Structure Determination of Tigridial, an Iridopentaene from *Tigridia pavonia* (Iridaceae)

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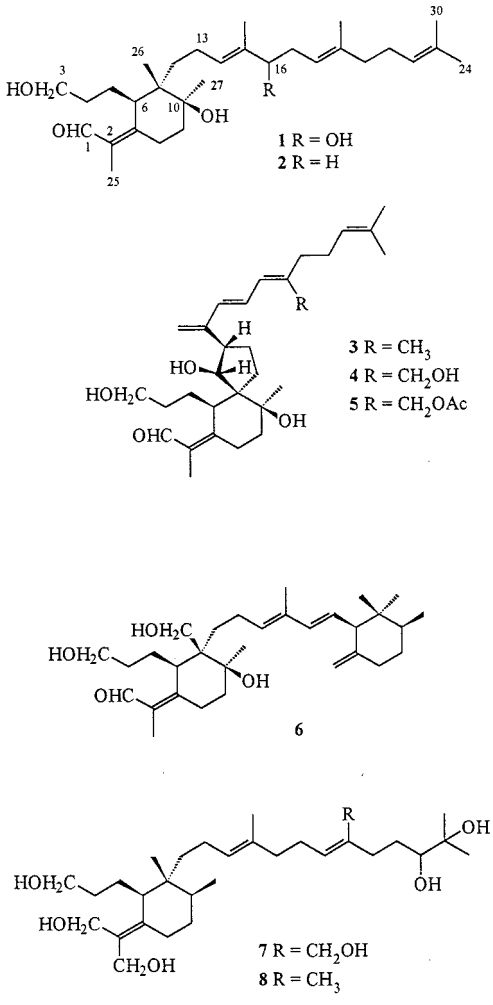
Besides numerous known iridals, which are unusual mono- or bicyclic triterpenoids of Iridaceae, lipid extracts of *Tigridia pavonia* bulbs contain tigridial (**17**), a new spirobicyclic hemiacetal with a conjugated pentaene moiety in the terpenoid side chain. The isolated natural product decomposes rapidly despite exclusion of light and oxygen. In contrast, derivatives of **17** formed by Diels–Alder reaction with 4-phenyl-3*H*-1,2,4-triazole-3,5-(4*H*)-dione (**13**) are stable, as are the products of a subsequent reduction with $NaBH_4$. The products **15a,b** and **16a,b** of this reaction sequence were isolated and their structures elucidated by spectroscopic techniques. From these results structure **17** was inferred for the new natural product, which was confirmed by 1D and 2D NMR spectroscopy of a mixture of **11** and **17**, in which the latter had been stabilized by addition of hydroquinone. The stereochemistry of **17** was deduced from 2D NOE experiments and comparison with known compounds.

Introduction

Within the last two decades numerous iridals, unusual phytogenic triterpenoids with mono-, spiro- or bicyclic structures (e.g. **1**–**6**), have been isolated from lipid extracts of sword lilies.[1] The compounds are derived from the cyclization of epoxysqualene to a bicyclic intermediate, which subsequently rearranges to give the iridal ring system characteristic of this family of natural products.[1] Except for crystallopicrin **7** and its deoxy-derivative **8**, which were isolated from toadstools,[2] triterpenoids showing this structural feature have only been found in Iridaceae. Plants of the genus *Iris*, in general, are a very rich source for these compounds, but they also have been found in other genera such as *Belamcanda*[3] and *Hermodactylus*,[1] whereas the investigated species of *Gladiolus* and *Crocus* contain only minute amounts.[4] In our ongoing studies on the chemistry and biochemistry of these triterpenoids we examined lipid extracts of *Tigridia pavonia*, which is a bulbous member of the Iridaceae, originally derived from Middle America but for long a popular garden plant in Europe. We report here on the composition of this oil and the structure elucidation of a new iridal.



