[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. CLVII. Sapogenins. LXIX.[1] Isolation and Structures of Thirteen New Steroidal Sapogenins. New Sources for Known Sapogenins

BY RUSSELL E. MARKER, R. B. WAGNER, PAUL R. ULSHAFER, EMERSON L. WITTBECKER, DALE P. J. GOLDSMITH AND CLARENCE H. RUOF

Due to the pressure of war work, the publication of our studies on the sapogenins of over three hundred plants from Mexico and United States will necessarily be delayed. In the present paper we are reporting some of our results in preliminary form.

Earlier work on the biogenesis of the steroidal hormones and related compounds has focused our attention on this problem in both animals[2] and plants. In the present paper we report the isolation and structures of thirteen new steroidal sapogenins, thus more than doubling the number of these substances known. We have also found new sources for many of the previously known sapogenins.

The extensive collections by the senior author were made possible only because of the kind and painstaking coöperation of the Botany Departments, Experimental Stations and Range Ecology Departments of the following Institutions: Texas A. and M. College, University of Arizona, University of California, New Mexico State College, North Carolina State College, Coker College, University of Mexico, Sol Russ College, University of Florida, Waco College, Purdy Botanical Gardens (Ukiah, Calif.), Laredo Cactus Gardens (Laredo, Texas), Missouri Botanical Gardens, and Huntington Botanical Gardens (San Marino, Calif.). We thank Dr. Lester Mallory and Mr. Douglas Crawford, United States Embassy, Mexico City, Ing. Eduardo Morillo Safa, oficial Mayor Departamento de Agricultura y Fomento, Mexico City, and The Ministry of Public Health of Mexico for making the Mexican collections possible. We thank especially the following botanists (listed alphabetically) for aiding in the collection and identification of the plants: Dr. G. Benevides, Dr. Lyman Benson, Dr. A. B. Conner, Dr. and Mrs. V. L. Cory, Dr. Ladd Cuitak, Dr. C. J. Epling, Dr. A. B. Hershey, Dr. Wm. Hertrick, Dr. H. Lewis, Dr. H. B. Parks, Dr. L. N. Pultz, Dr. B. E. Smith, Dr. O. T. Sperry, and Dr. J. J. Thornber. We express our appreciation to Dean Frank C. Whitmore and Dr. Oliver Kamm for their encouragement in this project. We thank Dr. H. M. Crooks and Dr. G. H. Fleming. We thank Parke, Davis and Company for their assistance.

(1) For previous papers in this series see Sterols. CLVI. Sapogenins. LXVIII, THIS JOURNAL, **65**, 1248 (1943).

(2) Marker, *ibid.*, **60**, 1725 (1938).

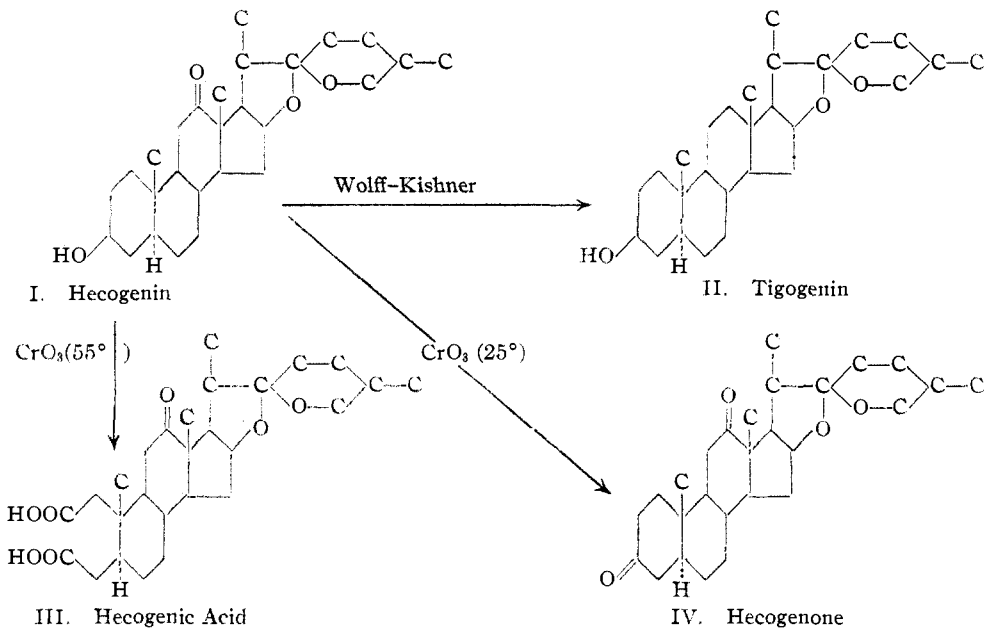
New Steroidal Sapogenins

1. **Hecogenin.**—In the course of our plant studies, we first isolated from *Hechtia texensis* (S. Wats.) a new steroidal sapogenin having the composition $C_{27}H_{42}O_4$, m. p. 245°, 253° and 268°, which we have named hecogenin (I). *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.5; H, 9.9. When refluxed with acetic anhydride, it forms a monoacetate having two forms, m. p. 243 and 252°. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.9; H, 9.3. Although hecogenin (I) forms a monosemicarbazone, it is unaffected by the conditions of a mild Clemmensen reaction, indicating the inert position of the carbonyl group. Wolff–Kishner reduction removes the carbonyl group giving tigogenin (II), m. p. and mixed m.p., 206°. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.5; H, 10.4. Vigorous oxidation with chromic anhydride in acetic acid gives hecogenic acid (III), m. p. 268° dec. *Anal.* Calcd. for $C_{27}H_{40}O_7$: C, 68.0; 8.4. Found: C, 68.3; H, 8.3. The latter is not identical with chlorogenic acid (IX), digitogenic acid or digitoic acid. Furthermore, the dimethyl ester of III, m. p. 187°, does not correspond to any of the dimethyl esters of the above acids. Mild oxidation of hecogenin (I) with chromic anhydride gives hecogenone (IV), m. p. 240°. *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.6; H, 9.5. Found: C, 75.3; H, 9.2. The latter is not identical with either chlorogenone or 7-ketotigogenone prepared from diosgenin. We propose structure I for hecogenin.

It is noteworthy that no previously known steroidal sapogenin having the spiro-ketal side-chain and established structure has a carbonyl group.

We have processed on an average of one hundred pounds of each species in the accompanying lists in the manner described before.[3] The re-

(3) Marker, Wagner and Ulshafer, *ibid.*, **64**, 1283 (1942).



I. Hecogenin II. Tigogenin

III. Hecogenic Acid IV. Hecogenone

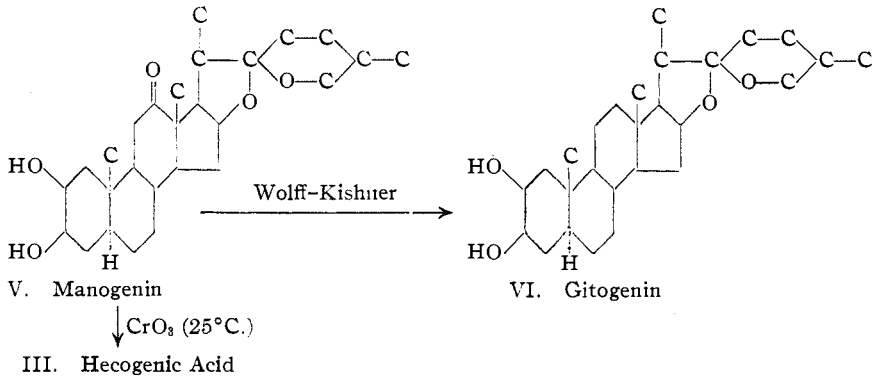
sults and geographical locations of the plants are given. The other *sources of hecogenin*, immediately following, make this substance readily available.

