The failure to find any clue to explain the unique activity of the A and O substances in terms of their sugar constituents points to the need to explore further the amino acid portions of these substances. Suggestive preliminary evidence that the amino acids may be involved in the specificity of the blood group substances has been obtained by Morgan[25] who found that, when allowed to act on a mixture of A and O substances from hog gastric mucin, a crude enzyme preparation containing enzymes splitting both blood group A and O substances inactivated the A substance and liberated primary amino groups and α-amino acids in addition to causing a change from dextro to levorotation, a fall in relative viscosity and the appearance of about 10% of reducing sugars. It is possible, however, that quantitative differences or differences in configurations of the carbohydrate moiety are also determinants of the specificity of the blood group substances.

(25) W. T. J. Morgan, *Nature*, **158**, 759 (1946).

Summary

1. Individual hog stomachs have been shown to yield purified substances showing either blood group A or blood group O specificity. Certain individual stomachs showed both A and O specificity in varying proportions. The products previously isolated from this laboratory and shown to be inactive with respect to blood group A activity have been identified as the blood group O substance.

2. Forssman antigen activity was exclusively associated with blood group A activity. No evidence of antibody production to the A or O substance in rabbits was obtained.

3. Blood group A and O substances were identical in electrophoretic mobility and derivatives of *l*-fucose, *d*-galactose and *d*-glucosamine were isolated from hydrolyzates of these substances.

NEW YORK, N. Y. RECEIVED MARCH 8, 1947

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

Steroidal Sapogenins[1a]

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With the establishment of the structure of the steroidal sapogenins and the demonstration of the conversion of some of them to certain sex hormones, a search was begun in this Laboratory for naturally occurring sapogenins oxygenated in the 11-position as possible starting materials for the synthesis of various cortical hormones.

While to date no 11-oxygenated sapogenins have been isolated, we have, in an investigation of over 40,000 kilograms of plants, comprising more than 400 species collected mainly in Mexico and the Southern United States, (1) found new sources for almost all the known sapogenins, including diosgenin, the starting material for the synthesis of certain sex hormones, (2) isolated and identified twelve new sapogenins, (3) isolated and identified two new steroids which apparently are the progenitors of the sapogenins, and (4) noted a correlation between plant cycle and saponin content which suggests a biogenetic relationship.

Hitherto only notes on this work have been published.[2-7] This paper presents in more detail the results of our investigations on the new steroidal sapogenins and their biogenetic interrelationships.

Previously, we reported the isolation of steroidal sapogenins from plants belonging to the families *Liliaceae* and *Dioscoreaceae*. Steroidal sapogenins also occur in plants of the *Scrophulariaceae* family. It is noteworthy that until the present work all the known sources of these compounds were confined to these three families. Most of the sources are plants belonging to genera of the *Liliaceae* family. For example, we have shown recently that diosgenin occurs in four different genera of this family. Sarsasapogenin has been shown to occur in two more of its genera, *Smilax* and *Asparagus*. In the present work we have extended our studies to include not only plants of the above families but some from the related family, *Amaryllidaceae*. Over four hundred species in the amount of forty thousand kilograms have been obtained as a result of several extensive botanical collection trips by the senior author in Mexico and southern United

(1) (a) This is Paper 160 in the Sterol Series and 72 in the Sapogenin Series from this Laboratory. For the preceding paper see THIS JOURNAL, **68**, 1658 (1943); (b) present address: Hotel Geneve, Mexico City, Mexico; (c) present address: Ciba Pharmaceutical Products, Inc., Summit, N. J.; (d) present address: Rayon Dept., E. I. du Pont de Nemours and Co., Buffalo, N. Y.; (e) present address: Research and Development Dept., Merck and Co., Rahway, N. J.

(2) Marker, Wagner, Goldsmith, Ulshafer and Ruof, THIS JOURNAL, **65**, 739 (1943).

(3) Marker, Wagner, Ulshafer, Wittbecker, Goldsmith and Ruof, *ibid.*, **65**, 1199 (1943); *cf. ibid.*, **69**, 2242 (1947); for supplementary tables address American Documentation Institute, 1719 N Street, N. W., Washington, D. C., requesting Document 2384, and remitting 50¢ for microfilm or $2.10 for photocopies.

(4) Marker, Wagner, Ulshafer, Goldsmith and Ruof, *ibid.*, **65**, 1247 (1943).

(5) Marker, Wagner, Goldsmith, Ulshafer and Ruof, *ibid.*, **65**, 1248 (1943).

(6) Marker, Wagner, Ruof, Goldsmith and Ulshafer, *ibid.* **65**, 1434 (1943).

(7) Marker, Wagner, Ruof, Ulshafer and Goldsmith, *ibid.*, **65**, 1658 (1943).

States. About two hundred of the species studied have not yet yielded sapogenins in our extraction experiments.

The steroidal sapogenins of determined structure from other laboratories and their sources are as follows

Sapogenin	Plant
Chlorogenin	*Chlorogalum pomeridianum* Kunth[8,9,10]
Digitogenin	*Digitalis purpurea*[11-14]
Diosgenin	*Dioscorea tokora* Makino[15]
Gitogenin	*Chlorogalum pomeridianum* Kunth[10]
	Digitalis species[13,16]
Neotigogenin	*Chlorogalum pomeridianum* Kunth[8]
Sarsasapogenin	*Smilax medica*[17]
	S. officinalis L.[18,19]
	S. ornata Hook[20]
Smilagenin	*Smilax ornata* Hook[17]
Tigogenin	*Chlorogalum pomeridianum* Kunth[8,9,10]
	Digitalis lanata[21]
	Digitalis purpurea[11]

The new steroidal sapogenins and pro-sapogenins isolated in this work and their acetates with the corresponding formulas and melting points are summarized in Table I.

TABLE I

A COMPARISON OF THE FORMULAS AND MELTING POINTS OF THE NEW STEROIDAL SAPOGENINS AND PRO-SAPOGENINS AND THEIR ACETATES

	Genin	M. p., °C.	Acetate	M. p., °C.
Agavogenin	$C_{27}H_{44}O_5$	242	$C_{33}H_{50}O_6$	228
Hecogenin	$C_{27}H_{42}O_4$	245, 253, 268[a]	$C_{29}H_{44}O_5$	243 and 252
Kammogenin	$C_{27}H_{46}O_5$	242	$C_{31}H_{44}O_7$	243 and 260
Kryptogenin	$C_{27}H_{42}O_4$	189	$C_{31}H_{46}O_6$	153
Lilagenin	$C_{27}H_{42}O_4$	246	$C_{31}H_{46}O_6$	155
Manogenin	$C_{27}H_{42}O_5$	243, 254, 264	$C_{31}H_{46}O_7$	215, 243, 255
Mexogenin	$C_{27}H_{42}O_5$	246	$C_{31}H_{46}O_7$	208
Nologenin	$C_{27}H_{44}O_5$	265	$C_{31}H_{48}O_7$	180
Pennogenin	$C_{27}H_{42}O_4$	247	$C_{29}H_{44}O_5$	199
Rockogenin	$C_{27}H_{44}O_4$	210 and 220	$C_{31}H_{48}O_6$	206
Samogenin	$C_{27}H_{44}O_4$	212	$C_{31}H_{48}O_6$	198
Texogenin	$C_{27}H_{44}O_4$	172	$C_{31}H_{48}O_6$	172
Yamogenin	$C_{27}H_{42}O_3$	201	$C_{29}H_{44}O_4$	182
Yuccagenin	$C_{27}H_{42}O_4$	246 and 252	$C_{31}H_{46}O_6$	178

[a] The multiple melting points probably correspond to polymorphic forms.

T plant sources for these new compounds, along with data on yields and locality of collection have been reported previously in our preliminary paper.[3] The most probable structures for these new sapogenins are shown in formulas 1, 3, 4 and 6 to 16.

STRUCTURE STUDIES

Hecogenin

Hecogenin was first isolated from the extracts of two species of the *Bromeliaceae* family, namely, *Hechtia texensis* S. Wats. and a botanically undescribed species native to Mexico. It was later found to occur in numerous species of the *Agaves*. In our present study we have shown its most probable structure to be 12-ketotigogenin (3).

The empirical formula of hecogenin and its acetate and its reaction with 2,4-dinitrophenylhydrazine and (more difficultly) with semicarbazide acetate, indicate a sapogenin containing one hydroxyl and one carbonyl group.

Semicarbazone.—To a solution of 0.1 g. of hecogenin in 5 cc. of ethanol and 5 cc. of pyridine was added 0.1 g. of semicarbazide acetate. The mixture was allowed to stand at room temperature for four days, after which it was diluted with water and filtered. The precipitate was washed thoroughly with water and dried at 60° *in vacuo*. It was crystallized from ethanol, m. p. 195-200° dec.

Anal. Calcd. for $C_{28}H_{45}O_4N_3$: N, 8.6. Found: N, 7.2.

2,4-Dinitrophenylhydrazone.—To a solution of 0.2 g. of hecogenin acetate in 30 cc. of hot ethanol was added a solution of 0.2 g. of 2,4-dinitrophenylhydrazine in 50 cc. of ethanol containing 1 cc. of concentrated hydrochloric acid. Within a few minutes needles started to form. After standing at room temperature for three hours, the crystals were filtered and washed with cold ethanol, m. p. 275–276° dec. Recrystallizations from ethanol gave orange needles, m. p. 281–282° dec.

Anal. Calcd. for $C_{35}H_{48}O_8N_4$: C, 64.4; H, 7.4. Found: C, 63.9; H, 7.2.

Relationship between Hecogenin and Tigogenin.—The carbonyl group in hecogenin (3) is readily removed by a modified Wolff–Kishner reduction which eliminates the customary use of the semicarbazone, difficult to prepare in this case. The product is tigogenin (17) further identified as its acetate and by its oxidation to tigogenone (18).

Wolff–Kishner Reduction of Hecogenin.—To a solution of 3 g. of sodium in 60 cc. of absolute ethanol contained in a bomb-tube was added 0.5 g. of hecogenin acetate and 5 cc. of 85% hydrazine hydrate. The tube was sealed and heated for twelve hours at 200°. After cooling it was opened and the reaction mixture was poured into water. The precipitate was taken up in ether. The ethereal solution was washed with water, dilute hydrochloric acid and water, and then concentrated until crystals started to appear. After cooling, the crystalline material was filtered, m. p. and mixed m. p. with tigogenin, 203–206°; yield 0.3 g.

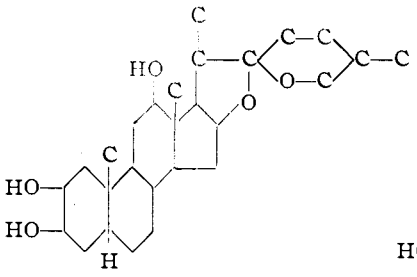
Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 77.5; H, 10.4.

An acetic anhydride solution of this material was refluxed for twenty minutes. The solvent was removed *in vacuo* on the steam-bath and the residue was crystallized from methanol as white needles, m. p. and mixed m. p. with tigogenin acetate, 204–206°.

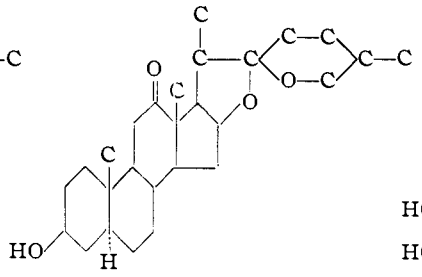
Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.0; H, 10.0.

To a solution of 0.1 g. of the unacetylated reaction product prepared above in 15 cc. of acetic acid was added a solution of 0.1 g. of chromic anhydride in 5 cc. of 80% acetic acid. The mixture was allowed to stand at room

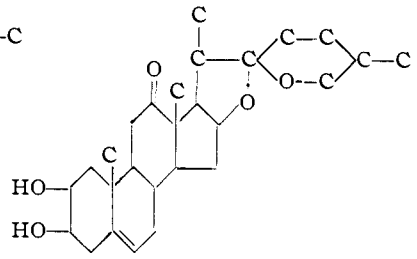
(8) Goodson and Noller, THIS JOURNAL, **61**, 2420 (1939).
(9) Liang and Noller, *ibid.*, **57**, 525 (1935).
(10) Noller, Goodson and Synerholm, *ibid.*, **61**, 1707 (1939).
(11) Jacobs and Fleck, *J. Biol. Chem.*, **88**, 545 (1930).
(12) Kiliani, *Ber.*, **24**, 339 (1891).
(13) Windaus and Schneckenberger, *ibid.*, **46**, 2628 (1913).
(14) Schmiedeberg, *Arch. exp. Path. Pharmakol.*, **3**, 16 (1875).
(15) Fujii and Matsukawa, *J. Pharm. Soc., Japan*, in German, **56**, 59 (1936); in Japanese, **56**, 408 (1936); *Chem. Zentr.*, **107**, II, 3305 (1936); *C. A.*, **30**, 6747 (1936).
(16) Windaus and Linsert, *Z. physiol. Chem.*, **147**, 275 (1925).
(17) Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936).
(18) van der Haar, *Rec. trav. chim.*, **48**, 726 (1929).
(19) Kaufman and Fuchs, *Ber.*, **56**, 2527 (1923).
(20) Power and Salway, *J. Chem. Soc.*, **105**, 201 (1914).
(21) Tschesche, *Ber.*, **69**, 1665 (1936).



