Phytochemistry, 1975, Vol. 14, pp. 1817–1820. Pergamon Press. Printed in England.

STEROIDAL SAPONINS OF *YUCCA FILAMENTOSA*: YUCCOSIDE C AND PROTOYUCCOSIDE C

IVAN P. DRAGALIN and PAVEL K. KINTIA

The Institute of Chemistry of the Academy of Sciences of the Moldavian SSR, Kishinev 277028, U.S.S.R.

(*Received* 25 *July*, 1974)

Key Word Index—*Yucca filamentosa*; Liliaceae; furostanol glycosides; spirostanol glycosides.

Abstract—Two new saponins, yuccoside C and protoyuccoside C, have been isolated from the methanolic extract of *Yucca filamentosa* root and their structures elucidated. Yuccoside C is 3-*O*-[α-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ol, whereas protoyuccoside C is 3-*O*-[α-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-26-0-[β-D-glucopyranosyl]-(25*S*)-5β-furostan-3β,22α,26-triol.

INTRODUCTION

The following genins have been reported [1, 2] in *Yucca filamentosa*: tigogenin, sarsasapogenin, gitogenin, hecogenin and chlorogenin. The isolation of saponins of diosgenin and tigogenin from the methanolic extract of *Y. filamentosa* root has been reported previously [3]. This paper describes the structural elucidation of two other saponins isolated from this plant. Spirostanol and furostanol glycosides are found in plants [4, 5] and it is assumed that the furostanol glycosides are synthesized first, and then transformed into spirostanol glycosides.

RESULTS AND DISCUSSION

Column chromatography of the saponins of *Y. filamentosa* on silica gel gave yuccoside C (**1**) and protoyuccoside (**2a**) while an additional product, 2b, was obtained in methanolic solvent systems. Protoyuccoside C (**2a**) showed only one spot on TLC. Using the Ehrlich reagent only protoyuccoside C gave a positive colour test.

After acid hydrolysis of **1**, **2a** and **2b**, the aglycone was identified as sarsasapogenin by mp, $[\alpha]_D$, IR, MS and chromatographic mobility.

GLC analysis of the aldonenitryl derivatives of the sugars obtained from the yuccoside C (**1**) hydrolysate showed the presence of galactose and glucose in the ratio of 1:2. In protoyuccoside C (**2a**) the galactose and glucose ratio was 1:3.

Under the influence of β-glucosidase of *Helix pomatia* protoyuccoside C (2a) was converted into yuccoside C (**1**) when kept in aqueous solution at room temperature for 24 hr.

The type of glycosidic linkage in compound **1** was proved by methylation [6]. The permethylated product was hydrolysed with $HClO_4$ and the methyl monosaccharides were chromatographed on silica gel to give three compounds. By TLC and GLC one of the products was identified as methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranoside (**3**). The other compounds were identified by MS [7] and NMR spectrometry [8] as methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside (**4**) and methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside (**5**).