Plant	Location	Hecogenin, g. per kg. (dry)
Agave americana (L.)	San Antonio, Tex.	0.4
A. americana var.	Tucson, Ariz.	3.0
A. asperrima (Jacobi)	Monterey, Mexico	0.1
A. crysantha (Peebles)	Devils Canyon, Ariz.	1.2
A. deserti (Engelm.)	Palm Springs, Calif.	0.6
A. deserti var.	Goldroad, Ariz.	3.3
A. endlichiana (Trel.)	Orizaba, Mexico	0.6
A. expansa	Tucson, Ariz.	2.0
A. fourcroydes (Baker)	Victoria, Mexico	0.1
A. gracilipes (Trel.)	El Capitan Mt., Tex.	7.0
A. huachucensis (Baker)	Fort Huachuca, Ariz.	0.3
A. murpheyi (Gibson)	Globe, Ariz.	1.0
A. palmeri (Engelm.)	Tucson, Ariz.	0.2
A. parviflora (Torr.)	Nogales, Ariz.	3.0
A. shawii (Engelm.)	Lower California, Mexico	2.5
A. toumeyana (Trel.)	Superior, Ariz.	13.0
Hechtia texensis (S. Wats.)	Big Bend, Tex.	0.1
Hesperaloe funifera (Koch)	Huasteca Canyon, Mexico	1.3
Maguey el ojital	El Ojital, Mexico	0.1
Maguey espadin	Zitacuaro, Mexico	0.5
Maguey tequilla manso	Tanaquila, Mexico	0.1
Manfreda maculosa (Hook.)	San Antonio, Tex.	3.0

The identity of the hecogenin was established in each case by analysis of the genin and its acetate along with mixed melting point determinations on both. Hecogenin has three polymorphic forms melting at 245, 253 and 268°, and its acetate, resembling sarsasapogenin acetate and smilagenin acetate, has two forms, m. p. 243 and 252°. In all cases the melting points and mixed melting points of the genin and acetate were not more than three degrees below the above temperatures and the analyses never varied more than three-tenths from the calculated carbon and hydrogen values.

2. **Manogenin.**—We have isolated from *Manfreda maculosa* (Hook.) a new keto-steroidal sapogenin, having the composition $C_{27}H_{42}O_5$, m. p. 241–243°, which we have named manogenin. *Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.5; H, 9.4. Boiling acetic anhydride formed a diacetate, m. p. 255°. *Anal.* Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.8. Found: C, 70.2; H, 8.6. The function of four of the five oxygens is shown by its conversion by the Wolff–Kishner method to gitogenin (VI), m. p. and mixed m. p., 266–268°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 75.2; H, 10.1. Manogenin (V) upon mild oxidation with chromic anhydride in acetic acid gave hecogenic acid (III), m. p. and mixed m. p., 268° dec. *Anal.* Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.4. Found: C, 68.3; H, 8.3. The identity was further established by the direct comparison of the dimethyl esters, m. p. and mixed m. p., 186–187°. The carbonyl group in manogenin like that in hecogenin cannot be removed under the conditions of a mild Clemmensen reaction. We propose structure V for manogenin.

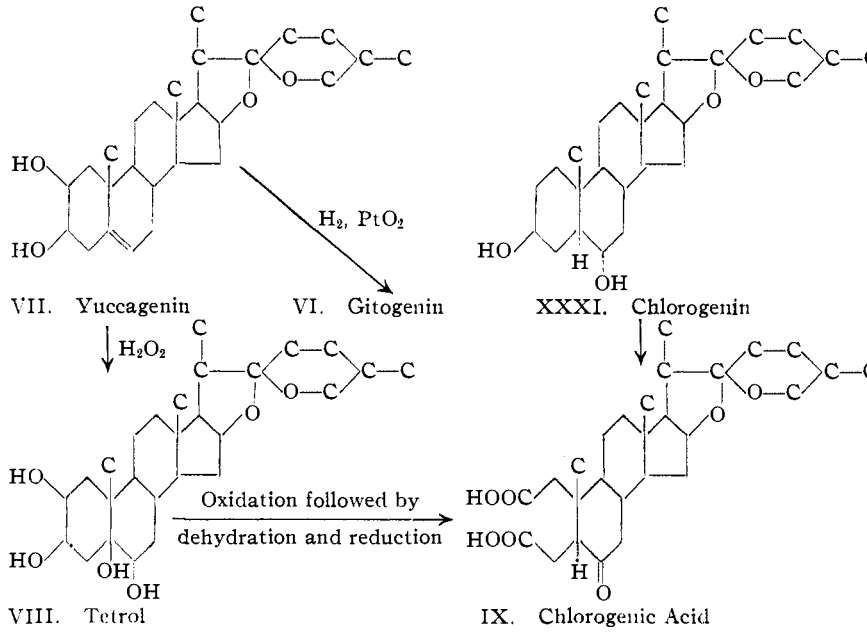
Although manogenin occurs abundantly in *Manfreda maculosa* (Hook.), the latter itself is scarce. We have found twenty-four additional sources, listed in the following table.



Plants	Location	Manogenin, g. per kg. (dry)
Agave atrovirens (Otto)	Tlaxcala, Mexico	0.1
A. bracteosa (Wats.)	Huasteca Canyon, Mexico	1.7
A. chisoensis	Big Bend, Tex.	0.7
A. crassispina (Trel.)	Bueno Vista, Mexico	0.9
A. ferox (Koch)	Portezuelo, Mexico	1.7
A. gracilipes (Engelm.)	El Capitan Mt., Tex.	0.6
A. havardiana (Trel.)	Davis Mts., Tex.	0.2
A. huachucensis (Baker)	Fort Huachuca, Ariz.	5.3
A. lehmanii	Portezuelo, Mexico	0.8
A. lophantha	Monterey, Mexico	0.5
A. mirabalis (Trel.)	Tacuro, Mexico	0.3
A. mitraeformis (Trel.)	Tehuacan, Mexico	0.6
A. parassana (Trel.)	Parras, Mexico	0.8
A. quiotefera (Trel.)	Saltillo, Mexico	0.3
A. salmiana (Otto)	Portequelo, Mexico	1.1
A. scabra	Iron Mt., Tex.	0.2
A. striata (Zucc.)	Ixmilquilpan, Mexico	1.0
A. utahensis (Engelm.)	St. George, Utah	1.3
Maguey cacaya	Orizaba, Mexico	0.5
M. canasto	Jacala, Mexico	0.8
M. ceniso	Zitacuaro, Mexico	1.2
M. cimmarron	Zitacuaro, Mexico	1.0
M. cuchacamba	Tanaquilla, Mexico	0.5
Manfreda tigrina (Engelm.)	Bonneau, S. Car.	0.3

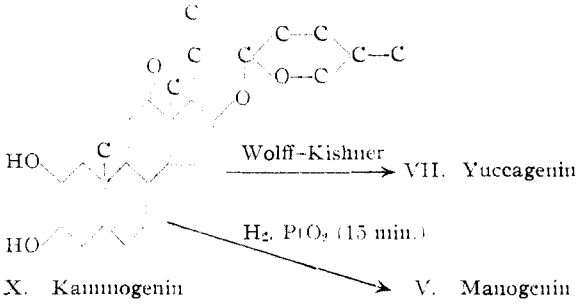
The identity of the manogenin was established in each case by analysis of the genin and its acetate along with mixed melting point determinations on both. Manogenin, resembling hecogenin, has three polymorphic forms melting at 243, 254 and 264°, and its acetate, resembling sarsasapogenin acetate and smilagenin acetate, has three forms melting at 215, 242 and 255°. In all cases the analyses never varied more than three-tenths from the calculated carbon and hydrogen values.

