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Iridal glycosides from *Iris spuria* (Zeal), cultivated in Egypt

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Abstract

Fractionation of a methanol extract obtained from rhizomes of *I. spuria* (Zeal) resulted in the isolation of seven iridalglycosides **5a**,**b**, **6a**–**c**, **7**, **8**. The structures of the aglycons and the position of the glycosidic bonds were elucidated by spectroscopic analyses. The nature of the sugar moieties was determined as glucose from capillary GC and GC/MS analyses after hydrolysis of the compounds, reduction and derivatization of the resulting hexitols in comparison to authentic standards. Methylation analysis revealed the glycosyl-linkage composition. The number and position of the glucose residues was determined by ozonolysis. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Iris spuria (Zeal); Iridaceae; Iridals; Iridal glycosides; Triterpenoids

1. Introduction

Numerous Iridaceae have been studied within the last two decades and more than thirty different iridals, unusual phytogenic triterpenoids with mono-, bi- or tricyclic structures (e.g. **1**–**3**), were isolated from their essential oils (Marner, 1997; Marner and Hanisch, 2001). The only derivatives of the iridals found to date are their fatty acid esters at C-3.[1] For the first time now iridal glycosides were found in extracts of *I. spuria* (Zeal), cultivated in Egypt. We report here on the isolation and structure elucidation of these natural products.

2. Results and discussion

An extract of *I. spuria* (Zeal) rhizomes, won with $MeOH/H_2O$ (70:30), was successively extracted with solvents of increasing polarity. The most polar extract, obtained by treatment of the oil with n-BuOH, was subjected to repeated chromatography on Sephadex LH20. Final purification by preparative HPLC gave nine fractions, six of which (3c, 4c, 6b, 7c, 10c and 1112a) appeared to contain homogeneous compounds, as seen by HPLC analyses, whereas the remaining three fractions need further purification and are still under investigation.

The UV spectra of all six compounds were identical and showed a single chromophore with $\lambda_{max} = 254$ nm, which is typical for the iridal ring system holding an α,β-unsaturated aldehyde group. Since the compounds were considerably more polar than iridals or iridal esters they were tentatively identified as iridal glycosides. Mass spectra, recorded with electrospray ionization (ESI) under addition of NaOAc showed quasimolecular ions $[M+Na]^+$ at m/z 1015 for the first three compounds (**3c**, **4c** and **6b**), thus establishing a molecular weight of 992. The successive loss of three hexose units was seen, when MS^n experiments were carried out, which pointed to the presence of three sugar units and an aglycon with M_r 506. In the spectra of the remaining three fractions (7c, 10c and 1112a) two quasimolecular ions $[M+Na]^+$ at m/z 853 and 855 were found, which contain two hexose units each. Therefore, two aglycons with M_r 506 and M_r 508 were to be expected. An aliquot of all fractions was hydrolyzed and

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[1] The carbon skeleton of all iridals and isoiridals is numbered in analogy to squalene.

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after reduction with $NaBH_4$ and silylation (Kilz et al., 2000) submitted to capillary GC and GC/MS. Since exclusively glucitol was found, the nature of the hexose in all compounds was established to be D-glucose (Glc).