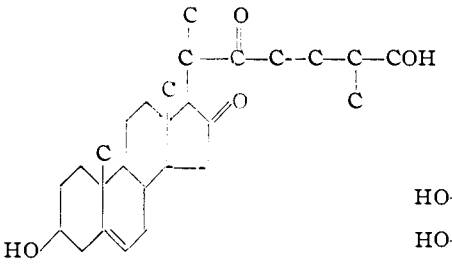
1. Agavogenin



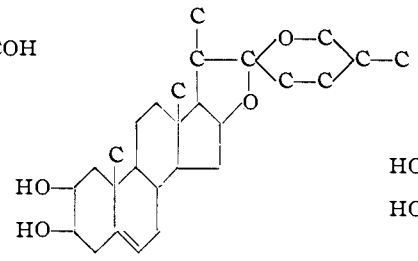
3. Hecogenin



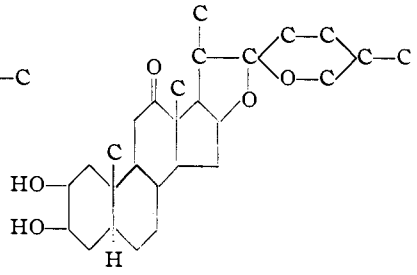
4. Kammogenin



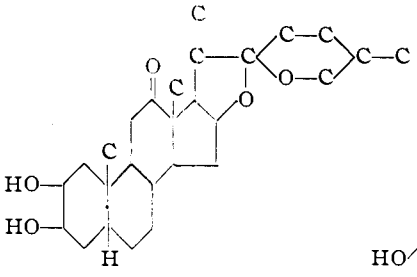
6. Kryptogenin



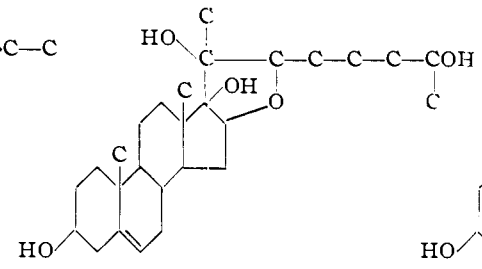
7. Lilagenin



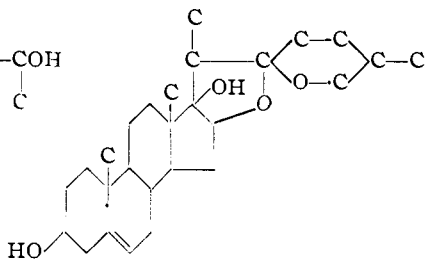
8. Manogenin



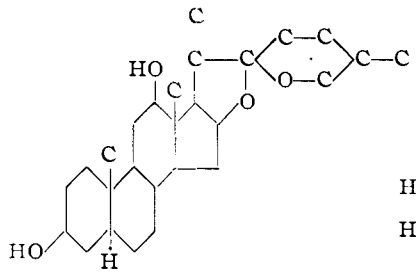
9. Mexogenin



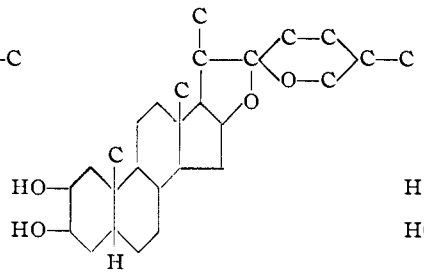
10. Nologenin



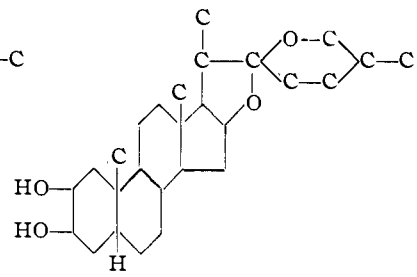
11. Pennogenin



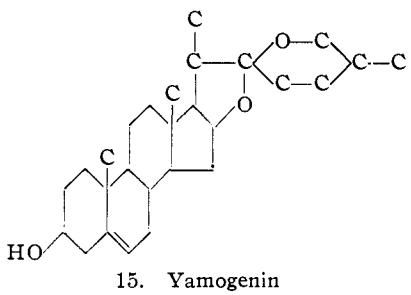
12. Rockogenin



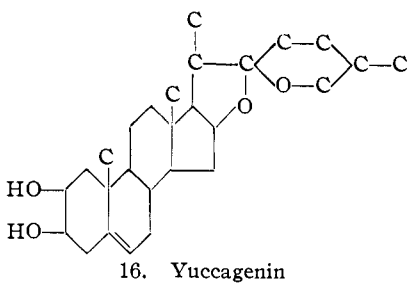
13. Samogenin



14. Texogenin

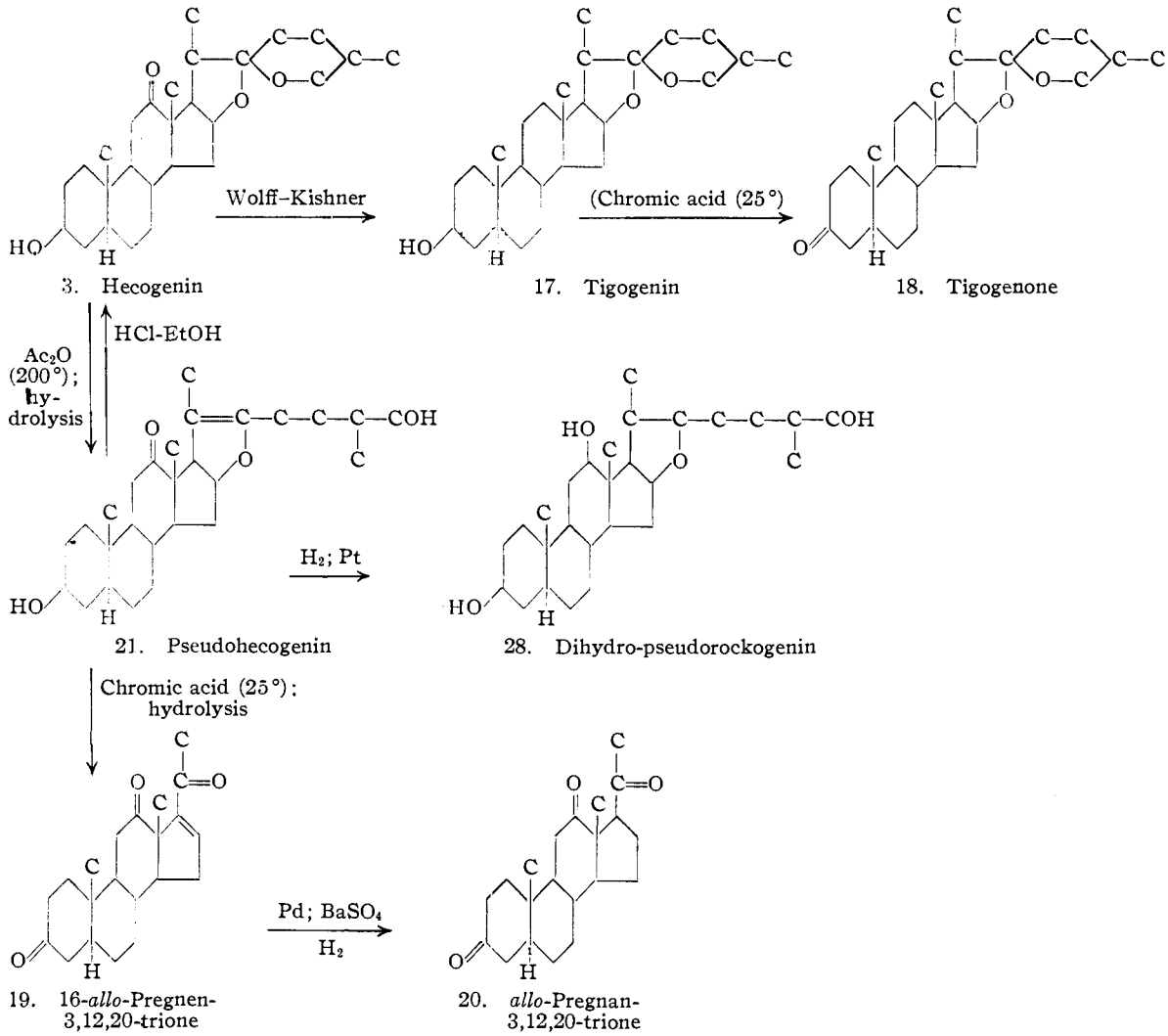


15. Yamogenin



16. Yuccagenin

temperature for one hour after which it was diluted with water and the precipitate extracted with ether. After washing with water, 5% sodium bicarbonate and water, the ether was evaporated and the residue was crystallized from acetone, m. p. and mixed m. p. with tigogenone, 204–206°.



3. Hecogenin

17. Tigogenin

18. Tigogenone

21. Pseudohecogenin

28. Dihydro-pseudorockogenin

19. 16-*allo*-Pregnen-3,12,20-trione

20. *allo*-Pregnan-3,12,20-trione

Much of the structure for hecogenin is elucidated through this degradation to tigogenin. Tigogenin has the cholestane or *allo*-configuration of the C-5 hydrogen atom,[22,23a-e] a C-3 hydroxyl group having the *beta*-configuration[23a,24] and the *iso*-configuration of the side-chain. Since the conditions of the Wolff–Kishner reaction used here on hecogenin[3] have no effect on these configurations, the nuclear structure, the position of the hydroxyl group and the side-chain configuration in hecogenin are thus settled. Finally, this experiment eliminates the possibility of unsaturation which the analysis might not exclude.

Pseudohecogenin and Pregnane Derivatives.—The fact that the ketonic group is not located in the side-chain of the molecule is indicated by the degradation of hecogenin to 16-*allo*-pregnen-3,12,20-trione (19) and *allo*-pregnan-3,12,20-trione (20) *via* pseudohecogenin (21) by the series of reactions 3 → 21 → 19 → 20.

When hecogenin (3) is heated with acetic anhydride it is isomerized to the crystalline diacetate of pseudohecogenin (21). This structure (21) has been assigned since its mode of preparation and reactions are completely analogous to the preparative method and reactions of the other pseudosapogenins and their nuclear derivatives. As is characteristic of the other pseudosapogenins,[25] pseudohecogenin (21) is easily converted to heco-

Pseudohecogenin.—A sealed tube containing 5 g. of hecogenin and 15 cc. of acetic anhydride was heated at 200° for ten hours. The solvent was removed *in vacuo* and the solid residue was treated with a solution of 5 g. of potassium hydroxide in 500 cc. of ethanol for thirty minutes. The product was ether extracted and the ethereal solution was washed and evaporated. The acetone solution of the residue after treatment with Norite was concentrated and cooled to give white needles, m. p. 189–191°; yield 2.6 g.

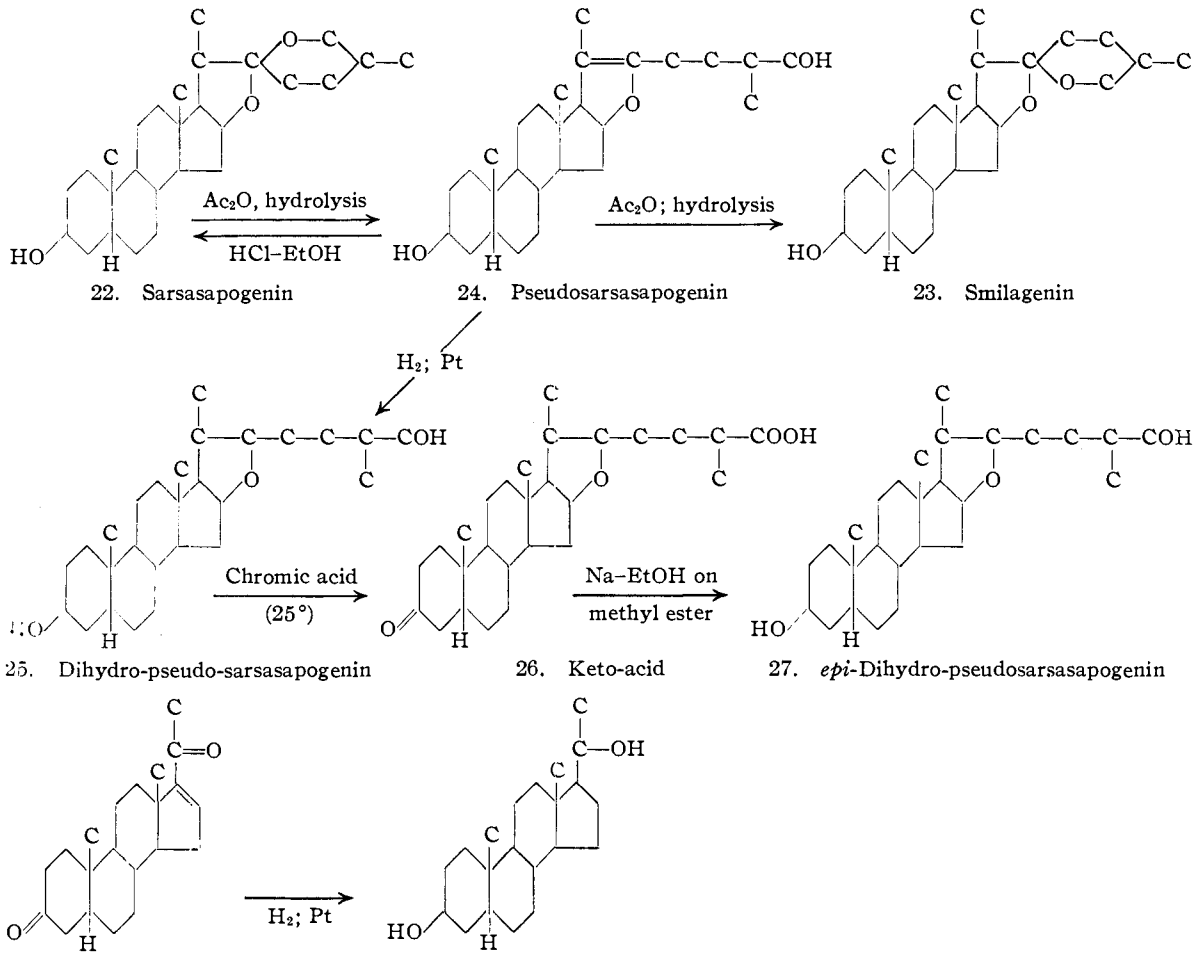
Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.2; H, 9.9.

(22) Tschesche and Hagedorn, THIS JOURNAL, **68**, 1412 (1935).

(23) (a) Marker and Rohrmann, *ibid.*, **61**, 1291 (1939); (b) **61**, 2722 (1939); (c) **62**, 76 (1940); (d) **62**, 518 (1940); (e) **62**, 521 (1940); (f) **62**, 900 (1940).

