crystallized from methanol and from ether, m. p. 184–186°; yield 8.8 g. When mixed with an authentic sample of 4,16-pregnadiendione-3,20 there was no depression in melting point. We failed to find any progesterone in the mother liquors of crystallization.

Anal. Calcd. for $C_{21}H_{28}O_2$: C, 80.7; H, 9.0. Found: C, 80.4; H, 8.8.

Oxidation of Pseudodiosgenin Diacetate by the Method of Crooks and Jones.[2]—A solution of 25 g. of pseudodiosgenin diacetate in 500 cc. of glacial acetic acid was allowed to react at room temperature with a solution of 12.5 g. of chromium trioxide in 125 cc. of 60% acetic acid for a period of ninety minutes. At the end of this time, 3 g. of zinc bromide and 30 g. of zinc dust were added to the solution. The solution was heated on a steam-bath for one hour with stirring. At the end of this time the solid excess zinc was filtered from the solution and the filtrate was evaporated *in vacuo*. The residue was extracted with ether and washed well with water and dilute sodium bicarbonate solution. It was then crystallized from methanol to give a product m. p. 85°; yield 16.2 g. This product gave no depression in melting point with a sample of the known oxidation product of pseudodiosgenin diacetate.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.1; H, 8.8. Found: C, 70.0; H, 9.0.

The above product was refluxed for thirty minutes with an excess of alcoholic potassium carbonate solution. Water was added and the product was extracted with ether. The ether was removed and the residue was refluxed for thirty minutes with 25 cc. of acetic anhydride. Upon cooling the product crystallized. It was recrystallized from methanol to give a product m. p. 176–178° which gave no depression in melting point with an authentic sample of the acetate of 5,16-pregnadien-3(β)-ol-20-one; yield 11.0 g.

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.5; H, 9.1. Found: C, 77.8; H, 9.4.

Summary

Furcogenin has been found to be a mixture of smilagenin and hecogenin. Kappogenin has been found to be a mixture of nologenin and pseudodiosgenin.

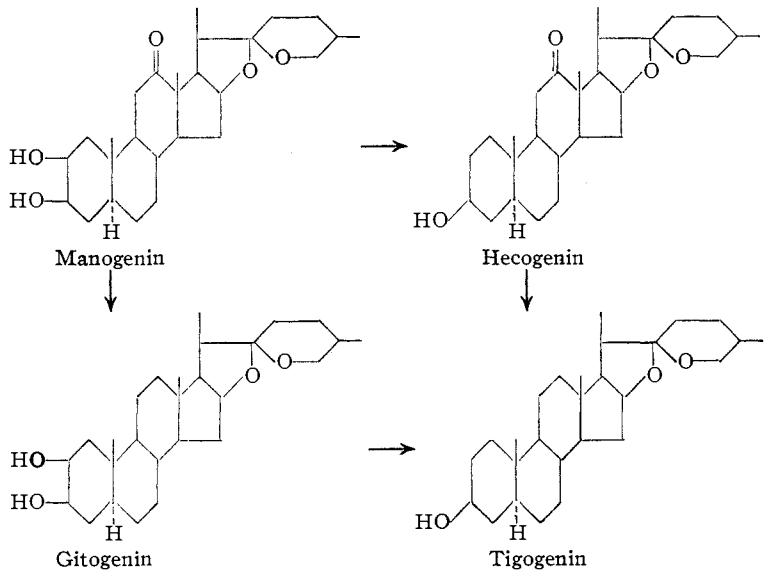
TEXCOCO, MEXICO RECEIVED MARCH 21, 1946

[CONTRIBUTION FROM THE LABORATORY OF BOTANICA-MEX., S. A.]

Steroidal Sapogenins. No. 163. The Biogenesis of Steroidal Sapogenins in Plants

BY RUSSELL E. MARKER AND JOSEFINA LOPEZ

We have previously reported on the biogenesis of the simpler steroidal sapogenins from the more complex ones using as examples the sapogenins derived from *Yucca schottii*, *Samuela carnerosana*, and *Agave striata*.[1] It was shown in the case of *Yucca schottii* and *Samuela carnerosana* that as the flowering and fruiting season approached the complex steroidal sapogenins were converted into the more simple ones which were discarded in the flower stalks and fruit.



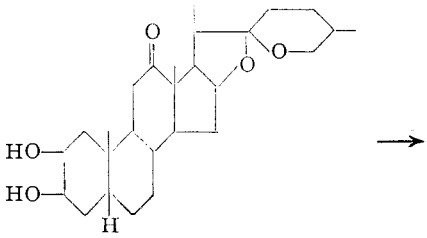
We have now extended this study to the Agave specie of plants and find that these follow the same generalizations as does the biogenesis of the simpler sapogenins from the more complex in the Yucca and Samuela. The Agaves differ from the Yucca and Samuela in that the latter flower and fruit annually, whereas the Agaves flower only after many years of age and then the whole plant dies. For this reason we have studied the sapogenins, first, in very young plants and compared them to the sapogenins present in the plants which had started to flower. For this work the entire plant was used.