^1H, ^{13}C and 2D-NMR spectra (H,H-COSY, HMQC, HMBC, ROESY) led to the structure elucidation of the aglycons. Homogenous compounds were found in fractions 3c and 6b. The spectra of fraction 6b, recorded in methanol-d_4, showed the appropriate signals of an iridal ring system: The exocyclic α,β-unsaturated aldehyde gives rise to a ^1H signal at δ_H 10.18 and the corresponding ^{13}C resonance at δ_C 192.3. The quaternary carbons of the acrolein unit appear at 134.1 (C-2) and 166.7 (C-7). The methine group at C-6 shows δ_H 3.38 and δ_C 45.0. A NOE cross peak between H-1 and H-6 in the ROESY spectrum manifests the usual conformation of the exocyclic double bond. C-10 gives rise to a signal at 75.4 and C-11 appears at 46.1. The CH_2O group of the propanol side chain shows the usual ^1H resonance at 3.5/3.8 but an unusually low field ^{13}C signal at 71.2, pointing to a glycosylation of the primary alcohol. Indeed, cross signals to the doublet of a glycosidic methine at δ_H 4.2/4.22 (δ_C 104.3) were seen in the long range spectrum. Appropriate couplings show that the iridal ring is substituted at C-11 with a methyl group (δ_H1.1, δ_C 18.4) and a homofarnesyl side chain. In contrast to the monocyclic iridals known to date, however, this terpenoid chain holds only two olefinic double bonds, which are located at C-14/15 and C-18/19. The two equivalent methyl groups C-24 and C-30 show long range coupling to a keto group at δ_C 216.7 and cross peaks with a quaternary carbon at δ_C 77.9, thus providing evidence that the terminal double bond has been oxidized to a carbonyl group at C-22 and a tertiary alcohol at C-23. The methine signals at δ_H 3.87 and δ_C 87.9 can be appointed to C-16. Again, the low field resonance and cross peaks with a glycosidic CH at δ_H 4.22/4.23 (δ_C 103.2) prove the connection to a glucose. These results establish the compound as 22-oxo-16,23-dihydroxy-iridal, glycosylated at C-3 and C-16. The third hexose residue, observed in the mass spectrum, is connected to the terminal CH_2O of one of the first two glucose moieties as seen by cross signals of its glycosidic CH at δ_H 4.36/4.37 (δ_C 104.8) with a CH_2 group at δ_H 3.77/4.11 (δ_C 69.8) in the HMBC spectrum. This result was confirmed by methylation analysis (Waeghe, 1983), clearly indicating the 1→6 connectivity of two glucose units. From the coupling constants of the glycosidic protons a β-linkage of all three sugars was established. The resolution of the NMR spectra was not sufficient to allocate all glucose signals and unambiguously assign the disaccharide substitution to C-3 or C-16. Also, recording the NMR spectra in pyridine-d_5 (data not shown) did not solve the problem, since only a downfield shift but no better resolution of the glucose signals was achieved. Therefore, a small amount of the compound was submitted to microozonization (Beroza and Bierl, 1967), followed by reductive workup with triphenylphosphine (Ph_3P). Analysis of the reaction mixture, dissolved in $MeOH/H_2O$/NaOAc, by ESI/MS showed two signals at m/z 643 and 675. The latter apparently was derived from the addition of MeOH to the molecular ion, as it disappeared, when MeOH was exchanged against CH_3CN and upon use of EtOH it was shifted to m/z 689. A successive loss of two glucose moieties was observed upon MS^n of the quasimolecular ion. Hence, due to the low temperature and the short reaction time during the ozonolysis the unreactive exocyclic α,β-unsaturated aldehyde remained intact and the product was the diglycoside **4a**. The mono-glycosylated fragment, derived from cleavage of the C-14/15 and C-18/19 double bonds, was not unambiguously identified, as it has the same M_r as Ph_3PO. However, no pseudomolecular ion $[M+Na]^+$ at m/z 463 was found, which had to be expected, if the diglycoside was positioned at C-16. Thus, the iridal glycoside in fraction 6b is the 22-oxo-23-hydroxyiridal-3-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside]-16-β-D-glucopyranoside **5a**.

The spectra of the compound in fraction 3c show some characteristic differences: The proton of the methine group C-6 appears at δ_H 2.8 (δ_C 48.4) and the C-8 methylene at δ_H 3.28/2.6 (δ_C 21.0), pointing to an opposite configuration of the exocyclic double bond, which is confirmed by NOE's of H-6 with a methyl group and the aldehyde proton with H-8. Therefore, an isoiridal is at hand. In addition the ^{13}C resonance of C-23 is shifted downfield to δ_C 83.5 and shows long range coupling to a glycosidic methine at δ_H 4.4/4.43 (δ_C 99.0), proving that the three glucose moieties are bound to C-3, C-16 and C-23. Consequently, the main product of the microozonolysis is the monoglucoside **4b**, giving quasimolecular ions $[M+Na]^+$ at m/z 481 and $[M+CH_3OH+Na]^+$ at m/z 513 in the ESI/MS, which upon CID lose one hexose unit. Thus, the compound is the 22-oxo-isoiridal-3,16,23-tri-β-D-glucopyranoside **6a**. The complete set of NMR data and their assignments are shown in Tables 1 and 2.

