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Tuliposides from *Tulipa sylvestris* and *T. turkestanica*

Lars P. Christensen

Department of Fruit, Vegetable and Food Science, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark

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Abstract

The investigation of leaves/stems and flowers of *Tulipa turkestanica* afforded, in addition to 6-tuliposide A, 1-tuliposide A, tuliposide D and the lactonized aglycones tulipalin A and (−)-tulipalin B, two new tuliposides. The structure of the new tuliposides were determined by spectral methods to be 1-(4-hydroxy-2-methylenebutanoate)-6-((*S*)-3,4-dihydroxy-2-methylenebutanoate)-β-D-glucopyranose (tuliposide F) and 6-((*S*)-3,4-dihydroxy-2-methylenebutanoate)-D-glucopyranose (6-tuliposide B), the latter being a new acyl derivative of the known 1-tuliposide B. The investigation of *Tulipa sylvestris* gave 6-tuliposide A and B, tuliposide D and tulipalin A and B. The possible biosynthesis of the isolated compounds is briefly discussed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Tulipa; Liliaceae; 6-Tuliposide A; 1-Tuliposide A; 6-Tuliposide B; Tuliposide D; Tuliposide F; Tulipalins

1. Introduction

The first tuliposides were isolated from *Tulipa gesneriana* by Tschesche et al. and identified as 1-tuliposide A and 1-tuliposide B (Tschesche, Kämmerer, Wulff, & Schönbeck, 1968; Tschesche, Kämmerer, & Wulff, 1969), and since then a few investigations have shown that tuliposide A and B are widely distributed in the Liliaceae although the position of the acyl group was not determined in all investigations (Slob, 1973; Slob, Jekel, de Jong, & Schlatmann, 1975; Slob & Varekamp, 1977). Tuliposides have also been found in Alstroemeriaceae were they occur regularly in the genera *Alstroemeria* and *Bomarea*. The tuliposides isolated from the Alstroemeriaceae have been identified as 6-tuliposide A, tuliposide D and E (Slob, 1973; Slob et al., 1975; Santucci, Picardo, Iavarone, & Trogolo, 1985; Christensen & Kristiansen, 1995a, 1995b, 1995c; Christensen, 1995a, 1995b; Kristiansen & Christensen, 1998). Tulips and *Alstroemeria* are widely used as cut flowers and they are responsible for several cases of allergic contact dermatitis (Santucci et al., 1985; Mijnssen Verspyck, 1969; Hausen, 1982; Hausen, Prater, & Schubert, 1983; Marks, 1988; Thiboutot, Hamory, & Marks, 1990; Gette & Marks, 1990; Lamminpää, Estlander, Jolanki, & Kanerva, 1996). Contact dermatitis to tulips and *Alstroemeria* is caused by exposure to recently damaged plant parts, e.g. during handling of cut flowers, and gives rise normally to a severe eczematous dermatitis of the hands, a condition known as 'tulip fingers' (Mijnssen Verspyck, 1969; Hausen, 1982; Hausen et al., 1983; Santucci et al., 1985; Marks, 1988; Thiboutot et al., 1990; Gette & Marks, 1990; Lamminpää et al., 1996). The causative agents have been identified as tuliposide A and its lactonized aglycone, tulipalin A (Mijnssen Verspyck, 1969; Hausen, 1982; Hausen et al., 1983; Santucci et al., 1985; Marks, 1988; Thiboutot et al., 1990; Gette & Marks, 1990; Lamminpää et al., 1996). Tuliposide B which only seem to occur in Liliaceae is non-allergenic (Slob et al., 1975; Hausen et al., 1983), whereas its lactonized aglycone (−)-tulipalin B, has been shown to sensitize guinea pigs and cross-react with tulipalin A (Barbier & Benezra, 1986; Papageorgiou, Stampf, & Benezra, 1988). Tuliposide D and E have so far not been investigated for their allergenic activity, although the structurally similarities to tuliposide A strongly suggests allergenic properties (Christensen & Kristiansen, 1995b, 1995c; Christensen, 1995b; Kristiansen & Christensen, 1998). Tuliposides and tulipalins have also shown to be strong antibiotics and they have been suggested to play a role in disease resistance (Bergman, Beijersbergen, Overeem, & Sijpesteijn, 1967; Bergman & Beijersbergen, 1968; Tschesche et al., 1968, 1969; Schönbeck & Schroeder, 1972; Hutchinson, 1974). Finally tuliposides may prove to be important in chemotaxonomic studies due to their limited distribution in the plant kingdom. As part of our continued investigations of plant species for potential allergenic and antibiotic tuliposides we have examined *Tulipa turkestanica* Regel

