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

Steroidal Sapogenins. No. 164. Nologenin and its Degradation Products

BY RUSSELL E. MARKER AND JOSEFINA LOPEZ

Kryptogenin has been isolated in varying amounts from diosgenin or neodiosgenin containing plants upon the acid hydrolysis of the glycoside mixture.[1] Accompanying this in relatively small amounts was nologenin, pennogenin and fesogenin. During the course of our studies it has been our observation[2] that these plants when freshly collected and processed yield more kryptogenin than the aged ones with a corresponding increase of the diosgenin or neodiosgenin content on aging. As an example, freshly collected Beth root gave approximately equal quantities of kryptogenin and diosgenin, but root which had been stored for many years before processing gave no kryptogenin but double the amount of diosgenin. This then showed a conversion of the saponide from which the kryptogenin was derived into the glycoside of diosgenin.

We have now isolated the saponide from which kryptogenin, nologenin, pennogenin, fesogenin and bethogenin are derived and have studied its various acid hydrolysis products. As this is the glycoside of nologenin we have named this product nolonin. This occurs together with the glycoside of neodiosgenin in *Dioscorea mexicana*, and its presence accounts for all of the above products which are obtained on the acid hydrolysis of saponide mixtures containing this product.

When nolonin was hydrolyzed with alcoholic hydrochloric acid under the standard conditions for hydrolysis of the steroidal glycosides, only kryptogenin was isolated from the hydrolysis mixture. However, when it was hydrolyzed by long heating with water at high temperatures only nologenin was obtained.

Mild acid treatment of nologenin gives pennogenin which has only one acetylatable hydroxyl group. Stronger acid treatment of pennogenin causes a dehydration of the 17-hydroxyl group to form a ketone group at C-16 with the side chain ring opening to give kryptogenin.

Bethogenin is obtained by refluxing either nolonin, nologenin, pennogenin or kryptogenin with methanolic hydrochloric acid.

Fesogenin is the result of the action of alkali on kryptogenin. It was originally isolated from the mother liquor sterols from Beth root[1] only after they had been treated with alkali. Kryptogenin behaves in the same manner with alkalies as does sarsasapogenoic acid,[3] which has the same 16,22-diketone system. As a further check on these transformations we have prepared the 5,6-dihydro derivatives of the above compounds and find that they pursue the same course on acid treatment.

Thus we have demonstrated that pennogenin, kryptogenin, fesogenin, and bethogenin are not present in the plant as naturally occurring glycosides as previously reported,[1] but are the result of acid or alkali treatment of their parent compound, nolonin, and its hydrolysis products.

Experimental Part

Nolonin.—The alcoholic extract from 25 kg. of freshly collected dried and finely powdered *Dioscorea mexicana* was distilled to a volume of 8 liters. To the hot solution was added a boiling solution of 300 g. of cholesterol in 4 liters of ethanol. The mixture was well stirred for fifteen minutes and then 4 liters of water was added. After standing for one week at room temperature the precipitate was centrifuged and washed with water and alcohol. The saponides were dried and pulverized. The dried saponides were dissolved in 4 liters of pyridine and heated for three hours on a steam-bath. The solution was filtered and to the clear filtrate was added 4 liters of ether and the saponides were filtered and washed thoroughly with ether. When dried their weight was 1.1 kg. These were dissolved in 10 liters of alcohol, filtered and the filtrate was concentrated to about 2 liters. To this was added 500 cc. of water and the product was allowed to stand in a refrigerator for one week. The solution was filtered, and the product was crystallized twice more from a small amount of 95% alcohol, wt. 196 g., m. p. 280–285° dec.

To a solution of 50 g. of nolonin in 1 liter of boiling ethyl alcohol was added 200 cc. of concentrated hydrochloric acid and the product was refluxed for three hours. At the end of that time water was added and the product was extracted with ether. The ethereal solution was washed well with water and dilute sodium hydroxide solution and the solvent was removed. The residue, 10.8 g., was refluxed with 15 cc. of acetic anhydride for twenty minutes. Upon cooling in a refrigerator overnight the product crystallized and was separated by filtration. One crystallization from methanol gave 7.8 g., m. p. and mixed m. p. with kryptogenin diacetate, 152–154°.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.5; H, 9.0.

Hydrolysis of the above product with alcoholic potassium carbonate gave a product m. p. and mixed m. p. with kryptogenin 184–185°.

