Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.7; H, 10.0.

Hydrolysis of this acetate and crystallization from methanol gave smilagenin, m. p. and mixed m. p. 184°.

Sapogenins from the Leaves of Samuela Carnerosana.—The sapogenin fraction from 5 kg. of the leaves of *Samuela carnerosana*, which were collected from plants which had just borne fruit, was treated with Girard reagent to separate into ketonic and non-ketonic sterols. There were no non-ketonic sapogenins present. The total sapogenin fraction was acetylated by refluxing for thirty minutes with acetic anhydride. The excess acetic anhydride was distilled and the residue was crystallized from ether to give the diacetate of kammogenin, m. p. and mixed m. p. 258–260°. Yield was 2.3 g.

Anal. Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.4; H, 8.6.

Hydrolysis of the above acetate with alcoholic potassium hydroxide gave kammogenin, m. p. and mixed m. p. 242°.

The ether soluble fraction from the mother liquors of crystallization of the diacetate of kammogenin were hydrolyzed with alcoholic potassium hydroxide and the product was crystallized from ether. The ether insoluble fraction was acetylated by boiling with acetic anhydride for thirty minutes. The acetate was crystallized from methanol to give 5.8 g. of mexogenin diacetate, m. p. and mixed m. p. 208°.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 70.1; H, 8.7.

Hydrolysis of the above acetate with potassium hydroxide gave mexogenin, which was crystallized from ether, m. p. and mixed m. p. 247°. No other sapogenins could be isolated from the leaves.

Sapogenins from Caudex and Roots of Samuela Carnerosana after Fruiting.—The sapogenins from 20 kg. of dried caudex and roots of *Samuela carnerosana* after fruiting were separated into a ketonic and a non-ketonic fraction by means of Girard reagent.

The ketonic fraction was acetylated and the product was crystallized from pentane to give 23 g. of the diacetate of mexogenin, m. p. and mixed m. p. 208°.

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

Hydrolysis of the above acetate and crystallization from ether gave mexogenin, m. p. and mixed m. p. 247°. No other ketonic sapogenins could be isolated from the caudex and roots.

The non-ketonic fraction from the caudex and roots was crystallized from ether to give samogenin, m. p. and mixed m. p. 206°; yield 18.3 g. Acetylation of this product and crystallization from methanol gave samogenin diacetate, m. p. and mixed m. p. 200°.

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

Sapogenins from Dioscorea Mexicana Leaves.—The crude sapogenins were isolated from 10 kg. of freshly dried leaves of *Dioscorea mexicana*. These were crystallized once from a small amount of ether to remove oily material. The total weight, 36 g., was refluxed for thirty minutes with 80 cc. of acetic anhydride. Upon cooling to room temperature the crystalline material was filtered. This consisted chiefly of neodiosgenin acetate. The mother liquors were evaporated and the residue was hydrolyzed with strong alcoholic potassium hydroxide. The sapogenin fraction was extracted with a large volume of ether, washed well and the solvent was removed to about 150 cc. Upon standing a week in a refrigerator the crystalline material was filtered, wt. 7.8 g. This was recrystallized several times from ether and from methanol to give a product, 2.1 g., m. p. and mixed m. p. with neokammogenin, 230°. Acetylation with acetic anhydride and crystallization from methanol gave the diacetate of neokammogenin, m. p. and mixed m. p. 203–205°.

Anal. Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.6; H, 8.6.

When the neokammogenin was refluxed for seventy hours with an alcoholic solution of hydrochloric acid it gave a product which upon acetylation and crystallization from methanol was identical with kammogenin diacetate, m. p. 258–260°.

The mother liquors from the isolation of neokammogenin were treated with Girard reagent to remove ketones. From these a small additional quantity of neokammogenin was obtained. The non-ketonic fraction was crystallized from ether to give 1.7 g. of neoyuccagenin, m. p. and mixed m. p. 247°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.0; H, 9.7.

Acetylation and crystallization from methanol gave the diacetate of neoyuccagenin, m. p. and mixed m. p. 157–159°.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.0; H, 9.0.