Scheme 1

Results and Discussion

HPLC analysis of the essential oil of *Tigridia pavonia* bulbs with diode array detection revealed the presence of several known iridals, namely 16-hydroxyiridal **1** (10%), iridal **2** (5%), spiroiridal **3** (31%), 26-hydroxyirid-16-enal **9** (7%), and 28-acetoxyspiroiridal (belamcandal) **11** (14%), which were identified by comparison with authentic standards.[1][3] Compounds with the α,β-unsaturated aldehyde moiety of the iridal ring system as the only chromophore

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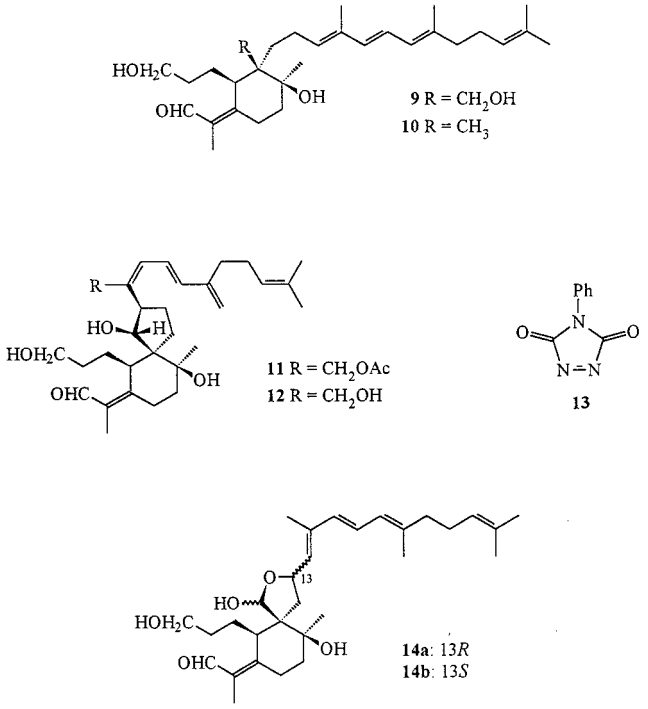
(e.g. **1** and **2**) show a UV absorption at λ_{max} = 254 nm. The iridotrienes (e.g. **3**, **9** and **11**) are easily recognized by their typical spectrum of a conjugated triene (λ_{max} = 278 nm) with a shoulder at 254 nm.



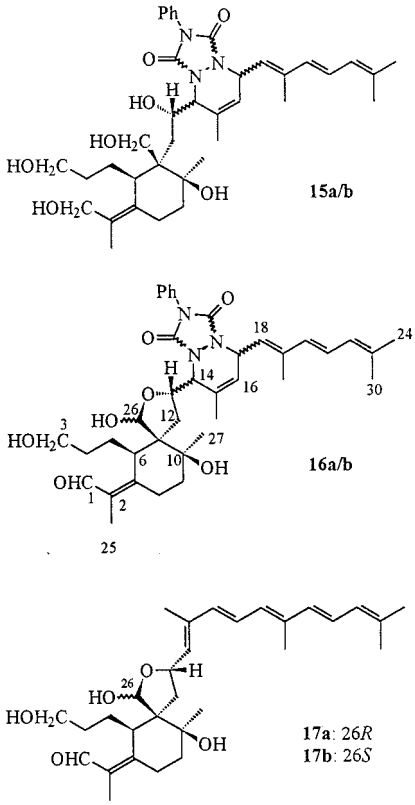
Scheme 2

In contrast, the UV spectrum of the main component (33%), eluted on reversed phase (RP) columns immediately after, but not completely resolved from, **11** showed two maxima at 254 and 340 nm, the latter with a fine structure characteristic of a polyene chain with side maxima at 324 and 357 nm. Therefore, it was highly probable that an iridal with a conjugated pentaene moiety was present. A molecular weight of 484 was determined for the compound by HPLC/MS: by use of a particle beam interface and NCI a value for the M^- ion at *m/z* = 484 was found, whereas APCI in the positive mode showed an $[M - H_2O + H]^+$ ion at *m/z* = 467. All attempts to isolate this compound failed, since it began to decompose with the formation of insoluble polymers almost immediately after the chromatographic separation of the extract despite all efforts to exclude light and oxygen. Similar problems upon the isolation of the iridotriene **10** have been overcome by Diels–Alder reaction of the conjugated double bonds with 4-phenyl-3*H*-1,2,4-triazole-3,5-(4*H*)-dione (PTAD, **13**).[5] In the course of this study the spiroiridal compound **5** was also treated with this dienophile. In both cases the formation of only two main products was observed; these proved to be the diastereomeric adducts of PTAD to the two double bonds adjacent to the ring systems. The pentaene system showed a similar behavior and semipreparative RP HPLC separation of the reaction mixture, obtained by treating the appropriate fraction with PTAD after MPLC separation of the crude extract, gave three fractions, which were stable enough for spectroscopic analyses.