3. **Yuccagenin.**—During the course of our investigation of the *Yucca* for steroidal sapogenins, we have isolated from *Y. schottii* (Engelm.), *Y. elata* (Engelm.) and *Y. flaccida* (Haw.) a new steroidal sapogenin having the composition $C_{27}H_{42}O_4$, m. p. 246° and 252°, which we have named yuccagenin (VII). *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.3; H, 9.8. Boiling acetic anhydride formed a diacetate, m. p. 178°. *Anal.* Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.4; H, 9.0. Catalytic hydrogenation (Adams catalyst) of yuccagenin (VII) in ether containing several drops of acetic acid gave gitogenin (VI), m. p. and mixed m. p., 264–266°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 75.2; H, 10.0. Treatment of yuccagenin (VII) with hydrogen peroxide in acetic acid gave a tetrol (VIII), m. p. 350°. *Anal.* Calcd. for $C_{27}H_{44}O_6$: C, 69.8; H, 9.5. Found: C, 69.4; H, 9.6. The latter upon mild oxidation with chromic anhydride in acetic acid followed by dehydration and zinc–acetic acid reduction gave chlorogenic acid (IX), m. p. and mixed m. p. 233–234°. *Anal.* Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.4. Found: C, 68.1; H, 8.5. The identity was further established by the direct comparison of the dimethyl esters, m. p.

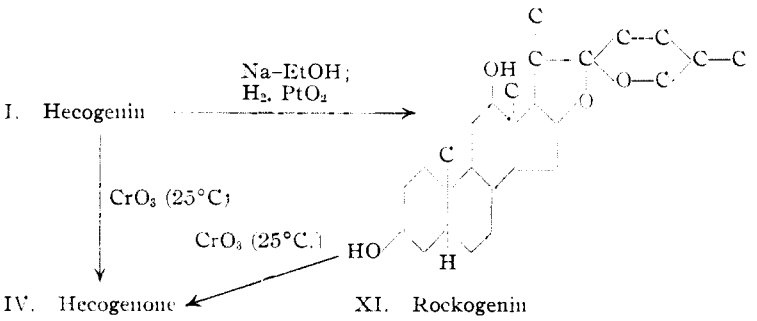


and mixed m. p., 163–164°. We propose structure VII for yuccagenin.

4. **Kammogenin.**—We have described above the isolation of yuccagenin (VII) from *Yucca schottii* (Engelm.) which accounted for the major portion of the total steroidal sapogenin fraction. From the mother liquors of the crystalline yuccagenin diacetate we obtained a new steroidal sapogenin having the composition $C_{27}H_{40}O_5$, m. p. 242°. *Anal.* Calcd. for $C_{27}H_{40}O_5$: C, 72.9; H, 9.1. Found: C, 72.6; H, 9.1. We have found other sources of this new substance, namely, *Samuela carnerosana* (Trel.), *Yucca brevifolia* (Engelm.) and *Yucca harrimanii* (Trel.). Boiling acetic anhydride formed a diacetate having two forms, m. p. 243° and 260°. *Anal.* Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.5; H, 8.5. Although kammogenin (X) forms a monosemicarbazone, it is unaffected by the conditions of a mild Clemmensen reaction, indicating the inert position of the carbonyl group. The reduction of kammogenin (X) by the Wolff-Kishner method gives yuccagenin (VII), m. p. and mixed m. p., 245–246°. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 74.8; H, 9.9. The identity was further established by direct comparison of the diacetates, m. p. and mixed m. p., 176–178°. Catalytic reduction (Adams catalyst) of kammogenin diacetate in ether containing several drops of acetic acid for fifteen minutes gave manogenin diacetate (V), m. p. and mixed m. p., 241–242°. *Anal.* Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.8. Found: C, 70.1; H, 8.7. We propose structure X for kammogenin.

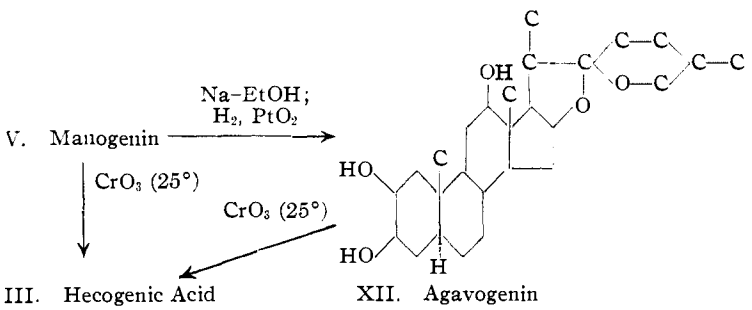


5. **Rockogenin.**—A new steroidal sapogenin, rockogenin, has been isolated from the steroidal fraction of *Agave gracilipes* (Trel.) along with large amounts of hecogenin (I). It is not surprising to find that their structures are closely related. Thus, we have shown by a comparison of the analyses and melting points along with mixed melting point determinations that rockogenin, m. p. 221°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.5; H, 10.2, and its acetate, m. p. 206°. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.9; H, 9.2, are identical with 12-dihydrohecogenin, m. p. and mixed m. p., 220° and its acetate, m. p. and mixed m. p., 206°. The latter (XI) is formed either by the catalytic reduction (Adams catalyst) or sodium–ethanol reduction of hecogenin (I). Mild oxidation of rockogenin (XI) with chromic anhydride in acetic acid gave hecogenone (IV), m. p. and mixed m. p., 240°. *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.6; H, 9.5. Found: C, 75.4; H, 9.4. We propose structure XI for rockogenin.