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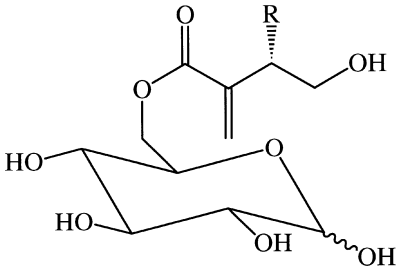
and *Tulipa sylvestris* L. This paper describes the isolation and structure elucidation of two new tuliposides, tuliposide F and the 6-acyl derivative of 1-tuliposide B (6-tuliposide B), from these plants.

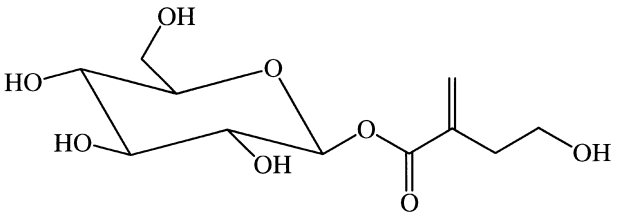
2. Results and discussion

Frozen leaves/stems and flowers of *T. turkestanica* were extracted with distilled water and the combined water extracts were subjected to column chromatography and prep. HPLC to afford 6-tuliposide A (**1**), 6-tuliposide



1 R = H

2 R = OH



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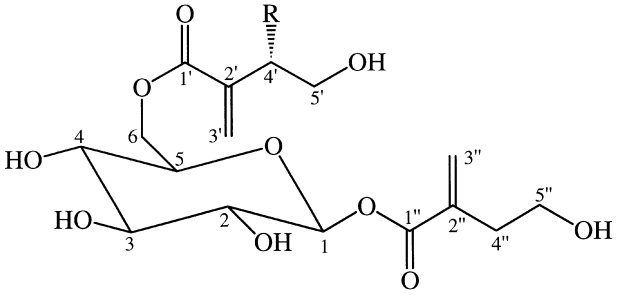
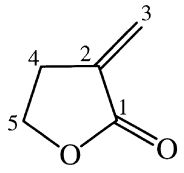


4 R = H

5 R = OH

B (**2**), 1-tuliposide A (**3**), tuliposide D (**4**) and tuliposide F (**5**) and the lactonized aglycones tulipalin A (**6**) and (−)-tulipalin B (**7**). *T. sylvestris* afforded **1**, **2**, **4**, **6** and **7**.



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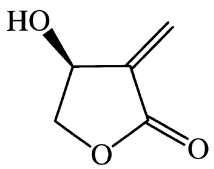

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Compounds **2** and **5** are to the best of my knowledge new natural products whereas **1** and **4** occur regularly in Alstroemeriaceae (Santucci et al., 1985; Christensen & Kristiansen, 1995a, 1995b, 1995c; Christensen, 1995a, 1995b; Kristiansen & Christensen, 1998). However, this is the first report of **1** and **4** in Liliaceae.

Compound **2** was obtained as a syrup and its FAB-mass spectrum exhibited a $[M+H]^+$ at m/z 295 and a $[M+Na]^+$ at m/z 317 in good agreement with the mass calculated for $C_{11}H_{19}O_9$. *S*-(−)-Tulipalin B (**7**) and D-glucose were obtained by acid hydrolysis of **2**, indicating the presence of a 3,4-dihydroxy-2-methylenebutanoate moiety in **2** with an *S* absolute configuration for the chiral center (Tschesche et al., 1969). The ^{13}C NMR spectrum of **2** showed 18 signals of which 12 could be assigned to a α- and β-D-glucopyranose unit, respectively, by comparison with the ^{13}C NMR spectrum of **1**, whereas the remaining signals were assigned to a 3,4-dihydroxy-2-methylenebutanoate moiety (Table 1). The downfield resonance of the C-6 protons in the ^1H NMR spectrum of **2** (δ 4.31, 4.43 (α) and 4.28, 4.47 (β)) compared with the values observed for H-6 of α- and β-D-glucose (δ 3.63, 3.72 (α) and 3.60, 3.75 (β)) (Bock & Thøgersen, 1982) showed that the 4-hydroxy-2-methylenebutanoate moiety was linked at C-6, thus **2** is a mixture of α and β 6-((*S*)-3,4-dihydroxy-2-methylenebutanoate)-D-glucopyranose. The integrals from the anomeric H-1 (δ 4.61 (β) and 5.16 (α)) showed that the equilibrium between the α- and β-anomers of **2** in aqueous solution was ca. 2:3 which is in accordance with the ratio determined by analytical HPLC. Although the two isomers are also easily separated by prep. HPLC the equilibrium is readily adjusted and no attempt was therefore made to isolate them separately (see Section 3).