(24) Marker and Rohrmann, *ibid.*, **62**, 898 (1940).

(25) Marker and Rohrmann, *ibid.*, **62**, 896 (1940).



22. Sarsasapogenin 24. Pseudosarsasapogenin 23. Smilagenin

25. Dihydro-pseudo-sarsasapogenin 26. Keto-acid 27. *epi*-Dihydro-pseudosarsasapogenin

31. 16-*allo*-Pregnen-3,20-dione 39. *allo*-Pregnan-3(β), 20(β)-diol

genin (3) with acid; however, the diacetate of pseudohecogenin, as other pseudosapogenin acetates, is stable in acid.

Isomerization of Pseudohecogenin.—A solution of 0.5 g. of pseudohecogenin in 100 cc. of absolute ethanol was heated with 5 cc. of concentrated hydrochloric acid for ninety minutes and then allowed to stand overnight. The reaction mixture was diluted with water and the precipitated solid was filtered. Crystallization from methanol gave white plates, m. p. and mixed m. p. with the high melting form of hecogenin, 256–258°.

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

As a rule, the pseudosapogenins revert to sapogenins having the *iso*-side-chain. There is, however, a single exception. Sarsasapogenin (22) and smilagenin (23), having the normal and *iso* configuration at C-22, respectively, give the same pseudosapogenin (24); the latter goes back upon acid treatment to sarsasapogenin (22) and not to smilagenin (23). The possibility that the coprostane configuration at C-5 is responsible for this abnormal course is eliminated since we have shown that pseudotexogenin follows the general rule as discussed later.

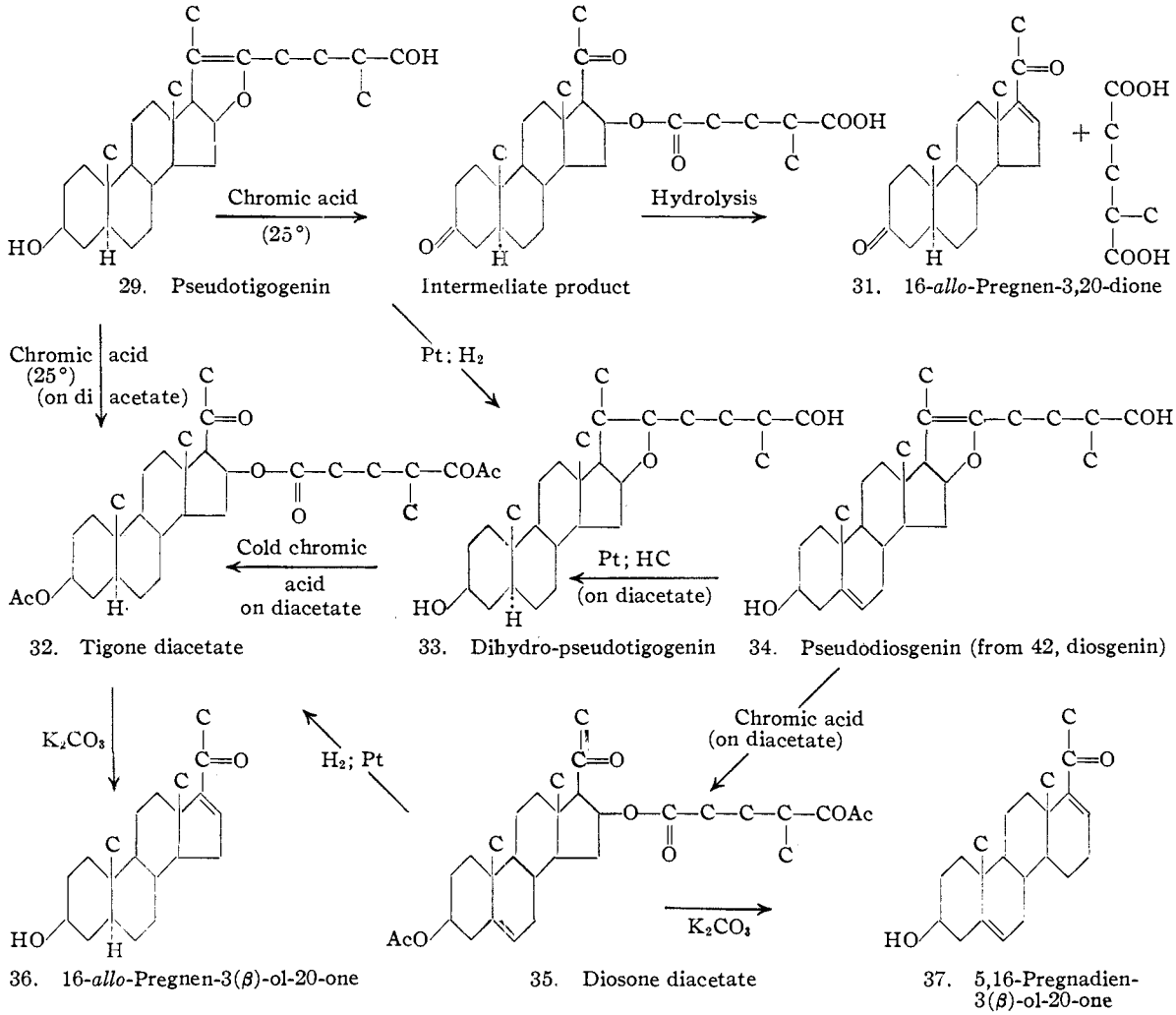
Upon catalytic reduction with Adams catalyst of the pseudosapogenins or their acetates, dihydropseudosapogenins or their acetates are formed.[26]

The function of the two alcohol groups as primary and secondary carbinols is shown by the oxidation[23d] of dihydro-pseudosarsasapogenin (25) at low temperature to a keto-acid (26). The corresponding hydroxy-acids are prepared by catalytic reduction. Bouveault reduction of the methyl ester of the keto-acid from dihydro-pseudosarsasapogenin gives *epi*-dihydro-pseudosarsasapogenin (27) also prepared by the reduction of *epi*-pseudosarsasapogenin.

Pseudohecogenin (21) also undergoes this reaction, forming dihydro-pseudorockogenin (28). The simultaneous reduction of its 12-carbonyl group forms a third alcoholic grouping.

Dihydropseudorockogenin from Pseudohecogenin.—An acetic acid solution of 1 g. of pseudohecogenin was shaken with hydrogen and 0.5 g. of Adams catalyst at room temperature and thirty pounds pressure for five hours. The mixture was filtered and the filtrate was evaporated *in vacuo* on the steam-bath. The residue was hydrolyzed with 100 cc. of 5% alcoholic potassium hydroxide solution for twenty minutes. The hydrolysis mixture was poured into water and the precipitated solid was filtered. After

(26) Marker, Turner and Ulshafer, THIS JOURNAL, **64**, 1655 (1942).



29. Pseudotigogenin — Intermediate product — 31. 16-*allo*-Pregnen-3,20-dione

32. Tigone diacetate — 33. Dihydro-pseudotigogenin — 34. Pseudodiosgenin (from 42, diosgenin)

36. 16-*allo*-Pregnen-3(β)-ol-20-one — 35. Diosone diacetate — 37. 5,16-Pregnadien-3(β)-ol-20-one

treatment with Norite, it was crystallized from acetone, m. p. 224–226°; yield, 0.3 g.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.7; H, 11.1.

Mild oxidation of pseudosarsasapogenin (24)[23d,27] and pseudotigogenin (29) at 25° with chromic acid followed by hydrolysis gives 16-pregnen-3,20-dione (30) and 16-*allo*-pregnen-3,20-dione (31), respectively, and α-methylglutaric acid. A crystalline ester intermediate (32) is obtained when the diacetate of 29 is oxidized.[28,29] The product (32) is identical with the substance obtained by similar oxidation of the diacetate of dihydro-pseudotigogenin (33). It is referred to as tigone diacetate. It is also obtained from the diacetate of pseudodiosgenin (34) by hydrogenation (Adams catalyst) of the crystalline oxidation product (35) called diosone diacetate. Upon hydrolysis with alcoholic alkali or acids, tigone and diosone diacetates yield 16-*allo*pregnen-3(β)-ol-20-one (36) and 5,16-pregnadien-3(β)-ol-20-one (37), respectively, Simultaneous dehydration of the unstable β-hydroxy ketones formed on hydrolysis results in the formation of the 16-double bond.

Mild oxidation followed by hydrolysis of unacetylated pseudohecogenin (21) gives 16-*allo*-pregnen-3,12,20-trione (19). The ester intermediate was not isolated.

16-*allo*-Pregnen-3,12,20-trione.—To a solution of 1 g. of pseudohecogenin in 40 cc. of acetic acid at 15° was added 0.9 g. of chromic anhydride dissolved in 10 cc. of 80% acetic acid. A dark brown solid precipitated. Within twenty minutes this solid material dissolved. The mixture was allowed to stand at 25° a total of ninety minutes with frequent shaking. The product was extracted with ether and the ethereal solution was washed thoroughly with water to remove the acetic acid. The ether was removed and the solid residue was refluxed with 100 cc. of a 2% alcoholic potassium hydroxide solution for thirty minutes. The mixture was cooled and ether extracted. After washing and concentrating, the ethereal solution was cooled to give fine white plates, m. p. 256–258°; yield 120 mg.

Anal. Calcd. for $C_{21}H_{28}O_3 \cdot H_2O$: C, 72.8; H, 8.7. Found: C, 72.6; H, 8.8.

By this series of reactions 3 → 21 → 19 → 20.

(27) Marker and Rohrmann, THIS JOURNAL, **61**, 3592 (1939).

(28) Marker, Turner, Wagner, Ulshafer, Crooks and Wittle, *ibid.*, **63**, 774 (1941).

(29) *Ibid.*, **63**, 779 (1941).

the side-chain of hecogenin (3) has been removed leaving a nucleus which still retains the original carbonyl oxygen. Thus, the presence of the latter in the nucleus is established. The structure of the resulting pregnenetrione is based upon its mode of formation and is assigned by analogy to the other 16-pregnene compounds.

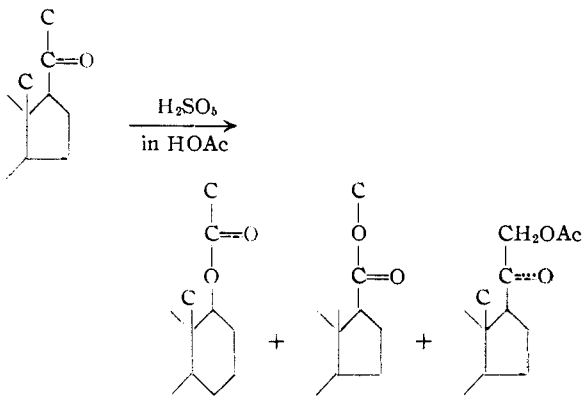
The structure proof for these pregnene compounds has been accumulated by relating them to previously known products of the pregnane series and by characteristic reactions. Thus, 16-pregnen-3,20-dione (30) from pseudosarsasapogenin (24) on reduction with sodium and ethanol gives pregnan-3(α),20(α)-diol (38), identical with the product from urine.[27,30] The complete reduction of a double bond and carbonyl group simultaneously by this method can be accomplished only when these groups are in conjugation. Reduction using Adams catalyst and hydrogen in acid medium, however, gives the 20-epimerides. Thus, catalytic hydrogenation of 16-*allo*-pregnen-3,20-dione (31) gives principally *allo*-pregnan-3(β),20(β)-diol (39). The pregnan-3,20-diols obtained by the sodium–ethanol reduction method are assigned the 20(α)-configuration and those by catalytic reduction in acid medium, the 20(β)-configuration. Reduction with hydrogen in the presence of 3% palladium–barium sulfate catalyst in neutral solvents, such as ethanol and ether, saturates the 16-double bond but does not affect the carbonyl group. In this manner, 16-pregnen-3,20-dione (30) and 5,16-pregnadien-3(β)-ol-20-one (37) give pregnan-3,20-dione (40) and 5-pregnen-3(β)-ol-20-one (41), respectively. Here again, the double bond and carbonyl group must be in conjugation in order to have reaction. The pregnene compound (19) from hecogenin has been reduced, but only in neutral medium (palladium–barium sulfate) to give *allo*-pregnan-3,12,20-trione (20).

allo-**Pregnan-3,12,20-trione.**—A solution of 50 mg. of 16-*allo*-pregnen-3,12-20-trione in 300 cc. of ether was shaken with hydrogen and one gram of 3% palladium–barium sulfate catalyst at room temperature and 3 atm. for two hours. The mixture was filtered through kieselguhr and the filtrate was concentrated to give white crystals, m. p. 261–264°. This material was insoluble in both hot and cold aqueous potassium hydroxide.

Anal. Calcd. for $C_{21}H_{30}O_3$-H_2O: C, 72.4; H, 9.3. Found: C, 72.9; H, 9.1.

Many of the pregnane compounds, readily available from the steroidal sapogenins, have been converted to progesterone and other steroidal hormones. The formation of pregnan-3,20-dione (40) and 5-pregnen-3(β)-ol-20-one (41) from sarsasapogenin (22) and diosgenin (42), respectively, takes place in excellent yields and very simply since it is not necessary to isolate the intermediates 24 and 34. The side-chain oxidation of 34 proceeds so rapidly that it can be conducted without protection of the 5–6 double bond. The former (40) is best converted to progesterone (43) by the method of Butenandt and Schmidt[31a,b,c] and the latter (41) by Oppenauer oxidation. Oxidation of 5-pregnen-3(β),20(α)-diol from (37) also furnishes progesterone.

