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TULIPOSIDES FROM *ALSTROEMERIA REVOLUTA*

LARS P. CHRISTENSEN

Research Group for Plant Breeding and Propagation, Department of Ornamentals, Research Center Årslev, Danish Institute of Plant and Soil Science, Kirstinebjergvej 10, DK-5792 Årslev, Denmark

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Key Word Index—*Alstroemeria revoluta*; Alstroemeriaceae; 1,6-di-(4-hydroxy-2-methylenebutanoate)-β-D-glucopyranose; tuliposide D; 6-tuliposide A.

Abstract—The investigation of *Alstroemeria revoluta* afforded a new tuliposide, tuliposide D, along with the known 6-tuliposide A. The structures of the isolated compounds were established by spectral methods.

INTRODUCTION

Contact dermatitis from *Alstroemeria* (Alstroemeriaceae) and *Tulipa* (Liliaceae) species is a well-known occupational dermatosis in the field of plant production. These ornamental plants normally cause an allergic skin disease known as 'tulip fingers' [1–3]. The allergens responsible for this contact dermatitis have been identified as tuliposide A and α-methylene-γ-butyrolactone (tulipalin A) [1–6]. Tuliposide A and tulipalin A have also been shown to possess antibiotic properties [7–9] and they are probably involved in the disease resistance of the plants. Tuliposide A and its β-hydroxy derivative (tuliposide B) are widely distributed in the Alstroemeriaceae (tuliposide A) [2, 5, 6, 10] and in the Liliaceae (tuliposide A and B) [6–8, 10] and, therefore, they seem to be useful in chemotaxonomic evaluations. As part of our continued investigations of *Alstroemeria* species for tuliposides [5] we have examined *Alstroemeria revoluta* Ruiz et Pavon. This paper describes the isolation and structure elucidation of a new tuliposide, named tuliposide D,* from this plant.

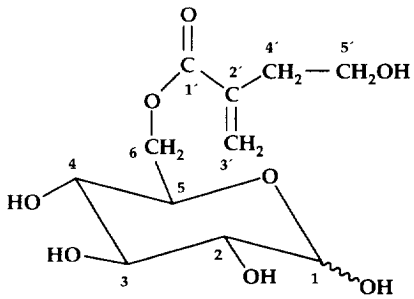
RESULTS AND DISCUSSION

Frozen flowers and leaves/stems of *A. revoluta* were extracted with distilled water and the combined water extracts were subjected to column chromatography. The tuliposides, 6-tuliposide A (**1**) and tuliposide D (**2**) were isolated together with large amounts of monosaccharides (D-glucose and D-fructose). 6-Tuliposide A has previously been detected in *A. revoluta* [5], whereas **2**, to the best of my knowledge, is a new natural product. Although the spectral data of **1** are in accordance with literature values [2, 5, 7, 8] they are given in the Experimental for comparison.

*The name tuliposide C has previously been used for a compound detected in *Tulipa gesneriana* L. [7, 8], although the compound was not identified.

Compound **2** was obtained as a syrup, which yielded D-glucose and the unsaturated lactonized aglycone α-methylene-γ-butyrolactone (**3**) upon acid hydrolysis [7, 8], (**3** was identified by comparison with authentic material, TLC, GC-MS and NMR). This indicated the presence of a 4-hydroxy-2-methylenebutanoate moiety in **2** [7, 8], which was supported by the IR absorptions at 1718 cm^{-1} (OCOR) and 1632 cm^{-1} (C=C) and the FAB-mass spectrum showing ions at m/z 377 $[M + H]^+$, 261 $[M - OCO(C{=}CH_2)CH_2CH_2OH]^+$ and m/z 115 $[C_5H_7O_3]^+$. The ^{13}C NMR spectrum of **2** showed 16 signals of which six could be assigned to a β-D-glucopyranose unit. The remaining 10 signals were assigned to two 4-hydroxy-2-methylenebutanoate moieties by comparison with the ^{13}C NMR spectrum of **1** (see Experimental). The presence of two 4-hydroxy-2-methylenebutanoate moieties in **2** was further supported by the ^1H NMR signals at δ2.57 (2H, *t*, $J = 6.5$ Hz), 2.60 (2H, *t*, $J = 6.5$ Hz), 3.75 (4H, *t*, $J = 6.5$ Hz), 5.81 (1H, *s*), 5.92 (1H, *s*), 6.29 (1H, *s*) and 6.44 (1H, *s*). The β-nature of **2** was confirmed by the ^1H NMR signal at δ5.64 (1H, *d*, $J = 7.6$ Hz). The downfield resonance of this anomeric proton, compared with the value (δ4.69) observed for the β-anomeric H-1 in **1**, is evidently owing to an ester linkage at this position, indicating the presence of a 4-hydroxy-2-methylenebutanoate moiety at C-1. Also, the downfield resonance of the C-6 protons in **2** (δ4.35–4.65, 2H, *m*), compared with the values (δ3.60 and 3.75) observed for H-6 of β-D-glucose [11] is owing to an ester linkage at this position, indicating that the second 4-hydroxy-2-methylenebutanoate moiety is linked at C-6. From the above data it was concluded that the structure of **2** was 1,6-di-(4-hydroxy-2-methylenebutanoate)-β-D-glucopyranose (tuliposide D).