The CI mass spectra of all three fractions were identical, showing that a single molecule of PTAD had reacted with the pentaene moiety, to give rise to an $[M + H]^+$ ion at *m/z* = 660 in the positive and an M^- ion at *m/z* = 659 in the negative mode. This finding was confirmed by the UV spectra, which still indicated the presence of a conjugated triene system with absorption maxima at 274 (sh), 284 and 294 (sh) nm, and a shoulder at 254 nm pointing to the unchanged acrolein subunit. The NMR spectra of the main fraction, however, showed that it contained a mixture of at least four components. Thus, in the range δ = 10.5–11 in the ^1H NMR spectrum four singlets of aldehyde protons, with different intensities, were seen. Despite their complexity the ^1H, ^{13}C and 2D NMR spectra clearly pointed to a spirobicyclic hemiacetal as a partial structure, as previously found in the 13*R*-spirotriene **14a**, isolated from *Iris pseudacorus*[1][6] and its *S* epimer at C13, **14b**, which has recently been isolated from *Belamcanda chinensis*[7] and *I. fulva*.[8] Thus, the characteristic low field resonances of the acrolein moiety at the iridal ring and of the hemiacetal section could be seen. The resolution of the olefinic and aliphatic regions, however, was insufficient for a complete structure elucidation.

Since it is well established that the hemiacetal ring of compound **14** is a mixture of the two possible anomers, it seemed probable that the same held true for the compounds at hand. Therefore it was anticipated that reductive cleavage of the hemiacetal ring would reduce the mixture to two components. Indeed, after reduction with $NaBH_4$ only two compounds **15a** and **15b** (3:1) were left, which were easily separated by semipreparative RP HPLC. In their UV spectra the shoulder at 254 nm had disappeared, indicating the reduction of the α,β-unsaturated aldehyde, whereas the conjugated triene still gave maxima at 273 (sh), 283 and 293 (sh) nm. NCI MS showed an M^- ion at *m/z* = 663, confirming the reduction of two carbonyl groups. ^1H, ^{13}C, H,H-COSY, HMQC and HMBC NMR experiments established the isomeric structures **15a,b** for the two products. Thus, the spectra of **15a** showed an AB system for the α,β-unsaturated carbinol group at δ_H = 3.83/4.59 and the corresponding ^{13}C resonance at δ_C = 63.3. The quaternary carbons of the former acrolein unit appear at δ = 135.6 (C2) and 129.5 (C7). C10 gives rise to a signal at δ = 75.2 and the CH_2OH group of the propanol side chain at δ = 3.58–3.69 in the ^1H NMR spectrum and at δ = 62.6 in the ^{13}C NMR spectrum. The reductive cleavage of the hemiacetal ring forms a primary alcohol at C26 (δ = 4.15/4.30, 63.8) and a secondary alcohol at C13 (δ = 4.51, 71.6). The tetrahydropyridazinyl ring formed by addition of PTAD to the adjacent double bonds substitutes the latter carbon as shown by appropriate long-range couplings (H12–C14, H14–C13). The conjugated triene moiety is connected to the opposite side of the six-membered ring and its NMR resonances and coupling constants (Tables 1 and 2) are in agreement with an all-*E* geometry. Therefore, the compound can be assigned structure **15a**.

The second reduction product, compound **15b**, proved to be an isomer, as it showed almost identical NMR spectra



Scheme 3

(Table 1). Slight differences were seen only around the ring system formed by the Diels–Alder reaction. Considering the hindered inversion at the bridge nitrogens, from the addition of PTAD to a diene with prochiral centers the formation of four different diastereoisomers (two *exo* and two *endo* adducts) can be expected, provided that the starting diene system had a uniform (e.g. *E*,*E*) geometry. Therefore, **15a,b** represent two of these products.

