

## SAPOGENINS OF *YUCCA GLAUCA* SEED PODS

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**Key Word Index**—*Yucca glauca*; Liliaceae; fruiting pods: sapogenins; neo-tigogenin; hecogenin; gitogenin; manogenin;  $\Delta^9$ -manogenin; sarsasapogenin;  $\Delta^2$ -desoxysarsasapogenin.

**Abstract**—From the fruiting pods of *Yucca glauca* Nutt. devoid of seeds the following sapogenins have been isolated and identified: neo-tigogenin, hecogenin, gitogenin, manogenin,  $\Delta^9$ -manogenin and sarsasapogenin. A small amount of a material believed to be  $\Delta^2$ -desoxysarsasapogenin was also isolated and is apparently an artifact arising from the hydrolysis and extraction procedure. Manogenin and  $\Delta^9$ -manogenin have not been previously detected in *Y. glauca*, and  $\Delta^9$ -manogenin has not been reported in any *Yucca* species.

### INTRODUCTION

The seeds of *Yucca glauca* contain sarsasapogenin, markogenin, tigogenin, neo-tigogenin, neo-gitogenin, hecogenin, gloriogenin, and possibly diosgenin [1]. Sarsasapogenin is the predominant sapogenin isolated from the seeds [1]. Undifferentiated tissue cultures derived from germinating *Y. glauca* seeds contain gitogenin, and small amounts of markogenin [2]. The steroidal sapogenin content of leaves of *Y. glauca* has also been determined, and sarsasapogenin, tigogenin, neo-tigogenin, smilagenin, and possibly hecogenin, gitogenin, and neo-gitogenin have been found [3]. The roots and rhizomes of *Y. glauca* contain the same sapogenins and possibly diosgenin [3]. We have extended the studies on the sapogenin content of *Y. glauca* by examining the steroidal sapogenin content of the fruiting pods from which the seeds have been removed.

### RESULTS AND DISCUSSION

The sapogenin and sterol fraction of *Y. glauca* seed pods was isolated by  $\text{CHCl}_3$  extraction of the powdered, defatted and acid hydrolyzed material, and constituted less than 1% of the dry weight. The mixture was fractionated on a silica gel column and all fractions were examined by TLC. Isolated sapogenins were identified by direct comparison with authentic materials.

Neo-tigogenin constituted the major sapogenin of the seed pods, with *ca* 800 mg of this material being isolated. Sarsasapogenin has been previously shown to be the major constituent of *Y. glauca* seeds [1]. Blunden and Hardman have shown that in individual *Y. glauca* plants either sarsasapogenin, neo-tigogenin or tigogenin can be the principal sapogenin in the leaves [2]. The maximum obtainable quantity of sapogenin from the leaves of many *Yucca* species is *ca* 1–2% [4–8], while the sapogenin content of seeds has been reported to be as high as 12% [9]. Both neo-tigogenin and tigogenin have been isolated from *Y. glauca* seeds [1], while no tigogenin was detected in the seed pods.

Hecogenin constituted the second most abundant sapogenin in the fruiting pods and has been previously isolated from *Y. glauca* seeds [1], and tentatively identified in the leaves [3]. Hecogenin has also been isolated from both the leaves and seeds of a number of *Agave* species [4–6].

Approximately 95 mg of gitogenin was isolated. Both gitogenin and neo-gitogenin have been tentatively identified from *Y. glauca* leaves, roots and rhizomes [3] and we have previously isolated neo-gitogenin but not gitogenin from *Y. glauca* seeds [1]. We have also isolated gitogenin from undifferentiated tissue cultures derived from germinating *Y. glauca* seeds, but detected no neo-gitogenin [2]. The reason that only one of the two isomers appears to be present in detectable amounts in tissue cultures [2], seeds [1], and seed pods is not known.

Manogenin and  $\Delta^9$ -manogenin were isolated from the seed pods in small amounts. Previous investigations indicate that these two sapogenins are usually present together [10]. Neither of these two sapogenins was previously isolated from *Y. glauca* leaves, roots, rhizomes [3], or seeds [1]. Manogenin has been previously reported in *Y. filamentosa* and *Y. gloriosa* leaves [4–6], while both manogenin and  $\Delta^9$ -manogenin have been found in many *Agave* species [4–6].