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

A mixture of 50 g. of nolonin, 1 liter of water and a few drops of sodium hydroxide solution was heated under pressure at 250° for ten hours. The bomb was cooled and the product was extracted with a large amount of ether. The ethereal solution was filtered and concentrated to a small volume. The solution was filtered and the product was crystallized from ether and from acetone, m. p. and mixed m. p. with nologenin, 265–267°, wt. 6.3 g.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.3; H, 9.9. Found: C, 72.4; H, 9.9.

The above product was acetylated by refluxing for twenty minutes with acetic anhydride. The solvent was removed and the residue was crystallized from methanol as needles, m. p. and mixed m. p. with nologenin diacetate, 179–180°.

Anal. Calcd. for $C_{31}H_{48}O_7$: C, 69.9; H, 9.1. Found: C, 70.0; H, 9.0.

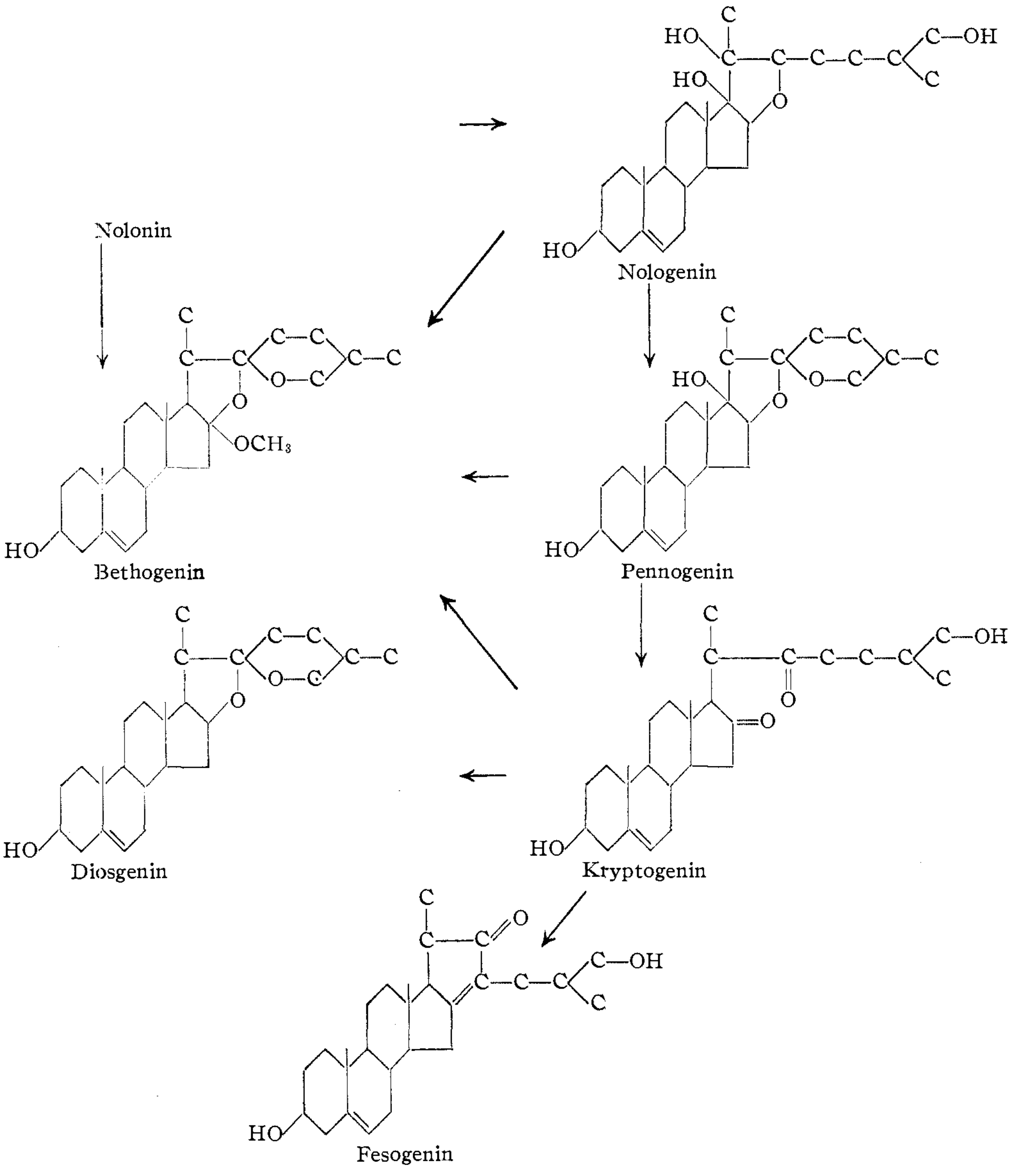
Kryptogenin from Nologenin.—A solution of 2 g. of nologenin in 200 cc. of ethanol containing 20 cc. of con-

---

[1] Marker and co-workers, THIS JOURNAL, 65, 1199 (1943).

