based on these results the possible significance of these compounds for the plants is discussed.

Introduction

The iridals and cycloiridals are products of a hitherto unknown metabolism of squalene in higher plants and are present in lipid extracts of sword-lilies. Up to now more than 20 different compounds of this family, some of which are shown in Scheme 1, were isolated from different *Iris* species [1–3]. In addition to the underivatized iridals their esters at the hydroxypropyl side chain of ring B with fatty acids are present in the extracts. During our studies on the occurrence and structure of the spiroiridals **13** and **16** from *I. pseudacorus* we observed that different parts of the plants show a completely different pattern of iridals or their esters [3]. Thus, the hemiacetal **13** is main component in rhizomes whereas the roots of this *Iris* species contain mainly the acetate **16** and its ester with capric acid **18.** This raised the question whether this phenomenon is a common feature of the sword-lilies or limited to *Iris pseudacorus*. Furthermore we were interested to find out if there is a seasonal change in content or composition of iridals and iridal esters in the plants. By these investigations we hoped to get some insight into the biosynthesis of these compounds and their function.

Materials and Methods

Plant material

The plants used in this study are grown in the garden of the institute. *I. germanica, I. pseudacorus* and *I. pallida* "BS" were originally purchased from a nursery (Bornträger & Schlemmer, Offstein). The other variety of *I. pallida* was obtained several years ago from the garden of the Pharmacological Institute, University of Bonn, and is named after this origin. Both varieties can be identified as *I. pallida* by their appearance according to [4].

Sample preparation

For the monthly analysis one plant of each species was harvested and carefully cleaned. Roots, rhizomes and leaves were separated, cut and extracted twice with $CHCl_3$/MeOH (2:1, v/v). After evaporation of the solvent, water and ether (50 ml each) were added and the aqueous phase was extracted twice with ether (50 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo*. For HPLC analysis of the iridals and their esters 10 mg of the crude extract dissolved in 1 ml of methanol were applied to 400 mg of reversed phase material (RP 18 on silica 30–40 µ) in a Pasteur pipette. The triterpenoids were eluted with 4 ml of methanol. The solvent was evaporated, the residue dissolved in 1 ml of methanol and supplied for HPLC analysis.

Analysis

HPLC: Kontron model 200 HPLC equipped with a Hewlett-Packard model 1040 A diode array detector; column: Lichrocart RP 18 (125 mm × 4 mm i.d., Merck); elution profile: 1 ml/min, MeOH/H_2O 7:3 (5 min) gradient within 15 min to 100% MeOH, 100% MeOH (20 min).

The iridals were identified by comparison of their retention time and their UV spectra with ap-

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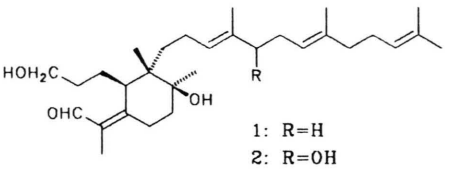

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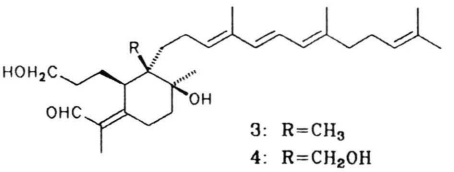
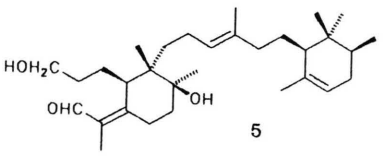
2: R=OH



3: R=CH_3
4: R=CH_2OH



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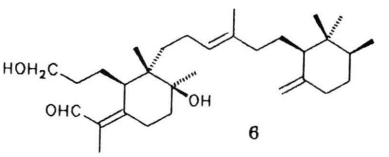

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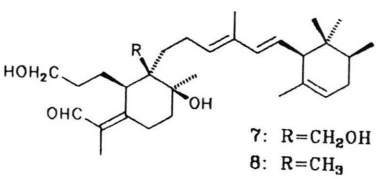
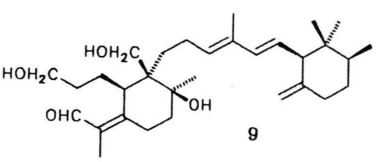


7: R=CH_2OH
8: R=CH_3



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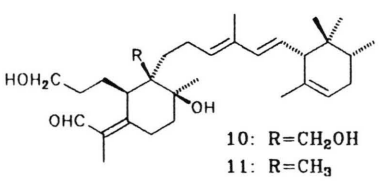
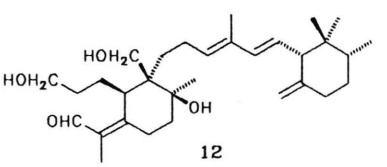