Summary

A study has been made of the seasonal variation of the steroidal content of *Agave striata, Yucca schottii, Samuela carnerosana* and *Dioscorea mexicana*.

TEXCOCO, MEXICO RECEIVED APRIL 6, 1946

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

Steroidal Sapogenins. No. 162. Kappogenin and Furcogenin

BY RUSSELL E. MARKER AND JOSEFINA LOPEZ

A preliminary announcement appeared in This Journal[1] on the isolation and tentative structures of a number of new steroidal sapogenins which were obtained from various plant sources. At the time it was submitted insufficient work was done on two of the products, "kappogenin" and "furcogenin," for complete characterization and structure proof.

A more careful study on "furcogenin" has shown it to be a mixture of smilagenin and hecogenin. Oxidation of "furcogenin" gave a diketone which is identical with hecogenone.

"Kappogenin" has been separated into nologenin and pseudodiosgenin. The latter is formed in small amounts upon refluxing diosgenin with acetic anhydride for thirty minutes. Its presence in the crude sapogenin mixture is accounted for by the fact that these were separated by means of their acetates. Its structure previously[1] was assumed because oxidation of the acetate gave 5,16-pregnadien-3-ol-20-one and treatment with

(1) Marker and co-workers, THIS JOURNAL, **65**, 1206 (1943).

hydrochloric acid gave diosgenin. Both these reactions are caused by the pseudodiosgenin present in the mixture.

Crooks and Jones[2] reported that the oxidation of "kappogenin" as its dibromide followed by a unique reduction of the ester group with zinc and acetic acid gave progesterone. These workers evidently failed to observe that "kappogenin" was a mixture of pseudodiosgenin and nologenin. For that reason the work of these investigators was repeated using identical conditions and it was found that the only non-acidic product resulting was Δ^4-diosgenone-3 which comes from the pseudodiosgenin in the "kappogenin." This is accounted for by the fact that only a small amount of acid is necessary to cause a rearrangement of pseudodiosgenin to diosgenin and oxidation of the latter as the dibromide gives Δ^4-diosgenone. In this experiment the double bond was protected by bromine which gave the acid necessary for the rearrangement of the side-chain to diosgenin. As proof of this a sample of pure pseudodiosgenin was brominated using identical conditions, and the resulting product was debrominated with zinc in acetic acid. This gave diosgenin as the only crystallizable product.

In the same patent, Crooks and Jones give other experiments to support their new and unique method of reduction of an ester group to a methylene group by heating with zinc dust and acetic acid for an hour on a steam-bath. These were repeated because of their close relationship to their supposedly oxidation of "kappogenin" to progesterone. These workers oxidized pseudodiosgenin diacetate with chromic anhydride and heated the resulting oxidation product with zinc bromide, zinc dust and acetic acid for one hour on a steam-bath. They claim to have obtained pure Δ^5-pregnen-3(β)-ol-20-one acetate. When this experiment was repeated using identical quantities and process it was found that no reduction of the ester group took place. Better than 50% yield of the known oxidation product of pseudodiosgenin diacetate[3] was obtained. This upon hydrolysis to the β-hydroxy ketone, dehydrated to give approximately 50% yield of the doubly unsaturated compound, 5,16-pregnadien-3(β)-ol-20-one acetate. Likewise, these workers claim that the oxidation of pseudodiosgenone followed by treatment with zinc dust and acetic acid gave pure progesterone. This experiment was repeated using identical quantities and conditions. As was to be expected, there was no progesterone formed and 4,16-pregnadienedione-3,20 was the only product resulting from this oxidation.

We are unable to explain the reason in the discrepancy of our results with those reported by Crooks and Jones and their failure to observe that "kappogenin" is a mixture of pseudodiosgenin and nologenin.

(2) Crooks and Jones, U. S. Patent 2,383,472, assigned to Parke, Davis and Company, Detroit, Mich.

(3) Marker and co-workers, THIS JOURNAL, **63**, 744 (1941).