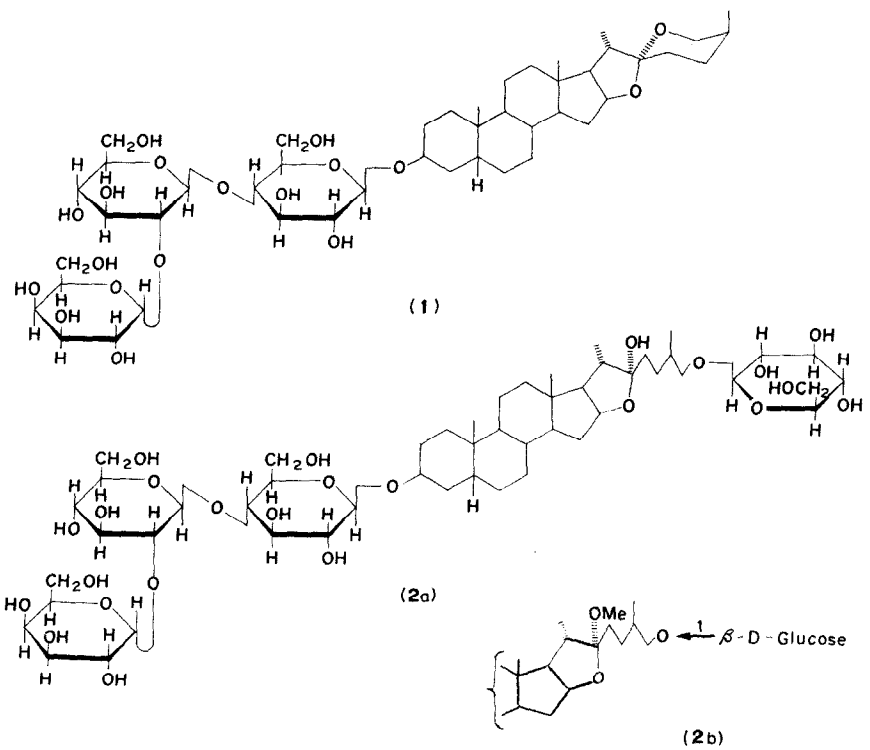
The sequence of the sugars in **1** was proved by partial hydrolysis with HCl which gave a monoglycoside (**6**) and a diglycoside (**7**). Acid hydrolysis of both the progenins gave glucose. After methylation of **7**, followed by hydrolysis, methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside were detected thus showing that compound **4** is directly linked to sarsasapogenin.

Compounds **2**a and **2**b, as well as their mixture (**2**a plus **2**b) gave the same product after methylation. Hence, the mixture of **2**a plus **2**b was used to determine the type of linkages between the monosaccharides in protoyuccoside C. Acid hydrolysis of permethylated protoyuccoside C gave four compounds, two of which were identified by TLC and GLC as methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranoside respectively. The other two partially methylated products were identified by MS and NMR as methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside and methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside. Periodate oxidation of **1** and **2** confirmed the conclusions of the methylation experiments.

Partial hydrolysis of protoyuccoside C (**2**a) led to the formation of **1**, **6**, **7** and sarsasapogenin. Peracetylated $\Delta^{20(22)}$-protoyuccoside C (**8**) was oxidised [9] with CrO_3 in acetic acid at room temp. followed by hydrolysis to form two products (**9**) and (**10**). δ-Hydroxy-γ-methyl-*n*-valeric acid glucoside (**9**), after acetylation followed by methylation with diazomethane, yielded the tetraacetylglucoside methyl ester of δ-hydroxy-γ-methyl-*n*-valeric acid (**11**) which showed the characteristic MS peaks [10, 11] for acetylated glucose, as well as fragment peaks at *m/e* 129 ($C_7H_{13}O_2$) and 97 (129-MeOH) for the acidic residue. Acid hydrolysis of **10** gave 3β-hydroxy-5β-pregn-16-en-20-one which, after acetylation, was identical by IR and UV to 3β-acetoxy-5β-pregn-16-en-20-one. The monosaccharide composition of the glycoside **10** was the same as that of yuccoside C (**1**).

The configurations at C-1 of the glycosidic residues was determined with the help of the NMR spectra of the methylated progenins and the original glycoside [12]. In the NMR spectra of the methylated derivatives of **6** and **7** the one proton doublets at δ 3·97 (J 8·9 Hz) and 4·05 (J 7·9 Hz) indicated the β-configuration of the glycosidic linkages in the monoglycoside (**6**) and diglycoside (**7**) respectively. For **1**, as well as for the glycoside obtained from the enzymic hydrolysis of **2**, an additional one proton doublet was found at δ 4·62 (J 3·5 Hz) proving the α-configuration at C-1 of the galactose residue. This was also proved by Klyne's rules [13].