10: R=CH_2OH
11: R=CH_3



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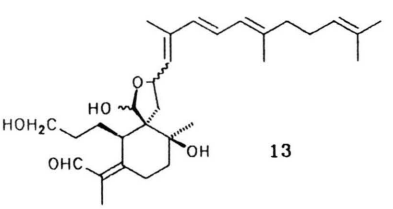

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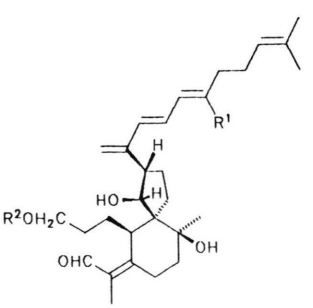

14: $R^1=CH_3$ $R^2=H$
15: $R^1=CH_2OH$ $R^2=H$
16: $R^1=CH_2OAc$ $R^2=H$
17: $R^1=CH_3$ $R^2=C_9H_{19}CO$
18: $R^1=CH_2OAc$ $R^2=C_9H_{19}CO$

Scheme 1. Iridals identified in the *Iris* species studied.

propriate standards. The typical UV spectra also served for the assignment of so far unidentified components as iridals or their fatty acid esters. Measurements of the molar extinction coefficients at 254 nm showed identical values (ε_{254} = 14,000) for several pure iridals (**2, 6, 7, 13** and the capric acid ester of **2**) independent of their absorption maxima. Consequently, equimolar solutions of the compounds give rise to identical peak areas in the HPLC chromatogram recorded at 254 nm, which therefore served as basis for the quantitative analyses. An average molecular weight of 470 for the hitherto unidentified iridals respectively of 700 for the unknown iridal esters (the fatty acids represent a mixture of C_{10} to C_{18} [5]) was assumed.

Results and Discussion

The quantitative determination of the total iridal content showed an interesting annual cycle of the triterpenoids and their esters produced or stored in the various parts of the plants. At the average the compounds amount to 0.1–0.2% of the fresh weight of the roots, whereas the values found in rhizome and leaf extracts are different for the various species. Thus, depending on the season, the rhizomes of both *I. pallida* varieties contain 0.1–1.1%, of *I. germanica* 0.1–0.5% and of *I. pseudacorus* 0.01–0.15% of iridals and their esters. From the leaves 0.1–0.7% *(I. pallida)*, 0.05–0.3% *(I. germanica)* or 0.01–0.4% *(I. pseudacorus)* of the substances were extracted. The instability of most of the compounds precluded measurements on the basis of dry weight of the plant material. The total amount of the iridals and iridal esters in the non-polar lipid fraction of the extracts is depicted in Fig. 1. The same trend shown here is found, when basing the calculation for the triterpenoids or the sum of extractable lipids on the fresh weight of the material. Thus, the possibility that differences in moisture or total lipid content account for the alterations is eliminated. Although, due to the limited number of plants available, only one specimen per species was analyzed each month, it is clearly visible that the iridal contents in all species studied show a similar pattern throughout the year. For this reason and since, except for the roots, which exhibit rather random variations, the tendency of changes continues for several months, the significance of the curves, which follow for leaves and rhizomes a very specific seasonal course, is not questioned. Apparently, in rhizomes the triterpenoids reach maximal values two different times during the year: in spring and in fall. The monthly position of