The side-chain in the 20-ketosteroids may be removed[32–35] by employing Caro's acid, a general reaction of von Baeyer and Villiger.[36] The oxidation proceeds to introduce an oxygen atom between C-17 and C-20 or between C-20 and C-21 or introduces a new C-21 hydroxyl group:



Thus, *allo*-pregnan-20-one gives androstan-17(α)-ol acetate, *etio-allo*-cholanic acid and *allo*-pregnan-21-ol-20-one acetate; pregnan-3(α)-20-one acetate gives *etio*-cholan-3(α),17(α)-diol diacetate and *etio*-lithocholic acid; *allo*-pregnan-3(β)-ol-20-one acetate gives androstan-3(β),17(α)-diol diacetate (dihydroisoandrosterone), the acetate of 3(β)-hydroxy-*etio-allo*-cholanic acid and the diacetate of *allo*-pregnan-3(β),21-diol-20-one; and *allo*-pregnan-3(α)-ol-20-one acetate gives androstan-3(α), 17(α)-diol diacetate (dihydroandrosterone). Pregnan-3,20-dione (40) oxidized as the 4-bromodiketone with Caro's acid gives, after elimination of hydrogen bromide with pyridine, the acetate of testosterone (44) plus desoxycorticosterone (45), isolated as 3-keto-4-*etio*-cholenic acid.

Scarcity of materials and lack of time prohibited the carrying out of most of these interesting reactions on our new sapogenins.

Reactions of the Carbonyl Group in Hecogenin.—The position of the carbonyl group in hecogenin is limited to the nucleus. It has been established at C-12 by characteristic reactions and by a systematic elimination of the other possible positions.

As mentioned previously, hecogenin readily yields a pure 2,4-dinitrophenylhydrazone but not a semicarbazone, particularly when treated with semicarbazide acetate in boiling ethanol. This is indicative of a C-12 ketone, as has been shown by

(30) Marker, Kamm, Crooks, Oakwood, Lawson and Wittle, THIS JOURNAL, **59**, 2297 (1937).

(31) (a) Butenandt and Schmidt, *Ber.*, **67**, 1893 (1934); (b) *ibid.*, **67**, 1901 (1934); (c) *ibid.*, **67**, 2088 (1934).

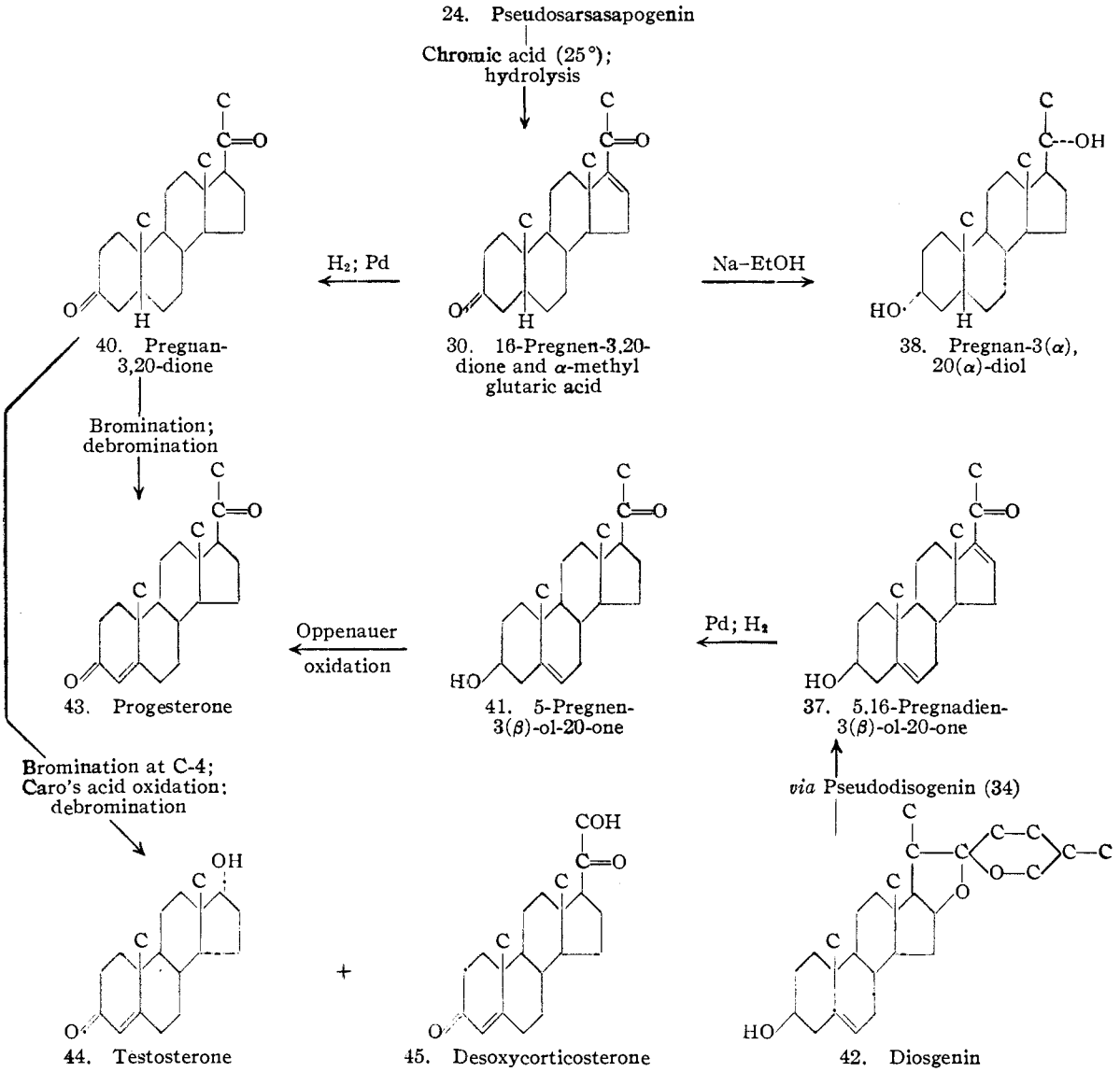
(32) Marker, Rohrmann, Wittle, Crooks and Jones, THIS JOURNAL, **62**, 650 (1940).

(33) Marker, *ibid.*, **62**, 2543 (1940).

(34) Marker, *ibid.*, **62**, 2621 (1940).

(35) Marker and Turner, *ibid.*, **62**, 3003 (1940).

(36) von Baeyer and Villiger, *Ber.*, **32**, 3625 (1899); **33**, 858 (1900).



the work of Dutcher and Wintersteiner.[37] These investigators found that the same treatment of 12-ketocholanic acid and certain bile acids having a 12-keto group, such as dehydrocholic acid (3,7,12-triketocholanic acid) and dehydrodesoxycholic acid (3,12-diketocholanic acid) with semicarbazide acetate is also unsatisfactory. However, they found that a mixture of the reactants in pyridine–alcohol according to the procedure of Haller and LaForge[38] gives better results. Hecogenin (3) also reacts more favorably under these conditions although the semicarbazone formation is incomplete as shown by the low nitrogen content of the analytical sample. In contrast to the relative inertness of 12-keto groups, 3- and 7-keto groups react readily.

Mild catalytic or chemical reduction of hecogenin (3) or its acetate forms a second hydroxy group. This hydroxyl group can be acetylated by *prolonged* refluxing with acetic anhydride to give the diacetate of 12-dihydrohecogenin (rockogenin) (12).

Rockogenin from Hecogenin.—1. An ethereal solution of 0.3 g. of hecogenin containing several drops of acetic acid was shaken with hydrogen and 0.2 g. of Adams catalyst for two hours at room temperature and three atms. After filtering, the solvent was removed. The oily residue was refluxed with acetic anhydride for one hour. The acetic anhydride was removed *in vacuo* on the steam-bath and 12-dihydrohecogenin diacetate was crystallized from methanol as long needles, m. p. 204–206°; yield, 0.12 g. A mixture with natural rockogenin diacetate (206°) melted 204–206°.

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

When hydrolyzed with 5% alcoholic potassium hydroxide for twenty minutes on the steam-bath, the diacetate was converted to rockogenin which crystallized from methanol as thick needles, m. p. 208–210°. Re-

(37) Dutcher and Wintersteiner, THIS JOURNAL, 61, 1998 (1939).
(38) Haller and LaForge, *J. Org. Chem.*, 1, 38 (1936).

peated crystallizations from ether gave material melting 217–220°.

A mixture with rockogenin (220°) from *Agave gracilipes* Trel. melted at 218–220°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.5; H, 10.2.

2. An ethereal solution of 0.5 g. of hecogenin acetate was shaken with hydrogen and Adams catalyst as described above. 12-Dihydrohecogenin 3-monoacetate crystallized from methanol as white plates, m. p. 211–213°,

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.4; H, 9.8. Found: C, 73.8; H, 9.7.

When refluxed with acetic anhydride for one hour it formed 12-dihydrohecogenin diacetate, m. p. and mixed m. p. 203–205°.

3. To a solution of 300 mg. of hecogenin in 100 cc. of absolute ethanol was added 7 g. of sodium in small strips over a period of thirty minutes. The mixture was cooled and poured into water. The precipitated solid was extracted with ether and the ethereal solution was washed with water, 10% hydrochloric acid and water. The ethereal solution after concentration and cooling gave white crystals, m. p. 238-244°, wt. 160 mg. Recrystallization from ether gave starting material melting 260-262°; a mixture with hecogenin (265°) melted 262-265°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 74.9; H, 9.8.

The first mother liquor was evaporated to dryness. The residue was recrystallized from methanol as white crystals, m. p. and mixed m. p. with 12-dihydrohecogenin, 209–210°; yield 80 mg.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.9; H, 10.4.

The acetate was prepared with boiling acetic anhydride and the product was crystallized from methanol as white needles, m. p. and mixed m. p. with 12-dihydrohecogenin diacetate, 205–207°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.2; H, 9.4.

The fact that the newly formed hydroxyl group is acetylated with difficulty is noteworthy. Wieland and Kapitel[39] have shown that cholic acid can be partially acetylated whereby the 3- and 7-hydroxyl groups are readily attacked, but the 12-hydroxyl is unchanged. Their experiment illustrates the decreased reactivity of the hydroxyl group at C-12 compared to one at C-7 or C-3. In fact the order of reactivity $C_3 > C_7 > C_{12}$ in the bile acid series holds not only for acetylation but also for hydrolysis, oxidation, reduction and hydrogenation.[40] The acetylation and other reactions of 12-dihydrohecogenin (12) give support to this relationship. Steiger and Reichstein[41a,b] have shown that a carbonyl or hydroxyl group at C-11 is unreactive. Since hecogenin (3) and 12-dihydrohecogenin (12) form a semicarbazone and a diacetate, respectively, position-11 is improbable for the carbonyl or hydroxyl group.[42]

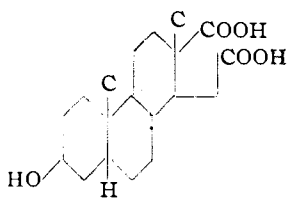
Catalytic hydrogenation of 12-dihydrohecogenin (12) under more vigorous conditions gives dihydrorockogenin (46).

Dihydrorockogenin.—A solution of 2 g. of hecogenin acetate in 125 cc. of acetic acid was shaken for nine hours at 70° with hydrogen (3 atm.) and 1 g. of Adams catalyst. After filtering, the acetic acid was removed *in vacuo* on the steam-bath and the residue was hydrolyzed with 100 cc. of 10% alcoholic potash for twenty minutes. The hydrolysis mixture was poured into water and ether extracted. The ethereal solution was washed with water and evaporated to dryness. The residue was then crystallized from acetone to give dihydrorockogenin, m. p. 212–215°.

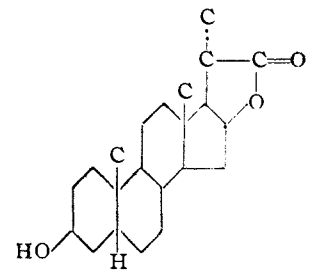
Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.5; H, 10.6.

This material formed a non-crystalline acetate and benzoate.

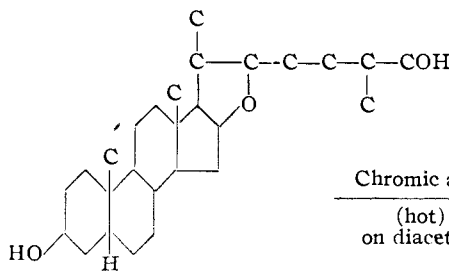
The structure assigned to dihydrorockogenin (46) is analogous to the structures of other dihydrosapogenins such as dihydrotigogenin (100), prepared in like manner.[42a] This substance (47) forms a dibenzoate and gives a monoketo-acid upon mild oxidation, proving the presence of a pri-



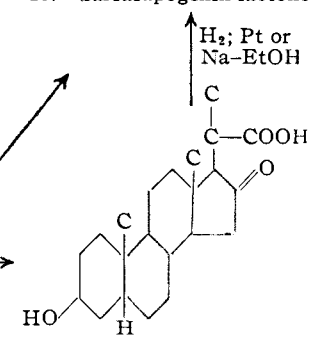
50. 3(β)-Hydroxy-*etio*-bilianic acid



48. Sarsasapogenin lactone



47. Dihydrosarsasapogenin



49. C_{22}-16-keto-acid

(39) Wieland and Kapitel, *Z. physiol. Chem.*, **212**, 269 (1932).

(40) Fieser, "Chemistry of Natural Products Related to Phenanthrene," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1937.

(41) (a) Steiger and Reichstein, *Helv. Chim. Acta*, **20**, 817 (1937); (b) *ibid.*, **20**, 1163 (1937).

(42) Recently it has been reported by Gallagher and Long (*J. Biol. Chem.*, **162**, 511, 521 (1946)) that the unreactivity of the 11-OH group in the bile acid series depends upon its spatial configuration. Thus the 11-(α) hydroxy compounds form acetates readily, while the *beta*-epimers are resistant to acetylation.