6. **Agavogenin.**—We have processed 1360 kg. of *Agave huachucensis* (Baker) obtaining almost two kilograms of crude sapogenins. We have found that manogenin (V) accounted for 22% of the total crystalline sapogenin fraction. From the mother liquors of the crystalline manogenin diacetate was obtained gitogenin diacetate (VI) (50% of the sapogenins) and hecogenin acetate (I) (22% of the sapogenins) and a new steroidal sapogenin having the composition $C_{27}H_{44}O_5$, m. p. 242°, which we have named agavogenin. *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.2; H, 9.9. Found: C, 72.0; H, 9.8. The yield of the latter was 5% of the total sapogenin fraction. Three of the five oxygens are present as hydroxyl groups, acetylated with boiling acetic anhydride, forming a triacetate, m. p. 228°. *Anal.* Calcd. for $C_{33}H_{50}O_6$: C, 68.9; H, 8.8. Found: C, 68.9; H, 8.8. We have found that agavogenin and its triacetate are identical, respectively, with 12-dihydromanogenin, m. p. and mixed m. p., 240°, and its triacetate, m. p. and mixed m. p., 228°. The latter is formed either by the catalytic reduction

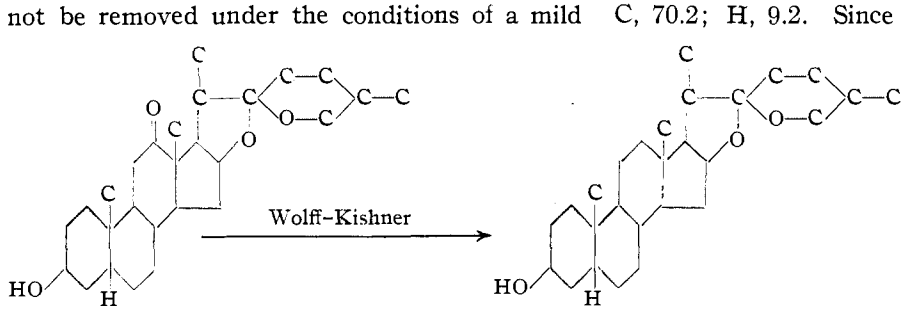
(Adams catalyst) or sodium–ethanol reduction of manogenin (V). The identity was further established by the mild chromic anhydride oxidation of agavogenin (XII), giving hecogenic acid (III), m. p. and mixed m. p., 268° dec. *Anal.* Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.4. Found: C, 68.2; H, 8.2. We propose structure XII for agavogenin.



V. Manogenin

III. Hecogenic Acid XII. Agavogenin

7. **Furcogenin.**—We have isolated as the principal constituent of the steroidal fraction from *Furcraea selloa* a new steroidal sapogenin having the composition $C_{27}H_{42}O_4$, m. p. 225°, which we have named furcogenin. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.4; H, 10.0. In the steroidal fraction from *Yucca flaccida* (Haw.), however, it occurs along with a large quantity of smilagenin (XIV) and smaller quantities of yuccagenin (VII). It gave a precipitate with alcoholic digitonin, indicating a C-3 hydroxyl group having the *beta* configuration. Boiling acetic anhydride formed a monoacetate, m. p. 225°. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.8; H, 9.2. The function of three of the four oxygens is shown by its conversion by the Wolff–Kishner method to smilagenin (XIV), m. p. and mixed m. p., 183°. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.8; H, 10.5. The carbonyl group in furcogenin (XIII) like that in manogenin (V) and hecogenin (I) cannot be removed under the conditions of a mild Clemmensen reaction, indicating its inert position. We suggest structure XIII for furcogenin.



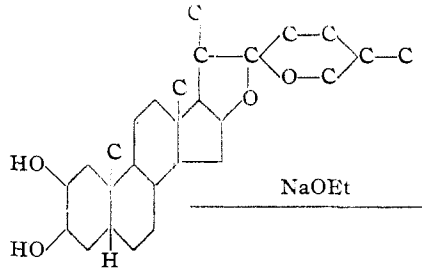
XIII. Furcogenin XIV. Smilagenin

8. **Samogenin.**—We have found that the steroidal fraction from *Samuela carnerosana* (Trel.) contains as its principal constituent a substance, isomeric with gitogenin (VI), melting 210–212°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.9; H, 10.2. It also occurs in the steroidal fraction from *Yucca schottii* (Engelm.). This material forms a precipitate in alcoholic digitonin indicating a 3(*β*)-hydroxyl group. When refluxed with acetic anhydride, it is converted into a diacetate, isomeric with gitogenin diacetate, melting 195–198°. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.9; H, 9.1. Both the free and acetylated compounds are entirely different from any of the known steroidal sapogenins and their acetates. We propose the name samogenin for this new substance.

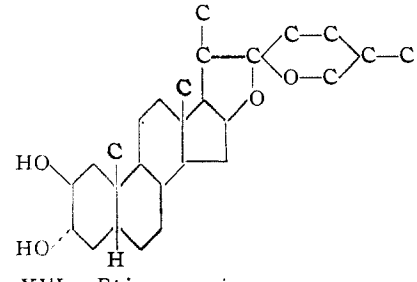
Samogenin (XV) is unaffected by treatment with hydrochloric acid in ethanol at the boiling point, indicating that it has the iso-configuration of the side-chain.[4] It readily undergoes other reactions characteristic of the steroidal sapogenins. The epimer (XVI) of samogenin (XV) is formed when the latter is heated with sodium ethoxide in a sealed tube at 200° for ten hours, m. p. 235–237°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.7; H, 10.1. *Epi*-samogenin (XVI) like other steroids having the *alpha* configuration of the hydroxyl group at C-3 is not precipitated by digitonin in aqueous alcohol. Mild oxidation of samogenin (XV) with chromic anhydride in acetic acid gave good yields of a dicarboxylic acid (XVII), thus indicating two adjacent hydroxyl groups. Samogenic acid (XVII), isomeric with gitogenic acid (XVIII), melts 264° dec. *Anal.* Calcd. for $C_{27}H_{42}O_6$: C, 70.1; H, 9.2. Found: C, 70.2; H, 9.2. Since one hydroxyl group has been assigned to C-3, the other is probably at C-2 by analogy to the other dihydroxy-steroidal sapogenins. We suggest structure XV for samogenin.

9. **Mexogenin.**—We have described above the isolation of samogenin from *Samuela carnerosana* (Trel.) which

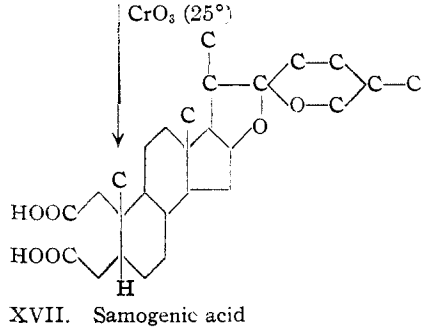
(4) Marker and Rohrmann, THIS JOURNAL, **62**, 647 (1940).



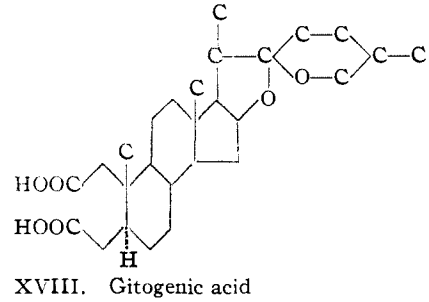
XV. Samogenin



XVI. *Epi*-samogenin



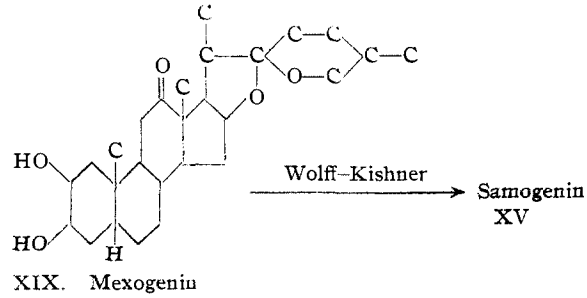
XVII. Samogenic acid



XVIII. Gitogenic acid

accounted for the major portion of the total steroidal sapogenin fraction. From the mother liquor of the crystalline samogenin was obtained a new steroidal sapogenin, isomeric with manogenin (V), melting 246°. *Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.9; H, 9.6. It also occurs in the steroidal fraction from *Yucca schottii* (Engelm.). Boiling acetic anhydride formed a diacetate, isomeric with manogenin diacetate, melting 208°. *Anal.* Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.8. Found: C, 70.3; H, 9.0. We propose the name mexogenin for the new substance.