From the above results it follows that yuccoside C (**1**) is 3-*O*-[α-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl (1 → 4)-β-D-glucopyranosyl]-



(25*S*)-5β-spirostan-3β-ol, whereas protoyuccoside C (**2a**) is 3-*O*-[α-D-galactopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-26-*O*[β-D-glucopyranosyl]-25*S*-5β-furostan-3β,22α-26-triol.

EXPERIMENTAL

Chromatography was on Si gel, Sephadex G-25, neutral alumina and FN-3 paper. The following solvents were employed: system A, $CHCl_3$–MeOH–H_2O (13:5:2, lower phase); system B, *n*-BuOH–EtOH–H_2O (5:1:1); system C, $CHCl_3$–MeOH–H_2O (65:35:7); system D, C_6H_6–Me_2CO (2:1); system E, C_6H_6–Et_2O (7:3); system F, *n*-BuOH–C_6H_6–C_5H_5N–H_2O (5:1:3:3).

On the Si gel TLC the glycosides were detected by Sannie reagent [14] and conc H_2SO_4; sugars were located on PC by anilinephthalate. GLC of the aldonenitryl derivatives of sugars and methylated sugars was on a column (2 m) of 5% XE-60 with helium carrier-gas (45 ml/min).

Separation of Y. filamentosa *saponins.* The saponin mixture (20 g) obtained from a BuOH extract [3] was purified by chromatography first on alumina (system B) and then on Sephadex G-25 eluted with H_2O. The pure saponins were applied to a Si gel column and eluted with system A to give 1 g yuccoside C (**1**), mp 276–278°; $[\alpha]_D^{20}$ − 24° (MeOH, C 1·65); IR (in KBr): characteristic absorption bands for spirostanol saponins [15, 16]; and 5 g protoyuccoside C (**2a**), mp 182–184°; $[\alpha]_D^{20}$ −30° (MeOH; C 2·00), the IR spectrum showed a wide band at 900 cm^{-1} characteristic of furostanol glycosides.

Hydrolysis of **1** *and* **2a** *plus* **2b**. 50 mg of **1** or **2a** plus **2b** were hydrolysed with 2·5% H_2SO_4 at 120° for 24 hr. Sarsasapogenin was obtained from both glycosides and purified by TLC (system E); mp 198–199°; $[\alpha]_D^{20}$ −73° ($CHCl_3$; C 0·90); MS: *m/e* 416 (M^+). The IR spectrum showed 912 > 892 cm^{-1}, which is characteristic of the (25*S*)-configuration [15].

Galactose and glucose were identified in the hydrolysate from both glycosides by PC (system F). GLC of the aldonenitryl derivatives of the sugars [17] showed the proportions of galactose and glucose were 1:2 for **1**, whereas they were 1:3 for **2a** and **2b**.

Methylation and hydrolysis of permethylated products. 0·5 g Yuccoside C (**1**) was methylated by the Kuhn method to yield permethylated yuccoside C, mp 74–76°; $[\alpha]_D^{20}$ −164° ($CHCl_3$; C 0·61); NMR: δ 4·62 (*J* 3·5 Hz); 3·85 (*J* 7·0 Hz). 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 (system D) gave three compounds. These compounds (**3**, **4** and **5**) were separated by chromatography on a column of Si gel. Compound **3** was identified by GLC, with the aid of an authentic sample, as methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranoside. Compound **4** was characterized as methyl 2,3,6-tri-*O*-methyl-β-D-glucopyranoside MS: *m/e* 71, 73, 75, 88, 101, 161 [7]; NMR: δ 3·42 (3H, *s*, C-1 OMe), 3·48 (3H, *s*, C-2 OMe), 3·51 (3*H*, *s*, C-3 OMe), 3·28 (3H, *s*, C-6 OMe), 4·05 (1H, *d*, *J* 7·42 Hz, C-1) [8]. Compound **5** was identified as methyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside, MS: *m/e* 71, 74, 75, 87, 88, 101, 102, 161 [7]; NMR: δ 3·28 (3H, *s*, C-1 OMe), 3·41 (3H, *s*, C-3 OMe), 3·39 (3H, *s*, C-4 OMe), 3·23 (3H, *s*, C-6 OMe), 4·48 (1H, *d*, *J* 3·82 Hz, C-1) [8].