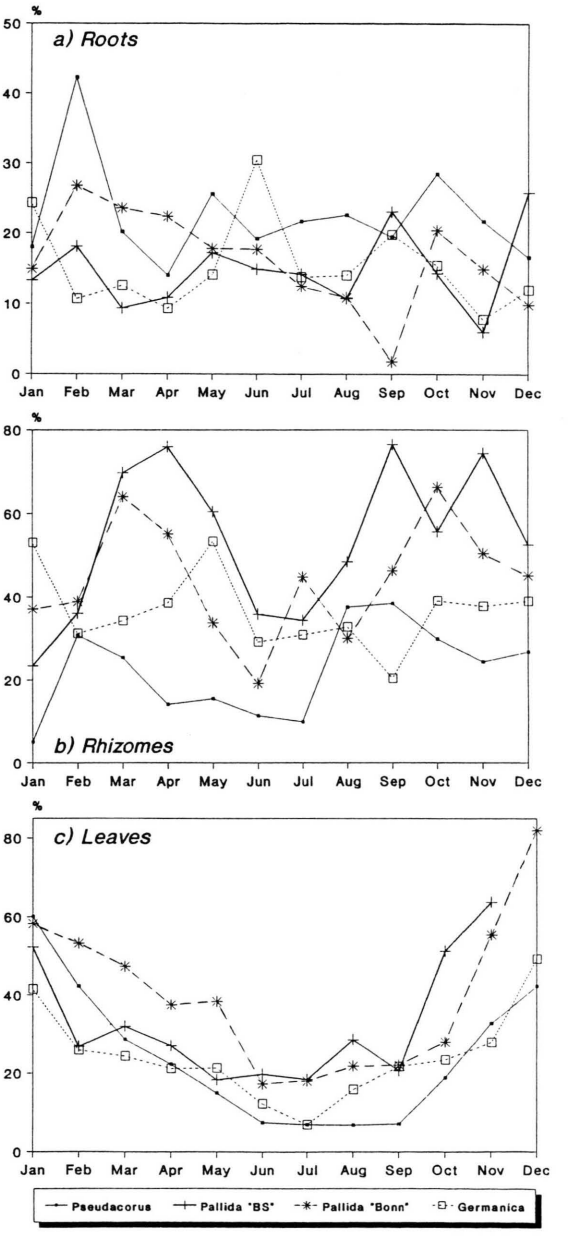

Fig. 1. Annual course of the content (weight %) of iridals and their esters in the neutral lipid fraction of a) roots, b) rhizomes and c) leaves of the various *Iris* species.

the peaks is different in the various species and does not show any coincidence with the climatic data shown in Fig. 2. Therefore, a direct influence of rainfall, duration of sunshine, temperature or combinations thereof on the production of the compounds can be excluded. The maxima may be connected, however, to two important events in the vegetation period, namely the growth of the leaves in spring and their senescence and decay in fall. Indeed, the growth of leaves was fastest in *I. pseudacorus,* followed by the two *I. pallida* varieties and *I. germanica.* Accordingly, the highest iridal values were reached at different times. The rather mild winter in 1989/1990 effected the relatively early start of the growing season. In contrast to the rhizomes, the spring and autumn maxima are not too distinct in the leaves. Instead, the iridal content rises in fall and winter to up to tenfold of the summer value and slopes down again in spring. Apparently, in leaves the decline of iridals runs parallel to the growth, whereas the decay of the old leaves and the formation of the new shoots which takes place in October/November leads to the increased formation or deposition of the triterpenoids.

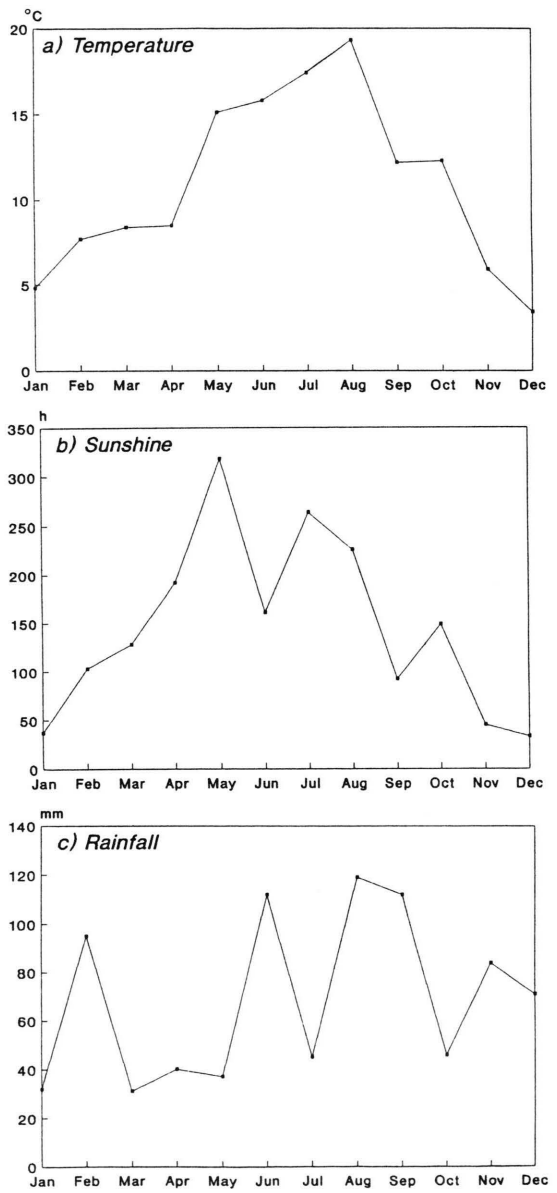

Fig. 2. a) Average temperature (°C), b) hours of sunshine per month and c) monthly rainfall (mm) in Cologne during 1990 (data courtesy of the Meteorological Institute, University of Cologne).

