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A FURTHER TULIPOSIDE FROM *ALSTROEMERIA REVOLUTA*

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Key Word Index—*Alstroemeria* species; Alstroemeriaceae; 6-tuliposide A; tuliposide D; tuliposide E; ^{1}H NMR; ^{13}C NMR.

Abstract—A reinvestigation of *Alstroemeria revoluta* afforded, in addition to tuliposides A and D, a new tuliposide. The structure of the new tuliposide was determined by spectral methods to be 1-[4-(4-hydroxy-2-methylenebutanoate)-2-methylenebutanoate]-6-(4-hydroxy-2-methylenebutanoate)-β-D-glucopyranose. The investigation of four other *Alstroemeria* species revealed only the presence of tuliposides A and D.

INTRODUCTION

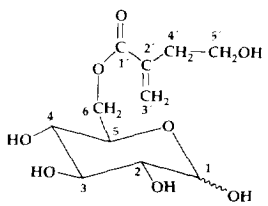
Tuliposide A and its β-hydroxy derivative tuliposide B are widely distributed in the Alstroemeriaceae (tuliposide A) [1–6] and the Liliaceae (tuliposides A and B) [5–7]. However, Kristiansen and Christensen have recently isolated a further tuliposide (tuliposide D) from *Alstroemeria*, which also seems to be widespread in this genus [2, 3]. I have now reinvestigated *A. revoluta* Ruiz et Pavon and four other *Alstroemeria* species. The investigation of *A. revoluta* gave, in addition to tuliposides previously isolated from this plant [1–3], a new tuliposide, named tuliposide E. This paper describes the isolation and structure elucidation of tuliposide E.

RESULTS AND DISCUSSION

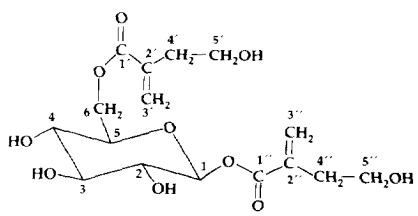
Frozen leaves plus stems and flowers of *A. revoluta* were extracted with water and the combined water extracts subjected to column chromatography to afford 6-tuliposide A (**1**), tuliposide D (**2**) and tuliposide E (**3**).

Alstroemeria angustifolia ssp. *angustifolia*, *Alstroemeria aurea* and *Alstroemeria ligtu* ssp. *ligtu* afforded **1** and **2**, whereas *A. ligtu* ssp. *simsii* gave only **1** (see Experimental and [1, 2]).

Compound **3** was obtained as a syrup. Its FAB-mass spectrum exhibited a $[M+H]^+$ at *m/z* 475 ($C_{21}H_{31}O_{12}$). The IR spectrum showed the presence of conjugated esters (1708 cm^{-1}) and double bonds (1632 cm^{-1}). The ^{13}C NMR spectrum of **3** showed 21 signals, of which six could be assigned to a β-D-glucopyranose unit by comparison with the ^{13}C NMR spectrum of **1** (see Experimental) and **2** (see Table 1). The remaining 15 signals were assigned to a 4-hydroxy-2-methylenebutanoate moiety and a 4-(4-hydroxy-2-methylenebutanoate)-2-methylenebutanoate moiety (Table 1). The presence of a 4-(4-hydroxy-2-methylenebutanoate)-2-methylenebutanoate moiety in **3** was further supported by the ^{1}H NMR signals at δ2.60 (2H, *t*, *J* = 6.5 Hz), 2.82 (2H, *t*, *J* = 6.5 Hz), 3.73 (2H, *t*, *J* = 6.5 Hz), 4.40 (2H, *t*, *J* = 6.5 Hz), 5.75 (1H, *d*, *J* = 1.3 Hz), 5.92 (1H, *d*, *J* = 1.1 Hz), 6.28



1



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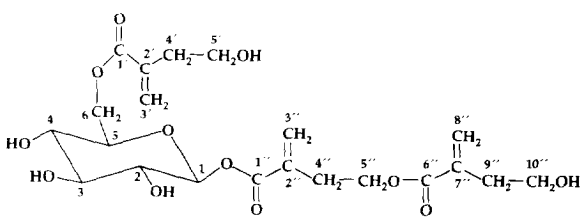