Protoyuccoside C (1·0 g) was methylated to give permethylated protoyuccoside C, mp 70–72°, $[\alpha]_D^{20}$ − 108° ($CHCl_3$; C 1·11). After hydrolysis methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and methyl 2,3,4,6-tetra-*O*-methyl-D-galactopyranoside were identified by GLC and methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside and methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside were characterized by MS and NMR.

Partial hydrolysis of yuccoside C and protoyuccoside C. 0·5 g of **1** or 1 g of **2a** plus **2b** mixture was heated in 20 ml 1·5 N HCl for 2 hr at 100°, then diluted with H_2O and extracted with 3 × 50 ml BuOH. The BuOH extracts were chromatographed on Si gel (system A) to obtain, from **1**, compound **6** mp 245–247°, $[\alpha]_D^{20}$ −66° (MeOH; C 0·70) and compound **7** mp 256–258°, $[\alpha]_D^{20}$ −67° (MeOH; C 0·95), as well as free sugars and sarsasapogenin. From **2a** plus **2b** yuccoside C (**1**) was obtained in addition to **6** and **7**. Hydrolysis of **6** and **7** with 3% H_2SO_4 for 24 hr at 120° gave glucose and sarsasapogenin.

Methylation of **6** *and* **7**. 0·1 g of each product was methylated as indicated above; 70 mg of the permethylated monoglycoside (NMR: δ 3·97, *J* 8·9 Hz) and 70 mg of the diglycoside (NMR: δ 4·05, *J* 7·9 Hz) were obtained. Hydrolysis of the permethylated diglycoside gave methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside.

Periodate oxidation of **1** *and* **2a** *plus* **2b**. 50 g of each product was dissolved in 50 ml aq. MeOH with 150 mg $NaIO_4$ and kept in the dark for 48 hr. Then ethanediol was added dropwise, and after 1 hr it was neutralized with resin, and evaporated at 50°. The residue was hydrolysed with H_2SO_4; no monosaccharides were detected in the hydrolysate by PC (system F).

Enzymic hydrolysis with β-glucosidase of Helix pomatia. 200 mg of **2a** plus **2b** in 20 ml H_2O were incubated with the enzyme for 24 hr at room temp. and the products checked by TLC (system C) After 24 hr the mixture was extracted 3× with 50 ml BuOH and the extract was chromatographed on a column of Si gel (system A) to yield 150 mg of **1**. Acid hydrolysis of the glycoside yielded galactose and glucose in the ratio of 1:2 and sarsasapogenin.

Synthesis of $\Delta^{20(22)}$*-protoyuccoside C* (**8**). 1 g of **2a** plus **2b** was stirred for 20 hr at 20° with 16 ml C_5H_5N and 13 ml Ac_2O to yield 1·4 g protoyuccoside C acetate. This was heated in HOAc for 75 min at 120° and the HOAc was then evaporated *in vacuo* with C_6H_6. The product was chromatographed on Si gel with $CHCl_3$–MeOH (97:3) to yield 1·4 g of **8**.