If more investigations show that tuliposide D (**2**) is present in further *Alstroemeria* species, it may be considered as characteristic for this genus and, therefore, of chemotaxonomic importance. Compound **2** is probably



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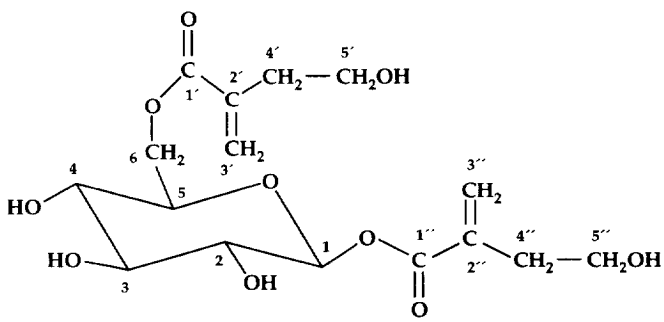
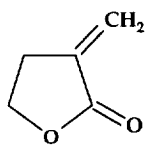


2



3

biosynthesized from the β-anomer of 6-tuliposide A. However, it cannot be excluded that tuliposide D is biosynthesized in one step from β-D-glucose and that tuliposide D may undergo enzymic hydrolysis followed by cyclization to yield 6-tuliposide A and the highly antibiotic tulipalin A [7–9]. From a medical point of view tuliposide D could be a further allergen in *Alstroemeria*, which most likely cross-reacts with tuliposide A.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were measured at 250 and 62.5 MHz, respectively, in D_2O, with dioxane as internal standard. FAB-MS: in glycerol. CC was carried out on silica gel 60 (Merck, 70–230 mesh) and TLC was performed on silica gel 60 plates (Merck, 0.25 mm, ART. 5721). Spots on TLC were visualized with a solution of aniline and diphenylamine in acidified acetone (Sigma No. A 8142) followed by heating.

Plant material. Specimens of *Alstroemeria revoluta* Ruiz et Pavon were produced from seeds in a greenhouse and identified according to Bayer [12]. A voucher specimen is deposited at the Department of Ornamentals, Research Center Årslev, Danish Institute of Plant and Soil Science. Flowers and leaves/stems of *A. revoluta* were harvested in May and frozen (− 20°) until use.

Extraction and isolation. Frozen flowers (54 g) were ground and extracted with distilled H_2O (600 ml) for 24 hr at 4°. The extraction was repeated and the combined extracts filtered and evapd, under red. pres. (35°), to give a brownish syrup (5.2 g). CC of the crude extracts on silica gel, using a $CHCl_3$–MeOH–H_2O gradient (80:20:1; 35:15:1; 15:10:1; 15:10:2 and 5:5:1) as eluent, gave **2** (105 mg) and **1** (510 mg) and the monosaccharides D-glucose and D-fructose (identified by TLC and HPLC). Leaves/stems (65 g) of *A. revoluta* afforded 455 mg **1** and 95 mg **2** and monosaccharides (D-glucose, D-fructose).