Fraction 4c predominantly consisted of two compounds. Although the NMR spectra were recorded in pyridine-d_5 (Tables 1 and 2), it was easily determined that the minor component (40%) is identical with **6a**. The major product proved very similar to the triglycoside **5a**, with one glucose substitution at C-16 and a glucosylglucoside at C-3, but again the resonances of C-6 (δ_H 2.83, δ_C 48.3) and C-8 (δ_H 3.25/2.75, δ_C 20.7) and the appropriate NOE's of H-6 with a CH_3 and H-8 with the aldehyde proton pointed to an isoiridal ring system, which lead to structure **6b**.

The NMR spectra showed that the diglycoside fractions 7c, 10c and 1112a contained two compounds each, which were not separated by HPLC. The main product in fraction 7c (60%) proved to be the 22-oxo-23-

Table 1
^{1}H NMR chemical shifts (δ) of the iridalglucosides and isoiridalglucosides[a]

	5a[b,d]	**5b**[b,d]	**6a**[b,e]	**6b**[c,e]	**6c**[c,e]	**7**[c,e]	**8**[b,d]
H-1	10.18 *s*	10.19 *s*	1.78 *s*	1.95 *s*	1.97 *s*	n.d.	10.19 *s*
H-3	3.5 *m*/3.8 *m*	3.53 *m*/3.83 *m*	3.5 *m*/3.82 *m*	3.48 *m*/4.09 *m*	3.58 *m*/4.05 *m*	n.d.	n.d.
H-4	1.33 *m*	1.25–1.45 *m*	1.4 *m*	1.45–1.55 *m*	1.45–1.65 *m*	n.d.	n.d.
H-5	1.8 *m*/2.14 *m*	1.82 *m*/2.15 *m*	1.85 *m*/2.2 *m*	2.0 *m*/2.6 *m*	2.0 *m*/2.6 *m*	n.d.	n.d.
H-6	3.38 br*d* (10.8)	3.39 br*d* (10.8)	2.8 *m*	2.83 br*d* (12.0)	2.87 br*d* (11.8)	n.d.	n.d.
H-8	2.55 br*d* (13.3) 2.65 *td* (4.7, 13.3)	2.5–2.75 *m*	3.28 *m*/2.6 *m*	3.25 *m*/2.75 *m*	3.27 *m*/2.75 *m*	n.d.	n.d.
H-9	1.68 *m*/1.85 *m*	1.69 *m*/1.85 *m*	1.6–1.9 *m*	1.85 *m*	1.8–1.9 *m*	n.d.	n.d.
H-12	1.17 *m*/1.27 *m*	1.15–1.3 *m*	1.15–1.3 *m*	1.2–1.4 *m*	1.2–1.4 *m*	n.d.	n.d.
H-13	1.9–2.0 *m*	1.93 *m*	2.0 *m*	2.08 *m*	2.1 *m*	n.d.	n.d.
H-14	5.19 *t* (6.6)	5.19 *t* (6.9)	5.22 *t* (6.3)	5.44 *t* (6.8)	5.44 *t* (6.8)	n.d.	n.d.
H-16	3.88 br*d*	3.9 *m*	3.92 *m*	4.33 *m*	4.36 *m*	n.d.	n.d.
H-17	2.21 *dt* (7.3, 14.7)/2.38 d*t* (6.0, 14.7)	2.2 *m*/2.4 *m*	2.2 *m*/2.4 *m*	2.45 *m*/2.65 *m*	2.45 *m*/2.65 *m*	n.d.	n.d.