(42a) Marker and Rohrmann, THIS JOURNAL, **61**, 1516 (1939).

mary and a secondary hydroxyl group. The dihydrosapogenins are unaffected by bromine in acetic acid or by selenium dioxide, showing the absence of any ketone group. Vigorous oxidation studies[23a,43] further support their structure. Thus, the diacetate of dihydrosarsasapogenin (47) is stable to mild oxidation but at elevated temperature it is oxidized with chromic acid to sarsasapogenin lactone (48), 3(β)-hydroxy-16-ketobisnor-cholanic acid (49) and 3(β)-hydroxy-*etio*-bilianic acid (50). The first acid (49) is converted to the lactone (48) by reduction with sodium in alcohol or by catalytic hydrogenation, giving strong support for its structure. The 16-keto acid (49) also forms a monosemicarbazone and a monomethyl ester, establishing the presence of the corresponding functional groups.

Although the formulas for the dihydrosapogenins are the same as those used for the dihydropseudosapogenins, the two series of compounds have entirely different chemical properties. This difference is probably due to different stereochemical configurations of the side-chains.

Comparison of Hecogenone with Chlorogenone.—Mild oxidation of either 12-dihydrohecogenin (12) or hecogenin (3) gives hecogenone (51) which is non-identical but isomeric with chlorogenone (52).

Hecogenone.—A solution of 0.1 g. of hecogenin in 30 cc. of acetic acid was mixed with a solution of 0.1 g. of chromic anhydride in 5 cc. of 80% acetic acid. After standing thirty minutes at 25°, water was added and the product was extracted with ether. An alkali wash of the ethereal solution followed by acidification of the aqueous layer gave no acid fraction. The ethereal solution was concentrated and cooled to give white needles, m. p. 237–240°. A mixture with chlorogenone (237°) melted 228–231°.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.5; H, 9.4.

12-Dihydrohecogenin, 0.2 g., dissolved in 25 cc. of acetic acid was oxidized with a solution of 0.15 g. of chromic anhydride in 10 cc. of 80% acetic acid for thirty minutes at 20°. The product was isolated as described above and crystallized from ether as white needles, m. p. and mixed m. p. with hecogenone from above, 237–240°; yield 0.1 g.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.5; H, 9.4.

The semicarbazone was prepared by the alcohol-pyridine procedure and crystallized from ethanol, m. p. 200–210° dec. The following analysis of this compound indicates that it is not pure. Probably it is contaminated with some of the starting material.

Anal. Calcd. for $C_{29}H_{46}O_4N_6$: C, 64.2; H, 8.5. Found: C, 66.8; H, 8.9.

The degradation of hecogenin (3) to tigogenin (17) by the Wolff–Kishner method has been discussed. In a like manner chlorogenone (52) has been converted to this common product (17) with the exception that the disemicarbazone was treated.[44] These experiments are in accordance with the observations of Dutcher and Wintersteiner[45] that the Wolff–Kishner reduction of steroidal 3-semicarbazones under these conditions yields mainly the carbinols at C-3, whereas the Wolff–Kishner reaction on 6-, 7- and 12-semicarbazones yields methylene groups.

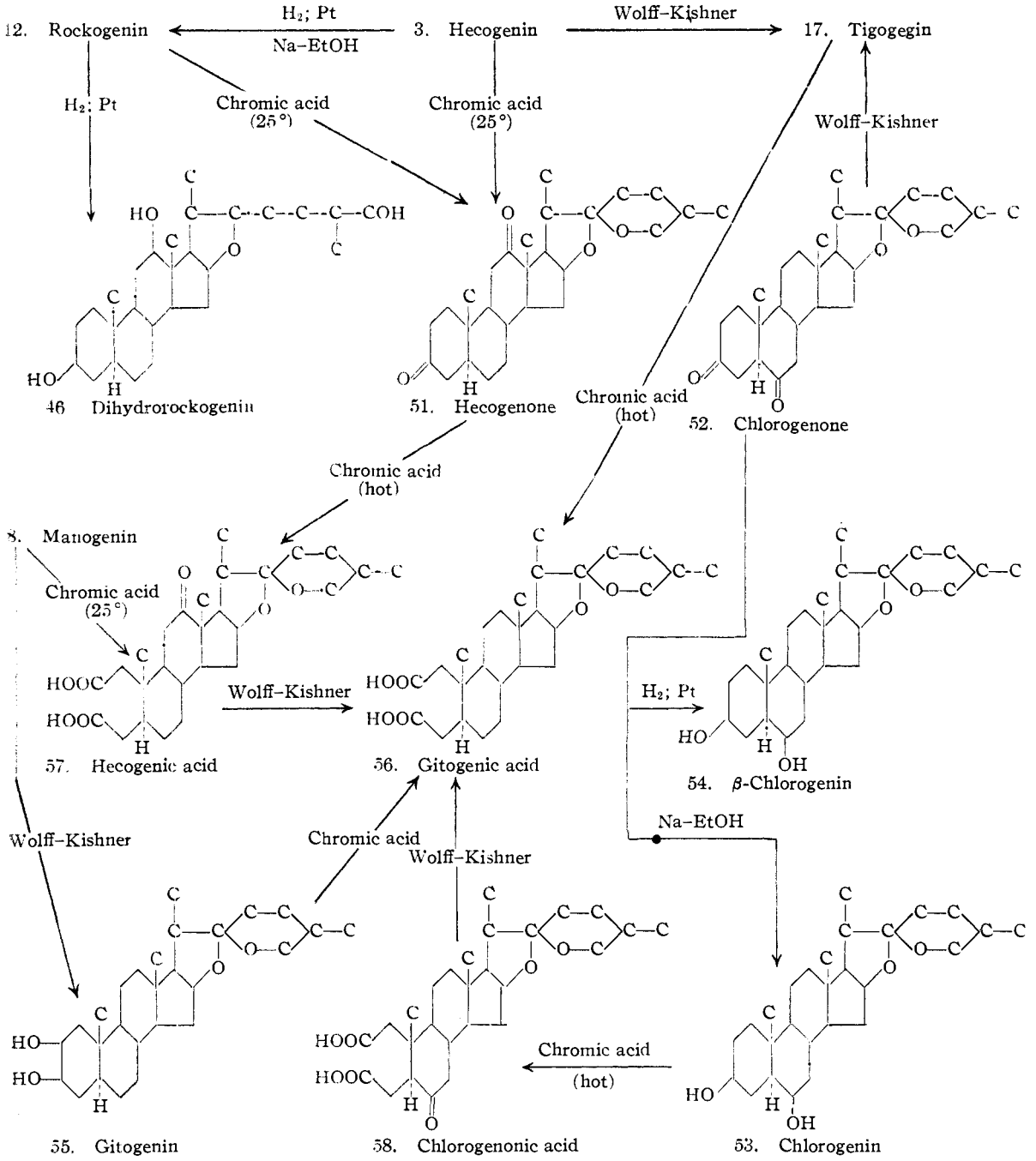
Since both hecogenin and chlorogenone can be converted to tigogenin, the position of one oxygen atom in each is established at C-3 and the compounds differ only in respect to the position of the second oxygen group. Since chlorogenone (52) has been shown to be a 3,6-diketo compound,[42-50] the position of the other hydroxyl group in hecogenin cannot be at C-6.

Reduction of the carbonyl group in hecogenin (3) by sodium and alcohol or catalytically takes the same stereochemical course resulting in both cases in the same product. Although no evidence has been obtained for the configuration of this second hydroxyl group, the fact that the C-3 hydroxyl group has the *beta* configuration makes it possible to compare 12-dihydrohecogenin with chlorogenin (53) and β-chlorogenin (54). These latter compounds are the respective epimeric C-6 hydroxy-tigogenins obtained by the sodium in alcohol or catalytic reduction of chlorogenone (52).[51] This comparison has been made and 12-dihydrohecogenin (12) or its acetate is not identical with either chlorogenin or *beta*-chlorogenin or their acetates. Accordingly, the non-identity of the diol (12), its diacetate and the diketone (51) from hecogenin (3) with the corresponding derivatives from chlorogenin (53) eliminates position-6 for the carbonyl oxygen in the former. These differences have been established by melting point and mixed melting point determinations. In addition a distinct melting point depression is observed when hecogenin (3) is mixed with 6-keto-tigogenin.

Ring Cleavage of Hecogenin; Comparison with Gitogenin.—Although 12-dihydrohecogenin (12) is isomeric with gitogenin (55), each gives entirely different products on mild oxidation. Gitogenin (55) having adjacent hydroxyl groups at C-2 and C-3 is readily oxidized by a cold chromic acid–acetic acid mixture to gitogenic acid (56) by a 2||3 cleavage of the ring. Further, it is logical to assume that oxidative cleavage of the ring would occur if the hydroxyl groups were at C-3 and C-4. Since there are no acid products formed in the mild oxidation of 12-dihydrohecogenin (12), the carbonyl group in hecogenin cannot be at C-2 or C-4.

Mild Oxidation of 12-Dihydrohecogenin 3-Monoacetate.—A solution of 0.2 g. of 12-dihydrohecogenin 3-monoacetate in 25 cc. of acetic acid was treated with 0.2 g. of chromic anhydride dissolved in 5 cc. of 80% acetic acid. After standing at room temperature for thirty minutes,

(43) Marker and Rohrmann, THIS JOURNAL, **61**, 3477 (1939).
(44) Marker, Turner and Ulshafer, *ibid.*, **62**, 3009 (1940).
(45) Dutcher and Wintersteiner, *ibid.*, **61**, 1992 (1939).
(46) Tsukamoto, Ueno, Ohta and Tschesche, *J. Pharm. Soc. Japan*, in German, **57**, 283 (1937); in Japanese, **57**, 985 (1937); *C. A.*, **32**, 2537 (1937).
(47) Marker and Rohrmann, THIS JOURNAL, **61**, 946 (1939).
(48) Marker and Rohrmann, *ibid.*, **61**, 3479 (1939).
(49) Marker, Jones and Krueger, *ibid.*, **62**, 2532 (1940)
(50) Marker, Jones, Turner and Rohrmann, *ibid.*, **62**, 3006 (1940).
(51) Marker, Turner and Wittbecker, *ibid.*, **64**, 221 (1942).



the product was precipitated with water and ether extracted. The ethereal solution was washed with 10% sodium bicarbonate and then water. Acidification of the alkaline wash gave no precipitate, indicating the absence of steroidal acids. The ethereal solution was concentrated and cooled to give white flat crystals, m. p. and mixed m. p. with the high or low melting form of hecogenin acetate, 246-248°; yield, 0.1 g.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.7; H, 9.2.

The oxidation of hecogenin (3) at higher temperatures gives, in addition to hecogenone (51), a mixture of acids which contains hecogenic acid (57).

Oxidation of Hecogenone to Hecogenic Acid.—To a solution of 7 g. of hecogenone in 400 cc. of glacial acetic acid was added a solution of 5 g. of chromic anhydride in 50 cc. of 90% acetic acid. The mixture was kept at 55° for two and one-half hours. Water was added and the product was extracted with ether. The ethereal solution was washed thoroughly with water and then with dilute alkali. The alkali wash was acidified and the precipitated hecogenic acid was collected in ether and crystallized from a small volume of ether, m. p. 267-268° dec.; wt. 0.5 g. A mixture with the diacid (269°) from manogenin melted 267-269° dec.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.5. Found: C, 68.3; H, 8.3.

A solution of 0.3 g. of the above acid in 100 cc. of ether was treated with excess diazomethane in ether at 0° for forty hours. The methyl ester crystallized from ether as white plates, m. p. 184–186°. A mixture with the dimethyl ester of the diacid from manogenin melted 184–186°.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.0; H, 8.8. Found: C, 69.3; H, 8.5.

Hecogenic acid is formed by the 2||3 cleavage of the ring in the same manner that gitogenic acid (56) is formed from tigogenin (17)[52] or chlorogenonic acid (58) from chlorogenin.[53] The opening of the ring at this position is common for the sterols and the bile acids of the *allo* series. For example, cholestan-3(β)-ol gives 2/3-cholestan-2,3-diacid,[54,55] whose structure has been established with certainty, and 3(β)-hydroxy-*allo*-cholanic acid gives Staden's acid (2/3-*allo*-cholan-2,3,24-triacid).[56] Chlorogenonic acid (58) has been reduced to gitogenic acid (56) by the Wolff–Kishner method.[57] In a like manner, hecogenic acid (57) has been converted to this same product (56). The two keto-acids, however, are not the same as shown by the wide difference in the melting points of the acids themselves and their dimethyl esters.

Gitogenic Acid from Hecogenic Acid.—Hecogenic acid, 0.5 g., was reduced by the Wolff–Kishner method under the conditions described for the reduction of hecogenin to tigogenin. The reaction mixture was acidified and extracted with ether. The ethereal solution was washed with water and evaporated. Gitogenic acid crystallized, m. p., and mixed m. p., 247°.

Non-identity of Hecogenin with 7-Ketotigogenin.—The remaining positions possible for the carbonyl group in hecogenin are C-7 and C-15. The latter position is improbable for reasons discussed under **agavogenin.** If hecogenin acetate had the carbonyl group at C-7, then it would be identical with 7-ketotigogenin acetate (59). We have now established the non-identity of these two compounds. For this purpose,[58] 7-keto-23-bromodiosgenin acetate (60) is prepared by the oxidation of 23-bromodiosgenin acetate (61) under the conditions employed for the preparation of 7-keto-Δ^5-steroids.[59] Because of the relative stability of the brominated side-chain to oxidation, 23-bromodiosgenin acetate (61) is more readily converted to the keto compound (60) than is diosgenin (42) acetate.[58] Selective hydrogenation of the α,β-unsaturated ketone system in 60 followed by debromination gives 7-ketotigogenin acetate (59), further identified by its hydrolysis product, oxidation product (62), and Wolff–Kishner reduction to tigogenin (17).

7-Ketotigogenin.—A solution of 1 g. of 7-keto-23-bromodiosgenin acetate in 150 cc. of ether was shaken with 2 g. of palladium–barium sulfate catalyst and hydrogen at three atm. for two hours. The solution was filtered and the filtrate evaporated to a volume of 20 cc. The bromo-compound crystallized as needles, m. p. 213° dec.; yield 450 mg. This product was dissolved in 50 cc. of acetic acid and heated with 3 g. of zinc dust for 15 hours. Water was added and the solid extracted with ether. The ether was washed with water, sodium carbonate solution and finally water. The 7-ketotigogenin acetate crystallized from a small volume of ether as plates, m. p. 216–218°; yield 70 mg. A mixture with hecogenin acetate melted 213–215°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.5; H, 9.2.