Only about 5 mg of sarsasapogenin was isolated from the seed pods, in contrast to the seeds where it constitutes the major sapogenin [1]. In addition, we isolated minute amounts of a compound believed to be the  $\Delta^2$ -desoxy derivative. Wall *et al* [11] have shown that prolonged refluxing of sarsasapogenin with HCl in EtOH results in the isomerization of sarsasapogenin to smilagenin as well as the formation of compounds believed to be the corresponding  $\Delta^2$ -desoxy derivatives.

Previous studies have indicated that the sapogenins present in leaves, roots, and rhizomes of the same plant are almost identical. However, marked differences exist in the detected sapogenin content of these plant organs in *Yucca* and also in the seeds, seed pods, and tissue cultures derived from seeds. Smilagenin has only been reported to be present in leaves, roots and rhizomes; manogenin and  $\Delta^9$ -manogenin only in fruiting pods; glorigenin only in seeds; and neo-tigogenin, sarsasapogenin and hecogenin in all but tissue cultures. Neo-gitogenin and tigogenin are apparently absent in fruiting pods and tissue cultures; and gitogenin is absent in seeds but is the primary sapogenin in seed-derived tissue cultures. Manogenin was detected only in seeds and seed-derived tissue cultures. The reason for the accumulation of the particular sapogenins in selected tissues is not apparent. No preferred biosynthetic pathway appears to be operative for any of the tissues.

#### EXPERIMENTAL

The fruiting pods of *Yucca glauca* were collected in late August and early September [1], the seeds removed, and the pods powdered. The powder (~ 500 g) was extracted in a Soxhlet apparatus with petrol for 27 hr, followed by  $C_6H_6$  for 24 hr. The powder was removed, allowed to dry, and moistened with a mixture of  $NH_4OH$ -MeOH- $CHCl_3$  (2:2:1). The moistened powder was packed in a Soxhlet apparatus,  $CHCl_3$  was added, and the mixture was allowed to macerate for 4 hr at 20°. The mixture was then Soxhlet extracted for *ca* 40 hr. The  $CHCl_3$

extract was removed, evaporated to dryness, and the residue examined for possible alkaloids. Only minute amounts of several alkaloidal components were detected by TLC and were not examined further. The defatted powder was hydrolyzed by refluxing for 3 hr with 10% HCl using 25 ml of acid per 2 g of powdered pod. After filtration the residue was washed with  $H_2O$  until the pH of the washings was *ca* 5. The residue was then dried at 60°, and extracted in a Soxhlet apparatus for *ca* 72 hr with  $CHCl_3$ . Evaporation of the solvent yielded 4.72 g of a mixture of steroids. A slurry of *ca* 400 g of silica gel in  $CHCl_3$  was packed into a column with an i.d. of 3.5 cm. The sapogenin fraction was loaded on to the column and 20 ml fractions were collected using a flow rate of 1 ml/min. The column was eluted sequentially with the following solvents:  $CHCl_3$ , 150 ml; 1% MeOH in  $CHCl_3$ , 200 ml; 2% MeOH in  $CHCl_3$ , 150 ml; and 1 l. of 3%, 5%, 10% and 50% MeOH in  $CHCl_3$ . The fractions were examined by TLC, using silica gel H plates developed in  $CHCl_3$ -MeOH (97:3) or petrol diisopropyl ether HOAc (30:10:1), and sprayed with anisaldehyde reagent. Identical fractions were pooled.

*Neo-tigogenin*. Fractions 167–194 yielded *ca* 867 mg of material which was further purified on a column of silica-gel by elution with  $C_6H_6$  and  $C_6H_6$  containing increasing proportions of  $Et_2O$ . The material co-chromatographed with neo-tigogenin in the two solvent systems above as well as in diisopropyl ether petrol HOAc MeOH (70:30:1:5). It had a m.p., 194–195° (reported 196–199°); m.m.p. undepressed; MS identical to neo-tigogenin with a  $M^+ = 416$ . The IR spectrum was identical to that of authentic neo-tigogenin, with the  $920\text{ cm}^{-1}$  peak greater than the  $900\text{ cm}^{-1}$  peak, indicating the  $25\beta$  configuration.