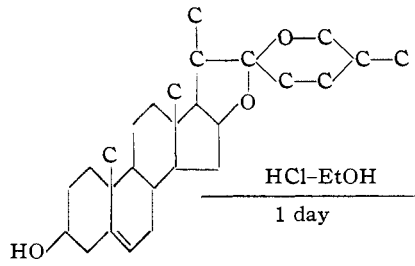
Although mexogenin (XIX) forms a monosemicarbazone, it is unaffected by the conditions of a mild Clemmensen reaction, indicating the inert position of the carbonyl group. In this respect, it is like the other 12-keto-steroidal sapogenins, namely, hecogenin (I), manogenin (V), furcogenin (XIII) and kammogenin (X). Wolff–Kishner reduction removes the carbonyl group, giving samogenin (XV), m. p. and mixed m. p., 210–212°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.9; H, 10.3. The identity of this degradation product (XV) was further established by its conversion to samogenin diacetate, m. p. and mixed m. p., 195–198°. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.0; H, 9.4. We suggest structure XIX for mexogenin.



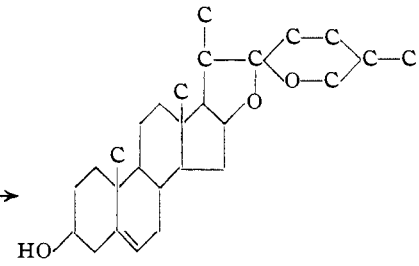
XIX. Mexogenin

10. Yamogenin.— We have obtained from *Dioscorea testudinaria* in good yields diosgenin, isolated as the acetate, m. p. and mixed m. p., 199–202°. *Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.1; H, 9.5. Hydrolysis of the acetate gave diosgenin, m. p. and mixed m. p., 206–209°. *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 77.8; H, 9.9. From the mother liquors of the crystalline diosgenin acetate was obtained a substance, isomeric with diosgenin acetate, melting 180–182°. *Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.3; H, 9.8. A mixture with diosgenin acetate melted 164–167°. Hydrolysis of this material gave the free genin, isomeric with diosgenin, melting 200–201°. *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.1; H, 10.3. We have named this new steroidal sapogenin, yamogenin.

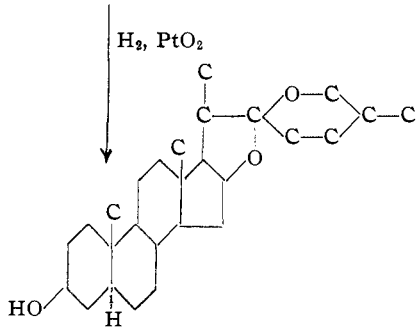
Treatment of yamogenin (XX) under the usual conditions employed for the isomerization of the sapogenin side-chain[4] gave diosgenin (XXI), m. p. and mixed m. p., 206°. Catalytic hydrogenation (Adams catalyst) of yamogenin acetate in ether containing several drops of acetic acid gave neotigogenin acetate (XXII), m. p. and mixed m. p., 179–180°. *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.4. Found: C, 76.0; H, 10.3. A mixture with the starting material melted 171–173°. The identity of XXII was further established by a direct comparison of the hydrolyzed product, m. p. and mixed m. p. with neotigogenin, 202–203°. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.8; H, 10.6. We propose structure XX for yamogenin.



XX. Yamogenin



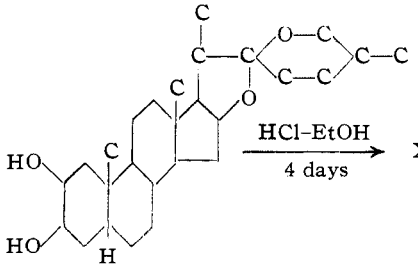
XXI. Diosgenin



XXII. Neotigogenin

We have found twelve new sources for yamogenin, listed below under diosgenin.

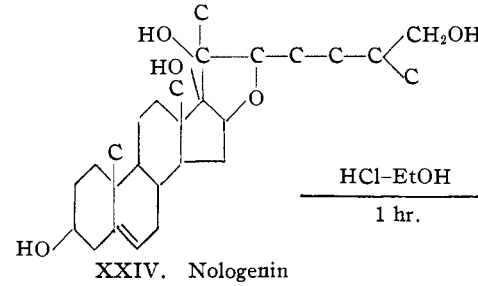
11. Texogenin.—We have processed 1364 kg. of *Yucca schottii* (Engelm.). We have found that yuccagenin (VII) accounted for 59% of the total crystalline sapogenin fraction. From its mother liquors was obtained smilagenin (XIV) (13% of the sapogenins), kammogenin (X) (13% of the sapogenins), samogenin (XV) (8% of the sapogenins), gitogenin (VI) (2% of the sapogenins), mexogenin (XIX) (1% of the sapogenins) and a new steroidal sapogenin, isomeric with samogenin (XV), melting 171–172°, which we name texogenin. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.7; H, 10.1. The yield of the latter was 4% of the total sapogenin fraction. Boiling acetic anhydride formed a diacetate, isomeric with samogenin diacetate, melting 170–172°. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.8; H, 9.3. Treatment of texogenin (XXIII) with ethanolic hydrochloric acid at the boiling point gave samogenin (XV), m. p. and mixed m. p., 210–212°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.8; H, 10.1. Thus, it differs from samogenin (XV) only in the configuration of the side-chain. In a like manner, sarsasapogenin (XXX) differs from smilagenin (XIV); the former can also be converted to the latter by ethanolic hydrochloric acid.[5] We propose structure XXIII for texogenin.



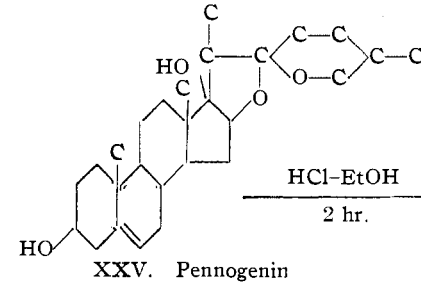
XXIII. Texogenin

XV. Samogenin

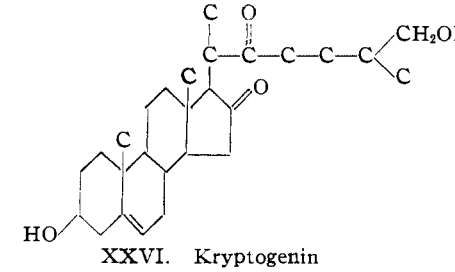
12. Nologenin.—Recently,[6a] we reported the isolation of nologenin, a new steroidal sapogenin, from *Beth* root, having the composition $C_{27}H_{44}O_5$, m. p. 265°. Four of



