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

Steroidal Sapogenins. No. 165. Structure of the Sapogenin Glycosides

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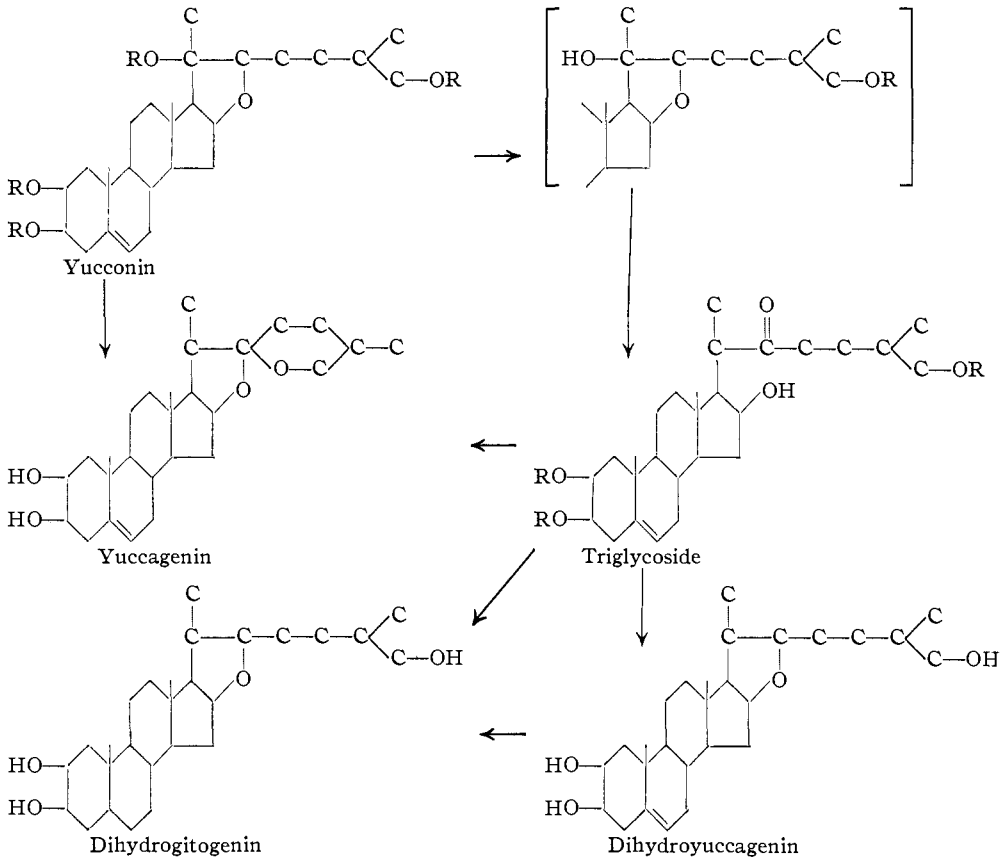
It has been shown that acid hydrolysis of the steroidal sapogenin glycosides in numerous cases causes a change in their structure[1] to produce aglucones other than those occurring as the original glycosides in nature. In order to study the biogenetic transformation of the more complex glycosides in the plant into the simpler ones, it is important to have a knowledge of the structure of the glycoside prior to acid treatment and possible rearrangement on the hydrolysis of the sugar groups with acid. Up to the present time our knowledge of the structure of these glycosides is very limited.



We have now isolated several of these glycosides and from this limited work in conjunction with that previously reported by other workers on the glycosides of sarsasapogenin, gitogenin, tigogenin and chlorogenin, we are able to obtain a view of the structure of these compounds. The sugars may differ in the glycosides of the same sapogenin when they occur in different plants. In this investigation we have not studied the nature of the sugar itself, but were merely interested in the structure of the aglucone as it is combined with the sugar in nature.

