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SAPOGENINS OF *YUCCA GLAUCA* SEEDS

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Abstract—From the seeds of *Yucca glauca* Nutt., sarsasapogenin, markogenin, tigogenin, neo-tigogenin, neo-gitogenin, hecogenin and gloriogenin have been isolated and identified. Sarsasapogenin was the predominant sapogenin. Markogenin and gloriogenin have not been reported in leaves, roots or rhizomes of this species before. Small amounts of a sapogenin believed to be either diosgenin or yamogenin were also detected.

INTRODUCTION

ALTHOUGH the genus *Yucca* is presently not an economic source of steroidal sapogenins, a number of species in this genus have been investigated, and many different steroidal sapogenins have been isolated.[1–5] Blunden and Hardman have recently investigated the steroidal sapogenin content of the leaves of *Yucca glauca* Nutt., and have found sarsasapogenin, smilagenin, tigogenin, and possibly hecogenin, gitogenin and neo-gitogenin.[6] The rhizomes and roots also contained the same sapogenins and possibly diosgenin.[6] Marker *et al.* first isolated sarsasapogenin from the leaves of this species,[7] and Blunden *et al.* subsequently isolated sarsasapogenin from the leaves of *Y. glauca* as well.[8] *Y. glauca* is one of the best known sources of sarsasapogenin.[9] However, Blunden and Hardman have shown that in individual plants either tigogenin, neo-tigogenin or sarsasapogenin can be the principal sapogenin in the leaves.[6] Studies on the sapogenin content of the leaves of many *Yucca* species indicate that 1–2% sapogenin is the maximal obtainable quantity.[1–5] Wall and Fenske[10] investigated the sapogenin content of the seeds of 12 *Yucca* species, none of which was *Y. glauca*, and found sapogenin contents as high as 12%.

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We have examined the steroidal sapogenin content of *Y. glauca* Nutt. seeds and have found two steroidal sapogenins to be present which have not previously been reported in leaves, roots or rhizomes of this species.

RESULTS AND DISCUSSION

The sapogenin and sterol fractions of *Y. glauca* seeds were isolated by CH_2Cl_2 extraction of the powdered, defatted and acid hydrolyzed seeds, and were fractionated by Silica Gel chromatography. All fractions were examined by TLC, and then identified by direct comparison with authentic materials.

Sarsasapogenin constituted the major sapogenin in *Y. glauca* seeds, with *ca.* 5·2 g of pure material being isolated from 14 g of crude mixture. Wall and Fenske[10] have previously isolated sarsasapogenin as the only identified sapogenin from the seeds of seven other *Yucca* species. The second most abundant sapogenin in the *Y. glauca* seeds was markogenin. We obtained *ca.* 150 mg of markogenin in pure form and over 1·0 g of impure material. Wall *et al.* first isolated markogenin from the leaves of *Y. faxoniana*, *Y. schidigera* and *Y. baccata*, and also from the fruiting pods of *Y. shidigera*.[11] A compound isolated by Marker *et al.*[12] called texogenin is believed to be identical to markogenin. Markogenin has not been previously isolated from *Yucca* seeds.

A total of 83 mg of a mixture of tigogenin and neo-tigogenin was isolated. Tigogenin has been previously isolated from seeds of *Y. whipplei* and *Y. peninsularis*, as well as *Y. glauca* leaves. Neo-tigogenin has not been previously found in *Yucca* seeds. Both tigogenin and neo-tigogenin have been isolated from *Y. glauca* leaves.[6]

From the neo-gitogenin fraction, 133 mg of crystalline compound was obtained. Blunden and Hardman[6] had tentatively identified neo-gitogenin as well as gitogenin from *Y. glauca* leaves, roots and rhizomes. We did not observe any gitogenin in the *Y. glauca* seeds.