3

(1H, *d*, $J = 1.3$ Hz) and 6.47 (1H, *d*, $J = 1.1$ Hz). The remaining signals in the ^{1}H NMR spectrum of **3** were assigned to a 4-hydroxy-2-methylenebutanoate moiety by comparison with the ^{1}H NMR spectrum of **1** and **2** (Table 2). The β-nature of **3** was confirmed by the ^{1}H NMR signal at δ5.63 (1H, *d*, $J = 7.9$ Hz). The chemical shift of this anomeric proton, compared with the chemical shift (δ4.59) observed for the β-anomeric H-1 in **1**, clearly indicates the presence of an ester linkage at C-1. The downfield resonance of the C-6 protons in **3** (δ4.36 and 4.57), compared with the values (δ3.60 and 3.75) observed for H-6 of β-D-glucose [8], is due to an ester linkage at C-6. By comparing the NMR spectral data (Tables 1 and 2) of **3** with those of **2** significant differences in the chemical shifts of only H-3″, H-4″ and H-5″ and C-1″, C-2″, C-3″, C-4″ and C-5″ could be observed. This clearly indicates that the 4-hydroxy-2-methylenebutanoate moiety is linked at C-6 and that the 4-(4-hydroxy-2-methylenebutanoate)-2-methylenebutanoate moiety is linked at C-1. From the above results the structure of **3** was determined to be 1-[4-(4-hydroxy-2-methylenebutanoate)-2-methylenebutanoate]-6-(4-hydroxy-2-methylenebutanoate)-β-D-glucopyranose.

Table 1. ^{13}C NMR spectral data (62.5 MHz, CD_3OD, δ-values) for compounds **2** and **3**

C	2*	3
1	96.0 *d*	96.1 *d*
2	76.1 *d*[a]	76.2 *d*[a]
3	77.8 *d*	77.9 *d*
4	71.3 *d*	71.3 *d*
5	73.8 *d*[a]	73.9 *d*[a]
6	64.7 *t*	64.7 *t*
1′	168.2 *s*	168.3 *s*
2′	138.5 *s*	138.6 *s*[b]
3′	128.0 *t*	128.0 *t*
4′	36.3 *t*	36.3 *t*
5′	61.6 *t*	61.6 *t*
1″	166.9 *s*	166.6 *s*
2″	138.1 *s*	137.6 *s*
3″	129.2 *t*	129.6 *t*
4″	36.1 *t*	32.3 *t*
5″	61.4 *t*	64.2 *t*
6″	–	168.3 *s*
7″	–	138.7 *s*[b]
8″	–	127.8 *t*
9″	–	36.2 *t*
10″	–	61.6 *t*

*^{13}C NMR (D_2O) is given in [3].
[a,b]Assignments in the same column are interchangeable.

The incidence of contact dermatitis from *Alstroemeria* has increased in recent years, probably because of extended production of *Alstroemeria* as cut flowers [9–11]. One of the causative agents has been identified as 6-tuliposide A (**1**) [4]. Although the allergenic properties of tuliposide D (**2**) and tuliposide E (**3**) have not been investigated, their structural relationship to the allergenic 6-tuliposide A clearly indicates that they are possible allergens. If so, tuliposide D and E most probably cross-react with 6-tuliposide A. The amounts of tuliposide E in *A. revoluta* are, however, too small to contribute significantly to the allergenic properties of this species. However, tuliposide D occurs regularly in the genus *Alstroemeria* and, in some cases, in relatively large amounts (see Experimental and refs [2, 3]), which seems to indicate that tuliposide D could be a further causative agent of allergic contact dermatitis in *Alstroemeria*. Tuliposides

Table 2. ^{1}H NMR spectral data (250 MHz, CD_3OD, δ-values) for compounds **1–3**

H	1*	2†	3
1	4.59 *d* (β); 5.19 *d* (α) (7.7)‡; (3.7)	5.63 *d* (β) (7.9)	5.63 *d* (β) (7.9)
2–5	3.20–4.10 *m*	3.40–3.90 *m*	3.40–3.90 *m*
6	4.30–4.65 *m*	4.36 *dd*; 4.57 *dd* (5.6, 12.0); (2.2, 12.0)	4.36 *dd*; 4.57 *dd* (5.6, 12.0); (2.2, 12.0)
3′	5.79 *br s*; 6.34 *d* (1.3)	5.78 *d*; 6.33 *d* (1.3); (1.3)	5.78 *d*; 6.33 *d* (1.3); (1.3)
4′	2.63 *t* (6.5)	2.62 *t* (6.5)	2.62 *t* (6.5)
5′	3.76 *t* (6.5)	3.76 *t* (6.5)	3.76 *t* (6.5)
3″	–	5.89 *d*; 6.44 *d* (1.1); (1.1)	5.92 *d*; 6.47 *d* (1.1); (1.1)
4″		2.65 *t* (6.5)	2.82 *t* (6.5)
5″	–	3.77 *t* (6.5)	4.40 *t* (6.5)
8″	–	–	5.75 *d*; 6.28 *d* (1.3); (1.3)
9″	–	–	2.60 *t* (6.5)
10″	–	–	3.73 *t* (6.5)

*^{1}H NMR (D_2O) is given in [1, 3, 4].
†^{1}H NMR (D_2O) is given in [3].
‡J in Hz in parentheses.

are probably storage products for D-glucose and a defence against fungial attack, as they may undergo enzymic hydrolysis to yield D-glucose and the highly antibiotic and allergenic α-methylene-γ-butyrolactone (tulipalin A) [4, 5, 7, 11–13].