We have previously reported the isolation of kammogenin from *Yucca schottii* leaves; yuccagenin from the roots; smilagenin from the flowers and sarsasapogenin from the fruit.[2] The first three compounds have the iso-sapogenin side chain, whereas the last one has the normal or neosapogenin side chain. We have now isolated the saponins of these products.

Yucconin contains four sugar groups. Catalytic reduction gives gitonin, which upon acid hydrolysis gives gitogenin. Yucconin was unaffected by reduction using sodium and alcohol. When yucconin was treated under mild hydrolytic conditions a triglycoside was obtained, which upon further hydrolysis gave yuccagenin. The triglycoside of yuccagenin upon mild catalytic hydrogenation (using the same conditions whereby yucconin yielded gitonin) absorbed 2 moles of hydrogen. Acid hydrolysis of this product gave

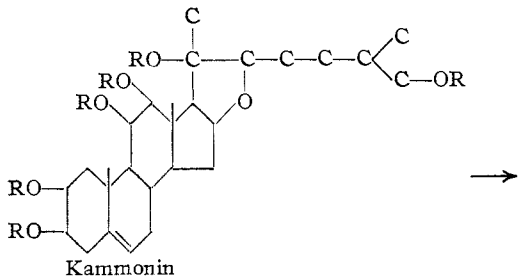
(1) Marker and Lopez, THIS JOURNAL, 69, 2386 (1947).

(2) Marker and Lopez, *ibid.*, 69, 2375 (1947).

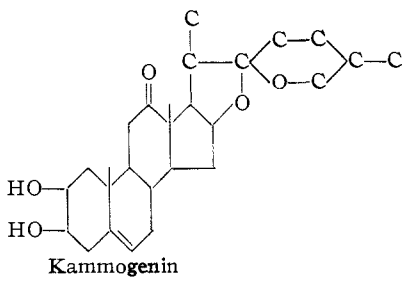
dihydrogitogenin. Whereas yucconin was unaffected by reduction using sodium and alcohol, the triglycoside was readily reduced. This product followed by acid hydrolysis gave dihydroyuccagenin, which upon catalytic reduction gave dihydrogitogenin. This indicates that a ketone group was formed in the mild acid hydrolysis of yucconin to form the triglycoside of yuccagenin, and the above formulation of the reaction is proposed. It also shows that the saponin does not have the sapogenin side chain, but that it exists in the open chain with glycoside groups attached to the hydroxyls. Further hydrolysis of the glycoside on the terminal chain carbon atom with ring closure would give in acid solution the isosapogenin side chain such as is found in tigogenin and smilagenin.

Smilonin was isolated from the flowers of *Yucca schottii*. The same product was also isolated from the old plants of *Agave funkiana* and *Agave lophantha*. Although this product contains but one hydroxyl group in the nucleus, there are five glycoside groups present in the molecule. This product resists both catalytic hydrogenation and reduction by sodium in alcohol. Hydrolysis gives smilagenin which has the isosapogenin side chain.

Kammonin contains six sugar groups and upon acid hydrolysis yields kammogenin which contains a ketone group in the 12 position.[3] The ketone group at C-12 is readily reduced by catalytic hydrogenation or by reduction with sodium in alcohol. However, when such reductions were tried on the parent saponide it was unaffected. Catalytic reduction of kammonin gave mannonin which upon acid hydrolysis gave manogenin with a 12 ketone group. The ketone group on kammogenin can be removed by the Wolff–Kishner method, but when kammonin was treated by the same method it was unaffected. This indicates that compounds which we have isolated containing a ketone group at C-12 do not exist as such in the form of their glycosides. On the basis of the above we suggest the above saponin structure for kammonin.



Kammonin



Kammogenin

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

Sarsasaponin contains but three sugar groups. Hydrolysis with acid gives sarsasapogenin which differs from smilagenin in the configuration of its side chain. It is unaffected by reduction with sodium in alcohol. However, catalytic reduction at elevated temperatures followed by acid hydrolysis of the sugar groups produced dihydrosarsasapogenin. This indicates that the saponide exists with its side chain already formed and all three of the sugar groups must therefore be attached to the hydroxyl at C-3.