H-18	5.01 *t* (6.6)	5.01 *t* (6.9)	5.06 *t* (6.5)	5.39 *t* (7.6)	5.40 *t* (6.5)	5.46 *t* (6.8)	5.08 *t* (6.9)
H-20	2.16 *t* (7.6)	2.16 *t* (7.6)	2.1–2.2 *m*	2.41 *t* (7.4)	2.42 *t* (7.6)	2.6 *m*	1.8–1.9 *m*
H-21	2.72 *t* (7.3)	2.72 *t* (7.8)	2.7–2.9 *m*	3.04 *t* (7.5)	3.06 *t* (7.6)	1.65–1.85 *m*	1.28–1.35 *m*
H-22	–	–	–	–	–	3.7 *m*	3.2 *m*
H-24	1.28 *s*	1.28 *s*	1.36 *s*	1.50 *s*	1.52 *s*	1.45[f] *s*	1.12[f] *s*
H-25	1.81 *s*	1.80 *s*	10.18 *s*	10.45 *s*	10.47 *s*	n.d.	n.d.
H-26	1.10 *s*	1.10 *s*	1.09 *s*	1.21 *s*	1.24 *s*	n.d.	n.d.
H-27	1.12 *s*	1.12 *s*	1.13 *s*	1.27 *s*	1.29 *s*	n.d.	n.d.
H-28	1.52 *s*	1.52 *s*	1.59 *s*	1.84 *s*	1.86 *s*	1.84 *s*	1.54 *s*
H-29	1.59 *s*	1.59 *s*	1.59 *s*	1.59 *s*	1.60 *s*	1.66 *s*	1.59 *s*
H-30	1.28 *s*	1.28 *s*	1.36 *s*	1.50 *s*	1.52 *s*	1.49[f] *s*	1.15[f] *s*
H-1′ (Glc′→C-3)	4.20/4.22 *d* (8.3)	4.20/4.21 *d* (7.8)	4.20/4.22 *d* (7.6)	4.69/4.71 *d* (7.6)	4.78/4.81 *d* (7.6)	n.d.	n.d.
H-2′	3.1 *m*	3.15 *m*	3.15 *m*	3.90 *m*	4.00 *m*	n.d.	n.d.
H-1″ (Glc″→C-16)	4.22/4.23 *d* (7.8)	4.22/4.23 *d* (7.8)	4.23/4.25 *d* (7.9)	4.91/4.93 *d* (8.0)	4.92/4.95 *d* (7.6)	n.d.	4.22/4.24 *d* (7.8)
H-2″	3.14 *m*	3.15 *m*	3.15 *m*	4.00 *m*	4.05 *m*	n.d.	n.d.
H-1‴ (Glc‴→C-6′)	4.36/4.37 *d* (7.8)	–	–	5.05/5.07 *d* (7.9)	–	–	–
H-1‴ (Glc‴→C-23)	–	–	4.40/4.43 *d* (7.6)	–	–	–	–
H-2‴	3.2 *dd* (7.8, 8.8)	–	3.25 *m*	4.00 *m*	–	–	–
H-3′, H-3″, H-3‴	3.2–3.4 *m*	3.21–3.34 *m* 3.18–3.4 *m*	3.0–3.4 *m* 3.1–3.4 *m* 3.0–3.6 *m*	3.6–4.4 *m* n.d.	3.8–3.95 *m* 4.15–4.3 *m* n.d.	n.d.	n.d.
H-4′, H-4″, H-4‴ H-5′, H-5″, H-5‴							
H-6′ (Glc‴→ C-6′)	3.77 *dd* (2.0, 9.8) 4.11 *dd*	–	–	4.3 *m*/4.8 *m*	–	–	–
H-6′(Glc‴→ C-23)	–	–	3.6 *m*/3.9 *m*	–	–	–	–
H-6″, H-6‴ (triglucosides)	3.65 *m*/3.85 *m* 3.63 *m*/3.75 *m*	–	3.6 *m*/3.9 *m*	4.3 *m*/4.5 *m*	–	–	–
H-6′, H-6″ (diglucosides)	–	3.63 *m*/3.83 *m* 3.62 *m*/3.74 *m*	–	–	4.37 br*d* (5.3)/4.52 *dd* (2.4, 11.8), 4.33 br*d* (5.3)/4.44 *dd* (2.1, 11.5)	n.d.	n.d.