Approximately 32 mg of gloriogenin was isolated. Gloriogenin is a sapogenin very recently isolated by Gonzalez *et al.*[13] from the leaves of *Y. gloriosa*, the only plant from which it had been isolated to date. These investigators were able to conclusively demonstrate that the struture of this compound is (25*R*)-5β-spirostan-3β-ol-12-one, which is the 12-keto derivative of smilagenin and also the 25α isomer of willagenin. Willagenin was isolated by Kenney and Wall from *Y. filitera* in low yields.[14]

Approximately 15 mg of hecogenin was isolated in crystalline form. Hecogenin was isolated by Marker and coworkers from the leaves of a number of *Agave* species.[12] It has been isolated from the seeds of several *Agave* species as well.[10] Blunden and Hardman tentatively identified hecogenin in *Y. glauca* leaves.[6]

A very small amount of an eighth sapogenin was detected, but was present in insufficient amounts to be isolated. This compound had an R_f identical to diosgenin and yamogenin, which are C-25 isomers. Blunden and Hardman detected small amounts of a sapogenin in *Y. glauca* roots and rhizomes which their data suggested was diosgenin.[6]

Although no previous studies have been made on the sapogenin content of *Y. glauca* seeds, the seeds of this species has been shown to be capable of hemolizing red blood cells

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of all blood types.[15] Wall and Fenske examined the steroidal sapogenin content of the seeds of 12 *Yucca* species, none of which was identified as *Y. glauca*.[10] They identified either sarsasapogenin or tigogenin as the only sapogenin in all of the species. We have identified five and possibly a sixth sapogenin which they did not detect in the seeds of any of the *Yucca* species which they examined, namely markogenin, neo-gitogenin, neo-tigogenin, gloriogenin, hecogenin and possibly diosgenin.

EXPERIMENTAL

Seed extraction. *Yucca glauca* seeds were collected in late August and early September in the Devil's Nest area of northern Nebraska along the Missouri River. Some plants were grown from these seeds for positive identification purposes. *ca.* 790 g *Yucca* seeds were ground in a Wiley mill, using a No. 10 screen. The ground seeds were Soxhlet extracted with C_6H_6 for 16 hr. The seed powder was dried at 50° for 4 hr, and reground in a Wiley mill using a No. 30 screen. This seed powder was then Soxhlet extracted with $CHCl_3$ for 16 hr, and the powder air-dried for 24 hr. The defatted *Yucca* seed powder was acid hydrolyzed using 25 ml 10% HCl/g of powder, refluxing for 2 hr. The hydrolysis mixture was cooled, filtered, and the filter cake was washed successively with 0·5% NH_4OH and H_2O until the pH of the final wash was *ca.* pH 4–5. The acid hydrolyzed *Yucca* seed powder was dried at 60° for 24 hr, and ground in a Wiley mill using a No. 40 screen. The weight of this powder was *ca.* 244 g. This powder was Soxhlet extracted with CH_2Cl_2 for 24 hr, and the CH_2Cl_2 fraction was flash evaporated, yielding a white crystalline sterol and sapogenin containing material weighing *ca.* 14 g. The sapogenin and sterol mixture was fractionated on a silica-gel 60 column as described below. Various fractions isolated by column chromatography were examined on silica-gel G or H TLC plates developed in: CH_2Cl_2–MeOH (99:1); CH_2Cl_2–MeOH (97:3); hexane–EtOAc–Me_2CO (5:5:1); and diisopropyl ether–light petrol.–HOAc (70:30:1). Column fractions containing identical components were pooled. M.p.s are uncorrected.

Isolation of sarsasapogenin, markogenin and neo-gitogenin. A column (3·5 cm i.d.) of 380 g silica-gel 60 was prepared in $CHCl_3$. A mixture of *Yucca* sapogenins (8 g) was loaded onto the column, collecting 20 ml fractions at a rate of 0·8–1·0 ml/min. The following eluents were used: $CHCl_3$, 1300 ml; 1% MeOH in $CHCl_3$, 1870 ml; 2% MeOH in $CHCl_3$, 1530 ml; 3% MeOH in $CHCl_3$, 1730 ml; 5% MeOH in $CHCl_3$, 1000 ml; 10% MeOH in $CHCl_3$, 1000 ml; and 50% MeOH in $CHCl_3$, 950 ml. Sarsasapogenin was present in fractions 143–156 (fraction A) together with other sapogenins and the *Yucca* sterols, while compound K (markogenin) was found singly in fractions 221–230 (fraction B), and compound M (neo-gitogenin) was present in fractions 246–260 (fraction C). Fractions 230–246 contained a mxiture of K and M. A second column (3·5 cm i.d.) of 300 g silica-gel 60 was prepared as above and used in the chromatography of another 6 g of the mixture of *Yucca* sapogenins and sterols. The development was accomplished with: $CHCl_3$, 1340 ml; 1% MeOH in $CHCl_3$, 640 ml; 3% MeOH in $CHCl_3$, 1000 ml; 5% MeOH in $CHCl_3$, 1000 ml; 10% MeOH in $CHCl_3$, 710 ml; and 50% MeOH in $CHCl_3$, 1000 ml. Sarsasapogenin was obtained in fractions 32–45 (fraction D), together with small amounts of several other sapogenins and sterols. Compound K (markogenin) was obtained in fractions 46–50 (fraction E) while compound M (neo-gitogenin) was obtained in fractions 47–74 (fraction F). Fractions 51–56 contained a mixture of K and M.