Compound **5** was obtained as a syrup which yielded D-glucose and the unsaturated lactonized aglycones **6** and **7** in the ratio 1:1 upon acid hydrolysis. This indicated the presence of a 4-hydroxy-2-methylenebutanoate and a (*S*)-3,4-dihydroxy-2-methylenebutanoate moiety in **5** (Tschesche et al., 1969), which was further supported by the NMR data (Tables 1 and 2) and its FAB-mass spectrum exhibiting a $[M+H]^+$ at m/z 393 and a $[M+Na]^+$ at m/z 415 in good agreement with the mass calculated for $C_{16}H_{25}O_{11}$. The ^{13}C NMR spectrum of **5** showed 16 signals of which six could be assigned to a β-D-glucopyranose unit by comparison with the ^{13}C NMR

Table 1
^{13}C NMR spectral data (75 MHz, D_2O, δ-values) for compounds **1**–**5**

Assignments	**1**[a]	**2**	**3**	**4**[b]	**5**
C-1	96.9 (β) d	96.9 (β) d	95.2 d	95.1 d	95.1 d
	93.0 (α) d	93.0 (α) d	–	–	–
C-2	74.9 (β) d	74.9 (β) d	72.7 d	72.7 d	72.7 d
	72.2 (α) d	72.2 (α) d	–	–	–
C-3	76.4 (β) d	76.4 (β) d	76.3 d	76.1 d	76.1 d
	73.4 (α) d	73.4 (α) d	–	–	–
C-4	70.4 (β) d	70.4 (β) d	70.0 d	70.3 d	70.3 d
	70.0 (α) d	70.0 (α) d	–	–	–
C-5	74.2 (β) d	74.2 (β) d	77.6 d	75.2 d	75.2 d
	70.6 (α) d	70.6 (α) d	–	–	–
C-6	64.5 (β)[c] t	64.6 (β)[c] t	61.2 t	64.1 t	64.1 t
	64.4 (α)[c] t	64.5 (α)[c] t	–	–	–
C-1′	169.5 s	167.9 s	–	169.3 s	167.9 s
C-2′	137.1 s	139.8 s	–	137.0 s	139.8 s
C-3′	130.0 (β)[d] t	129.2 (β)[d] t	–	130.0 t	129.2 t
	129.9 (α)[d] t	129.1 (α)[d] t	–	–	–
C-4′	34.9 t	71.2 d	–	35.0 t	71.2 d
C-5′	60.8 t	65.2 t	–	60.8 t	65.2 t
C-1″	–	–	167.9 s	167.7 s	167.7 s
C-2″	–	–	136.4 s	136.3 s	136.4 s
C-3″	–	–	131.4 t	131.4 t	131.5 t
C-4″	–	–	34.7 t	34.7 t	34.7 t
C-5″	–	–	60.7 t	60.7 t	60.7 t

[a] ^{13}C NMR in accordance with lit. values (Santucci et al., 1985; Christensen & Kristiansen, 1995a, 1995b; Christensen, 1995a).
[b] ^{13}C NMR in accordance with lit. values (Christensen, 1995a; Christensen & Kristiansen, 1995b).
[c,d] Assignments in the same column are interchangeable.