The remaining products **16a,b** were present in the two minor fractions, obtained from the Diels–Alder reaction mixture. Detailed analysis of their ^{1}H, ^{13}C and 2D NMR spectra (H,H- and H,C-COSY, HMBC) showed that they were indeed the two additional stereoisomers. Thus, the fully substituted α,β-unsaturated aldehyde of **16a** gave rise to signals at $\delta_H = 10.29$ (**16b**: $\delta = 10.71$) and $\delta_C = 190.2$, 132.0 and 161.4 (**16b**: $\delta = 189.6$, 132.8, 160.5). The primary carbinol group appeared at $\delta_H = 3.58$ (3.53) and $\delta_C = 61.4$ (62.2), and C10 gave a resonance at $\delta_C = 72.5$ (72.1). The presence of the hemiacetal ring was established by signals in the ^{1}H NMR spectrum at $\delta = 5.58$ (5.38, H-26) and 4.06 (4.00, H-13) and the corresponding ^{13}C resonances at $\delta =$ 99.4 (99.9, C-26) and 80.9 (81.2, C-13). Again, direct and long range couplings indicated that the double bonds next to the hemiacetal ring had reacted with PTAD, whereas the terminal triene moiety was unchanged. The complete assignment of the NMR spectroscopic data can be found in Tables 1 and 2. Surprisingly, the NMR spectra of **16a** and **16b** showed the presence of single compounds with no signs of anomeric mixtures. Presumably the hemiacetal ring is stabilized by hydrogen bonding with the PTAD moiety, or steric hindrance prevents the protonation of the ring oxygen and thus the formation of the isomer by cleavage of the five-membered ring.

From the nature of **15a,b** and **16a,b** the structure of a 26-hydroxy-13-oxaspiroirido-16,20-dienal **(17)** can be assigned to the natural product from *Tigridia pavonia* which has been named tigridial. Comparison of the spectroscopic data with the values found for other iridals, and biosynthetic reasons,[1] suggest a 6*R*,10*S*,11*R* configuration. At this point no attempts were made to elucidate the stereochemistry at

Table 1. ^{1}H NMR data (δ) of **11** and **15–17**[a]