Oxidation of compound **8** [10]. 1 g of product **8** was dissolved in 11 ml HOAc and 220 mg NaOAc was added. This was followed by 447 mg CrO_3 in 1·65 ml 80% HOAc and 2·2 ml H_2O, added dropwise to the mixture at 15°, during 15 min [9]. The reaction mixture was finally extracted with $CHCl_3$. The oxidized product (1·1 g) was hydrolysed in 35 ml *t*-BuOH with 1·2 g KOH in 1·4 ml H_2O under N_2 with stirring at 30° for 3·5 hr and at room temp. for 30 min. Then 20 ml H_2O were added and the *t*-BuOH evaporated *in vacuo*. The residue was extracted with *n*-BuOH to give a BuOH phase A and an aq. phase B. Phase B was acidified to pH3 and extracted twice with *n*-BuOH and $CHCl_3$. Then phase B was neutralized with 2 N NaOH and evaporated to give **9**. The residue, containing salts, was acetylated with Ac_2O–C_5H_5N. The product was methylated with CH_2N_2 and 40 mg δ-hydroxy-γ-methyl-*n*-valeric acid tetraacetylglucoside methyl ester (**11**) was obtained. The MS of **11** showed the characteristic peaks for completely acetylated glucose at *m/e* 331, 243, 242, 200, 169, 157, 145, 141, 115, 109, 103 and 98, as well as characteristic peaks for the acidic residue at *m/e* 129, 97, 89 and 81. The BuOH phase A was washed several times with H_2O and evaporated *in vacuo*. The residue was purified on a column of silica gel (system A) to give 75 mg of **10**, mp 122–124° $[\alpha]_D^{20}$ + 7° (MeOH; C 2·40).

The 3β-hydroxy-5β-pregn-16-en-20-one 3-glycoside (**10**, 5 mg) was hydrolysed in 3 ml 4N HCl and 3 ml C_6H_6 for 3 hr at 80°. The pregnenolone obtained was acetylated in 4 ml Ac_2O-C_5H_5N (2:3), then purified on Si gel to give 60 mg 3β-acetoxy-5β-pregn-16-en-20-one; IR ($CHCl_3$): ν_{max} 1735, 1660 characteristic of Δ^{16}-20-one [18], 958, 920, 895, 820 cm^{-1}; UV: λ_{max}^{MeOH} nm (log ε) 239 (3·97). Galactose and glucose were detected in the hydrolysate by PC (system F).

REFERENCES

1. Madaeva, O. S. (1958) *Z h. Obshch. Khim.* **28,** 551.
2. Waclaw-Rozkrutova, B. (1969) *Dissert. Pharm. Pharmacol.* **21,** 425.
3. Kintia, P. K., Dragalin, I. P. and Chirva, V. Ja. (1972) *Khim. Prir. Soedin.* **5,** 615.
4. Tschesche, R., Kottler, R. and Wulff, G. (1966) *Ann. Chem.* **699,** 212.
5. Tschesche, R., Lüdke, G. and Wulff, G. (1969) *Chem. Ber.* **102,** 1253.
6. Kuhn, R. and Trischmann, H. (1963) *Chem. Ber.* **96,** 284.
7. Heyns, K., Sperling, K. R. and Grützmacher, H. F. (1969) *Carbohyd. Res.* **9,** 79.
8. Gagnaire, D. and Odier, L. (1969) *Carbohyd. Res.* **11,** 33.
9. Wall, M. E., Kenney, H. E. and Rothman, E. S. (1955) *J. Am. Chem. Soc.* **77,** 5665.
10. Heyns, K. and Scharmen, H. (1963) *Ann. Chem.* **667,** 183.
11. Biemann, K., De Yongh, D. C. and Schnoes, H. K. (1963) *J. Am. Chem. Soc.* **85,** 1763.
12. Parkhurst, R. M., Thomas, D. W. and Skinner, W. A. (1973) *Phytochemistry* **12,** 1437.
13. Klyne, W. (1950) *Biochem. J.* 41. **47.**
14. Sannie, M. Ch. and Lapin, H. (1952) *Bull. Soc. Chim. France* 1082.
15. Tschesche, R., Wulff, G. and Ballé, G. (1962) *Tetrahedron* **18,** 959.
16. Wall, M. E., Eddy, C. R., McClennan, M. L. and Klumpp, M. E. (1952) *Analyt. Chem.* **24,** 1337.
17. Krochmaliuk, V. V., Kintia, P. K. and Chirva, V. Ja. (1975) *Izv. Akad. Nauk Mold SSR Ser. Biol. Khim.* **1,** 85.
18. Jones, R. N., Humphries, P. and Dobriner, K. (1949), *J. Am. Chem. Soc.* **71,** 241.