The above observations indicate that the steroidal sapogenins which are isolated with the iso-side chain such as tigogenin, gitogenin, yuccagenin, chlorogenin, kammogenin, etc., occur as saponides with an open side chain. Hydrolysis of the glycosides brings about ring closure with the iso-configuration. Substantiating this is the fact that all the sapogenins with the sarsasapogenin side chain can be converted into those of the iso-configuration by long boiling with alcoholic hydrochloric acid. It also indicates that the sapogenins which are isolated with the sarsasapogenin side chain, such as neo-tigogenin, neo-diosgenin, etc., have had this side chain formed in nature by enzymatic hydrolysis of the side chain glycoside groups. This accounts for the fact that the *Yucca schottii* flowers gives smilonin which has 5 glycoside groups, yet when the fruit is formed a few weeks later the saponide has been changed to sarsasaponin, with the loss of two glycoside groups in the side chain followed by its closing to the normal sapogenin side chain ring structure.

TABLE I

Aglucone	Saponin
Sarsasapogenin from *Smilax ornata*	3 sugars
Sarsasapogenin from *Yucca schottii*	3
Tigogenin from *Digitalis*	5
Smilagenin from *Yucca schottii, Agave lophantha* and *Agave funkiana*	5
Chlorogenin from *Chlorogalum pomeridianum*	6
Gitogenin from *Digitalis*	4
Yuccagenin from *Yucca schottii*	4
Kammogenin from *Yucca schottii*	6

The above table includes the present work combined with that previously published. It is then indicated that sarsasaponin contains three sugar groups all of which are attached to the C-3 atom and with the sapogenin side chain already established in nature. Tigonin and smilonin each have 5 sugar groups, three of which are probably attached to C-3 to conform with sarsasapogenin which has a mono-hydroxy nucleus, and the other two are in the side chain as described for yucconin. The saponin of chlorogenin contains 6 sugar groups. Three of these can be placed on the C-3 atom, one on the C-6 and the remaining two in the

sapogenin side chain. It is interesting to note that both yucconin and gitonin, although their aglucones contain one more hydroxyl group than either smilagenin or tigogenin, have but four sugar groups in the molecule. This suggests that the compounds having a hydroxyl group at both C-2 and C-3 are present as the saponides with monoglycosides on each of these carbon atoms with two glycosides in the side chain. Kammonin has six sugar groups, probably one each on C-2 and C-3 to conform with yucconin and gitonin, and one each on C-11 and C-12 (which on acid hydrolysis gives a ketone at C-12 instead of the C-11,12 diol) and two glycoside groups in the side chain.

Experimental Part

Sarsasaponin from Yucca Schottii Fruit.—Five kilos of the dried fruit was pulverized and thoroughly extracted with alcohol. The solvent was removed by distillation and the residue was shaken several times with ether to remove fats. The residual ether was removed by heating on a steam-bath. The residue was dissolved in 1 liter of alcohol and 500 cc. of this was distilled. To the residue remaining was added 100 cc. of water and the product was allowed to stand in an open vessel in a cold room for two months. The crystalline mass was stirred with a little cold alcohol and filtered. It was crystallized to a constant melting point from 80% alcohol, m. p. 241–245° dec.

Anal. Calcd. for $C_{27}H_{44}O_3(C_6H_{10}O_5)_3$: C, 59.9; H, 8.2. Found: C, 59.8; H, 8.0.

Dihydrosarsasapogenin from Sarsasaponin.—A mixture of 2 g. of sarsasaponin and 100 cc. of acetic anhydride was refluxed for fifteen minutes. The solvent was removed *in vacuo* and the residue was dissolved in 500 cc. of acetic acid. To this was added 500 mg. of platinum oxide catalyst and the product was shaken with hydrogen at 45 pounds pressure and 70° for six hours. The solution was filtered and the solvent was removed *in vacuo*. To the residue was added 300 cc. of alcohol and 60 cc. of concentrated hydrochloric acid. It was refluxed for two hours, water was added and the product was extracted with ether. It was crystallized from ether and from dilute acetone, m. p. and mixed m. p. with dihydrosarsasapogenin, 163–164°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 77.4; H, 11.1. Found: C, 77.4; H, 11.0.