Hydrolysis of the acetate with alcoholic potassium hydroxide solution gave 7-ketotigogenin which crystallized from a small volume of ether as tiny plates, m. p. 214–215°.

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

7-Ketotigogenone.—To a solution of 0.2 g. of 7-ketotigogenin was added 0.18 g. of chromic anhydride in 10 cc. of 90% acetic acid. After standing at room temperature for thirty-five minutes, the reaction mixture was worked up in the usual manner. 7-Ketotigogenone crystallized from ether as plates, m. p. 240-242°; a mixture with hecogenone melted 216-219°.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.5; H, 9.2.

Since the elimination of the C-7 position for the carbonyl group in hecogenin (3) depends so much on its non-identity with 7-ketotigogenin, we have further established the source of the latter. For this purpose, 7-keto-23-bromodiosgenin acetate (60) is debrominated to 7-ketodiosgenin acetate (63). The latter is identical with the product obtained by the direct oxidation of diosgenin acetate.[58]

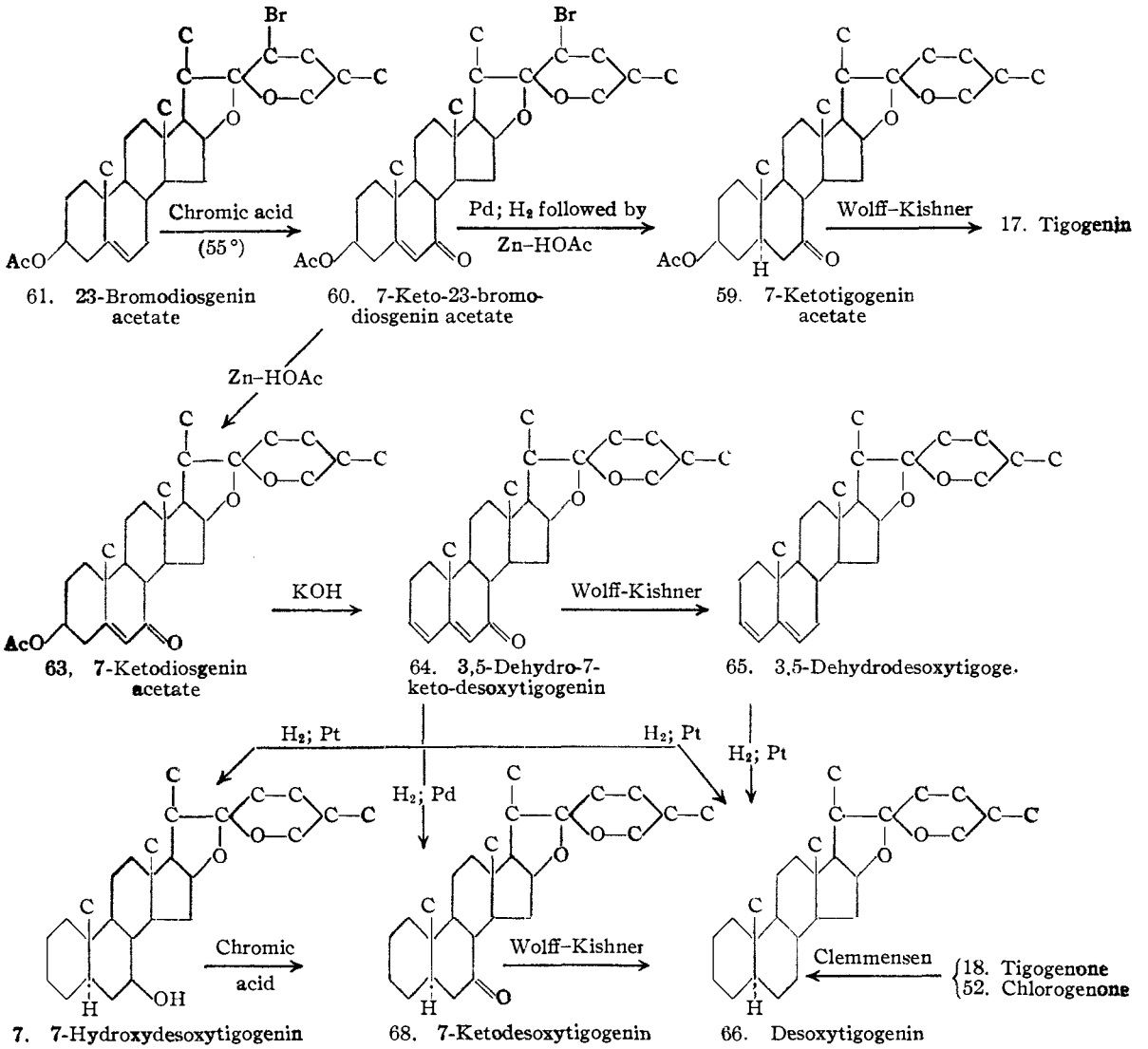
7-Ketodiosgenin Acetate.—A solution of 0.5 g. of 7-keto-23-bromodiosgenin acetate in 50 cc. of acetic acid was treated with 3 g. of zinc dust at steam-bath temperature for five hours. The reaction mixture was extracted with ether and the ethereal solution was washed thoroughly with water, 10% sodium bicarbonate and water. The product was crystallized from pentane, m. p. and mixed m. p. with 7-ketodiosgenin acetate prepared by the oxidation of diosgenin acetate 197°; yield 0.2 g.

Anal. Calcd. for $C_{29}H_{42}O_5$: C, 74.0; H, 9.0. Found: C, 73.8; H, 9.0.

It has been shown that treatment of 7-ketodiosgenin acetate (63) with ethanolic potash results in dehydration with the formation of 3,5-dehydro-7-ketodesoxytigogenin (64). This is analogous to the behavior of 7-ketocholesteryl acetate which gives a cholestadien-7-one[60] assigned the structure 7-keto-3,5-cholestadiene by Stavely and Bergmann[61] who reduced it by the Wolff-Kishner method to 3,5-cholestadiene. By analogy the Wolff-Kishner reduction of the ketodiene (64) from 7-ketodiosgenin gives 3,5-dehydrodesoxytigogenin (65),

3,5-Dehydro-7-ketodesoxytigogenin.—A solution of 0.15 g. of 7-ketodiosgenin acetate in 45 cc. of ethanol containing 7 g. of potassium hydroxide was heated on the steam-bath for fifteen minutes. During the first few minutes the mixture turned from a light amber to a dark green color. The reaction mixture was extracted with

(52) Tschesche and Hagedorn, *Ber.*, **68**, 1090 (1935).
(53) Noller, THIS JOURNAL, **59**, 1092 (1937).
(54) Windaus, *Ann.*, **447**, 233 (1926).
(55) Windaus, van Staden and Seng, *Z. physiol. Chem.*, **117**, 146 (1921).
(56) Wieland, Dane and Martius, *ibid.*, **215**, 15 (1933).
(57) Noller and Lieberman, THIS JOURNAL, **63**, 2131 (1941).
(58) Marker, Turner, Shabica and Ulshafer, *ibid.*, **63**, 1032 (1941).
(59) Windaus, Lettré and Schenck, *Ann.*, **520**, 98 (1935).
(60) Mauthner and Suida, *Monatsh.*, **17**, 579 (1896).
(61) Stavely and Bergmann, *J. Org. Chem.*, **1**, 567 (1936).



ether and the solution was washed thoroughly with water. The product was crystallized from an ether–pentane mixture and then washed with cold pentane, m. p. and mixed m. p. with 3,5-dehydro-7-ketodesoxytigogenin, 206–208°.

Anal. Calcd. for $C_{27}H_{38}O_3$: C, 79.0; H, 9.3. Found: C, 78.7; H, 9.3.

The identity of 3,5-dehydro-7-ketodesoxytigogenin (64) is further established by its catalytic reduction to a mixture of desoxytigogenin (66) and 7-hydroxydesoxytigogenin (67). For their separation, the mixture is treated with succinic anhydride. The non-carbinol fraction contains the former and the carbinol fraction obtained by subsequent hydrolysis of the half-succinate gives the latter. 7-Hydroxydesoxytigogenin (67) is readily oxidized to 7-ketodesoxytigogenin (68). This same material is the principal product from the hydrogenation (palladium–barium sulfate) of 3,5-dehydro-7-ketodesoxytigogenin (64). It is further identified by conversion by the Wolff–Kishner method to desoxytigogenin (66), obtained by the Clemmensen reductions of chlorogenone (52) and tigogenone (18). It is not identical with 12-keto-desoxytigogenin (69), the product obtained from the mild Clemmensen reduction of hecogenone (51). This fact is further proof that the carbonyl group in hecogenin is not at C-7.

7-Ketodesoxytigogenin.—An ethereal solution of 3,5-dehydro-7-ketodesoxytigogenin from the reaction of 0.9 g. of 7-keto-diosgenin acetate with alcoholic potash was shaken with hydrogen and 1 g. of palladium–barium sulfate catalyst at room temperature and 3 atm. for three hours. The reaction mixture was filtered and the product which separated from ether was crystallized from acetone as white plates, m. p. 202–204°; yield 0.4 g.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 77.9; H, 9.8.

Wolff–Kishner Reduction of 7-Ketodesoxytigogenin.—A mixture of 0.27 g. of 7-ketodesoxytigogenin, sodium ethylate and hydrazine hydrate reacted as described previously for hecogenin. Desoxytigogenin was crystallized from acetone as white plates, m. p. and mixed m. p., 173–175°.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.9; H, 11.1. Found: C, 81.2; H, 11.0.

Reduction and Subsequent Oxidation of 3,5-Dehydro-7-ketodesoxytigogenin.—A solution of 3,5-dehydro-7-ketodesoxytigogenin, 1 g., in 150 cc. of ether acidified with several drops of acetic acid was shaken with 0.5 g. of Adams catalyst and hydrogen at three atm. for two hours. The solution was filtered and the solvent removed from the filtrate.

To a solution of the crystalline residue in 75 cc. of acetic acid cooled to 20° was added a solution of 0.2 g. of chromic anhydride in 20 cc. of 90% acetic acid. The temperature was maintained at 25° for forty minutes. Water was then added and the solid which precipitated was extracted with ether. The ether was washed with water and the acids were removed with dilute potassium hydroxide. After washing with water, the ethereal solution was evaporated and the residue weighing 386 mg. was treated with 2 g. of Girard's reagent T in 50 cc. of ethanol. This was worked up in the usual manner to give 165 mg. of ketonic material which crystallized from a small volume of acetone as large, thin plates, m. p. 194–195°; a mixture with 7-ketodesoxytigogenin (204°) from above melted 200–204°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.1; H, 10.1.

In addition, 200 mg. of non-ketonic material was obtained which crystallized from acetone, m. p. and mixed m. p. with desoxytigogenin, 170–171°.

The products from the hydrogenation of our ketodiene (64) are analogous in structure to those obtained from the catalytic hydrogenation of 7-keto-3,5-cholestadiene. Hydrogenation[62] of the latter using palladium catalyst gives cholestan-7-one while hydrogenation[63] using Adams catalyst gives cholestane.

Clemmensen Reduction and Bromine Derivatives of Hecogenin.—Hecogenin is not affected by treatment with unamalgamated zinc and hydrochloric acid in ethanol. This mild Clemmensen reaction reduces 7-keto groups to the methylene group. For example, cholestan-7-one is readily reduced to cholestane.[48] In a like manner, the 6-keto group is removed. Thus, cholestan-3,6-dione reacted under these conditions gives cholestane[64] and chlorogenone (52) is converted to desoxytigogenin (66). On the other hand, a carbonyl group at C-12 is not reduced under these mild conditions. Windaus and Schlichting[65] reduced the 3,12-diketo acid, dehydrodesoxycholic acid, to 12-ketocholanic acid using unamalgamated zinc. In the same manner, hecogenone (51) upon mild Clemmensen treatment retains the 12-carbonyl oxygen to give 12-ketodesoxytigogenin (69). These experiments again show the relative decreased reactivity of a C-12 substituent as compared with C-3 and C-7.

Attempted Mild Clemmensen Reaction with Hecogenin.—To an alcoholic solution of 1.2 g. of hecogenin in 200 cc. of ethanol was added 20 g. of 20-mesh zinc. The mixture was heated under a reflux and treated with 5 cc. portions of concentrated hydrochloric acid every thirty minutes for ten hours. The reaction mixture was poured into water and ether extracted. After washing with water, the ether was concentrated and cooled to give crystals, m. p. and mixed m. p. with hecogenin (253°), 250–253°; wt. 0.9 g.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.4; H, 9.9.

Manogenin (8) and mexogenin (9) when treated in the the same manner were unchanged.

Clemensen Reduction of Hecogenone.—To a solution of 10 g. of hecogenone in 200 cc. of ethanol was added 100 g. of 20-mesh zinc and 20 cc. of concentrated hydrochloric acid. The mixture was refluxed on the steam-bath for one hour and then 10 cc. of concentrated hydrochloric acid was added every hour for five hours. An additional 100 g. of zinc and 200 cc. of ethanol was added and 20 cc. of acid was added every hour for another five hours. The reaction mixture was filtered and the filtrate was diluted with water. The precipitate was filtered and heated with 10 g. of Girard reagent T in the usual manner. The ketone fraction was crystallized from acetone to give 12-ketodesoxytigogenin m. p. 196–198°; yield 1 g. A mixture with 7-ketodesoxytigogenin melted 170–175°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.2; H, 10.1.

Hecogenin (3) reacts with bromine to give 23-bromohecogenin (70) which is readily reconverted to hecogenin by reaction with zinc and acetic acid.

23-Bromohecogenin Acetate.—To a solution of 2 g. of hecogenin acetate in 20 cc. of chloroform was added at room temperature 1.36 g. of bromine in 10 cc. of chloroform. The chloroform was evaporated *in vacuo* at room temperature and the product was crystallized from ether as crystals, m. p. 234° dec. Yield was 1.6 g.