Isolation of tigogenin, neo-tigogenin, gloriogenin and hecogenin. The mother liquors from the sarsasapogenin crystallization (from fractions A and D above) were combined, dried and subjected to column chromatography on 130 g of silica-gel 60 packed with benzene in a column, 1·9 cm in dia. Solvents used for elution were: C_6H_6, 250 ml; C_6H_6–Et_2O (17:3), 1970 ml; C_6H_6–Et_2O (3:1), 950 ml; C_6H_6–Et_2O (7:3), 375 ml; and C_6H_6–Et_2O (13:7), 500 ml. An initial fraction of 180 ml, was followed by 20 ml fractions. Sarsasapogenin was isolated in fractions 26–40 (fraction G) together with the *Yucca* sterols. Tigogenin and neo-tigogenin were isolated in fractions 41–65 (fraction H). Gloriogenin was isolated in fractions 95–122 (fraction J) while hecogenin was found in fractions 123–140 (fraction P).

Recrystallization and purification of the various genins. Sarsasapogenin. Fractions A, D and G, were each recrystallized from $CHCl_3$ or $CHCl_3$ containing a few drops of acetone to obtain crystalline needles of sarsasapogenin. Each was recrystallized from the same solvent to obtain pure sarsasapogenin, m.p. 194–196° (lit. 198–200°); acetate, m.p. 141–142° (lit. 144–145°); m.m.p. with standard sarsasapogenin, undepressed. The MS had $M^+ = 416$, and the fragmentation pattern corresponded with that of authentic sarsasapogenin. The IR spectrum shows the characteristic four bands of the spiroketal system at 987, 917, 896 and 870 cm^{-1}. The intensity of the band at 917 cm^{-1} was much more pronounced than that of the band at 896 cm^{-1} indicative of the 25-β configuration as in sarsasapogenin. The 3 fractions co-chromatographed in 3 solvents on TLC with authentic sarsasapogenin. A reference sample was kindly provided by Drs. A. G. Gonzalez and G. Wulff.

Compound K (markogenin). Markogenin was obtained from fractions B and E by recrystallization from $CHCl_3$ containing a small amount of MeOH. The product obtained was recrystallized from the same solvent to obtain

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pure compound K with m.p. 250–252° (lit. 255–257°); no depression of m.p. was observed on m.m.p. with markogenin standard: acetate, m.p. 183·5–185°; MS $M^+ = 432$; IR showed the spiroketal bands at 985, 920, 896 and 867 cm^{-1}. The 25-β configuration was indicated by the band at 920 cm^{-1} being much more intense than that at 896 cm^{-1}. This compound gave a positive reaction with periodate-benzidine spray, indicative of a glycol system. The IR spectrum was superimposible on the spectrum of the authentic material, and the compound co-chromatographed with the standard in 3 solvents. A reference sample was kindly provided by Dr. M. E. Wall.

Compound M (neo-gitogenin). Compound M was isolated from fractions C and F by recrystallization from $CHCl_3$ containing an excess of acetone. The product was recrystallized from the same solvent to obtain pure compound M with m.p. 242–246° (lit. 246–248°); no depression of m.p. with authentic material; acetate, m.p. 226–227°; MS $M^+ = 432$. The IR spectrum showed the characteristic spiroketal bands at 980, 920, 898 and 867 cm^{-1}. The relative intensity of the 920 cm^{-1} band to the 898 cm^{-1} band indicated the 25-β configuration. A glycol system was indicated by a positive periodate-benzidine test. This compound co-chromatographed with neo-gitogenin in the TLC solvent systems given above. The IR spectrum was similar to that of gitogenin except for the inversion of the intensities of the 920 and 898 cm^{-1} peaks. A reference sample of neo-gitogenin was obtained from Dr. G. Blunden, and gitogenin was obtained from Syntex Laboratories.