Table 2
^{1}H NMR spectral data (300 MHz, D_2O, δ-values) for compounds **1**–**5**

H	**1**[a,b]	**2**[a]	**3**	**4**[c]	**5**
1	4.61 d (β) (7.9)[d]	4.61 d (β) (7.9)	5.59 d (β) (7.6)	5.60 d (β) (7.6)	5.59 d (β) (7.6)
	5.16 d (α) (3.7)	5.16 d (α) (3.7)			
2–5	3.2–4.0 m	3.2–4.0 m	3.4–3.9 m	3.4–3.9 m	3.4–3.9 m
6	4.34 dd (α) (5.6; 12.2)	4.31 dd (α) (5.6; 12.2)	3.6--3.8 m	4.32 dd (β) (5.6; 12.2)	4.32 dd (β) (5.6; 12.2)
	4.41 dd (α) (2.2; 12.2)	4.43 dd (α) (2.2; 12.2)		4.49 dd (β) (2.0; 12.2)	4.50 dd (β) (2.0; 12.2)
	4.29 dd (β) (5.6; 12.2)	4.28 dd (β) (5.6; 12.2)			
	4.46 dd (β) (2.2; 12.2)	4.47 dd (β) (2.2; 12.2)			
3′	5.75 s	5.97 s	–	5.75 s	5.98 s
	6.26 br s	6.39 br s	–	6.25 s	6.39 s[e]
4′	2.52 t (6.5)	4.60 m	–	2.51 t (6.5)	4.58 br dd (3.4; 6.5)
5′	3.68 t (6.5)	3.52 dd (6.5; 11.5)	–	3.68 t (6.5)	3.52 m
		3.69 dd (3.4; 11.5)			3.70 dd (3.4; 11.5)
3″	–	–	5.86 s	5.88 s	5.86 s
			6.39 s	6.39 s	6.38 s[e]
4″	–	–	2.53 t (6.5)	2.53 t (6.5)	2.52 t (6.5)
5″	–	–	3.69 t (6.5)	3.69 t (6.5)	3.69 t (6.5)

[a] The ratio between the α- and β-anomer was ca. 2:3.
[b] ^{1}H NMR in accordance with lit. values (Santucci et al., 1985; Christensen & Kristiansen, 1995a, 1995b; Christensen, 1995a).
[c] ^{1}H NMR in accordance with lit. values (Christensen, 1995a; Christensen & Kristiansen, 1995b).
[d] Coupling constants J (in Hz) in parentheses.
[e] Assignments may be interchanged.

spectra of compounds **1**, **2** and **4** (see Table 1). The remaining 10 signals were assigned to a 3,4-dihydroxy-2-methylenebutanoate and a 4-hydroxy-2-methylenebutanoate moiety (Table 1). The presence of a 4-hydroxy-2-methylenebutanoate moiety in **5** was further supported by the ^{1}H NMR signals at δ 2.52 (2H, t, $J=6.5$ Hz), 3.69 (2H, t, $J=6.5$ Hz), 5.86 (1H, s) and 6.38 (1H, s) whereas the signals at δ 3.52 (1H, m), 3.70 (1H, dd, $J=3.4$ and 11.5 Hz), 4.58 (1H, br dd, $J=3.4$ and 6.5 Hz), 5.98 (1H, s) and 6.39 (1H, s) confirmed the presence of a 3,4-dihydroxy-2-methylenebutanoate moiety. The β-configuration of **5** was further verified by the ^{1}H NMR signal at δ 5.59 with a coupling constant of 7.6 Hz as in compounds **3** and **4**. For an α-configuration, one would have expected a value of 3–4 Hz as seen in compounds **1** and **2** (Table 2). The downfield resonance of the β-anomeric proton, compared with the value (δ 4.61) observed for the β-anomeric H-1 in **1** and **2**, was evidently owing to an ester linkage at C-1. By comparing the chemical shift of the C-3″ methylene protons in **5** occurring at δ 5.86 and 6.38 with the corresponding protons in compounds **1**, **3** and **4**, clearly indicated that the 4-hydroxy-2-methylenebutanoate moiety in **5** is linked at C-1 (Table 2). This is also accordance with the ^{13}C NMR data (Table 1). Furthermore the downfield resonance of the C-6 protons in **5** (δ 4.32 and 4.50) compared with the values (δ 3.60 and 3.75) observed for H-6 of β-D-glucose (Bock & Thøgersen, 1982) is due to an ester linkage at C-6, indicating that the 3,4-dihydroxy-2-methylenebutanoate moiety is linked at this position, which is also in accordance with the ^{13}C NMR data (Table 1). From the above results the structure of **5** was determined to be 1-(4-hydroxy-2-methylenebutanoate)-6-((*S*)-3,4-dihydroxy-2-methylenebutanoate)-β-D-glucopyranose (tuliposide F).