Anal. Calcd. for $C_{29}H_{43}O_5Br$: C, 63.1; H, 7.9. Found: C, 63.0; H, 7.8.

A solution of 1 g. of 23-bromohecogenin acetate in 100 cc. of acetic acid was heated with 6 g. of zinc dust for thirty minutes. The reaction mixture was then cooled, filtered and diluted with ether. The acetic acid was removed with an alkali wash and the ethereal solution was evaporated to yield hecogenin acetate, m. p. and mixed m. p., 240–241°.

Hydrolysis of the acetate gave hecogenin which crystallized from ether, m. p. and mixed m. p., 245–247°.

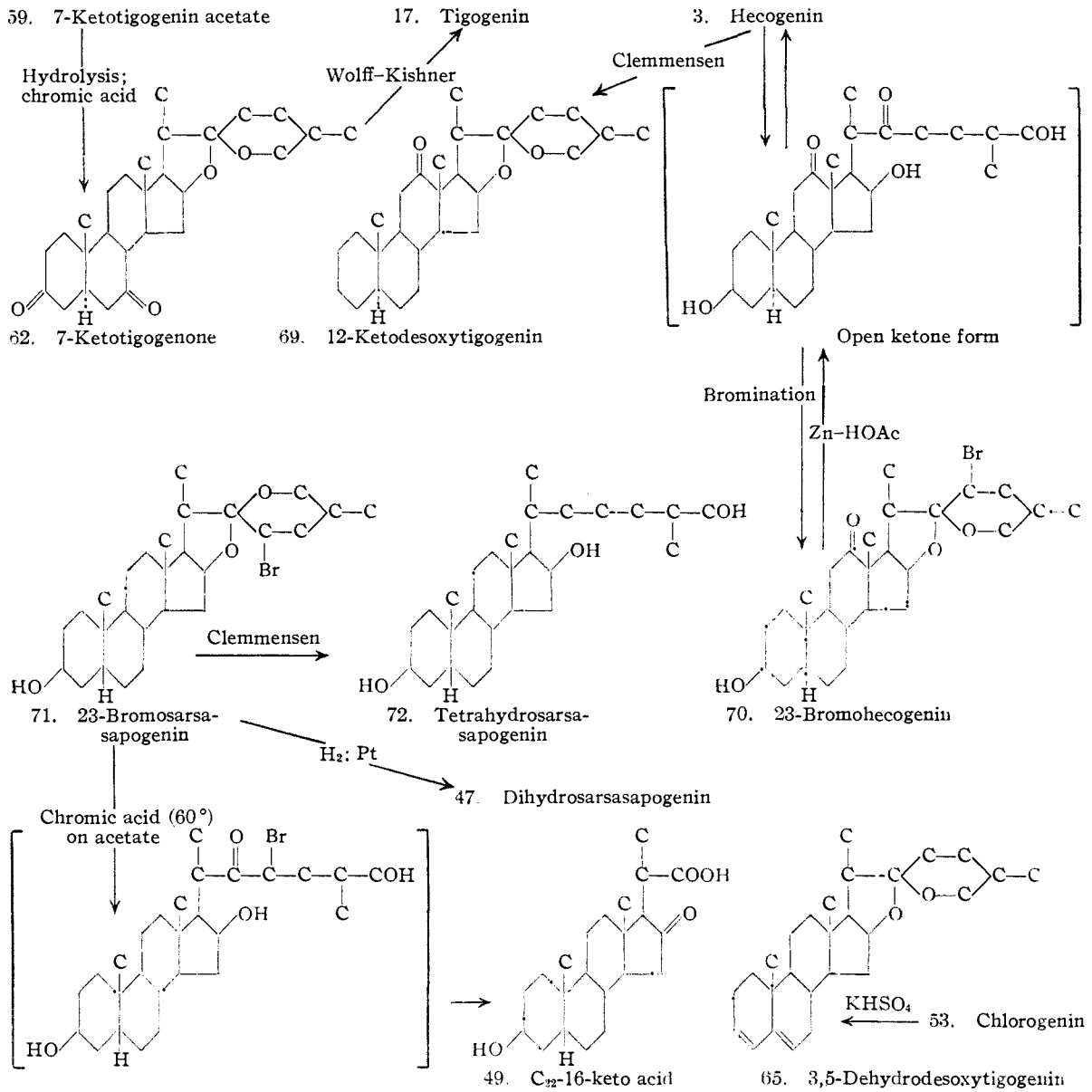
The bromo compound was unaffected by treatment with boiling pyridine for eight hours.

This reaction is characteristic of the other steroidal sapogenins in which the substitution of bromine takes place in the activated position adjacent to the potential carbonyl group at C-22. It is very unlikely that the bromine entered at the 11-position, since bromination here requires a higher temperature or longer time. The bromosapogenins show the usual side-chain reactions.[66] Thus, catalytic hydrogenation of 23-bromosarsasapogenin (71) acetate followed by hydrolysis gives dihydrosarsasapogenin (47) and the Clemmensen reduction gives tetrahydrosarsasapogenin (72). Oxidation[58] of this bromosapogenin with chromic acid at 60° gives a good yield of 3-β-hydroxy-16-keto-*bisnor*cholanic acid (49).

Rockogenin

Rockogenin (12) is isolated from the steroidal fraction of *Agave gracilipes* Trel. along with large amounts of hecogenin. It is not surprising then to find that their structures are closely related. A comparison of the analyses and melting points along with mixed melting point determinations show that rockogenin (12) and its diacetate are identical with 12-dihydrohecogenin and its diacetate. Mild oxidation of rockogenin (12) with

(62) Windaus and Resau, *Ber.*, **48**, 851 (1915).
(63) Windaus and Kirchner, *ibid.*, **53**, 614 (1920).
(64) Windaus, *ibid.*, **50**, 133 (1917).
(65) Windaus and Schlichting, *Z. physiol. Chem.*, **150**, 267 (1925).
(66) Marker and Rohrmann, THIS JOURNAL, **61**, 846 (1939).



chromic acid in acetic acid gives hecogenone (51), further establishing its identity.

Manogenin

Manogenin (8), first isolated from *Manfreda maculosa* Hook., is a common constituent of the steroidal fraction from numerous *Agaves*. It forms a 2,4-dinitrophenylhydrazone.

2,4-Dinitrophenylhydrazone.—Manogenin diacetate, 0.2 g., was treated with the ketone reagent as described under hecogenin. The 2,4-dinitrophenylhydrazone of manogenin diacetate crystallized from ethanol as orange needles, m. p. 218–221°.

Anal. Calcd. for $C_{37}H_{40}O_{10}N_4$: C, 62.5; H, 7.1. Found: C, 62.6; H, 7.2.

Gitogenin from Manogenin.—The function of four of the five oxygen atoms is shown by its conversion by the Wolff–Kishner method to gitogenin ($C_{27}H_{44}O_4$) (55). Thus, manogenin (8) differs from gitogenin (55) only in possessing a carbonyl group. Just as gitogenin is readily oxidized to gitogenic acid (56), so manogenin is oxidized to hecogenic acid (57). Furthermore, even

Gitogenin from Manogenin Diacetate.—The procedure and quantities of reactants were identical with those used for the Wolff–Kishner reduction of hecogenin acetate. Gitogenin was crystallized from ether as needles, m. p. and mixed m. p., 266–268°; yield 0.4 g. This depressed the melting point of the starting material (264°) to 245–250°.

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

Gitogenin diacetate crystallized from methanol as white needles, m. p. 238–242°; a mixture with gitogenin diacetate (242°) melted 238–242°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.4; H, 9.5.

as gitogenic acid is the dibasic acid obtained by the oxidation of tigogenin (17), so hecogenic acid (57) is the diacid from the oxidation of hecogenin (3).

Hecogenic Acid from Manogenin.—To a solution of 0.3 g. of manogenin in 25 cc. of acetic acid was added 0.3 g. of chromic anhydride dissolved in 5 cc. of 80% acetic acid. The reaction mixture was allowed to stand at room temperature for thirty minutes and was then diluted with water and ether extracted. The acid products were removed with sodium carbonate solution. The latter was acidified and the precipitated solid was dissolved in ether. The ether was removed and the residue was crystallized from 80% acetic acid as white crystals, m. p. 264° dec.; yield 0.15 g. Recrystallization from dilute acetic acid raised the melting point to 269° dec. A mixture with hecogenic acid melted 267-269° dec.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.5; neut. equiv., 238.2. Found: C, 68.1; H, 8.3; neut. equiv., 234.0.

The dimethyl ester of the diacid was prepared as described before and was crystallized from ether, m. p. and mixed m. p. with dimethyl ester of hecogenic acid, 184–186°.

Accordingly, the carbonyl group in manogenin (8) is at the same position as the carbonyl group in hecogenin, namely, C-12.

Comparison of 12-Dihydromanogenin with Digitogenin.—Mild catalytic or sodium and alcohol reduction forms a third hydroxyl group which can be acetylated by prolonged boiling with acetic anhydride. This material is 12-dihydromanogenin identical with agavogenin (1).

Agavogenin.—1. An ethereal solution of 0.3 g. of manogenin containing several drops of acetic acid was shaken with hydrogen and Adams catalyst for three hours at room temperature and three atm. Agavogenin was crystallized from aqueous methanol, m. p. 238–240°. A mixture with the starting material (240°) gave a melting point elevation, 242-247°. A mixture with natural agavogenin (240°) melted 240°.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.3; H, 9.9. Found: C, 72.2; H, 9.9.

The triacetate was prepared by refluxing a solution of the triol in acetic anhydride for one hour. The solvent was removed and the residue was crystallized from methanol as plates, m. p. and mixed m. p. with natural agavogenin triacetate, 226–228°.

Anal. Calcd. for $C_{33}H_{50}O_8$: C, 68.9; H, 8.8. Found: C, 68.5; H, 8.7.

2. To a solution of one gram of manogenin diacetate in 300 cc. of absolute ethanol was added 23 g. of sodium over a period of thirty minutes. After the sodium had reacted the reaction mixture was diluted with water and ether extracted. The ethereal solution was washed free of alkali with 10% hydrochloric acid and evaporated. Agavogenin crystallized from ether as white needles, m. p. 238–240°; yield 0.25 g. Mixtures with natural agavogenin and with material from (1) melted 240°.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.3; H, 9.9. Found: C, 72.0; H, 9.8.

The triacetate was prepared by refluxing the triol with acetic anhydride for one hour. It was crystallized from methanol as plates, m. p. and mixed m. p. with natural agavogenin triacetate or triacetate from (1), 226–228°.

Anal. Calcd. for $C_{33}H_{50}O_8$: C, 68.9; H, 8.8. Found: C, 68.5; H, 8.8.

The carbonyl group in manogenin like that in hecogenin cannot be removed under the conditions of a mild Clemmensen reaction.

Attempted Mild Clemmensen Reduction of Manogenin.—Manogenin, 1 g., was treated in a like manner as described for hecogenin. The product crystallized from ether as needles, m. p. and mixed m. p. with starting material, 245–248°; wt. 0.42 g. The diacetate was prepared and crystallized from methanol as white needles, m. p. and mixed m. p. with manogenin diacetate, 242–243°.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 70.4; H, 9.0.

More vigorous catalytic hydrogenation of manogenin (8) gives dihydroagavogenin (73), closely resembling the formation of dihydrorockogenin (46) from hecogenin (3).

Dihydroagavogenin.—A solution of manogenin, 2 g., in acetic acid was shaken with hydrogen and Adams catalyst for eight hours at 70° and forty-five pounds pressure. The reaction mixture was worked up as described for dihydrorockogenin. Dihydroagavogenin was crystallized from ether and then acetone, m. p. 191–193°; yield, 1.5 g.

Anal. Calcd. for $C_{27}H_{46}O_5$: C, 72.0; H, 10.3. Found: C, 71.9; H, 10.3.

This material formed a non-crystalline acetate and benzoate.

The fact that 12-dihydromanogenin (1) and its triacetate are isomeric but not identical with digitogenin (74) and its triacetate lends additional support to the assigned C-12 position for the oxygen atom in the many interrelated new sapogenins by eliminating C-15 as a possible location for it. The difference between agavogenin (1) and digitogenin (74) is shown not only by the wide variation in melting points but also by the chemical behavior of the corresponding keto-dicarboxylic acids. Thus, hecogenic acid (57) is unaffected by alkali, whereas under these conditions digitogenic acid (75) is converted to the isomeric acid, digitoic acid (76).[67,68] The latter also is non-identical with hecogenic acid (57). The dimethyl esters are also different. Because of the isomerization of digitogenic acid to digitoic acid, it has been suggested[69] that one hydroxyl group in digitogenin is at position-6, adjacent to the center of asymmetry at C-5. The conversion 75 → 76 would be analogous to the formation of 3,6-diketo-*allo*-cholanic acid from the corresponding acid in the regular series, *i.e.*, α-dehydrohyodesoxycholic acid. The positions of the other hydroxyl groups at C-2 and C-3 have been ascertained by Tschesche who reduced digitogenic acid (75) to gitogenic acid (56).[52]

Experiments carried out in this Laboratory[70] have shown that digitogenin (74) cannot have a hydroxyl group at C-6. If this were the case, then digitogenic acid (75) or digitoic acid (76) would have to be identical with chlorogenonic acid (58). The latter is readily obtained from chlorogenin (53) and has the same basic structure as digitogenic acid (75) since it too has been converted to gitogenic acid (56). Lieberman and Noller[57] have proved the non-identity of these diacids by a direct comparison of the diacids and their dimethyl esters. Furthermore, it has been shown[70] that

(67) Kiliani, *Arch. Phar.*, **231**, 448 (1894).
(68) Kiliani and Merk, *Ber.*, **34**, 3562 (1901).
(69) Windaus and Weil, *Z. physiol. Chem.*, **117**, 146 (1921).
(70) Marker, Turner and Ulshafer, THIS JOURNAL, **64**, 1843 (1942).

the chemical behavior of these acids (75) and (58) is different. Thus, chlorogenonic acid, like 6-keto-2/3-cholestan-2,3-diacid and 6-keto-2/3-*allo*-cholan-2,3,24-triacid (77), gives a characteristic lactone analogous to 78 upon catalytic reduction. On the other hand, catalytic reduction of digitogenic and digitoic acids gives the same hydroxy-diacid which does not lactonize, indicating that the carbonyl group in 75 must be in a position remote from the carboxyl group. Digitogenin is recovered unchanged after distillation with potassium bisulfate. This treatment readily causes dehydration of chlorogenin (53) and hyodesoxycholic acid to 3,5-dehydrodesoxytigogenin (65) and hyodesoxycholadienic acid, respectively.