Experimental Part

"Furcogenin."—The furcogenin used in these experiments was isolated from *Yucca flaccida* and melted at 222–225°. This was a portion of the sample originally reported on and brought to Mexico for further study. To a solution of 1 g. of furcogenin in 100 cc. of acetic acid was added a solution of 1 g. of chromic anhydride in 2 cc. of water and 20 cc. of acetic acid. After standing at room temperature for thirty minutes water was added and the product was extracted with ether. Upon evaporation of the ether to a small volume needles were obtained which were recrystallized from ether to a constant m. p. of 237–240°. When mixed with chlorogenone, m. p. 237°, there was a depression of melting point to 225–230°; when mixed with hecogenone prepared by the oxidation of hecogenin, m. p. 237–240°, there was no depression in melting point.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.4; H, 9.2.

To a solution of 2 g. of furcogenin in 100 cc. of ethanol was added an excess of Girard reagent. The mixture was heated on a steam-bath for forty-five minutes, cooled and extracted with ether and water. The aqueous layer was warmed on a steam-bath for fifteen minutes with hydrochloric acid and extracted again with ether. The ether was evaporated to a small volume and the product was cooled in ice–salt mixture. The crystals which appeared were filtered and recrystallized from ether, m. p. 250–253°; when mixed with hecogenin, m. p. 250–253°, there was no depression in melting point.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.1; H, 10.0.

The above product was converted to the acetate by refluxing with acetic anhydride for twenty minutes. The anhydride was removed and the residue was crystallized from ethyl acetate, m. p. and mixed m. p. with hecogenin acetate, 242°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 74.0; H, 9.4.

The ether extract of the non-ketonic fraction from treatment with Girard reagent was evaporated and the residue was crystallized from ethanol, m. p. and mixed m. p. with smilagenin, 182–184°.

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

The above product was refluxed for twenty minutes with acetic anhydride. The excess acetic anhydride was removed and the residue was crystallized from methanol, m. p. and mixed m. p. with smilagenin acetate 149–151°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.7; H, 10.0.

"Kappogenin."—The kappogenin used in these experiments was isolated from the mother liquors of the preparation of diosgenin acetate from 20 kg. of crude diosgenin containing sapogenins. After filtering the diosgenin acetate from the acetic anhydride, the filtrate was concentrated to a small volume, cooled and the other insoluble sterol acetates were removed by filtration. The acetic anhydride was removed from the filtrate by distillation *in vacuo* and the residue was hydrolyzed by refluxing with an alcoholic potassium hydroxide solution. The product was extracted with ether and upon concentration fine needles appeared. This was further crystallized from acetone to give a product, m. p. 230–232°. Yield was 27 g. A mixed melting point with the original product[1] previously reported gave no depression.

"Kappogenin" acetate was prepared and crystallized from aqueous methanol to a constant melting point of 178–180°. Mixed with the original gave no depression in melting point.

Anal. Found: C, 71.8; H, 9.1.

Separation of Kappogenin Diacetate into Nologenin Diacetate and Pseudodiosgenin Diacetate.—A solution of 10 g. of "kappogenin" acetate in 100 cc. of absolute ethanol was allowed to stand at room temperature for one

week. The needles which separated were recrystallized from ethanol, m. p. 180°. Yield was 2.8 g. A mixed melting point with an authentic sample of nologenin diacetate, m. p. 180°, gave no depression.

Anal. Calcd. for $C_{31}H_{48}O_7$: C, 69.9; H, 9.1. Found: C, 70.1; H, 9.1.

A solution of 2 g. of the above acetate was hydrolyzed by refluxing with an alcoholic solution of potassium hydroxide. The product was precipitated with water, filtered and crystallized from ether from which it is very insoluble, m. p. 266–268°. A mixture with an authentic sample of nologenin, m. p. 268°, gave no depression.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.3; H, 9.9. Found: C, 72.1; H, 10.0.

As a further identity of the above product a solution of 1 g. of nologenin in 100 cc. of ethanol containing 5 cc. of concd. hydrochloric acid was heated on the steam-bath for ninety minutes. The product was extracted with ether and crystallized from acetone, m. p. 184–186°. Mixed with kryptogenin it gave no depression in m. p.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.1; H, 9.9.