It was found that young *Agave parassana* plants gave manogenin only as the steroidal sapogenin, whereas when the plant was at its flowering stage this had disappeared entirely and the sapogenins present consisted of a mixture of hecogenin, gitogenin and tigogenin.

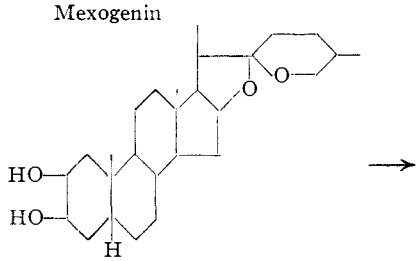
Agave funkiana young plants contained a mixture of mexogenin and samogenin only, whereas when the plant was at the flowering stage these sapogenins had been converted into smilagenin, which was the only product present.

The only sapogenins found in the young plants of *Agave roezliana* were a mixture of neomexogenin and neosamogenin. These two products differ from mexogenin and samogenin in the configuration of their side-chain oxy-

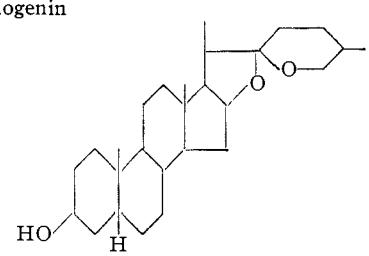
(1) Marker and Lopez, THIS JOURNAL, 69, 2375 (1947).



Mexogenin

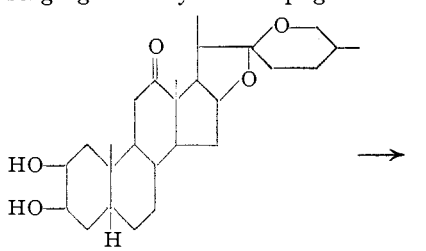


Samogenin

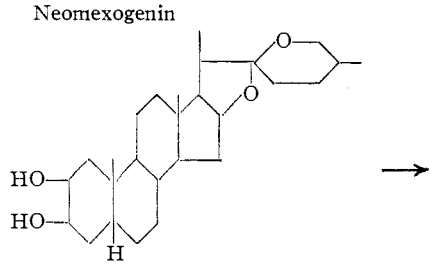


Smilagenin

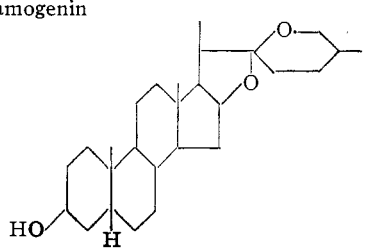
genated atom. An examination of the sapogenins when the plant reached the flowering stage gave only sarsasapogenin.



Neomexogenin



Neosamogenin



Sarsasapogenin

Unlike the *Yucca schottii*, no unsaturated compounds at C-5 were obtained. It might be suggested that the formation of the above products in their biogenesis may also originate in kammogenin and neokammogenin, but that immediately upon their formation in the plant the biogenesis is so rapid that they are converted into their saturated compounds with both the allo and coprostane configurations at C-5. Thus kammogenin on reduction of the double bond first gave in young *Agave parrasana* manogenin, of the alloconfiguration, and in *Agave funkiana* its C-5 isomer, mexogenin, whereas neokammogenin would give neomanogenin in young plants of *Agave striata*[1] and neomexogenin in *Agave roezliana*.

We have picked the above as typical examples of our studies of the biogenesis of sterols in the Agave specie. These follow in complete detail the scheme proposed in our previous publication on the biogenesis.[1]

It should be pointed out that because of this biogenesis, any study of steroidal sapogenins in plants should be made with care at the time of their selection. It was previously reported[2] that *Agave parrasana* contained only manogenin, *Agave funkiana* only smilagenin and *Agave roezliana* only sarsasapogenin. This depends entirely upon the age of the plants collected and their proximity to the flowering season. In numerous other cases[2] when it was previously reported that manogenin was the only steroidal sapogenin present in the plant we have found that the sterols of older plants contained no manogenin, but that this had been converted to gitogenin, hecogenin and tigogenin. In the same manner, in the plants in which it was previously reported that the only sterol present was hecogenin, we have found that if young plants were used there was no hecogenin present and the only sterol isolated was manogenin.[3]

These results then substantiate our previously proposed scheme of the plant biogenesis of the steroidal sapogenins. From this it can be assumed that if a plant is worked up and tigogenin or smilagenin or sarsasapogenin is found, its origin is kammogenin or its reduction products at C-5. Examination of the plant at other ages or seasons or other parts of the plant should give the more complex sterols from which these simpler ones originated unless the biogenesis is so rapid that these complex ones are converted into the simpler as soon as they are formed. This appears to be the case of the formation of the saturated sterols, manogenin, mexogenin, neomanogenin and neomexogenin from kammogenin and neokammogenin in the Agave specie. Therefore, it is obvious that a study of the steroidal sapogenins in plants is meaningless unless their age cycle is taken into consideration.

(2) Marker and co-workers, THIS JOURNAL, **65**, 1206 (1943).
(3) Unpublished results.