XXIV. Nologenin



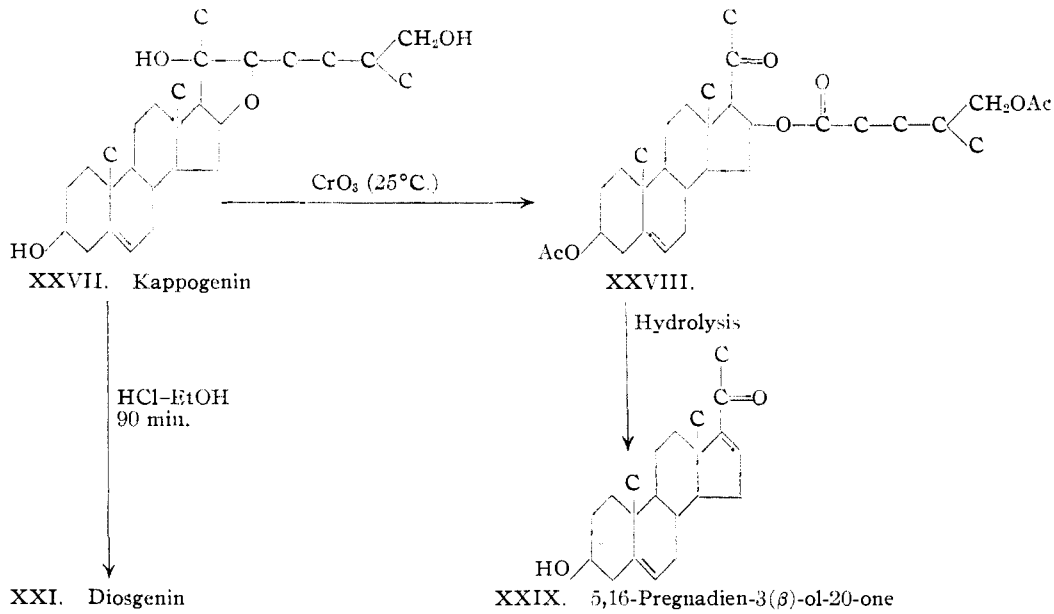
XXV. Pennogenin



XXVI. Kryptogenin

(5) Marker and Rohrmann, THIS JOURNAL, **61**, 896 1939).

(6) (a) Marker and co-workers, *ibid.*, **65**, 1248 (1943); (b) **65**, 739 (1943).



XXVII. Kappogenin

XXVIII.

XXI. Diosgenin

XXIX. 5,16-Pregnadien-3(β)-ol-20-one

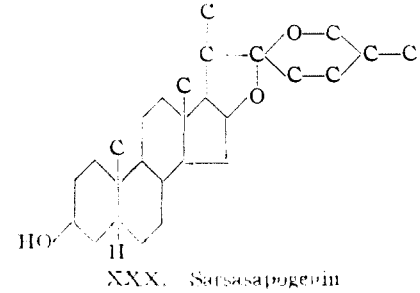
the five oxygens are present as hydroxyl groups, two of which are acetylated with boiling acetic anhydride forming a diacetate ($C_{31}H_{48}O_7$), m. p. 200°. Mild treatment of nologenin (XXIV) with ethanolic hydrochloric acid gives pennogenin (XXV)[6a] isolated as the monoacetate, m. p. and mixed m. p., 198–199°. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.8; H, 9.4. Prolonged treatment of this product (XXV) with ethanolic hydrochloric acid gives kryptogenin (XXVI).[6b] We propose structure XXIV for nologenin.

13. **Kappogenin.**—We have reported the isolation and structures of two new steroidal sapogenins from *Beth* root (*Trillium erectum*), namely, nologenin[6a] and kryptogenin.[6b] These are peculiar in having an open side-chain instead of the usual spiro-ketal side-chain possessed by the other sapogenins. Furthermore, their close structural relationship is shown by the conversion of the former to the latter by prolonged ethanolic hydrochloric acid treatment.

We have isolated from the mother liquor of nologenin (XXIV) another sapogenin having an open side-chain, which we have named kappogenin, m. p. 230°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.3. Found: C, 75.0; H, 10.4. Two of the three hydroxyl groups react with boiling acetic anhydride forming a diacetate, m. p. 178°. *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.0; H, 9.4. Found: C, 72.2; H, 9.4. Treatment of kappogenin (XXVII) for ninety minutes with boiling 2 *N* ethanolic hydrochloric acid gives diosgenin (XXI) isolated as the monoacetate, m. p. and mixed m. p., 198–200°. *Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.5; H, 9.6. This reaction is analogous to the conversion of nologenin (XXIV) to pennogenin (XXV). Mild oxidation of XXVII followed by hydrolysis of the intermediate ester (XXVIII) gives 5,16-pregnadien-3(β)-ol-20-one (XXIX), isolated as its acetate, m. p. and mixed m. p., 176°. *Anal.* Calcd. for $C_{23}H_{32}O_3$: C, 77.4; H, 9.1. Found: C, 77.1; H, 9.0. We propose structure XXVII for kappogenin.

New Sources for Known Sterols

1. **Sarsasapogenin.**—We have described above the isolations and structures of nine new steroidal sapogenins, isolated from either *Yuccas* or *Agaves*. We have found that these plants are particularly good sources for other steroidal sapogenins. Thus, we have found twenty-six new sources for sarsasapogenin (XXX). Previous to this work only three sources were known.



XXX. Sarsasapogenin

Plant	Location	Yield, g. per kg. (dry)
Agave attentua (Baker)	St. Louis, Mo.	0.2
Agave roezliana (Baker)	Puebla, Mexico	0.3
Lechuguilla espadilla morado	Tehuacan, Mexico	0.6
Maguey de la pena or *estrella*	Zitacuaro, Mexico	0.4
Smilax lancelata (L.)	South Carolina	5.0
Smilax rotundifolia	South Carolina	2.8
Yucca angustissima	S. W. Utah	1.5
Y. arizonica (McKel.)	Nogales, Ariz.	0.1
Y. baccata (Torr.)	Los Cruces, N. Mex.	0.8
Y. baleyi (Woot-Standl.)	Meteor Crater, Ariz.	0.9
Y. confinis (McKel.)	Douglas, Ariz.	1.0
Y. decipiens (Trel.)	Zitacuaro, Mexico	0.1
Y. endlichiana (Trel.)	Parras, Mexico	1.1
Y. elata (Engelm.)	Tucson, Ariz.	10.0
Y. elata cultivated young	Tucson, Ariz.	0.6
Y. glauca (Nutt.)	Northern Texas	5.0
Y. harrimanii (Trel.)	Zion Park, Utah	1.7
Y. jalicensis (Trel.)	La Primanera, Mexico	0.4
Y. rigida	Western Mexico	1.0
Y. schidigera (Roezl.)	Palm Springs, Calif.	2.0
Y. schottii (Engelm.) fruit	Sonoita, Ariz.	5.0
Y. thornberi (McKel.)	Tucson, Ariz.	0.7
Y. torreyi (Shafer)	Big Bend, Texas	2.0
Y. treculeana succ.	Sonora, Texas	2.7
Y. treculeana can. (Hook.)	Tamazuchane, Mexico	2.0
Y. valida	Western Mexico	3.0

The identity of the sarsasapogenin was established by analysis of the genin and its acetate along with mixed melting point determinations on both. In all cases the melting points, mixed melting points and analyses of the genin and its acetate were within the following ranges: genin, m. p. and mixed m. p., 199–202°. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.8 ± 0.3; H, 10.6 ± 0.3; acetate, m. p. and mixed m. p. 138–142° or 126–129°. *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.1 ± 0.3; H, 10.2 ± 0.3.