A 4-week old sample of compound **5** stored in an aqueous solution at 4°C was partly hydrolysed to compounds **2**, **3**, **6** and **7** as demonstrated by analytical HPLC. A similar result was obtained by heating **5** in water and/or methanol for 5 min at 100°C. The decomposition products of **5** clearly support the structure of this compound as the cleavage of **7** gives **3** whereas the cleavage of **6** affords **2**. Compounds **1**–**4** are more or less also hydrolysed in water with compound **1** being the most stable of the isolated tuliposides.

Tuliposides are most likely biosynthesized from D-glucose and 4-hydroxy-2-methylenebutanoic acid (HMBA) and/or S-3,4-dihydroxy-2-methylenebutanoic acid (DHMBA). Compounds **1** and **3** may be formed by a condensation of β-D-glucose and HMBA with the loss of water, whereas a condensation between D-glucose and DHMBA leads to compound **2**. Further condensation between compounds **1** or **3**, and HMBA gives **4**, whereas a condensation between compound **3** and DHMBA affords **5**. Compound **5** may also be biosynthesized via a condensation between **2** and HMBA. As compounds **2** and **3** are present in *T. turkestanica* both biosynthetic routes leading to **5** seem reasonable. HMBA and its oxidation product DHMBA are most likely biosynthesized from pyruvate and acetate (Hutchinson & Leete, 1970).

Based on the present and previous investigations of *Tulipa* species (Tschesche et al., 1968, 1969) it appears that both the 1- and the 6-acyl derivative of tuliposide A and B are produced in *Tulipa*, although some plants only seem to biosynthesize the 6-acyl derivative, whereas others produce both derivatives of tuliposide A and B. Further investigations may show which of the acyl derivatives are most common in the genus. The tuliposides **1** and **3**, and their degradation product tulipalin A (**6**) are strong sensitizers and it has been shown that these compounds are among the causative agents for allergic contact dermatitis (type IV allergy) in *Alstroemeria* and tulips (Mijnssen Verspyck, 1969; Hausen, 1982; Hausen et al., 1983; Santucci et al., 1985; Marks, 1988; Thiboutot et al., 1990; Gette & Marks, 1990; Lamminpää et al., 1996). Furthermore, it has been shown that a methylene group in α position is important for the allergenic activity (Santucci et al., 1985). This is also in accordance with chemical considerations because the methylene group in e.g. compounds **1**, **3** and **6** is activated by the electron-withdrawing carbonyl group, making it suitable for attack by nucleophils such as thiol and amino groups in skin proteins, thus generating sensitizing antigens (Roberts & Lepoittevin, 1998). For example the type of reaction which can occur between **6** and a protein nucleophile is one of the most common haptenation reactions found with naturally occurring sensitizers (Roberts & Lepoittevin, 1998). Although the allergenic activity of compounds **4** and **5** have not been investigated, their structural relationship to **1** and **3** clearly suggest that they have allergenic properties and most likely cross-reacts with **1** and **3**. However, if compounds **4** and **5** are not allergenic in the native form their presence still cannot be ignored due to their instability, since they will most likely be converted into e.g. the allergens **1**, **3** and **6** in the plants and after deposition on the skin.

Tulipalin A (**6**) and (−)-tulipalin B (**7**) have been shown to be strong antibiotics and they appear to function as defensive agents against infection of tulips by common pathogenic soil fungi such as *Fusarium oxysporum* (Bergman, 1966; Bergman et al., 1967; Bergman & Beijersbergen, 1968; Beijersbergen & Lemmers, 1971, 1972; Beijersbergen, 1972) and species of *Botrytis* (Bergman & Beijersbergen, 1968; Schönbeck & Schroeder, 1972). Although tuliposides also possess antibiotic properties (Tschesche et al., 1968, 1969) it has been shown that their content decreases while the content of tulipalins increases when tulips are infected with *F. oxysporum* (Beijersbergen & Lemmers, 1971, 1972) and *Botrytis cinerea* (Schönbeck & Schroeder, 1972), clearly indicating that tulipalins are liberated from tuliposides upon fungal attack (Schönbeck & Schroeder, 1972;

Beijersbergen & Lemmers, 1971, 1972). The isolated tuliposides **1**–**5** therefore seem to function as storage products for the highly antifungal compounds **6** and **7** (Schönbeck & Schroeder, 1972; Beijersbergen & Lemmers, 1971, 1972). Such a role may for example be suggested for compound **5** due to the relatively high amounts found in *T. turkestanica* (see Section 3).