	11[b,d]	**15a**[c,e]	**15b**[c,e]	**16a**[b,d]	**16b**[c,d]	**17a**[b,d]	**17b**[b,d]
H-1	10.16 s	3.83/4.59 AB-system (11)	3.84/4.59 AB-system (11)	10.29 s	10.71 s	10.19 s	10.16 s
H-3	3.44-3.67 m	3.58-3.69 m	3.59-3.71 m	3.58 m	3.53 m	3.44-3.67 m	3.44-3.67 m
H-4	1.24-1.45 m	1.39-1.55 m	1.40-1.54 m	1.10-1.30 m	1.20-1.40 m	1.24-1.45 m	1.24-1.45 m
H-5	1.85-2.05 m	2.04-2.19 m	2.06-2.22 m	1.90-2.00 m	2.00-2.20 m	1.85-2.05 m	1.85-2.05 m
H-6	3.55 brd (9)	3.38 brd (10)	3.4 brd (10.6)	3.79 brd (10)	3.96 brd (10)	3.64 brd (9)	3.13 brd (9)
H-8	2.49/2.66 2m	2.36/2.45 2m	2.34/2.47 2m	2.52-2.70 m	2.50-2.70 m	2.66/2.49 2m	2.66/2.49 2m
H-9	1.71-1.74/1.81-1.84 2m	1.39/1.69 2m	1.4/1.69 2m	1.10-1.30 m	1.10-1.30 m	1.71-1.74/1.81-1.84 2m	1.71-1.74/1.81-1.84 2m
H-12	1.23-1.34/1.46-1.53 2m	1.55/1.95 2m	1.54/1.96 2m	1.60-1.80 m	1.30-1.60 m	1.42-1.49/1.88-1.95 2m	1.24-1.32/1.95-2.01 2m
H-13	1.62-1.69/1.91-1.98 2m	4.51 m	4.50 m	4.06 q (8.5)	4.00 q (8)	4.82-4.91 m	4.99-5.08 m
H-14	3.07-3.10 m	4.51 m	4.50 m	4.48 d (8.5)	4.46 d (8)	5.63 d (11)	5.38 d (12)
H-16	6.22 d (11)	5.03 d (1)	5.10 d (1)	5.35 brs	4.92 brs	6.20 d (15)	6.19 d (15)
H-17	6.48 dd (11,15)	4.87 dd (1,9)	5.10 dd (1,8.3)	5.15 brd (9)	4-90 brd (9)	6.53 dd (11, 15)	6.53 dd (11, 15)
H-18	6.29 d (15)	5.57 d (9)	5.42 d (8.3)	5.27 d (9)	5.33 d (9)	6.07 d (11)	6.07 d (11)
H-20	2.18-2.26 m	6.39 d (15.2)	6.71 d (15)	6.12 d (15.3)	6.60 d (15)	6.17 d (15)	6.17 d (15)
H-21	2.14-2.20 m	6.60 dd (15.2,11)	6.63 dd (15, 10.6)	6.48 dd (15.3, 10.8)	6.63 dd (15, 11)	6.44 dd (11, 15)	6.44 dd (11, 15)
H-22	5.08 brt (7)	6.03 d (11)	6.08 d (10.6)	5.82 d (10.8)	6.10 d (11)	5.93 d (11)	5.93 d (11)
H-24	1.66 s	1.67 s	1.69 s	1.79 s	1.70 s	1.78 s	1.78 s
H-25	1.80 s	1.85 s	1.86 s	1.83 s	1.89 s	1.78 s	1.78 s
H-26	4.45 d (4)	4.15/4.30 AB-system (11)	4.15/4.31 AB-system (11)	5.58 s	5.38 s	5.65 s	5.47 s
H-27	1.25 (s)	1.15 s	1.14 s	1.18 s	0.77 s	1.26 s	1.50 s
H-28	4.72/4.55 AB-system (13.5)	1.69 s	1.63 s	1.79 s	1.30 s	1.78 s	1.78 s
H-29	5.07/5.03 2s	1.90 s	1.89 s	1.93 s	1.80 s	1.90 s	1.90 s
H-30	1.57 s	1.63 s	1.63 s	1.77 s	1.62 s	1.78 s	1.78 s
Phe-2,2'	-	7.81 d (8)	7.84 d (8)	7.46 m	7.70 d (8)		
Phe-3,3'	-	7.22 dd (8,8)	7.21 dd (8, 8)	7.45 m	7.20 m		
Phe-4	-	7.02 dd (8,8)	7.02 dd (8,8)	7.36 m	7.10 dd (8, 8)		
Acetyl-CH_3	2.08 s						

[a] *J* values (Hz) are given in parentheses. – [b] $CDCl_3$. – [c] [D_6]benzene. – [d] 300 MHz. – [e] 600 MHz.

Table 2. ^{13}C NMR data (δ) of **11** and **15**−**17** (n.d. = not determined)