EXPERIMENTAL

General. FAB-MS: in glycerol; CC: silica gel 60 (Merck, 70-230 mesh); TLC: silica gel 60 plates (Merck, ART. 5721). Spots on TLC were visualized with a solution of aniline and diphenylamine in acidified Me_2CO followed by heating [1–3].

Plant material. Alstroemeria species were produced from seeds in a greenhouse and identified according to Bayer [14]. Voucher specimens are retained at the Department of Ornamentals, Research Centre Årslev, Danish Institute of Plant and Soil Science. Flowers and leaves/stems were harvested in August and frozen (−20°) until use.

Extraction and isolation. Frozen leaves/stems and flowers of *A. revoluta* were ground and extracted with distilled H_2O for 24 hr at 4°. The extraction was repeated and the combined extracts filtered and evapd, under red. press., to give a brownish syrup [3]. CC of the crude extracts on silica gel, using a $CHCl_3$–MeOH gradient (8:2; 7:3; 3:2; 1:1; 3:7; 1:9) as eluent gave D-glucose and D-fructose [3], **1** and **2** and a mixture of **2** and **3**. Compound **3** was separated from **2** by CC on silica gel, using a $CHCl_3$–MeOH gradient (9:1; 1:4; 3:7; 2:3). Leaves/stems (120 g) of *A. revoluta* gave 986 mg **1**, 277 mg **2** and 22 mg **3** flowers (41 g) gave 321 mg **1**, 125 mg **2** and 14 mg **3**.

A. angustifolia Herbert ssp. *angustifolia*: Leaves/stems (72 g) gave 248 mg **1** and 66 mg **2**, whereas flowers (40 g) gave 409 mg **1** and 71 mg **2**.

A. aurea Graham: Leaves/stems (82 g) gave 420 mg **1** and 12 mg **2**, flowers (41 g) gave 442 mg **1** and 47 mg **2**.

A. ligtu L. ssp. *ligtu*: Leaves/stems (76 g) gave 136 mg **1** and less than 6 mg **2**, whereas flowers (46 g) gave 288 mg **1** and 14 mg **2**.

A. ligtu L. ssp. *simsii* (Sprengel) Bayer: Leaves/stems (92 g) gave 517 mg **1** and flowers (53 g) gave 331 mg **1**.

6-*Tuliposide A* (**1**). Syrup; R_f 0.56, $CHCl_3$–MeOH–H_2O (15:10:2); UV, IR and FAB-MS in accordance with lit. values [3]. ^{1}H NMR: Table 2; ^{13}C NMR (62.5 MHz, CD_3OD): δ36.3 (*t*, C-4′), 61.6 (*t*, C-5′), 127.8 (*t*, C-3′α)[a], 127.9 (*t*, C-3′β)[a], 138.7 (*s*, C-2′), 168.3 (*s*, C-1′α)[b], 168.4 (*s*, C-1′β)[b], α-D-glucose: 93.9 (*d*, C-1), 73.7 (*d*, C-2), 75.3 (*d*, C-3)[c], 70.7 (*d*, C-4), 71.9 (*d*, C-5), 65.2 (*t*, C-6), β-D-glucose: 98.2 (*d*, C-1), 76.1 (*d*, C-2)[c], 77.8 (*d*, C-3), 71.7 (*d*, C-4), 74.7 (*d*, C-5)[c], 65.1 (*t*, C-6). [a–c]Assignments may be interchanged.

Tuliposide D (**2**). Syrup; R_f 0.71, $CHCl_3$–MeOH–H_2O (15:10:2); UV, IR and FAB-MS in accordance with lit. values [3]. ^{1}H NMR: Table 2; ^{13}C NMR: Table 1.

Tuliposide E (**3**). Syrup; R_f 0.82, $CHCl_3$–MeOH–H_2O (15:10:2); UV λ_{max} (H_2O) nm (log ε): 208 (4.59); IR ν_{max} (film) cm^{-1}: 3409 (OH), 1708 (conjugated ester), 1632 (C=C); FAB-MS *m/z*: 497 $[M + Na]^+$, 475 $[M + H]^+$ ($C_{21}H_{31}O_{12}$); ^{1}H NMR: Table 2; ^{13}C NMR: Table 1.

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