Finally it has been shown that tuliposides might be useful in the efforts to arrive at a natural classification of the genus *Tulipa* (Slob & Varekamp, 1977). If more investigations show that compounds **4** and **5** are present in further *Tulipa* species and cultivars they may be considered as characteristics for *Tulipa* and therefore of importance in future chemotaxonomic studies of this genus.

3. Experimental

3.1. General

FAB-MS spectra (recorded on a Kratos MS50 TC double focusing mass spectrometer) were obtained in positive ion mode using glycerol as a matrix and ES-MS spectra (recorded on a Finnigan MAT TSQ 700) were obtained in positive ion mode using MeOH as solvent. ^{1}H and ^{13}C NMR spectra were measured at 300 and 75 MHz (Varian gemini 300), respectively, in D_2O with dioxane (δ_H 3.700 ppm, δ_C 64.700 ppm) as internal standard or in $CDCl_3$ with TMS as internal standard. CC was carried out on silica gel 60 (Merck, 70–230 mesh) and TLC was performed on silica gel 60 plates (Merck, 20×20 cm, 0.25 mm). Tuliposides and other sugars were visualized with a soln of aniline and diphenylamine in acidified Me_2CO (Christensen, 1995a; Christensen & Kristiansen, 1995b) followed by heating and tulipalins were visualized with 1 M $KMnO_4$.

3.2. Plant material

T. sylvestris L. and *T. turkestanica* Regel were obtained from the Royal Veterinary and Agricultural University, Copenhagen (Denmark), in May 1998 and frozen (-20°C) until use. Voucher specimens are deposited at the Royal Veterinary and Agricultural University.

3.3. Prep. HPLC

A Merck L-6200 intelligent pump and a Merck L-4200 UV–VIS detector were used. Prep. HPLC was performed on a Develosil ODS-HG-5 (RP-18, 250×20 mm i.d., Nomura Chemical Co., Japan) column protected with a guard cartridge (50×20 mm i.d.) packed with the same material as the column. Compounds to be separated were dissolved in 30 ml H_2O (injection volume). Separations were performed at 25°C at a flow rate of 7 ml/min. Compounds were detected at 208 nm. Mobile phases were degassed before use.

3.4. Extraction and isolation

Frozen leaves/stems of *T. turkestanica* were ground and extracted with distilled H_2O for 24 h at 4°C. The extraction was repeated and the combined extracts filtered and evapd, under red. pres. (25°C), to give a brownish syrup. CC of the crude extract (6.6 g) on silica gel, using a $CHCl_3$–MeOH–H_2O gradient (400 ml 65:35:2, 200 ml 30:20:1, 200 ml 25:25:1, 200 ml 45:55:2, 200 ml 35:65:2, 200 ml 25:75:2 and 400 ml 5:45:1) as eluent gave 36 frs containing a mixt. of compounds **6** and **7** (fr. 4–12), **1**, **3**–**5** (fr. 13–25), **1**–**3** (fr. 26–36), respectively. The crude frs were applied to prep. HPLC for further purification. Fr. 4–12 and fr. 26–36 were separated by isocratic elution with H_2O to afford compounds **6** (R_t 85–95 min), **7** (R_t 25–30 min) and D-glucose, D-fructose, sucrose (R_t 10–15 min), **1** (R_t 40–65 min), **2** (R_t 18–25 min), **3** (R_t 31–35 min), respectively, whereas fr. 13–25 were purified by a H_2O–MeOH gradient (250 ml H_2O; 150 ml 95:5; 100 ml 90:10; 400 ml 80:20; 200 ml 50:50) as eluent to afford compounds **1** (R_t 40–65 min), **3** (R_t 31–35 min), **4** (R_t 125–135 min) and **5** (R_t 88–100 min).

Leaves/stems (66 g, fr wt) of *T. turkestanica* gave 198 mg **1**, 109 mg **2**, 15 mg **3**, 40 mg **4**, 248 mg **5**, 5 mg **6** and 69 mg **7** and flowers (32 g, fr wt) gave 68 mg **1**, 40 mg **2**, 8 mg **3**, 22 mg **4**, 82 mg **5**, 5 mg **6** and 15 mg **7**.