The diacetate was prepared by refluxing with acetic anhydride. It was crystallized from acetone, m. p. 154°. Mixed with an authentic sample of kryptogenin diacetate gave no depression in m. p.

The mother liquors from the first separation of nologenin diacetate were evaporated to dryness and the residue weighing 5 g. was dissolved in 5 cc. of acetic anhydride and allowed to stand for a week, in a refrigerator. The needles which separated were filtered and recrystallized from methanol, m. p. 101°. Yield was 2.1 g. When mixed with an authentic sample of pseudodiosgenin diacetate there was no depression in melting point.

Anal. Calcd. for $C_{31}H_{46}O_5$: C, 74.6; H, 9.3. Found: C, 74.3; H, 9.2.

Hydrolysis of the above acetate with alcoholic potassium hydroxide, followed by crystallization from acetone and from methanol, gave a product, m. p. 193°. When mixed with an authentic sample of pseudodiosgenin there was no depression in melting point.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.4; H, 10.0.

A solution of 500 mg. of pseudodiosgenin isolated above, in 100 cc. of 95% ethanol and 25 cc. of concentrated hydrochloric acid, was warmed on a steam-bath for thirty minutes. Water was added to the reaction mixture and the product was extracted with ether. The solvent was removed and the residue was refluxed with 2 cc. of acetic anhydride for thirty minutes. Upon cooling the crystals were filtered, washed with methanol and crystallized from acetone, m. p. 202–204°; yield 0.3 g. A mixture with diosgenin acetate melted at 204°.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.4; H, 9.6.

To a solution of 0.5 g. of pseudodiosgenin diacetate, isolated above from kappogenin diacetate, was added a solution of 0.25 g. of chromic anhydride in 80% acetic acid. After standing for ninety minutes at room temperature water was added and the product was extracted with ether, washed free of acids and the ether was removed on a steam-bath. The residue was hydrolyzed with alcoholic potassium carbonate for thirty minutes at a reflux temperature. The hydrolysis mixture was extracted with ether, washed well with water and the solvent was removed on a steam-bath. The residue was boiled for fifteen minutes with 2 cc. of acetic anhydride. Upon cooling the product crystallized and was filtered. It was recrystallized from ethyl acetate, m. p. 176–178°. When mixed with an authentic sample of the acetate of 5,16-pregnadien-3(β)-ol-20-one there was no depression in melting point.

Anal. Calcd. for $C_{23}H_{32}O_3$: C, 77.5; H, 9.1. Found: C, 77.1; H, 9.2.

Treatment of Diosgenin Acetate with Acetic Anhydride.—A solution of 2 kg. of diosgenin acetate, m. p. 204°, in 5 liters of acetic anhydride was heated at reflux temperature for one hour. The product was cooled in a refrigerator overnight and the diosgenin acetate was filtered and washed with acetic anhydride. The mother liquors were evaporated to about 500 cc. and again cooled overnight in a refrigerator, filtered and washed with acetic anhydride. Again the mother liquors were concentrated to about 100 cc., cooled and filtered. The filtrate was evaporated to dryness and the residue was hydrolyzed with alcoholic potassium hydroxide solution. Water was added and the precipitate was filtered and crystallized from acetone and from methanol, m. p. 193°; yield 10.1 g. When mixed with an authentic sample of pseudodiosgenin there was no depression.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.6; H, 10.0.

Acetylation of the above product with acetic anhydride and crystallization of the resulting product from methanol gave material of m. p. 101° which gave no depression when mixed with pseudodiosgenin diacetate.