*Hecogenin*. Fractions 196–226 contained 320 mg of a material which co-chromatographed with hecogenin. This material was further purified by PLC, and recrystallized from  $Me_2CO$ . It had a m.p. 256–257° (reported 255–257°); m.m.p. undepressed. The MS was identical to that of authentic hecogenin, with a  $M^+ = 430$ . The IR spectrum was identical to that of the authentic material, with an  $898\text{ cm}^{-1}$  band greater than the  $917\text{ cm}^{-1}$  peak, indicating the  $25\alpha$  configuration.

*Gitogenin*. Fractions 296–313 yielded 95 mg of crystalline material which co-chromatographed with gitogenin in three TLC systems. The material was purified by PLC; m.p. 267–269° (reported 271–275°); m.m.p. undepressed;  $M^+ = 432$  with a MS identical to that of gitogenin; IR was identical to that of authentic gitogenin with the  $904\text{ cm}^{-1}$  band greater than the  $920\text{ cm}^{-1}$  band, indicative of the  $25\alpha$  configuration.  $AgNO_3$ -silica gel H TLC and  $3 \times$  developments with  $CH_2Cl_2$ - $Me_2CO$  (93:7), which is capable of resolving these isomers [1], failed to reveal the presence of neo-gitogenin.

*$\Delta^9$ -Manogenin*. Fractions 283–295 yielded *ca* 33 mg of material which co-chromatographed with  $\Delta^9$ -manogenin. This compound was further purified by PLC, and recrystallized from  $Me_2CO$ . It had a m.p. 235–240° (reported 239–241°); m.m.p. undepressed;  $M^+ = 444$ ; IR identical to that of the authentic material, with the  $900\text{ cm}^{-1}$  band greater than the  $920\text{ cm}^{-1}$  peak, indicating the  $25\alpha$  configuration. A reference sample was kindly provided by Dr. M. E. Wall as a mixture with manogenin and purified by TLC.

*Manogenin*. Fractions 330–343 contained 27 mg of a sapogenin which co-chromatographed with manogenin, and was purified by PLC. The product was recrystallized from  $Me_2CO$ ; m.p. 242–248° (reported 245–247°); m.m.p. undepressed;  $M^+ = 446$  with a MS corresponding to that of manogenin; IR identical to that of authentic manogenin. The  $900\text{ cm}^{-1}$  peak was greater than the  $920\text{ cm}^{-1}$  band, confirming the  $25\alpha$  configuration.

**Sarsasapogenin.** From fractions 27–34 of the eluted  $C_6H_6$ – $Et_2O$  eluate and fractions 167–194 of the initial  $CHCl_3$ – $MeOH$  eluate was obtained *ca* 5 mg of a sapogenin which co-chromatographed with sarsasapogenin. It was recrystallized from  $CHCl_3$ – $Me_2CO$ : m.p. 195–199° (reported 198–200°); m.m.p. undepressed;  $M^+$  = 416, IR identical to that of sarsasapogenin, having the  $25\beta$  configuration.

**Sarsasapogenin dehydration product ( $\Delta^2$ -desoxysarsasapogenin).** Fractions 22–26 of the  $C_6H_6$ – $Et_2O$  eluate and fractions 167–194 of the initial  $CHCl_3$ – $MeOH$  eluate contained less than 1 mg of a material which co-chromatographed in three TLC systems with the contaminant commonly found in commercial smilagenin. Smilagenin is prepared by prolonged refluxing of sarsasapogenin with  $HCl$  in  $EtOH$  [11]. The  $\Delta^2$  desoxy derivatives of both sarsasapogenin and smilagenin are believed to be formed under these conditions. The MS fragmentation patterns of both the sapogenin from fractions 22–26 and the smilagenin contaminant had an identical  $M^+$  = 398. Insufficient amounts of the material were available to determine by IR whether the  $25\alpha$  isomer,  $25\beta$  isomer or both were present. No smilagenin was detected in the seed pods, suggesting that we had formed and isolated the  $\Delta^2$  desoxy derivative of sarsasapogenin during the hydrolysis procedure.

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