[a] Coupling constants *J* (Hz) are given in parentheses.
[b] Methanol-d_4.
[c] Pyridine-d_5.
[d] 500 MHz.
[e] 300 MHz.
[f] Assignments may be interchanged. n.d. = not determined, signals show no significant difference to resonances of main component.

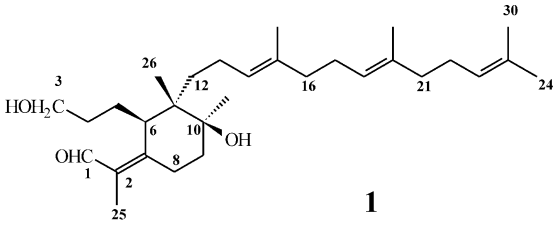
hydroxy-isoiridal-3,16-di-β-D-glucopyranoside **6c**, as seen by the appropriate resonances (Tables 1 and 2). The signals of the minor compound were significantly different only for the terpenoid side chain. Thus, instead of a ketone a secondary hydroxy group is located at C-22, giving resonance at δ_H 3.7 (δ_C 78.3) and the suitable cross peaks in the 2D spectra. This explains the presence of a quasimolecular ion $[M+Na]^+$ at m/z 855 in the ESI/MS and the compound is identified as 22,23-dihydroxy-isoiridal-3,16-di-β-D-glucopyranoside **7**.

Fractions 10c and 1112a contained identical compounds, however in slightly different amounts (10c: 60:40, 1112a: 70:30). It was easily seen from the spectroscopic data that, instead of the isoiridal ring of **6b** and **7**, they own an iridal structure. Thus, the structures of a 22-oxo-23-hydroxy-iridal-3,16-di-β-D-glucopyranoside

Table 2
^{13}C NMR chemical shifts (δ) of the iridalglucosides and isoiridalglucosides