6-*Tuliposide A* (**1**). Syrup; R_f 0.56, $CHCl_3$–MeOH–H_2O (15:10:2); UV $\lambda_{max}^{H_2O}$ nm (log ε): 208 (4.23); IR (see also ref. [7]) ν_{max}^{film} cm^{-1}: 3370 (OH), 1708 (OCOR), 1632 (C=C); FAB-MS *m/z* 279 $[M + H]^+$ ($C_{11}H_{19}O_8$); ^1H NMR (D_2O) (see also refs [2, 5, 7]); δ2.57 (2H, *t*, *J* = 6.5 Hz, H-4′), 3.25–4.10 (4H, *m*, H-2, H-3, H-4, H-5), 3.75 (2H, *t*, *J* = 6.5 Hz, H-5′), 4.30–4.60 (2H, *m*, H-6), 4.69 (*d*, *J* = 7.9 Hz, H-1β), 5.24 (*d*, *J* = 3.7 Hz, H-1α), 5.81 (1H, *s*, H-3′), 6.32 (1H, *s*, H-3′). The ratio between α(H-1) and β (H-1) was determined to be 2:3, at room temp. ^{13}C NMR (D_2O) (see also refs [2, 5]): δ35.0 (*t*, C-4′), 60.9 (*t*, C-5′), 129.8 (*t*, C-3′), 137.0 (*s*, C-2′), 169.3 (*s*, C-1′); α-D-glucose: 93.0 (*d*, C-1), 72.3 (*d*, C-2), 73.5 (*d*, C-3), 70.1 (*d*, C-4), 70.6 (*d*, C-5), 64.5 (*t*, C-6); β-D-glucose: 96.9 (*d*, C-1), 74.9 (*d*, C-2), 76.4 (*d*, C-3), 70.5 (*d*, C-4), 74.3 (*d*, C-5), 64.5 (*t*, C-6).

Tuliposide D (**2**). Syrup; R_f 0.71, $CHCl_3$–MeOH–H_2O (15:10:2); UV $\lambda_{max}^{H_2O}$ nm (log ε): 208 (4.49); IR ν_{max}^{film} cm^{-1}: 3392 (OH), 1718 (OCOR), 1632 (C=C); FAB-MS *m/z* (rel. int.): 377 $[M + H]^+$ ($C_{16}H_{25}O_{10}$) (8), 261 $[M - OCO(C=CH_2)CH_2CH_2OH]^+$ (17), 115 $[C_5H_7O_3]^+$ (40); EI-MS (70 eV) *m/z* (rel. int.): 261 $[M - OCO(C=CH_2)CH_2CH_2OH]^+$ (46), 243 (8), 230 (24), 212 (17), 163 (31), 145 (40), 127 (50), 117 (54), 115 (16), 103 (54), 98 (89), 85 (95), 71 (77), 68 (100); ^1H NMR (D_2O): δ2.57 (2H, *t*, *J* = 6.5 Hz, H-4′), 2.60 (2H, *t*, *J* = 6.5 Hz, H-4″), 3.50–4.00 (4H, *m*, H-2, H-3, H-4, H-5), 3.75 (4H, *t*, *J* = 6.5 Hz, H-5′, H-5″), 4.35–4.65 (2H, *m*, H-6), 5.64 (1H, *d*, *J* = 7.6 Hz, H-1β), 5.81 (1H, *s*, H-3′), 5.92 (1H, *s*, H-3″), 6.29 (1H, *s*, H-3′), 6.44 (1H, *s*, H-3″). ^{13}C NMR (D_2O): δ34.8 (*t*, C-4″)[a], 35.0 (*t*, C-4′)[a], 60.7 (*t*, C-5″)[b], 60.9 (*t*, C-5′)[b], 129.9 (*t*, C-3′), 131.4 (*t*, C-3″), 136.3 (*s*, C-2″), 137.0 (*s*, C-2′), 167.6 (*s*, C-1″), 169.2 (*s*, C-1′): β-D-glucose: 95.1 (*d*, C-1), 72.7 (*d*, C-2)[c], 76.2 (*d*, C-3), 70.3 (*d*, C-4), 75.2 (*d*, C-5)[c], 64.1 (*t*, C-6). [a–c]Assignments may be interchanged.

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