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NEW STEROIDAL SAPONINS OF *AGAVE AMERICANA*

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Abstract—Two new saponins, agavasaponin E and agavasaponin H have been isolated from the methanolic extract of *Agave americana* leaves and their structures elucidated. Agavasaponin E is 3-*O*-[β-D-xylopyranosyl-(1→2glc 1)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3 glc 1)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl]-(25*R*)-5α-spirostan-12-on-3β-ol, whereas agavasaponin H is 3-*O*-[β-D-xylopyranosyl-(1→2 glc 1)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3 glc 1)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*R*)-5α-furostan-12-on-3β,22α,26-triol.

INTRODUCTION

Hecogenin [1], chlorogenin and rocogenin [2, 3] have been reported in *Agave americana*. The isolation and structural elucidation of several saponins of hecogenin from a methanolic extract of *A. americana* leaves has been reported previously [4]. This paper describes the structural elucidation of two new saponins isolated from this plant.

RESULTS AND DISCUSSION

Column chromatography on silica gel of the mixture of saponins obtained from *A. americana* gave the previously reported saponins, agavasaponin E (**1**) and agavasaponin H (**2**) Both saponins gave only one spot on TLC. Using the Ehrlich reagent only agavasaponin H (**2**) gave a positive colour test [5].

After acid hydrolysis of **1** and **2** the aglycone was identified as hecogenin by mp [6], IR, MS, and chromatographic mobility. PC of the sugars obtained from both saponin hydrolysates showed the presence of rhamnose, xylose, glucose and galactose.

GLC analysis of aldonenitryl derivatives of the sugars obtained from the agavasaponin E (**1**) hydrolysate showed the presence of rhamnose, xylose, galactose and glucose in the ratio 2:1:1:2. In agavasaponin H (**2**) the ratio was 2:1:1:3.

In the presence of β-glucosidase of *Helix pomatia* agavasaponin H (**2**) was converted into agavasaponin E (**1**) when kept in aqueous solution at room temp. for 24 hr.

The type of glycosidic linkage in compound **1** was proved by methylation [7]. The permethylated product was hydrolysed with $HClO_4$ and the methyl monosaccharides were chromatographed on silica gel to give six compounds. All methylated products were identified by TLC and GLC. In addition methyl-2,3,6-tri-*O*-methyl-D-glucopyranoside (**3**), methyl-2,3,6-tri-*O*-methyl-D-galactopyranoside (**4**), and methyl 4,6-di-*O*-methyl-D-glucopyranoside (**5**) were identified by MS and NMR spectrometry [8–10]. After methylation compound **2** gave the same products and additionally methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (**6**).

The sequence of the sugars in **1** was proved by partial hydrolysis with HCl which gave a monoglycoside (**7**), diglycoside (**8**), triglycoside (**9**),

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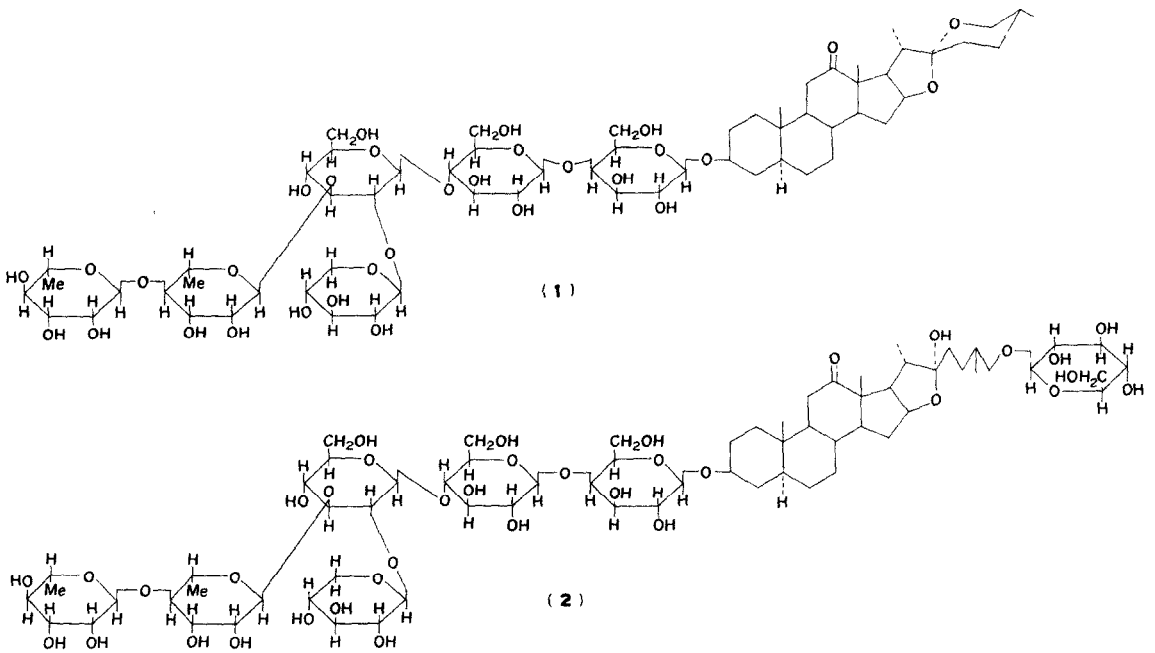