	5a[a,c]	**5b**[a,c]	**6a**[a,d]	**6b**[b,d]	**6c**[b,d]	**7**[b,d]	**8**[a,c]
C-1	192.3	192.4	12.1	11.8	12.3 [e]	n.d.	n.d
C-2	134.1	134.0	133.7	133.0	133.0	n.d	n.d
C-3	71.2	71.1	70.9	70.6 [e]	70.6	n.d	n.d
C-4	30.8	30.7	30.0	30.2	30.0	n.d	n.d
C-5	28.1	28.1	28.4	27.1	28.0	n.d	n.d
C-6	45.0	44.9	48.4	48.3	48.3	n.d	n.d
C-7	166.7	166.8	167.0	165.0	165.0	n.d	n.d
C-8	25.1	25.1	21.0	20.7	20.7	n.d	n.d
C-9	37.9 [e]	38.0 [e]	38.7	38.3	39.0	n.d	n.d
C-10	75.4	75.3	75.3	74.3	74.2	n.d	n.d
C-11	46.1	46.1	46.3	46.0	46.0	n.d	n.d
C-12	38.0 [e]	37.9 [e]	37.8	37.3	37.3	n.d	n.d
C-13	22.9	22.9	23.8	23.8	23.8	n.d	n.d
C-14	129.3	129.3	129.1	128.6	128.6	128.3	n.d
C-15	136.4 [f]	136.4 [f]	136.1	136.1 [f]	136.0	136.0	n.d
C-16	87.9	87.9	87.8	86.7	86.7	86.8	n.d
C-17	32.3	32.4	32.3	32.5	32.4	32.5	n.d
C-18	121.7	121.7	121.4	121.4	121.4	121.2	121.6
C-19	136.7 [f]	136.7 [f]	136.6	136.1 [f]	136.0	138.0	136.4
C-20	34.4	34.4	34.3	34.1	34.1	37.8	37.7
C-21	35.8	35.8	35.8	35.4	35.4	30.8	30.6
C-22	216.7	216.7	215.0	215.7	215.7	78.3	78.9
C-23	77.9	77.9	83.5	76.9	76.9	72.3	73.8
C-24	26.8	26.8	24.4 [e]	27.4	27.4	26.1 [e]	25.9
C-25	10.9	10.9	192.6	191.0	191.0	n.d	n.d
C-26	18.4	18.4	18.0	18.4	18.4	n.d	n.d
C-27	26.0	25.9	25.7	26.4	26.3	n.d	n.d
C-28	11.9	11.8	11.7	12.4	12.4 [e]	12.4	11.8
C-29	16.6	16.6	16.3	16.7	16.7	16.7	16.6
C-30	26.8	26.8	23.2 [e]	27.4	27.4	26.2 [e]	25.9
C-1′ (Glc′→C3)	104.3	104.4	104.1	104.9	105.0	n.d	n.d
C-2′	75.0 [g]	75.1 [g]	74.8 [f]	75.1 [g]	75.3 [f]	n.d	n.d
C-1″ (Glc″→C16)	103.2	103.3	103.1	103.7	103.7	n.d	103.3
C-2″	75.1 [g]	75.3 [g]	74.9 [f]	75.3 [g]	75.6 [f]	n.d	n.d
C-1‴ (Glc‴→C-6′)	104.8	–	–	105.7	–	–	–
C-1‴ (Glc‴→C23)	–	–	99.0	–	–	–	–
C2‴	75.3 [g]	–	75.1 [f]	75.7 [g]	–	–	–
C3′–C3‴	71.4/71.5/71.6/	71.5/71.6/	71.2/71.4/71.4/	71.6/71.7/	71.8/71.8/		
C4′–C4‴	77.1/77.7/77.9/	77.7/77.9	77.4/77.5/77.6	71.8/77.3/	78.3/78.6/	n.d	n.d
C5′–C5‴	78.0/78.0/78.2	78.1/78.2	77.8/77.9/77.9	78.4/78.6	78.8		
C-6′ (Glc‴→C-6′)	69.8	–		70.3 [e]	–	–	–
C-6′- C-6‴	62.7/62.8	62.7/62.8	62.4/62.5/	62.8/63.0/			
			62.6	63.1	62.9/63.0	n.d.	n.d

[a] Methanol-d_4.
[b] Pyridine-d_5.
[c] 500 MHz.
[d] 300 MHz.
[e] Assignments may be interchanged.
[f] Assignments may be interchanged.
[g] Assignments may be interchanged. n.d. = not determined, signals show no significant difference to resonances of main component.



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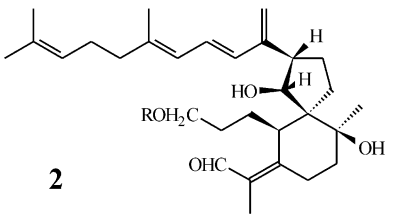

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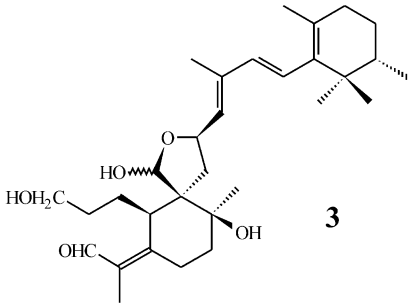

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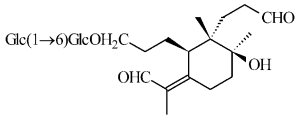
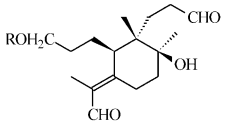
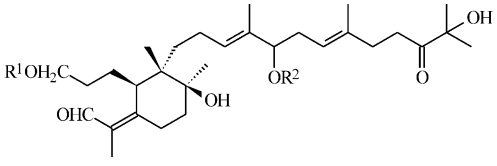