[2] Unpublished results.

[3] Marker and Shabica, THIS JOURNAL, 64, 147 (1942).



centrated hydrochloric acid was refluxed for three hours. The product was extracted with ether and crystallized from acetone to give kryptogenin, m. p. and mixed m. p. 184–186°; yield 0.9 g.

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

Acetylation gave kryptogenin diacetate, m. p. and mixed m. p. 153°.

Pennogenin from Nologenin.—A solution of 2 g. of nologenin in 200 cc. of ethanol containing 10 cc. of concentrated hydrochloric acid was refluxed for one hour on a steam-bath. The product was extracted with ether and the solvent was removed. The residue was refluxed for twenty minutes with 5 cc. of acetic anhydride. It was cooled and filtered, then crystallized from methanol and from acetone to give pennogenin acetate, m. p. and mixed m. p. 199°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.9; H, 9.4.

The acetate was hydrolyzed with alcoholic potassium hydroxide and the product was crystallized from ether, to give pennogenin, m. p. and mixed m. p. 240°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.5; H, 10.0.

Kryptogenin from Pennogenin.—A solution of 1 g. of pennogenin in 100 cc. of ethanol containing 10 cc. of concentrated hydrochloric acid was refluxed on a steam-bath for two hours. The product was extracted with ether and crystallized from acetone to give kryptogenin, m. p. and mixed m. p. 184–186°.

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

Acetylation gave kryptogenin diacetate, m. p. and mixed m. p. 153°.

Fesogenin from Kryptogenin.—A solution of 2 g. of kryptogenin in 100 cc. of methanol containing 10 g. of potassium hydroxide and 25 cc. of water was refluxed on a steam-bath for five hours. The product was extracted with ether and crystallized from acetone, m. p. and mixed m. p. with fesogenin 180°; yield 1.2 g. Mixture with kryptogenin gave a depression to 155–160°.

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.6; H, 9.8. Found: C, 78.7; H, 9.6.

Bethogenin from Nolonin.—To a solution of 50 g. of nolonin in 1 liter of boiling methanol was added 200 cc. of concentrated hydrochloric acid and the product was refluxed for three hours. At the end of that time water was added and the product was filtered and crystallized from methanol, m. p. and mixed m. p. with bethogenin 191–192°; yield 6.8 g. The same product was obtained when nologenin, pennogenin, or kryptogenin was refluxed with methanolic hydrochloric acid for three hours. When methanol was used instead of ethanol, bethogenin instead of kryptogenin was formed as described above.

Anal. Calcd. for $C_{28}H_{44}O_4$: C, 75.6; H, 10.0. Found: C, 75.6; H, 10.3.

The above product was acetylated with pyridine and acetic anhydride and crystallized from methanol, m. p. and mixed m. p. with bethogenin acetate, 230°.

Anal. Calcd. for $C_{30}H_{46}O_5$: C, 74.0; H, 9.5. Found: C, 74.2; H, 9.5.

Dihydronolonin.—A mixture of 5 g. of nolonin, 500 cc. of ethanol, 1 g. platinum oxide catalyst and a few drops of acetic acid was shaken with hydrogen at 3 atm. for two hours. The solution was filtered and the product crystallized from alcohol, m. p. 287–290° dec. When mixed with nolonin there was a depression to 262–270° dec.

5,6-Dihydronologenin.—A mixture of 1 g. of nologenin acetate, 500 mg. of platinum oxide catalyst, 400 cc. of ether and a few drops of acetic acid were shaken with hydrogen at 3 atm. for one hour. The solution was filtered and the solvent evaporated. The residue was crystallized from methanol to give dihydronologenin diacetate, m. p. 175–177°.

Anal. Calcd. for $C_{31}H_{50}O_7$: C, 69.6; H, 9.5. Found: C, 70.0; H, 9.5.

Hydrolysis with alcoholic potassium hydroxide gave dihydronologenin which was crystallized from methanol, m. p. 255–257°.