Sarsasapogenin from Sarsasaponin.—To a solution of 18 g. (0.02 mole) of sarsasaponin in 500 cc. of alcohol was added 100 cc. of concd. hydrochloric acid and the product was refluxed for two hours. At the end of that time the solvent was distilled *in vacuo* to about 100 cc. One liter of water was added and the precipitated aglucone was filtered, washed well with water and dried. Yield was 8.0 g. of sapogenin from 18.0 g. of sarsasaponin. Theoretical yield on the basis of three 6-carbon sugar groups in the molecule is 8.3 g. The product was crystallized from ether, m. p. and mixed m. p. with sarsasapogenin 198–200°. Acetylation gave sarsasapogenin acetate which was crystallized from methanol, m. p. and mixed m. p. 130°.

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.9; H, 10.1. Found: C, 75.7; H, 10.2.

Smilonin from Yucca Schottii Flowers.—An alcoholic extract of 3 kg. of *Yucca schottii* flowers was distilled until no more alcohol came off. The residue was washed thoroughly with ether and allowed to stand in a refrigerator with 80% alcohol until crystallization occurred. The product was filtered and recrystallized from alcohol, m. p. 235–237° dec. The same product was obtained from *Agave funkiana* old plants and *Agave lophantha* old plants.

Anal. Calcd. for $C_{27}H_{46}O_4(C_6H_{10}O_5)_5$: C, 54.9; H, 7.8. Found: C, 54.9; H, 7.7.

Smilonin could not be reduced catalytically or by sodium in alcohol.

Smilagenin from Smilonin.—Smilonin was hydrolyzed in the same manner as described for sarsasaponin. Yield was 7.9 g. of sapogenin from 25.3 g. of smilonin. Theoretical yield on the basis of five 6-carbon sugar groups in the molecule is 8.3 g. The product was crystallized from methanol, m. p. and mixed m. p. with smilagenin 184–186°. The acetate was 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, 75.6; H, 10.0.

Kammonin from Yucca Schottii Leaves.—Kammonin was isolated from the leaves of *Yucca schottii* in the same manner as described for the isolation of smilonin. The product was crystallized from alcohol, m. p. 310–315° dec.

Anal. Calcd. for $C_{27}H_{44}O_7(C_6H_{10}O_5)_6$: C, 52.1; H, 7.3. Found: C, 52.2; H, 7.3.

Kammogenin from Kammonin.—Kammonin was hydrolyzed in the same manner as described for sarsasaponin. Yield was 8.6 g. of sapogenin from 29 g. of kammonin. Theoretical yield on the basis of six 6-carbon sugar groups in the molecule is 9.0 g. The product was crystallized from ether, m. p. and mixed m. p. with kammogenin, 242°. The acetate was prepared and crystallized from ether, m. p. and mixed m. p. with the diacetate of kammogenin 258–260°.

Anal. Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.2; H, 8.5.

Manogenin from Kammonin.—A mixture of 3 g. of kammonin, 500 cc. of alcohol and 1 g. of platinum oxide catalyst was shaken with hydrogen at 45 pounds pressure for three hours. The catalyst was filtered and the filtrate was distilled to about 100 cc. To this was added 20 cc. of concentrated hydrochloric acid and the product was refluxed for two hours. Water was added and the precipitate was filtered and crystallized from ether, m. p. and mixed m. p. with manogenin, 254°.

Acetylation gave the diacetate of manogenin, m. p. and mixed m. p. 242°.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 70.4; H, 8.8.