tetraglycoside (**10**), pentaglycoside (**11**) and a trace of hecogenin. Acid hydrolysis of **7** gave galactose, **8** and **9** gave galactose and glucose in the ratios 1·1 and 1 2 respectively, **10** gave galactose, glucose and xylose in the ratio 1:2 1, and **11** gave galactose, glucose, xylose, and rhamnose in the ratio 1:2:1:1

Compounds **8–11** were next methylated and after hydrolysis with $HClO_4$ the following products were obtained. From **8**, methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (**6**) and methyl-2,3,6-tri-*O*-methyl-D-galactopyranoside (**4**); from **9** the same products as for **8** but in addition methyl-2,3,6-tri-*O*-methyl-D-glucopyranoside (**3**), from **10** compounds **3**, **4**, methyl-4,6-di-*O*-methyl-D-glucopyranoside (**5**) and 2,3,4-tri-*O*-methyl-D-xylopyranoside (**12**); from **11** the same products as for **10** and in addition methyl-2,3,4-tri-*O*-methyl-L-rhamnopyranoside (**13**).

Partial hydrolysis of agavasaponin H led to the formation of **1**, **7**, **8–11** and hecogenin.

Peracetylated $\Delta^{20(22)}$ agavasaponin H (**14**) was oxidized [11] with CrO_3 in acetic acid at room temp. followed by hydrolysis to form 2 products, **15** and **16**. δ-Hydroxy-γ-methyl-*n*-valeric acid glucoside (**15**), after acetylation and methylation with diazomethane, yielded the tetraacetylglucoside methyl ester of δ-hydroxy-γ-methyl-*n*-valeric acid (**17**) which showed the characteristic MS peak [11, 12] for acetylated glucose at *m/e* 331, as well as fragmentation peaks at *m/e* 129 ($C_7H_{13}O_2$) and 97 (129-MeOH) for the acidic residue. Acid hydrolysis of **16** gave 3β-hydroxy-5α-pregn-16-en-12,20-dione, which after acetylation was identical by IR and UV to 3β-acetoxy-5α-pregn-16-en-12,20-dione. The monosaccharide composition of the glycoside (**16**) was the same as that of agavasaponin E (**1**).

The configuration at C-1 of the monosaccharides was determined with the help of Klyne's rule [13]

From the above results it follows that agavasaponin E is: 3-*O*-[β-D-xylopyranosyl-(1→2 glc 1)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3 glc 1)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-(25*R*)-5α-spirostan-12-on-3β-ol. whereas agavasaponin H is: 3-*O*-[β-D-xylopyranosyl-(1→2 glc 1)-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3) glc 1)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*R*)-5α-furostan-12-on-3β,22α,26-triol

EXPERIMENTAL

Separation of Agave americana *saponins L and H* Dry leaves (700 g) of 4 *americana* were extracted with MeOH in a Soxhlet for 48 hr From the extract after evaporation of the solvent

35 g of a mixture of saponins were isolated by chromatography on Sephadex G-25. The saponin mixture (20 g) was further purified by chromatography on Si gel ($CHCl_3$–MeOH–H_2O, 65:35:7), yielding 2·5 g of saponin E mp 304–308°, $[\alpha]_D^{20°} = -130°$, (MeOH; *c* 0·83) and 3·1 g of saponin H mp 228–230°, $[\alpha]_D^{20°} = -113$ (MeOH; *c* 0·62).

GLC. Aldonenitryl and methyl derivatives of sugars were separated using a 2 m glass-column of 5% XE-60.

Hydrolysis of **1** *and* **2**. 20 mg of **1** or **2** were hydrolysed with 5% H_2SO_4 at 110° for 24 hr. Hecogenin was obtained from both glycosides and purified by TLC ($CHCl_3$–MeOH, 96:4) mp 244–247°, $[\alpha]_D^{20°} = +10$ ($CHCl_3$; *c* 0·9). MS: *m/e* 430 M^+. Monosaccharides were identified in the hydrolysate from both glycosides by PC and by GLC of their aldonenitryls [14].