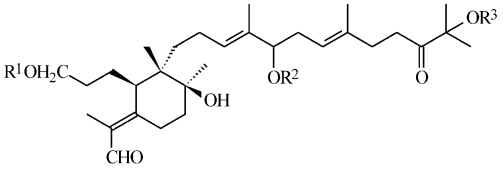
4a



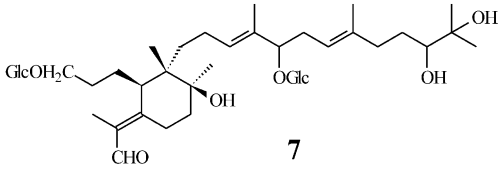
4b: R = Glc
4c: R = Glc(1→6)Glc



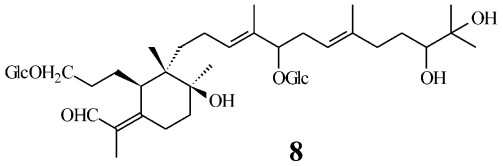
5a: R^1 = Glc(1→6)Glc; R^2=Glc
5b: R^1 = R^2 = Glc



6a: R^1 = R^2 = R^3 = Glc
6b: R^1 = Glc(1→6)Glc; R^2 = Glc; R^3 = H
6c: R^1 = R^2 = Glc; R^3 = H



7



8

5b and 22,23-dihydroxy-iridal-3,16-di-β-D-glucopyranoside **8** can be assigned.

Comparison of the spectral data with the values found for other iridals and biosynthetic reasons (Marner, 1997) suggest a 6*R*,10*S*,11*R*-configuration for the three chiral centers of all iridal and isoiridal ring systems. The stereochemistry at C-16 and at C-22 (in the 22-hydroxy-homologues) remain to be determined.

These are the first examples of glycosylated iridals found in Iridaceae. It is interesting to note that no glycosides were found in a variety of *I. spuria* grown in Germany nor in any other *Iris* species studied by the author (F.J.M.) to date. Therefore, their presence in the rhizomes of *I. spuria* (Zeal), investigated in this study, may be due to their habitat.

3. Experimental

3.1. General

CC: Sephadex LH-20 (25–100 μm, Pharmacia) column (100×3 cm). Preparative HPLC: Gilson-305 pump equipped with JASCO UV-970 detector and a YMC-PACK R&D prep. column (10 μm ODS 120 Å, 250×20 mm). TLC: silica 60 F_{254} (Merck, $CHCl_3$:MeOH 8:2) and KC_{18} RP plates (5×20 cm, 200 μm, Whatman, MeOH:H_2O 1:1), detection was carried out by spraying with 10% H_2SO_4 followed by heating. 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: Thermo Finnigan LCQ, equipped with ESI ion source. The compounds, dissolved in MeOH–H_2O (1:1)/1mM NaOAc, were injected with a microliter syringe. HR-ESI-MS: Finnigan MAT 900 ST (EB-Q-Trap configuration), conditions as reported for MS. GC: Fisons GC 8000 with FID, cap. column DB225 WCOT (30 m, 0.25 mm i.d., H_2: 2 ml/min) Temp. progr.: 140 °C (2 min.) → 180 °C (10 °C/min)–180 °C (2 min) → 250 °C (10 °C/min)–250 °C (7 min) → 280 °C (20 °C/min). GC–MS: Finnigan-MAT 4510 GC/MS (EI: 70 eV), column and conditions as indicated for GC. NMR: Bruker AM-300 (^{1}H: 300 MHz, ^{13}C: 75 MHz), Bruker AM-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz), spectra were recorded in methanol-d_4 or pyridine-d_5, respectively.

3.2. Plant material

Rhizomes of *I. spuria* (Zeal) were obtained during the flowering season in March 1999. The plants were cultivated in the Experimental Station of Medicinal Plants at the Faculty of Pharmacy, Assiut University, Egypt.