Tigogenin and neo-tigogenin. Fraction H was taken to dryness and crystallized from MeOH and acetone. The product was recrystallized from the same solvent to obtain the product, m.p. 180–185°; m.m.p. with either tigogenin or neo-tigogenin was undepressed (lit. tigogenin 190–191°, neo-tigogenin 179–184°). The spiroketal IR bands at 980, 920, 900 and 864 cm^{-1} were present. IR spectrum was similar to that of both tigogenin or neo-tigogenin. However, comparison of the relative intensities of the 920 cm^{-1} band to that of the 900 cm^{-1} band indicated that this product was a mixture of both tigogenin and neo-tigogenin. The mixture was separated on preparative silica gel $PF_{254+366}$ plates containing 2·1% silver nitrate. The plates were developed 3 × in CH_2Cl_2–MeOH (97:3). The bands from the plates containing each compound were extracted with CH_2Cl_2–MeOH (3:2), the organic extracts were washed with H_2O, and dried. The solvents were removed *in vacuo*, and IR spectra of the two compounds were prepared. The two spectra were superimposable with the respective spectra of reference tigogenin and neo-tigogenin. Authentic neo-tigogenin was obtained from Dr. G. Blunden, and tigogenin was purchased from K & K Laboratories, Inc.

Gloriogenin. This compound was obtained from fraction J by crystallization from a small vol. $CHCl_3$ with the addition of excess acetone. After recrystallization, the MS $M^+ = 430$; the IR showed a $\nu_{C=O}$ at 1705 cm^{-1}, and the four spiroketal bands at 982, 918, 900 and 865 cm^{-1}. The relative intensities of the 918 and the 900 cm^{-1} bands indicated the 25α configuration. The IR of an authentic sample was identical to the compound that we had isolated. The isolated compound co-chromatographed with authentic gloriogenin in the TLC solvent systems given above, as well as in $CHCl_3$–acetone–AcOH (16:4:1). A 2·0 mg sample was reduced by the Huang-Minlon modification of the Wolff–Kishner reaction.[16] The product of this reaction had an R_f identical to smilagenin on silica-gel H plates developed with $CHCl_3$–MeOH (9:1), and petrol–diisopropyl ether–HOAc (30:10:1), therefore verifying that the compound isolated was 12-ketosmilagenin or gloriogenin. An authentic sample of gloriogenin, m.p. 188–191°, was kindly provided by Dr. A. G. Gonzalez.

Hecogenin. Hecogenin was isolated from fraction P by recrystallization from acetone. It gave a m.p. 239–250° (lit. 255–257°); m.m.p. with standard hecogenin was undepressed; the MS had an $M^+ = 430$. The IR had a $\nu_{C=O}$ at 1708 cm^{-1}, and the four spiroketal bands at 982, 918, 898 and 863 cm^{-1}. The intensity of the 918 cm^{-1} band was lower than that of the 898 cm^{-1} band indicating the 25α configuration. The isolated compound also co-chromatographed with hecogenin in the above solvent systems. Authentic hecogenin was purchased from K & K Laboratories, Inc.

Diosgenin or yamogenin. ca. 0·55 g of the dried mother liquor from the sarsasapogenin isolation from fractions B and D was chromatographed on a 45 g silica-gel H column, eluted with the single solvent petrol–diisopropyl ether–AcOH (30:10:1). A total of 76 20-ml fractions were collected. Fractions 12–17 contained pure sterols. Fractions 29–45 contained primarily sarsasapogenin. Fractions 46–48 contained the unknown sapogenin plus sarsasapogenin. The unknown compound co-chromatographed with diosgenin in the three solvent systems given above as well as gave the same color reaction with 20% H_2SO_4. Yamogenin, the 25β- isomer of diosgenin will also produce the same R_f. Due to a lack of sufficient material, we were unable to provide a complete characterization.

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