	11[a,c]	**15a**[b,d]	**15b**[b,d]	**16a**[a,c]	**16b**[b,c]	**17a**[a,c]	**17b**[a,c]
C-1	191.2	63.3	63.3	190.2	189.6	190.7	190.2
C-2	132.9	135.6	135.8	132.0	132.8	132.6	132.9
C-3	61.9	62.6	62.2	61.4	62.2	61.9	61.9
C-4	31.1	31.9	31.5	29.6	31.5	31.1	31.1
C-5	28.2	26.4	26.2	27.4	28.5	28.2	28.2
C-6	42.4	44.4	43.9	42.1	43.7	43.5	48.4
C-7	163.0	129.5	129.8	161.4	160.5	161.8	161.1
C-8	23.9	21.6	21.5	23.8	23.9	23.9	23.7
C-9	37.9	38.3	38.3	39.0	39.0	38.7	38.7
C-10	74.2	75.2	75.5	72.5	72.1	72.9	74.2
C-11	57.7	47.6	48.0	61.0	62.0	60.9	56.3
C-12	35.2	37.3	37.1	39.6	40.0	42.4	45.7
C-13	25.2	71.6	71.6	80.9	81.2	76.6	74.0
C-14	47.9	62.7	63.1	62.9	63.2	135.4	132.0
C-15	134.0	130.3	130.3	129.9	130.2	135.8	135.5
C-16	130.3	121.6	121.9	122.6	123.3	136.4	135.9
C-17	123.0	54.1	53.0	55.0	55.0	125.6	125.3
C-18	136.0	126.8	125.1	125.1	125.5	130.7	130.5
C-19	145.6	137.7	136.3	138.0	135.4	137.0	137.0
C-20	32.3	134.2	126.7	132.0	127.1	134.8	134.8
C-21	26.8	126.4	128.1	126.2	126.7	126.5	126.4
C-22	123.8	126.2	126.5	125.0	126.4	129.1	129.0
C-23	132.1	136.2	137.4	137.1	137.1	136.2	136.2
C-24	25.67	26.2	26.1	26.3	26.2	26.3	26.2
C-25	11.07	17.1	17.0	11.2	11.2	11.0	11.0
C-26	77.7	63.8	63.5	99.4	99.9	99.4	105.2
C-27	27.9	27.0	26.7	27.6	27.3	27.4	28.0
C-28	67.6	21.4	21.0	22.0	21.5	12.8	12.8
C-29	117.3	13.3	20.8	13.2	20.5	13.0	12.9
C-30	17.8	18.4	18.4	18.6	18.4	18.6	18.5
Phe C-1		132.5	132.5	130.8	132.4		
Phe C-2/2'		125.7	125.6	125.6	125.6		
Phe C-3/3'		129.2	129.1	129.1	129.0		
Phe C-4		128.4	127.9	128.4	128.3		
PTAD C=O		150.8	151.0	152.0	n.d.		
		152.7	152.8	153.4			
Acetyl CH_3	21.0						
Acetyl C=O	171.6						

[a] $CDCl_3$ − [b] [D_6]benzene − [c] 75 MHz − [d] 150.9 MHz

C13 and the chiral centers of the tetrahydropyridazinyl rings of the four Diels−Alder adducts, as this would have been no help in solving the geometry of the original C14−C17 diene system. Instead, numerous experiments were carried out to stabilize tigridial before or after isolation from the crude extract by preparing derivatives without affecting the conjugated polyene chain. All these efforts were unsuccessful. It was, however, possible to delay the decomposition of the compound by addition of hydroquinone to fractions obtained after the initial reversed phase MPLC. This way a fraction was isolated consisting of 65% of the two tigridial epimers **17a** and **17b** (present in a ratio of 6:4) and 35% belamcandal (**11**), which was used to record ^1H, ^{13}C, HMBC, HMQC and ROESY spectra.

With these NMR spectroscopic data it was possible to clarify the stereochemistry and to unequivocally assign all ^1H and ^{13}C resonances of the two anomeric hemiacetals (Tables 1 and 2). The all-*E* geometry of the pentaene chain was proven by the appropriate H,H coupling constants (Table 1), strong allylic couplings H14/H28, H18/H29 and H22/H30, and NOE′s of H17 with H28 and H29, and of H21 with H29 and H30. The main isomer **17a** showed NOE cross peaks of H27 with H13 and H26, (Figure 1) whereas the latter is missing for the minor compound **17b**.

Therefore, C26 is *R*-configured in **17a** and *S* in **17b**, whereas the *S* configuration can be assigned for C13 of

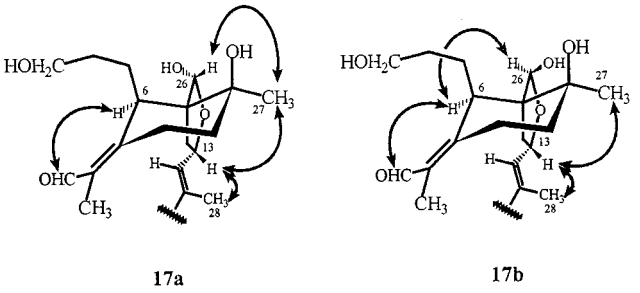


Figure 1. Selected NOE correlations of the tigridial epimers **17a** and **17b**

both isomers. It is remarkable that the instability of the compounds resembles that of the iridotriene hemiacetal **14b** with 13*S*-configuration,[8] whereas the corresponding 13*R* epimer **14a** is stable for many years if stored in the cold. This may be due to the close proximity of the C26 hydroxy group and the conjugated side chain in the 13*S* isomers, which can be shown by MM2 molecular model calculations. Presumably, a neighboring effect of the hydroxy group supports the decomposition of these compounds.