12-Dihydrokammogenin and 12-Dihydromanogenin.—To a boiling solution of 3 g. of kammogenin in 500 cc. of absolute ethanol was added 30 g. of sodium during one hour. Water was added and the product was extracted with ether. It was crystallized from ether and from acetone, m. p. 218°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.4; H, 9.5.

Reduction of manogenin with sodium and alcohol gave a product m. p. 240–242°. This is identical with the product obtained when 12-dihydrokammogenin was reduced catalytically with platinum oxide catalyst. The same product was obtained when either kammogenin or manogenin was reduced catalytically with hydrogen and platinum oxide catalyst in alcohol.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.3; H, 9.9. Found: C, 72.5; H, 10.0.

Acetylation gave a triacetate which was crystallized from methanol. This is identical with the triacetate of agavagenin, m. p. and mixed m. p. 225–228°.

Anal. Calcd. for $C_{33}H_{50}O_8$: C, 69.0; H, 8.8. Found: C, 68.7; H, 8.8.

Attempted Reduction of Kammonin.—When kammonin was reduced catalytically under the same conditions in which kammogenin was converted into 12-dihydromanogenin, it gave reduction only at the double bond producing manogenin after acid hydrolysis.

When kammonin was treated with sodium in alcohol under the conditions in which kammogenin is converted

into 12-dihydrokammogenin there was no change in the product. Acid hydrolysis gave kammogenin.

When kammonin was treated with sodium, hydrazine hydrate and alcohol at 200 degrees followed by acid hydrolysis only kammogenin was obtained. Under similar conditions kammogenin is converted almost quantitatively into yuccagenin with the loss of its ketone group.

These experiments indicate the absence of a ketone group in kammonin and that the ketone group is formed upon the acid hydrolysis of the glycosides.

Yucconin from Yucca Schottii.—Yucconin was isolated from the roots of *Yucca schottii* in the same manner as described for smilonin from its flowers. The product was crystallized from alcohol, m. p. 275–278° dec.

Anal. Calcd. for $C_{27}H_{44}O_5(C_6H_{10}O_5)_4$: C, 55.8; H, 7.8. Found: C, 56.2; H, 7.6.

Yuccagenin from Yucconin.—Yucconin was hydrolyzed in the same manner as described for sarsasaponin. Yield was 8.3 g. of sapogenin from 22 g. of yucconin. Theoretical yield on the basis of 4 6-carbon sugar groups in the molecule is 8.6 g. The product was crystallized from ether, m. p. and mixed m. p. with yuccagenin 248–250°. This was converted into its acetate which was crystallized from acetic anhydride and from methanol, m. p. and mixed m. p. with the diacetate of yuccagenin, 178–180°.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.4; H, 8.9.

Gitonin from Yucconin.—A mixture of 5 g. of yucconin, 500 cc. of alcohol and 1 g. of platinum oxide catalyst was shaken with hydrogen at 45 pounds pressure for two hours. The solution was filtered and the product was crystallized from alcohol, m. p. 275° dec. When mixed with yucconin it melted at 244–249° dec.

Anal. Calcd. for $C_{27}H_{46}O_5(C_6H_{10}O_5)_4$: C, 55.7; H, 7.9. Found: C, 55.9; H, 7.9.

Gitogenin from Gitonin.—The gitonin prepared above by the reduction of yucconin was hydrolyzed in the same manner as described for sarsasaponin. Yield was 8.4 g. from 22 g. of gitonin. Theoretical yield based on 4 6-carbon sugar groups in the molecule is 8.6 g. The product was crystallized from ether, m. p. and mixed m. p. with gitogenin, 268°. It was converted into its diacetate and this was crystallized from acetic anhydride and from methanol, m. p. and mixed m. p. with the diacetate of gitogenin, 242–244°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.1; H, 9.3.