Experimental Part

Agave parrasana was collected near Parras, Mexico; *Agave funkiana* near Pachuca, Mexico, and *Agave roezliana* at Tehuacan, Mexico. As the Agave specie take many years to reach the flowering age, young plants were collected at the same time of the year as that of old plants. In each specie a separate collection was made of very young plants, and old plants in which the flower stem had started to grow. In both cases 30 kg. of the entire plant was used. The saponins were extracted with alcohol and hydrolyzed to the sapogenins by refluxing for three hours with 2 *N* alcoholic hydrochloric acid. The crude sapogenins were extracted with ether, washed well with water and alkali and the solvent was removed. These were then dissolved in 95% alcohol and treated with Girard reagent in all cases to separate into ketonic and non-ketonic fractions.

Sapogenins from Agave Parrasana. Young Plants.—No crystalline non-ketonic fraction was obtained from the young plants. The ketonic fraction was crystallized from ether and acetylated by refluxing with acetic anhydride. The solvent was removed and the residue was crystallized from methanol, m. p. and mixed m. p. with manogenin diacetate, 242°; yield 21.6 g.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 70.3; H, 8.5.

Hydrolysis with alcoholic potassium hydroxide gave manogenin which was crystallized from ether, m. p. and mixed m. p. 254°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.9; H, 9.4.

Sapogenins from Agave Parrasana. Old Plants.—The ketonic fraction was acetylated and the product was crystallized from methanol to give hecogenin acetate, m. p. and mixed m. p. 242°; yield 5.6 g. No other products could be obtained from the ketonic fraction.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.5; H, 9.3.

Hydrolysis with alcoholic potassium hydroxide gave a product which was crystallized from ether, m. p. and mixed m. p. with hecogenin, 255–257°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.5; H, 9.8.

The non-ketonic fraction was crystallized from a small amount of ether to remove oily material; wt. 16.7 g. This was crystallized from methanol to give a product m. p. and mixed m. p. with tigogenin 206–208°; yield 7.4 g.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 77.9; H, 10.6.

Acetylation and crystallization from methanol gave tigogenin acetate, m. p. and mixed m. p. 202–204°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.0; H, 10.0.

The methanol mother liquors from the crystallization of tigogenin were evaporated and the residue was crystallized from ether to give gitogenin, m. p. and mixed m. p. 267–268°; yield 4.1 g.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 75.0; H, 10.0.

Acetylation gave the diacetate of gitogenin which was crystallized from methanol, m. p. and mixed m. p. 242°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.5; H, 9.7.

Sapogenins from Agave Funkiana. Young Plants.—The ketonic fraction was crystallized from ether, m. p. and mixed m. p. with mexogenin, 244–246°; yield 3.4 g.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.6; H, 9.7.

Acetylation gave a product which was crystallized from methanol, m. p. and mixed m. p. with the diacetate of mexogenin, 205–207°.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 70.4; H, 8.9.

The non-ketonic fraction was crystallized from ether, m. p. and mixed m. p. with samogenin, 210–212°; yield 9.8 g.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.7; H, 10.1.

Acetylation gave a product which was crystallized from methanol, m. p. and mixed m. p. with the diacetate of samogenin, 198°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.1; H, 9.7.

Sapogenins from Agave Funkiana. Old Plants.—No ketonic fraction was present in the old plants. The sapogenin fraction was crystallized from acetone and from methanol, m. p. and mixed m. p. with smilagenin, 184–186°; yield 14.5 g.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 77.6; H, 10.4.

The acetate was made and crystallized from methanol, m. p. and mixed m. p. with smilagenin acetate, 150–152°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.7; H, 9.9.

No other sapogenins could be isolated from the old plants.

Sapogenins from Agave Roezliana. Young Plants.—The ketonic fraction was acetylated by refluxing for thirty minutes with acetic anhydride. The excess anhydride was distilled *in vacuo* and the residue was crystallized from a small amount of methanol to give the diacetate of neomexogenin, m. p. and mixed m. p. 162–164°; yield 4.8 g.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 70.3; H, 9.0.

The above acetate was hydrolyzed with alcoholic potassium hydroxide and crystallized from dilute methanol and from ether, m. p. and mixed m. p. with neomexogenin, 221–222°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.6; H, 9.3.

The non-ketonic fraction was washed with a little ether to remove the oils and crystallized from acetone to give neosamogenin, m. p. and mixed m. p. 174–176°; yield 9.4 g.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 75.3; H, 10.6.

Acetylation and crystallization gave the diacetate of neosamogenin, m. p. and mixed m. p. 173–175°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.9; H, 9.2.

Sapogenins from Agave Roezliana. Old Plants.—No ketonic fraction was present in the old plants. The non-ketonic fraction was crystallized from ether, m. p. and mixed m. p. with sarsasapogenin, 198–200°; yield 16.5 g.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 78.1; H, 10.7.

Acetylation gave sarsasapogenin acetate which was crystallized from methanol, m. p. and mixed m. p. 130°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.7; H, 10.2.

Summary

The biogenetic relationship of the steroidal sapogenins in the Agave specie is discussed.

TEXCOCO, MEXICO RECEIVED APRIL 20, 1946