For the individual compounds no similar annual cycle and only small individual deviations were found. Table I shows the relative amounts of iridals and their esters in the extracts. It is obvious that remarkable differences between roots, rhizomes and leaves are not limited to *I. pseudacorus* but seem to be a general feature. In all plants the iridal esters are the predominant compounds in roots and leaves (it should be noted that **17** and **18** are iridal esters, too). Much smaller amounts, however, are found in the rhizomes. On the other hand, within a species, leaves and rhizomes have a very similar qualitative composition of underivatized iridals. Leaving aside the production of cycloiridals with enantiomeric E-ring [6, 7] such as **5–9** or **10–12** the phylogenetic relationship of *I. pallida* and *I. germanica,* both belonging to the bearded irises, is obvious. *I. pseudacorus,* however, shows a completely different iridal pattern. This may be an adaptation to the wet and anaerobic environment since *I. pseudacorus* occupies lakeside muds, whereas the bearded irises prefer well-drained soils. It has been shown earlier that *I. pseudacorus* is much more tolerant to anoxia than *I. germanica* and even more so to reexposure to air after prolonged anoxic treatment [8, 9]. Therefore, the presence of some special protective mechanism against peroxidation of membraneous lipids has been postulated for *I. pseudacorus.* Considering the chemical nature of the iridals and especially the conjugated trienes **13–18,** being the main iridals of this plant, they may very well play a

Table I. Relative amounts of iridals and their fatty acid esters (relative deviation ±20%) in roots, rhizomes and leaves ($\Sigma_{unid.}$ = total of unidentified iridals, Σ_{FAE} = total of iridal esters).

	I. pallida "Bonn"			*I. pallida* "BS"			*I. germanica*			*I. pseudacorus*		
Iridal	Roots	Rhizomes	Leaves	Roots	Rhizomes	Leaves	Roots	Rhizomes	Leaves	Roots	Rhizomes	Leaves
1	0.4			1.5		0.5						
2	1.2	4.8	2.4	2.2	5.9	3.1	1.6	6.4	3.7	1.5	2.9	2.7
3	0.6		0.9	1.5	5.4	2.5			3.4			
4	0.9	2.5	2.5	2.0	9.8	5.4	2.2	2.3	4.6		3.9	2.6
5					0.2			3.3				
6							2.7	6.4	2.2			
7	1.8	23.4	5.4					22.6	4.3			
8		2.8	0.7					0.9	0.7			
9								11.6	3.4			
10					6.8	1.1						
11					0.7	0.2						
12					15.3	2.5						
13		0.2	1.0	1.1	0.7	1.0		0.5	1.3	1.3	10.7	10.8
14	6.4	1.1	4.9	6.9	1.8	4.4	7.9	2.2	7.2	1.6		
15	0.7	1.3	1.8	0.5	1.7	1.7		1.1	1.4	0.9	0.2	0.5
16	3.7	1.6	3.1	2.2	2.3	2.9	2.7	1.9	4.3	6.6	2.1	2.7
17										9.0		
18										22.2		
$\Sigma_{unid.}$	10.3	9.8	9.7	17.9	7.2	9.9	16.1	9.8	13.3	16.6	26.5	18.7
Σ_{FAE}	74.0	52.5	67.6	64.2	42.2	64.8	66.8	31.0	50.2	40.3	53.7	61.4

role in this process. Indeed, these compounds are extremely sensitive and decompose rapidly on contact with air [2, 3]. Certainly, the addition of an oxygen radical to the conjugated portion of the side chain is the reason. In the much less sensitive cycloiridals **7–12** this reaction leads to the well known irones responsible for the violet-like scent of the *Iris* oil [10].

Another protective function of the iridals may be concluded from their ability to trap solvents which has been a very unpleasant phenomenon during isolation and structure elucidation. Thus, an X-ray analysis of compound **6** proved that it crystallized from methanol together with one molecule of the solvent [5]. Certainly water is as firmly held in place and this way the compounds may help in preventing desiccation of the plant. The extremely high content of iridals in the new leaves during the winter period and in the rhizomes of the bearded irises which are adapted to very dry habitats thus would find an explanation.

Finally, these triterpenoids may serve for the production of a protective cover upon wounding. Thus, in the course of the oxidative decomposition, mentioned above, the highly substituted B-ring of the compounds polymerizes. No definite oligomeric reaction products of this part of the molecule have been found so far. The polymer is insoluble in aqueous solvents and therefore would represent an effective coating, equivalent to the resin of trees or the latex of Euphorbiae.

Acknowledgements

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