3.3. Extraction and isolation

Fresh rhizomes of *I. spuria* (Zeal) (3 kg) were cleaned under running tap water, cut into small pieces, and extracted threefold with MeOH–H_2O (7:3) at room temperature. The extract was concentrated *i. vac.* at a temperature not exceeding 45 °C to give 250 g of extract. The MeOH extract was successively dissolved in petrol (40–60 °C), Et_2O, EtOAc and finally *n*-BuOH. The *n*-BuOH extract was evaporated *i. vac.* at a temperature below 55 °C to give a brown residue (25 g). 10 g of the extract were chromatographed on Sephadex LH-20 (MeOH–H_2O, 9:1) in 5 portions to give a fraction enriched in iridal glycosides (2 g), showing two major spots on TLC (silica and RP). The material was rechromatographed on Sephadex LH-20 (MeOH–H_2O, 9:1). Final separation was achieved by prep. HPLC (MeOH–H_2O, 1:1) to give 9 fractions, which were collected and freeze dried. Each fraction was repurified on Sephadex LH-20 (MeOH). The fractions 3c (21 mg), 4c (16 mg), 6b (20 mg), 7c (17 mg), 10c (18 mg) and 1112a (19 mg) appeared homogeneous by analytical HPLC and were submitted to structure elucidation. The three non-homogeneous fractions 2a, 5b and 1113a are still under investigation.

3.4. HRMS data

5a: $C_{48}H_{80}O_{21}$ *m/z* 1015.509 (calcd. 1015.5089 $[M+Na]^+$), **5b**: $C_{42}H_{70}O_{16}$ *m/z* 853.457 (calcd. 853.4561 $[M+Na]^+$), **6a**: $C_{48}H_{80}O_{21}$ *m/z* 1015.509 (calcd. 1015.5089 $[M+Na]^+$), **6b**: $C_{48}H_{80}O_{21}$ *m/z* 1015.510 (calcd. 1015.5089 $[M+Na]^+$), **6c**: $C_{42}H_{70}O_{16}$ *m/z* 853.457 (calcd. 853.4561 $[M+Na]^+$), **7**: $C_{42}H_{72}O_{16}$ *m/z* 855.471 (calcd. 855.4718 $[M+Na]^+$), **8**: $C_{42}H_{72}O_{16}$ *m/z* 855.472 (calcd. 855.4718 $[M+Na]^+$).

3.5. Sugar analysis

To a soln of 0.1 mg natural product in 0.1 ml 2 N trifluoracetic acid 5 μl of a *myo*-inositol standard (55.5 nmol/μl) were added and the soln was heated to 125 °C for 1 h. After cooling the hydrolysate was evaporated. To remove all traces of trifluoracetic acid evapn. was repeated twice after addition of 0.2 ml MeOH. To the residue 0.15 ml of a freshly prepared soln. of $NaBH_4$ (5 mg/ml MeOH) was added and reduction was carried out for 2 h at room temp. After hydrolysis with 0.2 ml 1% AcOH and evapn. the borate was removed by repeated evapn. at 55 °C after addition of 0.2 ml MeOH. The remainder was dried over P_2O_5 over night and derivatized by treatment with *N*-methyl-*N*-trimethylsilyltrifluoracetamide for 1 h at 100 °C. Sugar standards were treated equally. The TMS derivatives of the hexitols were analyzed by GC and GC/MS and the peaks identified by their retention time relative to *myo*-inositol

and their mass spectra. Determination of the glycosyl linkage composition was carried out by methylation analysis according to the procedure of Waeghe et al. (1983).

3.6. Microozonolysis

1 mg of the substance was dissolved in 1 ml MeOH. At −70 °C O_3, produced by a micro-ozonizer (Supelco) from O_2 (10 ml/min), following the procedure of Beroza and Bierl (1967), was passed through the soln. for 4 min. Excess O_3 was blown off with nitrogen. 1 mg of Ph_3P, dissolved in 100 μl MeOH, was added and after 2 min at −70 °C the soln was allowed to warm to room temp. 50 μl of the reaction mixture were diluted with MeOH–H_2O (1:1)/1 mM NaOAc and submitted to ESI/MS. **4a** (obtained from **5a**): *m/z* 643 $[M+Na]^+$, 675 $[M+CH_3OH+Na]^+$ **4b** (obtained from **6a**): *m/z* 481 $[M+Na]^+$, 513 $[M+CH_3OH+Na]^+$

Ozonolysis of fraction 4c gave a mixture of **4c** (main product) and **4b**, indicating the presence of **6b** as major and **6a** as minor components.

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