Methylation and hydrolysis of permethylated products. Agavasaponin E (1 g) was methylated by the Kuhn method to yield permethylated agavasaponin E, mp 106–110°, $[\alpha]_D^{20} = -75$ ($CHCl_3$; *c* 0·82). This was hydrolysed with 72% $HClO_4$ in MeOH (1:10) for 5 hr at 105°. After neutralization by anionic Dowex IX8 TLC on Si gel (Me_2CO–C_6H_6, 1:2) showed 6 compounds. These compounds were separated on a column of Si gel. Compounds **6**, **12** and **13** were identified by GLC, with the aid of authentic samples. Compound **3** was characterized as methyl-2,3,6-tri-*O*-methyl-β-D-glucopyranoside MS (*m/e*): 71, 73, 75, 88, 101, 161, [8], NMR: δ 3·42 (3H,*S*, C-1 OMe), 3·48 (3H,*S*, C-3 OMe), 3·28 (3H,*S*, C-6 OMe), 4·05 (1H,*d*,*J* 7·42 Hz, C-1) [10]. Compound **4** gave the same peaks but after demethylation gave galactose. After reaction with CD_3I by the Hakomori method [15] compound **5** was characterized as methyl-4,6-di-*O*-methyl-2,3-di-OGD_3-α-D-glucopyranoside, MS (*m/e*): 91, 94, 104, 107, 114, 152, 182, 190, 211, 225, [9], NMR: γ 3·21 (3H,*S*, C-1 OMe), 3·45 (3H,*S*, C-4 OMe), 3·23 (3H,*S*, C-6 OMe), 4·48 (1H,*d*,*J* 3·82 Hz, C-1) [10]. Agavasaponin H was methylated by the same method to yield permethylated agavasaponin H, pm 88–96° $[\alpha]_D^{20°}$ −102° ($CHCl_3$ *c* 0·82). After hydrolysis this gave the same products as above and additionally methyl-2,3,4,6-tetra-*O*-methyl-glucopyranoside.

Partial hydrolysis. 0·8 g of **1** or **2** was heated in 30 ml. 1·5 N HCl for 2 hr at 100°, with H_2O and extracted with 3 × 60 ml BuOH. BuOH extracts were chromatographed on Si gel ($CHCl_3$–MeOH–H_2O, 65:25:10) to obtain from **1**, compound **7** mp 220–223°, $[\alpha]_D^{20°}$ −113° (DMF; *c* 1·01), compound **8** mp 260–262°, $[\alpha]_D^{20°}$ −70° (DMF; *c* 1·00), compound **9** mp 275–278°, $[\alpha]_D^{20°}$ −55° (DMF; *c* 1·05), compound **10** mp 200–205°, $[\alpha]_D^{20°}$ −54° (MeOH; *c* 1·10), compound **11** mp 297–299° $[\alpha]_D^{20°}$ −60° (MeOH; *c* 1·33). From **2**, agavasaponin E (**1**) was obtained in addition to **7**, **8**–**11**. 0·05 g of each product (**7**–**11**) was methylated and the products identified by TLC and GLC.

Enzymic hydrolysis with β-glucosidase of Helix pomatia. 100 mg of **2** in 10 ml H_2O were incubated with the enzyme for 24 hr at room temp. After 24 hr the mixture was extracted 3× with 30 ml BuOH and the extract was chromatographed on a column of Si gel to yield 70 mg of **1**. Acid hydrolysis of the glucoside yielded the same monosaccharides as obtained from agavasaponin E (**1**) and hecogenin.

Oxidation of compound **2**. Acetylated compound **2**, obtained by reaction with acetic acid [12], was dissolved in 10 ml HOAc and 200 mg NaOAc was added. This was followed by 400 mg CrO_3 in 1·59 ml 80% HOAc and 2 ml H_2O added dropwise to the mixture at 15° during 15 min. The reaction mixture was finally extracted with $CHCl_3$. The oxidized product (650 mg) was hydrolysed in 30 ml *t*-BuOH with 1·0 g KOH in 1·2 ml H_2O under N_2 with stirring at 30° for 3·5 hr and at room temp. for 30 min. Then 18 ml H_2O were added and the *t*-BuOH evaporated *in vacuo*. Residue was extracted with *n*-BuOH to give a BuOH phase A and an aq phase B. From phase B, compound **15** was extracted and next acetylated and methylated with CH_2N_2. MS showed the characteristic peaks for completely acetylated glucose at *m/e* 331, 243, 242, 200, 169, 157, 145, 141, 115, 109 as well as characteristic peaks for the acidic residue at *m/e* 129, 97, 89 and 81. From phase A compound **16** was extracted and hydrolysed in 2 ml 4 N HCl and 2 ml C_6H_6 for 3 hr at 80°. The obtained derivative of pregnenolone was acetylated and purified on Si gel to give 30 mg 3β-acetoxy-5α-pregn-16-en-12,20-dione, mp 179–180° $[\alpha]_D^{20°}$ +125° ($CHCl_3$; *c* 1·00). The monosaccharides were detected in the hydrolysate by PC.

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