Oxidation of "Kappogenin" by the Method of Crooks and Jones.[2]—Ten grams of kappogenin was dissolved in 400 cc. of glacial acetic acid and cooled to 15° with stirring. To this was added slowly 23.3 cc. of a 1 molar solution of bromine in acetic acid. To this solution was added a solution of 10 g. of chromic anhydride in 100 cc. of 60% acetic acid, while the temperature was maintained below 40°. After ninety minutes of oxidation, 10 g. of zinc dust was added to the solution and the mixture was heated with stirring on a steam-bath for one hour. The excess zinc was filtered from the solution and the clear filtrate was evaporated *in vacuo*. The residue was dissolved in ether and water and washed well with water and sodium carbonate solution. Evaporation of the resulting neutral ether solution gave an oily material, wt. 2.8 g., which was crystallized from methanol to give a product melting at 186–188°; yield 1.2 g. This gave no depression in melting point when mixed with an authentic sample of diosgenone-3 (188°) prepared by the aluminum *t*-butylate oxidation of diosgenin. The mother liquors from this yielded no progesterone.

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.6; H, 9.8. Found: C, 78.6; H, 10.0.

Bromination and Debromination of Pseudodiosgenin.—Ten grams of pseudodiosgenin was brominated as described above. To the brominated solution was added 10 g. of zinc dust with stirring and the mixture was heated on a steam-bath for one hour. The excess zinc was filtered from the solution and the filtrate was concentrated *in vacuo* to about 100 cc. Water was added and the precipitated product was filtered. This was dried and refluxed with 25 cc. of acetic anhydride for thirty minutes. Upon cooling to room temperature the product crystallized and was filtered off. It was crystallized from acetone and from methanol, m. p. 202–204°; yield 3.0 g. A mixture with diosgenin acetate (204°) gave no depression in melting point.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.0; H, 9.5.

Oxidation of Pseudodiosgenone by the Method of Crooks and Jones.[2]—The pseudodiosgenone was prepared by heating diosgenone with acetic anhydride at 200° for ten hours. Twenty grams of pseudodiosgenone was dissolved in 400 cc. of glacial acetic acid and a solution of 17.5 g. of chromium trioxide in 175 cc. of 60% acetic acid was added while maintaining the temperature below 40°. After the oxidation had continued for ninety minutes, a solution of 2 g. of zinc chloride in 20 cc. of 50% acetic acid was added. This was followed by the addition of 15 g. of powdered zinc. The mixture was heated on a steam-bath for one hour. At the end of this time the excess zinc was filtered from the solution and the filtrate was evaporated to dryness. The residue was dissolved in 1 liter of ether and washed well with water and sodium carbonate solution. The ether was evaporated but the residue failed to crystallize. The product was refluxed with alcoholic potassium carbonate for thirty minutes, extracted with ether and the solvent was removed. The residue was

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.

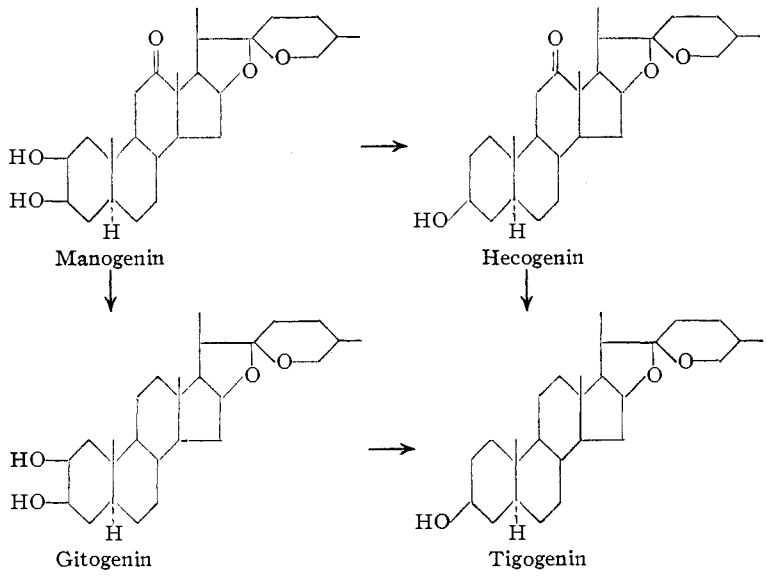
TEXCOCO, MEXICO RECEIVED MARCH 21, 1946

[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).