Partial Hydrolysis of Yucconin.—To a solution of 50 g. of yucconin in 2 l. of boiling alcohol was added 100 cc. of concentrated hydrochloric acid and the product was refluxed for twenty minutes after boiling started. It was cooled somewhat in ice and the acid was exactly neutralized to litmus with strong sodium hydroxide solution. The solution was filtered from the precipitated sodium chloride and concentrated to dryness in vacuum. The residue was pulverized and washed well with ether. It was then boiled with absolute alcohol and filtered. The filtrate was concentrated to about 150 cc. and 25 cc. of water was added. Upon standing for one week in an open flask in a refrigerator large plates crystallized from the solution. This was crystallized several times from a small amount of 90% alcohol, m. p. 268–270° dec. When mixed with yucconin it melted at 238–245° dec. Yield was 10.2 g. Analysis indicates the triglycoside of yuccogenin.

Anal. Calcd. for $C_{27}H_{44}O_5(C_6H_{10}O_5)_3$: C, 57.8; H, 8.0. Found: C, 58.0; H, 8.1.

Hydrolysis of the above product with alcoholic hydrochloric acid gave yuccagenin, m. p. and mixed m. p. 248–250°. Acetylation and crystallization from methanol gave the diacetate of yuccagenin, m. p. and mixed m p. 178–180°.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.0; H, 8.9.

Mild Catalytic Reduction of the Triglycoside of Yuccagenin.—A solution of 4 g. of the triglycoside of yuccagenin in 500 cc. of alcohol was shaken with 1 g. of platinum oxide catalyst and hydrogen at 45 pounds pressure for one hour. The solution was filtered and to the filtrate was added 100 cc. of concentrated hydrochloric acid and the mixture was refluxed for two hours. Water was added and the product was extracted with ether. The solvent was removed and the residue was crystallized from acetone and from ethyl acetate. This product was identical with dihydrogitogenin which was prepared by catalytic reduction of gitogenin diacetate in hot acetic acid for eight hours, m. p. and mixed m. p. 195–197°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.7; H, 10.8.

The above product was acetylated by refluxing with acetic anhydride and gave a triacetate which was crystallized from methanol, m. p. and mixed m. p. with the triacetate of dihydrogitogenin, 117–119°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.7; H, 9.4. Found: C, 70.6; H, 9.4.

Mild catalytic reduction of yuccagenin under identical conditions as above gave only gitogenin. Dihydrogitogenin could be obtained only by catalytic reduction at elevated temperatures of yuccagenin in acetic acid for a long period of shaking.

Reduction of the Triglycoside of Yuccagenin with Sodium in Absolute Alcohol.—To 5 g. of the triglycoside of yuccagenin in 300 cc. of hot absolute alcohol was added 20 g. of sodium in small portions. When the sodium had dissolved the reaction mixture was neutralized with concentrated hydrochloric acid and the sodium chloride was filtered and washed well with hot alcohol. This gave approximately 500 cc. of solution. To this was added 100 cc. of concentrated hydrochloric acid and the product was refluxed for two hours on a steam-bath. Water was added and the material was extracted with ether, the solvent removed and the residue was crystallized from ethyl acetate, m. p. 202–204°. This is exo-dihydroyuccagenin.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.4. Found: C, 75.0; H, 10.4.

The product obtained above was dissolved in alcohol and shaken with platinum oxide and hydrogen at 45 pounds pressure for one hour. The solution was filtered and the product was crystallized from methanol, m. p. and mixed m. p. with dihydrogitogenin, 195–197°. Acetylation gave the triacetate of dihydrogitogenin, m. p. and mixed m. p. 117–119°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.7; H, 9.4. Found: C, 70.9; H, 9.4.

The glycoside of yuccagenin (yucconin) was unchanged when treated with sodium in alcohol. In a like manner kammonin, sarsasaponin and smilonin was unaffected with sodium in alcohol. Catalytic reduction at elevated temperatures of kammonin, smilonin and yucconin did not affect the side chain on subsequent hydrolysis of the glycoside.

Summary

A study of the possible structure of the sapogenin glycosides has been made.

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