Leaves/stems (42 g, fr wt) of *T. sylvestris* gave 390 mg **1**, 109 mg **2**, 9 mg **4**, 9 mg **6** and 16 mg **7** and flowers (23 g, fr wt) gave 110 mg **1**, 62 mg **2**, 14 mg **6** and 14 mg **7**.

3.5. Acid hydrolysis of 6-tuliposide B (2) and tuliposide F (5)

A soln of compound **5** (240 mg) in 2 M HCl (10 ml) was heated at 100°C for 2 h. After cooling, the soln was extracted $\times 6$ with $CHCl_3$ (20 ml) and the combined $CHCl_3$ phases were dried over dry Na_2SO_4. Solvent evaporation and prep. HPLC afforded **6** (41 mg) and **7** (45 mg). Acid hydrolysis of compound **2** (200 mg) afforded **7** (56 mg). The aqueous phases consisted of D-glucose as shown by TLC and ^{1}H and ^{13}C NMR.

3.6. 6-Tuliposide A (1)

Syrup; R_f 0.55, $CHCl_3$–MeOH–H_2O (15:10:2); UV and FAB-MS in accordance with lit. values (Christensen & Kristiansen, 1995a, 1995b; Christensen, 1995a, 1995b; ^{1}H NMR: Table 2; ^{13}C NMR: Table 1.

3.7. 6-Tuliposide B (2)

Syrup; R_f 0.42, $CHCl_3$–MeOH–H_2O (15:10:2); UV λ_{max} (H_2O) nm ($\log \varepsilon$): 198 (4.23); FAB-MS m/z: 317

$[M+Na]^+$, 295 $[M+H]^+$ ($C_{11}H_{19}O_9$); ES-MS m/z: 317 $[M+Na]^+$; ^{1}H NMR: Table 2; ^{13}C NMR: Table 1.

3.8. 1-Tuliposide A (**3**)

Syrup; R_f 0.55, $CHCl_3$–MeOH–H_2O (15:10:2); UV λ_{max} (H_2O) nm ($\log\varepsilon$): 210 (4.24); FAB-MS m/z: 301 $[M+Na]^+$, 279 $[M+H]^+$ ($C_{11}H_{19}O_8$); ^{1}H NMR: Table 2; ^{13}C NMR: Table 1.

3.9. Tuliposide D (**4**)

Syrup; R_f 0.70, $CHCl_3$–MeOH–H_2O (15:10:2); UV and FAB-MS in accordance with lit. values (Christensen & Kristiansen, 1995b; Christensen, 1995a, 1995b; ^{1}H NMR: Table 2; ^{13}C NMR: Table 1.

3.10. Tuliposide F (**5**)

Syrup; R_f 0.56, $CHCl_3$–MeOH–H_2O (15:10:2); UV λ_{max} (H_2O) nm ($\log\varepsilon$): 209 (4.45); FAB-MS m/z: 415 $[M+Na]^+$, 393 $[M+H]^+$ ($C_{16}H_{25}O_{11}$); ES-MS m/z: 415 $[M+Na]^+$; ^{1}H NMR: Table 2; ^{13}C NMR: Table 1.

3.11. Tulipalin A (**6**)

Oily liquid; R_f 0.61 (Et_2O); UV, MS, ^{1}H NMR in accordance with lit. values (Bergman et al., 1967; Tschesche et al., 1968, 1969; Hutchinson, 1974); ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.0 (t, C-4), 65.0 (t, C-5), 121.8 (t, C-3), 133.4 (s, C-2), 170.4 (s, C-1).

3.12. S-(−)-Tulipalin B (7)

Oil; R_f 0.41 (Et_2O); UV λ_{max} (H_2O) nm ($\log\varepsilon$): 204 (3.96), MS, $[\alpha]_D$ and ^{1}H NMR in accordance with lit. values (Tschesche et al., 1968, 1969; Hutchinson, 1974; Tanaka & Yamashita, 1980); ^{13}C NMR (75 MHz, $CDCl_3$): δ 67.7 (t, C-5), 73.1 (d, C-4), 126.7 (t, C-3), 137.8 (s, C-2), 169.1 (s, C-1).

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