2. **Smilagenin.**—Previously,[7] there has been reported only one source for smilagenin (XIV), namely, *Smilax ornata* (Hooker). We have found twenty-eight new sources, listed in the table.

Plant	Location	Yield, g. per kg. (dry)
Agave funkiana (Koch-Bouche)	Victoria, Mexico	0.8
A. funkiana var. (Koch-Bouche)	Pachuca, Mexico	0.9
A. heterocantha (Zucc.)	Laredo, Texas	1.3
A. lechuguilla (Torr.)	Big Bend, Texas	5.0
A. lophantha	San Antonio, Texas	7.5
Dracena australis	Laredo, Texas	3.0
Yucca aloifolia (L.)	Galveston, Texas	3.0
Y. aloifolia var. (Naudin)	Beaumont, Texas	0.8
Y. arkansana (Trel.)	San Antonio, Texas	5.6
Y. australis (Engelm.)	Monterey, Mexico	0.2
Y. brevifolia (Engelm.)	San Bernardino, Calif.	0.1
New Yucca	Big Bend, Texas	0.1
Y. elephantipes (Regel)	Cordoba, Mexico	0.6
Y. filifera (Chaband)	Tehuacan, Mexico	0.5
Y. flaccida (Haw.)	Tryon, N. C.	8.0
Y. gloriosa (L.)	Gainesville, Florida	1.7
Y. jalicensis (Trel.)	Western Mexico	1.0
Y. louisianensis (Trel.)	Beaumont, Texas	1.0
Y. recurvifolia (Salisb.)	Beaumont, Texas	0.1
Y. reverchoni (Trel.)	Sonora, Texas	0.6
Y. rostrata (Engelm.)	Big Bend, Texas	1.6
Y. rupicola (Scheele)	S. Central Texas	1.1
Y. schottii (Engelm.) flowers	Sonoita, Ariz.	1.2
Y. tenuistyla (Trel.)	Beaumont, Texas	2.0
Y. treleaseana (MacBride)	Beaumont, Texas	0.5
Samuela carnerosana (Trel.)	Big Bend, Texas	0.5
Samuela faxoniana (Trel.)	Big Bend, Texas	0.7
Zygadenus glaberrimus (Michx.)	Myrtle Beach, S. C.	2.5
Zygadenus nutallii (Gray)	Central Texas	2.0

Smilagenin (XIV), which may be prepared by the action of ethanolic hydrochloric acid on sarsasapogenin (XXX), differs in structure from the latter only in the side-chain.[5] The identity of the smilagenin was established by analysis of the genin and its acetate along with mixed melting point determinations on both. Smilagenin acetate resembles sarsasapogenin acetate in showing polymorphic forms. These melt at 110, 130 and 152°. In all cases the melting points, mixed melting points and analyses of the genin and its acetate were within the following range: genin, m. p. and mixed m. p., 183–185°. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.8 ± 0.3; H, 10.6 ± 0.3.; acetate, m. p. and mixed m. p., 106–110°, 126–130° and 149–152°. *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.9 ± 0.3; H, 10.1 ± 0.3.

3. **Gitogenin.**—We have found sixteen new sources of gitogenin (VI), ten new sources of tigogenin (II) and two new sources for chlorogenin (XXXI). Previous to this work only three sources were known for each of these sapogenins. The *gitogenin* plants are:

Plant	Location	Yield, g. per kg. (dry)
Agave gracilipes (Trel.)	El Capitan Mt., Texas	0.3
A. huachucensis (Baker)	Fort Huachuca, Ariz.	0.3
A. lechuguilla (Torr.)	Big Bend, Texas	0.6
A. mescal (Koch)	Mexico City, Mexico	2.0
A. schottii (Engelm.)	Tucson, Ariz.	11.0
A. stricta glauca	Parras, Mexico	1.5
A. stricta nana	Saltillo, Mexico	1.1
A. stricta rosea	Laredo, Texas	1.1
Manfreda gigantea var.	Laredo, Texas	2.1
Manfreda virginica (L.)	South Carolina	10.0
Yucca filamentosa (L.)	South Carolina	11.0
Y. schottii (Engelm.)	Santa Catalina Mts., Ariz.	1.0
Y. whipplei caespitosa (Torr.)	Palmdale, Calif.	3.0
Y. whipplei intermedia	Santa Monica Mt., Calif.	0.5
Y. whipplei percursa	Santa Barbara, Calif.	2.0

The identity of the gitogenin was established by analysis of the genin and its acetate along with mixed melting point determinations on both. In each case the melting point of gitogenin was within the range 264–268° and the analyses never varied more than three-tenths from the calculated carbon and hydrogen values. The melting point of gitogenin diacetate was within the range 238–242° and the analyses never varied more than

(7) Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936).

two-tenths from the calculated carbon and hydrogen values.

4. **Tigogenin.**

Plant	Location	Yield, g. per kg. (dry)
Agave lophantha	Monterey, Mexico	1.5
A. stricta purpurea	Laredo, Tex.	2.5
A. schottii (Engelm.)	Tucson, Ariz.	1.4
Allium tricocum	Bradford, Penna.	0.1
Hesperaloe parviflora (Torr.)	San Antonio, Tex.	1.0
Maguey Canon del Abra	Victoria, Mexico	0.4
Solanum dulcamara (L.)	Commercial Product	0.1
Yucca whipplei intermedia (Torr.)	Santa Monica Mt., Calif.	1.0
Y. whipplei parishii	Canyon Pass, Calif.	4.4
Y. whipplei typica	San Bernardino, Calif.	8.8

In each case the melting point of tigogenin was within the range 205–208° and the analyses of the genins and their acetates were within the above limits. The melting point of tigogenin acetate was within the range 200–204°.

It is noteworthy that the subspecies of *Yucca whipplei* (Torr.) and *Agave stricta* contain different steroidal sapogenins. Thus, Yucca *whipplei* (Torr.) subsp. *caespitosa* and subsp. *percursa* contain gitogenin; subsp. *typica* and subsp. *parishii* contain tigogenin; and subsp. *intermedia* contains gitogenin and tigogenin. Furthermore, three of the four subspecies of *Agave stricta* contain gitogenin, the other contains tigogenin.

5. **Chlorogenin.**

Plant	Location	Yield, g. per kg. (dry)
Agave utahensis (Engelm.)	St. George, Utah	1.5
Maguey cacaya	Orizaba, Mexico	0.1

In each case the melting point of chlorogenin was within the range 276–278°, and the analyses of the genins and their acetates were within the above limits. The melting point of chlorogenin diacetate was within the range 153–155°.

6. **Diosgenin.**—We reported previously the isolation and structures of yamogenin (XX) and kryptogenin (XXVI) from *Dioscorea testudinaria* and *Beth* root,[6b] respectively. Both substances are structurally related to diosgenin, and it is of interest that all three occur in many of the *Dioscoreas*. With the present and the fourteen previously reported sources, diosgenin is the commonest steroidal sapogenin, having been isolated from forty-three plants.