It has frequently been shown that the unmodified iridals are usually accompanied by the corresponding fatty acid esters at C3.[1] The extracts of *Tigridia* bulbs contain only small amounts of these compounds. The main components were identified as palmitic acid esters of **1, 3** and **17** by combined LC/UV (DAD) and LC/MS (APCI) analyses.

In contrast to the bulbs, leaves of *Tigridia pavonia* contain only trace amounts of the pentaenes. Instead, the known iridals **1** (2%), **2** (5%), **3** (20%), **4** (6%), **5** (58%) and **12** (9%) are present in considerable amounts. Furthermore, in these extracts the underivatized iridals amount to only 40%, whereas their $C_{14:0}$ and $C_{16:0}$ esters account for the remaining 60% of the iridal fraction.

It has been demonstrated that dehydrogenation of iridal **2** rather than dehydration of a hydroxyiridal (e.g. **1**) leads to the formation of the iridotrienes.[9] Presumably, the pentaene moiety is also produced by the further action of a desaturase. The function of the iridals in the plants is still unknown. There are, however, strong indications that the compounds serve as membrane constituents,[10] and it has been inferred from their chemical nature and behavior that they protect the cells against environmental damage.[1] The high affinity of compound **17** towards oxidative degradation supports this theory. Further investigations on the occurrence of iridopentanes in other Iridaceae are in progress

Experimental Section

General Remarks: MPLC: Büchi model 681 chromatography pump, columns: 240 mm, 20 mm i.d., RP 18 14−40 μm. − Semipreparative HPLC: Altex 110 A pumps with controller Altex 420, column: Spherisorb 5 ODS 2 (240 mm, 5 mm i.d., Chromatographie Service). − Analytical HPLC: Kontron model 200, column: LiChrocart RP 18 (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 were recorded during the HPLC run. − MS: Finnigan-MAT 4510 GC/MS, solid probe (CI: NH_3). − HPLC/MS: Hewlett Packard 5988 LC/MS system equipped with Hewlett Packard 1090 HPLC and Hewlett Packard 59880A particle beam interface (NCI: NH_3), Finnigan MAT LCQ equipped with Hewlett Packard 1100 HPLC and APCI ion source. − NMR: Bruker AM-300 (^{1}H: 300 MHz, ^{13}C: 75 MHz), Bruker AM-600 (^{1}H: 600 MHz, ^{13}C: 150.9 MHz). All solvents were distilled and saturated with argon. Extracts and compounds isolated were stored under argon at −20°C.

Plant Material: Bulbs of *Tigridia pavonia* were obtained commercially from Küpper Mitteldeutsche Samen GmbH, D-37269 Eschwege in 1994, 1995 and 1997. Some plants were grown and the leaves were harvested for extraction after the flowering season.

Extraction and Separation: Bulbs (1000 g) and leaves (1000 g) were cut into pieces and extracted three times with MeOH/$CHCl_3$ (2:1 v/v). After evaporation of the solvent the residue was partitioned between Et_2O and H_2O. The organic phase was dried with $MgSO_4$ and evaporated to give the crude oil (bulbs: 4.5 g; leaves: 4 g). The extract of the bulbs was fractionated by MPLC using a MeOH/H_2O gradient (60:40 v/v to 100% MeOH). The fraction eluting with MeOH/H_2O (75:25 v/v) contained a mixture (99.5 mg, 2.2%) of the pentaenes **17a/b** and belamcandal (**11**) in a 65:35 ratio. For NMR spectroscopic studies of the underivatized compounds the solvent was evaporated and 10% hydroquinone was added as a stabilizer.

