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STEROIDAL GLYCOSIDES FROM *Allium rotundum*

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We have previously reported [1] the isolation of seven sapogenins from inflorescences and flower stalks of *Allium rotundum* (Alliaceae). Herein we present results from structural studies of five steroidal glycosides from this plant.

Exhaustive extraction of air-dried raw material with hot methanol isolated total saponins, which were separated by repeated chromatography into seven pure compounds called in order of increasing polarity glycosides **1**-**7**. According to color reactions [2] and IR spectroscopy, they were spirostanols.

The structures of the glycosides were elucidated based on chemical transformations and GC, IR, mass, and NMR spectroscopy.

Exhaustive methylation of the saponins by the Hakomori method [3] and subsequent methanolysis gave information about the genins; GC, about the monosaccharide compositions, the presence of branching centers, and the sites of attachment of the sugar units to each other (Table 1).

FAB mass spectrometry of the glycosides enabled their mass and that of the genins in them to be determined. The fragmentation pattern of the carbohydrate parts confirmed the GC data (Table 2).

The structures of the glycosides were studied further using very informative NMR methods (^{1}H and ^{13}C). Resonances were assigned using homo- and heteronuclear double resonance experiments. Experiments observing the heteronuclear Overhauser effect determined the spin—spin coupling constants J{^{13}C—^{1}H} of the anomeric C atoms of the sugars and, therefore, their configuration [4]. HMBC spectra found correlations of through-space couplings between anomeric protons and C atoms to which the corresponding sugar units were bonded.

Based on the results, the following conclusions could be made.

Glycoside 1 contained diosgenin and one β-D-glucopyranose, i.e., was (25*R*)-spirost-5-en-3β-ol 3-*O*-β-D-glucopyranoside or trillin [5].

Glycoside 2 consisted of β-chlorogenin, β-D-galactopyranose, and β-D-glucopyranose (terminal) and was identified as (25*R*,5α)-spirostan-3β,6β-diol 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside or dideglucoeruboside B [6].

Glycoside 4 had agigenin as the genin, one β-D-galactopyranose, one β-D-xylopyranose, and two β-D-glucopyranoses. The chemical structure was determined as (25*R*,5α)-spirostan-2α,3β,6β-triol 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside or aginoside [7].

Glycoside 5 contained β-chlorogenin, β-D-galactopyranose, and three β-D-glucopyranoses. The chemical structure corresponded to (25*R*,5α)-spirostan-3β,6β-diol 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside or eruboside B [8].

Glycoside 6 consisted of agigenin, β-D-galactopyranose, and three β-D-glucopyranoses (the composition of the monosaccharides and the nature of the branching were analogous to those of **5**). It was identified as (25*R*,5α)-spirostan-2α,3β,6β-triol 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside or yayoisaponin C [9].

Work on elucidating the structures of **3** and **7** is continuing.

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TABLE 1. Genin Composition and GC Data for Glycosides **1**, **2**, **4**-**6**

Compound	Genin	mp, °C	$[\alpha]_D^{22}$, deg	Monosaccharide composition	Methylsaccharides
1	Diosgenin	272-274	-102.8	Glc	2,3,4,6-Tetra-*O*-Me-Glc
2	*β*-Chlorogenin	292-294 dec.	-66.2 (*c* 0.9, MeOH)	Glc, Gal - 1:1	2,3,6-Tri-*O*-Me-Gal 2,3,4,6-Tetra-*O*-Me-Glc
4	Apigenin	271-274	-67.6 (*c* 1.18, CH_3-MeOH, 10:1)	Glc, Gal, Xyl -2:1:1	2,3,6-Tri-*O*-Me-Gal 2,6-Di-*O*-Me-Glc (A) 2,3,4,6-Tetra-*O*-Me-Glc (B) 2,3,4-Tri-*O*-Me-Xyl
5	*β*-Chlorogenin	274-277	-72.1 (*c* 1.2, CH_3-MeOH, 10:1)	Glc, Gal - 3:1	2,3,6-Tri-*O*-Me-Gal 2,6-Di-*O*-Me-Glc (A) 2,3,4,6-Tetra-*O*-Me-Glc (B and C)
6	Apigenin	-	-40.6 (*c* 0.28 C_5H_5N)	Glc, Gal - 3:1	2,3,6-Tri-*O*-Me-Gal 2,6-Di-*O*-Me-Glc (A) 2,3,4,6-Tetra-*O*-Me-Glc (B and C)

TABLE 2. FAB-Mass Sepctra and NMR Spectra of Studied Glycosides

Compound, empirical formula	Molecular ion and fragmentation pattern	J [^{13}C-^{1}H], Hz, and glycoside bond configuration	Through-space correlations in HMBC spectra
1, $C_{33}H_{52}O_8$	599 $[M+Na]^+$	159.6, *β*-Glc	H-1 (Glc) - C-3 of the genin
	437 $\{M+Na\}^+$-hexose		
2, $C_{39}H_{64}O_{14}$	779 $[M+Na]^+$	161.2, *β*-Gal	H-1 (Gal) - C-3 of the genin
	617 $[M+Na\}^+$-hexose	159.2, *β*-Glc	H-1 (Glc) - C-4 (Gal)
	455 $[M+Na\}^+$-hexose		
4, $C_{50}H_{82}O_{24}$	1089 $[M+Na]^+$	161.2, *β*-Gal	H-1 (Gal) - C-3 of the genin
	957 $[M+Na]^+$-pentose	162.0, *β*-Glc A	H-1 (Glc A) - C-4 (Gal)
	927 $[M+Na]^+$-hexose	159.5, *β*-Glc B	H-1 (Glc B) - C-2 (Glc A)
	471 $[M+Na]^+$-tetraose	158.4, *β*-Xyl	H-1 (Xyl) - C-3 (Glc A)
5, $C_{51}H_{84}O_{24}$	1103 $[M+Na]^+$	161.2, *β*-Gal	H-1 (Gal) - C-3 of the genin
	941 $[M+Na]^+$-hexose	162.0, *β*-Glc A	H-1 (Glc A) - C-4 (Gal)
	455 $[M+Na]^+$-tetraose	159.5, *β*-Glc B	H-1 (Glc B) - C-2 (Glc A)
		159.2, *β*-Glc C	H-1 (Glc C) - C-3 (Glc A)
6, $C_{51}H_{84}O_{25}$	1119 $[M+Na]^+$	161.2, *β*-Gal	H-1 (Gal) - C-3 of the genin
	957 $[M+Na]^+$-hexose	162.0, *β*-Glc A	H-1 (Glc A) - C-4 (Gal)
	471 $[M+Na]^+$-tetraose	159.5, *β*-Glc B	H-1 (Glc B) - C-2 (Glc A)
		159.2, *β*-Glc C	H-1 (Glc C) - C-3 (Glc A)

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