Plant	Location	Yield, g. per kg. (dry) Diosg.	Yamog.	Krypto.
Dioscorea bulbifera	Puebla, Mexico	4.5	0.2	0.3
D. capillaris	Oaxaca, Mexico	2.2		
D. composita	Oaxaca, Mexico	3.0		
D. cyphocarpa	Iguala Cañon, Mexico	2.0	0.1	0.2
D. dugessi	Guadalajara, Mexico	2.0	0.2	0.4
D. galeottiana	Oaxaca, Mexico	4.0		
D. grandifolia	Cuernavaca, Mexico	1.5	0.2	0.1
D. hirsuta	Iguala, Mexico	3.0		
D. hirsuticaulis	Cuernavaca, Mexico	1.5	0.2	0.1
D. jaliscana	Guadalajara, Mexico	3.1		
D. lobata	Cuernavaca, Mexico	5.0		
D. macrostachya	Vera Cruz, Mexico	2.9		
D. mexicana	Central Mexico	3.9		
D. militaris	Uruapan, Mexico	3.3	0.2	0.1
D. minima	Patzcuaro, Mexico	2.7	0.4	0.1
D. multinervis	Patzcuaro, Mexico	2.7	0.2	0.1
D. platycalpata	Iguala, Mexico	3.0	0.2	0.1
D. plumifera	Jalapa, Mexico	4.1		
D. pringlei	Guadalajara, Mexico	3.5		
D. remotiflora	Guadalajara, Mexico	3.7	0.7	0.6
D. subtomentosa	Cuernavaca, Mexico	4.4		
D. testudinaria	Guadalupe, Mexico	5.0	0.3	
D. ulinei	Cuernavaca, Mexico	4.0	0.2	0.3
D. urceolata	Cuernavaca, Mexico	4.6	0.1	0.2
Nolina erumpens	Alpine, Texas	0.5		
Nolina greeni	N. New Mexico	4.0		
Smilacena stellata	Westerly, R. I.	5.0		
Trillium stylosum	Greenville, S. C.	0.1		
T. sessile californicum	Ukiah, Calif.	0.1		

The identities of the above were established by the analyses of the genins and their acetates along with mixed melting point determinations on both. In each case the melting point of diosgenin, yamogenin and kryptogenin was within the ranges 200–202°, 199–201° and 187–189°, respectively, and the analyses never varied more than three-tenths from the calculated carbon and hydrogen values. The melting points of diosgenin acetate, yamogenin acetate and kryptogenin diacetate were within the ranges 200–202°, 180–182° and 150–153°, respectively, and the analyses never varied more than three-tenths from the calculated carbon and hydrogen values.

7. **Sitosterol.**—In the course of our investigation of plants for steroidal sapogenins, we have found that the following plants, listed with their geographical location, contained sitosterol as their principal steroidal constituent. No steroidal sapogenin could be isolated, except in the case of *Yucca arizonica* and *Trillium sessile* from which we obtained sarsasapogenin and diosgenin, respectively.

Plant	Location	Yield, g. per kg. (dry)
Agave melliflua (Trel.)	La Union, Mexico	0.1
A. palmaris (Trel.)	Amatitan, Mexico	0.3
Amaryllis	Apoka, Florida	0.1
Hechtia rosea	Tehuacan, Mexico	0.2
Hechtia scariosa	Hot Springs, Texas	0.1
Lily of the Valley	Commercial Product	0.1
Maguey mescal azul	Arenal, Mexico	0.2
M. mescal bermejo	Amatitan, Mexico	0.2
M. mescal chato	Amatitan, Mexico	0.2
M. mescal mana larga	Arenal, Mexico	0.4
M. tequila ticuis	Tanaquilla, Mexico	0.2

Plant	Location	Yield, g. per kg. (dry)
Nolina bigelovi (Torr.)	Goldroad, Ariz.	0.4
N. macrocarpo (Wats.)	Central Arizona	0.1
N. paraviflora (H. B. K.)	Tehuacan, Mexico	0.1
N. texana (Wats.)	Central Texas	0.2
Trillium sessile californicum	Uklah, Calif.	0.1
Yucca arizonica (McKel.)	Sonolta, Ariz.	1.4
Y. thompsoniana	Big Bend, Texas	0.1

The identification of sitosterol was established by the analyses of the sterol and its acetate along with mixed melting point determinations on both. Sitosterol melted 133–136° and its acetate melted 123–126°. In all cases the analyses never varied more than three-tenths from the calculated carbon and hydrogen values.

Of particular interest are those *Magueys* used for the manufacture of an intoxicating liquor known as tequila. These include *M. mescal azul*, *M. mescal bermejo*, *M. mescal chato* and *M. mescal mano larga*. In the course of time the natives have singled out these species as the best sources for its manufacture. Although many *Magueys* contain steroidal sapogenins, all of these above contain sitosterol.

Summary

1. Some three hundred additional plants have been investigated for steroidal sapogenins.

2. From sixty-six plants thirteen new sapogenins have been isolated. Structures are suggested.

3. From one hundred and sixty-two plants, eight previously known sapogenins and sitosterol have been isolated.

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Water-Soluble Derivatives of Menadione[1]

BY AMEL R. MENOTTI[1a]

The antihemorrhagic activity of sulfonated derivatives of 2-methylnaphthalene has been the subject of publications by Moore[2] and Baker, Davies, McElroy and Carlson.[3] The results of the present investigation provide further proof that (a) the primary product formed from the interaction of sodium bisulfite with menadione (2-methyl-1,4-naphthoquinone) is a monobisulfite addition product, and (b) that this addition product rearranges under the influence of heat to form the sulfonated derivative, 2-methyl-1,4-naphthohydroquinone-3-sulfonate. These two products have been characterized, and methods are herein described for their identification in pharmaceutical preparations.

Menadione, when shaken with a saturated aqueous sodium bisulfite solution at room or slightly elevated temperature, yielded a clear, yellow solution which, on cooling to 0°, deposited a white, crystalline, trihydrated salt having the characteristics of a bisulfite addition product. If the solutions were heated at 90–100°, the quantity of bisulfite addition product obtained on cooling to 0° decreased with increase in heating time. After heating for twenty to thirty hours, no crystalline organic salt precipitated on cooling or on the addition of organic solvents. However, if saturated potassium chloride solution was added, the cooled solution deposited crystals of a potassium salt which exhibited properties of an organic sulfonate. The optical properties of the sulfonate and its behavior toward dilute alkalies, *o*-phenanthroline-ferrous complex solution and ethyl cyanoacetate, differed radically from the menadione bisulfite addition product. The sulfonate could be detected in the mother liquid, by precipitation with *o*-phenanthroline-ferrous complex, when the bisulfite addition product was made from warmed solutions. The greater solubility of the sodium salt of the sulfonate in contrast with that of the addition product made possible a complete separation on cooling the reaction mixture. The crystallized, purified menadione-sodium bisulfite addition product was converted to 2-methyl-1,4-naphthohydroquinone-3-sulfonate in 20–30% yield by solution in water and heating at 100° for twenty-four hours in a closed vessel. No bisulfite addition product

(1) The Council on Pharmacy and Chemistry of the American Medical Association has accepted the name "Menadione" as a non-proprietary designation for 2-methyl-1,4-naphthoquinone" (*J. A. M. A.*, **116**, 1054 (1941)), (1a) Present address, Cheplin Biological Laboratories, Syracuse, N. Y., and the name "Menadione Bisulfite as a non-proprietary designation for the water soluble monosodium bisulfite addition product of menadione" described in this paper (*J. A. M. A.*, **121**, 839 (1943)).

(2) M. B. Moore, THIS JOURNAL, **63**, 2049 (1941).

(3) B. R. Baker, T. H. Davies, L. McElroy and G. H. Carlson, *ibid.*, **64**, 1096 (1942).