Reaction with PTAD: In a typical run the mixture of **11** and **17** (20 mg), immediately after the MPLC separation, was dissolved in MeOH (2 mL) and a solution (0.1%) of PTAD (Merck) in MeOH was added at −20°C in portions. The progress of the reaction was controlled by HPLC. When the reaction was complete, the solvent was evaporated and the crude product (24 mg) was separated by semipreparative HPLC (eluent: MeOH/H_2O 7:3 v/v, 1 mL/min). The main fraction, which was subsequently reduced with $NaBH_4$, amounted to 2 mg. In addition **16a** (0.6 mg) and **16b** (0.4 mg) were obtained.

Reduction with $NaBH_4$: The main product of the Diels−Alder reaction (15 mg) was dissolved in 2-propanol (0.5 mL) and reacted at room temp. for 24 h with an excess of $NaBH_4$. After hydrolysis with satd. NH_4Cl solution and extraction with Et_2O the crude product was separated by semipreparative HPLC (MeOH/H_2O 65:35 v/v) to give **15a** (4.5 mg, 30%) and **15b** (2.5 mg, 17%).

15a. − UV: λ_{max} = 273 (sh), 283, 293 (sh). − MS (solid probe, NCI): *m/z* = 663 [M$^-$]. − ^{1}H NMR ([D$_6$]benzene, 600 MHz): see Table 1. − ^{13}C NMR ([D$_6$]benzene, 150.9 MHz): see Table 2.

15b. − UV: λ_{max} = 273 (sh), 283, 293 (sh). − MS (solid probe, NCI): *m/z* = 663 [M$^-$]. − ^{1}H NMR ([D$_6$]benzene, 600 MHz): see Table 1. − ^{13}C NMR ([D$_6$]benzene, 150.9 MHz): see Table 2.

PTAD adduct 16a. − UV: λ_{max} = 254 (sh), 274 (sh), 284, 294 (sh). − MS (solid probe, PCI): *m/z* = 660 [M + H$^+$], (solid probe, NCI): *m/z* = 659 [M$^-$]. − ^{1}H NMR ($CDCl_3$, 300 MHz): see Table 1. − ^{13}C NMR ($CDCl_3$, 75 MHz): see Table 2.

PTAD adduct 16b. − UV: λ_{max} = 254 (sh), 274 (sh), 284, 294 (sh). − MS (solid probe, PCI): *m/z* = 660 [M + H$^+$], (solid probe, NCI): *m/z* = 659 [M$^-$]. − ^{1}H NMR ([D$_6$]benzene, 300 MHz): see Table 1. − ^{13}C NMR ([D$_6$]benzene, 75 MHz): see Table 2.

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[1] F.-J. Marner, *Current Organic Chemistry* **1997**, *1*, 153−186.
[2] [2a] W. Steglich in *Biologically Active Molecules* (Ed.: U.P. Schlunegger), Springer Verlag, Berlin, Heidelberg, **1989**, pp. 1−8. − [2b] W. Steglich, B. Steffan, T. Eizenhöfer, B. Fugmann, R. Herrmann, J.D. Klamann in *Bioactive Compounds From Plants*, Ciba Foundation Symposium 154, Wiley, Chichester, **1990**, pp. 56−65.
[3] F. Abe, R.-F. Chen, T. Yamauchi, *Phytochemistry* **1991**, *30*, 3379−3382.
[4] F.-J. Marner, unpublished.
[5] A. Littek, F.-J. Marner, *Helv. Chim. Acta* **1991**, *74*, 2035−2042.
[6] Y. Miyake, H. Ito, T. Yoshida, *Can. J. Chem.* **1997**, *75*, 734−741.
[7] H. Ito, S. Onoue, Y. Miyake, T. Yoshida, *J. Nat. Prod.* **1999**, *62*, 89−93.
[8] J.-P. Bonfils, F.-J. Marner, unpublished results.
[9] [9a] F.-J. Marner, I. Ritzdorf, G. Johnen, *Phytochemistry* **1993**, *33*, 573−579. − [9b] I. Ritzdorf, M. Bartels, B. Kerp, T. Kasel, S. Klonowski, F.-J. Marner, *Phytochemistry* **1999**, *50*, 995−1003.
[10] J.-P. Bonfils, Y. Sauvaire, L. Maurin, *Planta* **1996**, *200*, 353−357.

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