Anal. Calcd. for $C_{27}H_{46}O_5$: C, 71.7; H, 10.3. Found: C, 71.6; H, 10.3.

5,6-Dihydropennogenin.—A solution of 2 g. of 5,6-dihydronologenin in 200 cc. of ethanol containing 10 cc. of concentrated hydrochloric acid was refluxed for one hour on a steam-bath. The product was extracted with ether and the solvent was removed. The residue was refluxed for twenty minutes with 5 cc. of acetic anhydride. It was cooled and filtered, then crystallized from methanol and from acetone to give the acetate of 5,6-dihydropennogenin m. p. 178–189°. This gave no depression when mixed with the acetate of 5,6-dihydropennogenin prepared by the catalytic reduction of pennogenin.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.4; H, 9.8. Found: C, 73.4; H, 9.7.

Hydrolysis with alcoholic potassium hydroxide gave 5,6-dihydropennogenin which was crystallized from methanol, m. p. 225°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.8; H, 10.2.

5,6-Dihydrokryptogenin from 5,6-Dihydronologenin and 5,6-Dihydronolonin.—A solution of 1 g. of 5,6-dihydronologenin in 100 cc. of ethanol containing 10 cc. of concentrated hydrochloric acid was refluxed on a steam-bath for two hours. The product was extracted with ether and crystallized upon concentration. It was recrystallized from ether, m. p. 170–172°. The same product was obtained when 5,6-dihydropennogenin was treated in the above manner. This is identical with the product obtained on the catalytic hydrogenation of kryptogenin.

A solution of 3 g. of 5,6-dihydronolonin in 100 cc. of alcohol containing 20 cc. of hydrochloric acid was refluxed for three hours. Water was added and the product was filtered and crystallized from ether, m. p. 170–172°. This gave no depression in melting point when mixed with the above product.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 75.2; H, 10.1.

The diacetate was prepared by refluxing with acetic anhydride. It was crystallized from methanol, m. p. 124°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.4; H, 9.3.

5,6-Dihydrofesogenin from 5,6-Dihydrokryptogenin.—A solution of 2 g. of 5,6-dihydrokryptogenin diacetate in 100 cc. of methanol containing 10 g. of potassium hydroxide and 25 cc. of water was refluxed on a steam bath for five hours. The product was extracted with ether and crystallized from ether, m. p. 216–217°; yield 1.1 g.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.0; H, 10.3.

Acetylation with acetic anhydride and pyridine gave a product which was crystallized on long standing from dilute methanol, m. p. 78–80°.

Anal. Calcd. for $C_{31}H_{46}O_5$: C, 74.6; H, 9.7. Found: C, 74.7; H, 9.8.

5,6-Dihydrobethogenin.—A solution of 2 g. of 5,6-dihydronologenin in 100 cc. of methanol containing 20 cc. of concentrated hydrochloric acid was refluxed for three hours. At the end of that time water was added and the product was filtered. It was recrystallized from methanol, m. p. 210–212°. This is identical with the product formed when 5,6-dihydropennogenin and 5,6-dihydrokryptogenin was treated in the above manner with methanolic hydrochloric acid.

The identical product was obtained when 2 g. of 5,6-dihydronolonin was refluxed for three hours with 100 cc. of methanol containing 20 cc. of concentrated hydrochloric acid.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.3; H, 10.4. Found: C, 75.3; H, 10.2.

The acetate of 5,6-dihydrobethogenin was prepared with acetic anhydride and pyridine. It was crystallized from methanol, m. p. 195–197°.

Anal. Calcd. for $C_{30}H_{48}O_5$: C, 73.7; H, 9.9. Found: C, 73.4; H, 10.0.

Summary

Nolonin has been isolated from *Dioscorea mexicana*. Mild hydrolysis gives nologenin. Strong acid hydrolysis gives kryptogenin. Methanolic hydrochloric acid on nolonin, nologenin, pennogenin and kryptogenin gives bethogenin. Mild acid treatment of nologenin gives pennogenin and the latter on further treatment with acid gives kryptogenin. Alkali on kryptogenin gives fesogenin.

Pennogenin, kryptogenin, fesogenin and bethogenin are not naturally occurring glycosides but are formed in their isolation from plants by the action of acids or alkalies on nolonin or its hydrolysis products.

TEXCOCO, MEXICO

RECEIVED MAY 3, 1946