Although the location of the third hydroxyl group in digitogenin has not been definitely determined, evidence strongly suggests that it is at at C-15. It is not in the side-chain since digitogenin triacetate upon oxidation gives digitogenin lactone triacetate (79).[71] In order to account for the isomerization of digitogenic acid (75) to digitoic acid (76), this hydroxyl group must be adjacent to one of several asymmetric centers, namely, C-6 (eliminated), C-7, C-11 or C-15. Position-11 is improbable because the keto-dicarboxylic acids (75) and (76) readily form semicarbazones and digitogenin forms a triacetate easily. Such oxygenated groups at C-11 have been shown by Steiger and Reichstein[41b] to be unreactive in these respects. The C-11 carbinols, moreover, readily undergo dehydration with mineral acids.[72] Digitogenin resists this treatment.

Similarly, the location of the hydroxyl group at C-7 in 74 is improbable because it should be readily dehydrated by distillation over potassium bisulfate. For example, simple distillation of 3,7-dihydroxycholanic acid converts it to the diene acid.[73] Oxidation of substances containing a carbonyl at C-7 leads to the formation of dicarboxylic acids. Thus, cholestan-7-one yields cholestan-6,7-diacid[74] and 7-keto-cholanic acid yields thilobilianic acid (cholan-6,7,24-triacid).[75] Oxidation of digitogenic acid (75) or digitoic acid (76) gives oxydigitogenic acid, a tribasic acid (80).[12,76,77,78]

Direct proof for the elimination of a 7-carbonyl group in the keto-dibasic acid (75) or a 7-hydroxyl group in digitogenin is shown by the fact that neither of the keto-dibasic acids from digitogenin is identical with 7-keto-gitogenic acid (81). The non-identity is further established by a comparison of their dimethyl esters.

Thus, the only alternate position remaining for the hydroxyl group in digitogenin is C-15. There are indications that this is the case, as Dimroth and Jonnson[79] have recently shown the possibility of inversion of configuration with a carbonyl group adjacent to C-14. Digitogenin also appears to differ from the other sapogenins in the reactions of the side-chain. Thus, dihydrodigitogenin and bromodigitogenin are formed only with difficulty. Digitogenin is recovered unchanged when treated under the conditions of the Clemmensen reaction employed for the formation of the tetrahydrosapogenins. It also is largely recovered when heated with acetic anhydride in a sealed tube, the reaction for the formation of the pseudosapogenins. All of these characteristic reactions shown by sapogenins might be conditioned by a hydroxyl group at C-15. Thus, positions 7 and 15 seem improbable for the carbonyl group in hecogenin (3) and manogenin (8).

Pseudomanogenin.—Manogenin (8) undergoes the usual pseudo reaction of the steroidal sapogenins. Pseudomanogenin (82) can be acid isomerized to the original sapogenin and degraded to 16-*allo*-pregnen-2,3(β)-diol-12,20-dione (83).

Pseudomanogenin.—A mixture of 10 g. of manogenin diacetate and 15 cc. of acetic anhydride contained in a sealed tube was heated at 200° for ten hours. The product was crystallized from methanol as white needles, m. p. 168–171°; yield 6 g.

Anal. Calcd. for $C_{33}H_{48}O_8$: C, 69.2; H, 8.5. Found: C, 69.4; H, 8.3.

Acid Isomerization of Pseudomanogenin.—The triacetate of pseudomanogenin, wt. 1 g., was hydrolyzed with excess alcoholic potassium hydroxide for thirty minutes. The reaction mixture was neutralized and then acidified with 5 cc. of concentrated hydrochloric acid and further heated for two hours. Manogenin was ether extracted and crystallized from ether, m. p. 246–248°; a mixture with manogenin (254°) melted 249–254°; yield 0.36 g.

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

The diacetate was prepared and crystallized from methanol as needles, m. p. and mixed m. p. with manogenin diacetate, 249–253°.

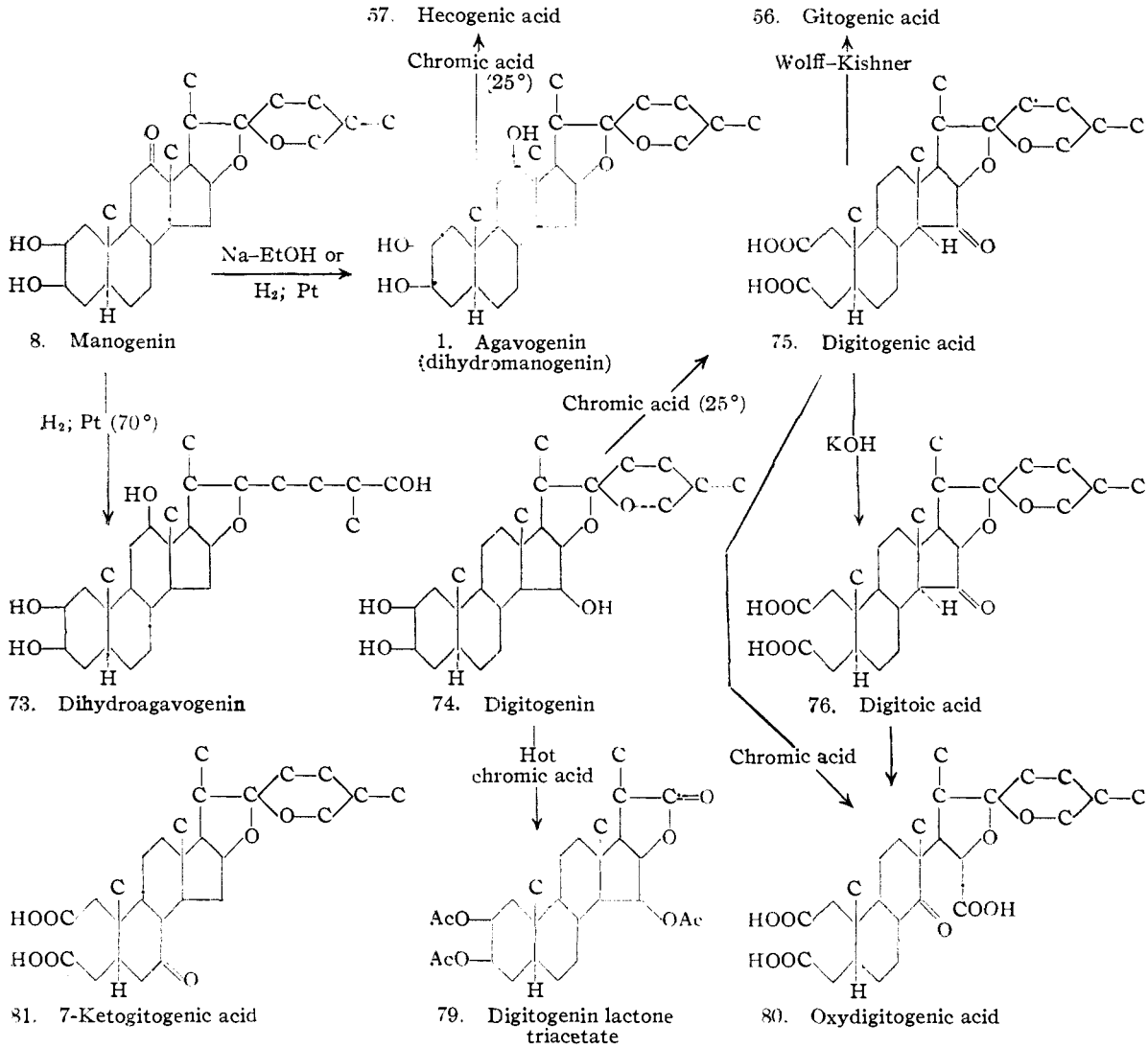
Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 70.1; H, 8.5.

16-*allo*-Pregnen-2,3(β)-diol-12,20-dione.—To a solution of 2 g. of pseudomanogenin triacetate in 30 cc. of acetic acid was added a solution of 1.2 g. of chromic anhydride in 10 cc. of 80% acetic acid. After the reaction mixture had stood at 20° for ninety minutes it was diluted with water and the product was extracted with ether. The ethereal solution was washed free from acetic acid, evaporated and the residue hydrolyzed with 2% alcoholic potassium hydroxide solution for thirty minutes. The hydrolysis mixture was diluted with an equal volume of water and extracted with 3 liters of ether. The ether was removed and the residue was acetylated and crystallized from aqueous methanol as white plates, m. p. 264–267°. This is 16-*allo*-pregnen-2,3(β)-diol-12,20-dione diacetate.

Anal. Calcd. for $C_{25}H_{34}O_6 \cdot H_2O$: C, 66.9; H, 8.1. Found: C, 66.8; H, 7.6.

Gitogenin (55), used as a model substance for the 2,3-dihydroxysapogenins, is easily converted to pseudogitogenin. It isomerizes back to the original sapogenin and can be degraded to the

(71) Marker and Rohrmann, THIS JOURNAL, **61**, 2724 (1939).
(72) Shoppee, *Helv. Chim. Acta*, **23**, 740 (1940).
(73) Wieland and Reverey, *Z. physiol. Chem.*, **140**, 186 (1924).
(74) Stange, *ibid.*, **218**, 74 (1933).
(75) Wieland and Dane, *ibid.*, **210**, 268 (1932).
(76) Kiliani, *Ber.*, **43**, 3562 (1910).
(77) Windaus and Weil, *Z. physiol. Chem.*, **121**, 62 (1922).
(78) Windaus and Willerding, *ibid.*, **143**, 33 (1925).
(79) Dimroth and Jonnson, *Ber.*, **74**, 520 (1941).



pregnane compound through 16-*allo*-pregnen-2,3-(β)-diol-20-one.

Pseudogitogenin.—Gitogenin, 10 g., was heated with 15 cc. of acetic anhydride in a sealed tube at 200° for twelve hours. The solvent was removed *in vacuo* on the steam-bath. The residue was treated with Norite in methanol and crystallized to give needles, m. p. 138–140°; yield, 6.5 g.

Anal. Calcd. for $C_{33}H_{50}O_7$: C, 70.9; H, 9.0. Found: C, 70.4; H, 9.0.

The above product when treated as described for pseudohecogenin gave gitogenin, m. p. and mixed m. p., 265–268°.

16-*allo*-Pregnen-2,3(β)-diol-20-one.—To a solution of 3 g. of pseudogitogenin triacetate in 45 cc. of glacial acetic acid was added a solution of 1.8 g. of chromic anhydride in 15 cc. of 80% acetic acid during ten minutes. The reaction mixture was allowed to stand at 25° for ninety minutes, and was then poured into water and worked up as described under 16-*allo*-pregnen-2,3(β)-diol-12,20-dione. The 16-*allo*-pregnen-2,3(β)-diol-20-one crystallized from ether, m. p. 228–230°; yield 0.5 g.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.8; H, 9.7. Found: C, 75.4; H, 9.8.

allo-**Pregnan-2,3(β)-diol-20-one.**—An ethereal solution of 0.15 g. of the above pregnene compound was shaken with hydrogen and 3% palladium-barium sulfate catalyst at room temperature and 3 atm. for two hours. Pregnan-2,3(β)-diol-20-one crystallized from ether, m. p. 238–240°; a mixture with starting material melted 200°.

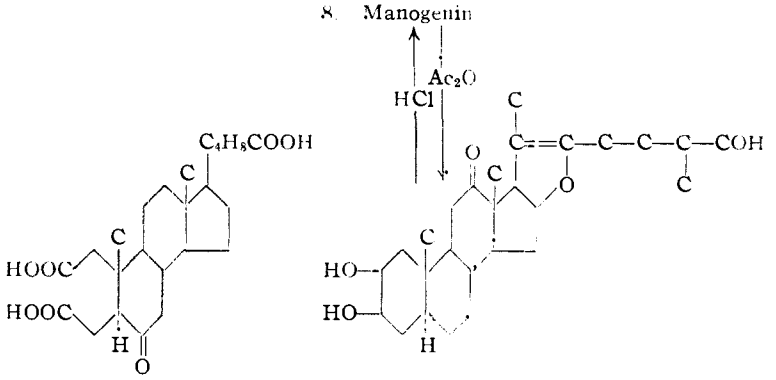
Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.4; H, 10.3. Found: C, 75.1; H, 10.3.

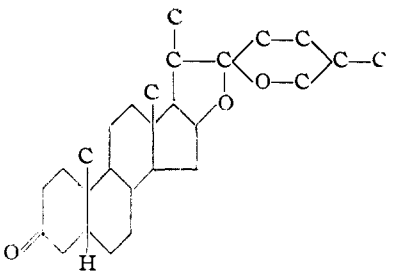
Agavogenin

Agavogenin (1), isolated from *Agave huachucensis* Baker in addition to manogenin and other sapogenins, is identical with 12-dihydromanogenin. The identity is established by the analysis of the genin and its acetate along with mixed melting point determinations on both. Mild oxidation gives hecogenic acid (57).

Yamogenin

Yamogenin (15) has been isolated along with diosgenin (42) from many of the *Dioscoreas*. It is





99. Smilagenone

interesting to find that these two sapogenins differ only in the configuration at C-22.

Treatment of yamogenin (15) with ethanolic hydrochloric acid gives diosgenin (42). Thus, the configurational difference of yamogenin and diosgenin is limited to the side-chain as will be shown later. Because of their simple interrelationship, however, the steroidal nature of yamogenin has been established.

Diosgenin from Yamogenin.—To a boiling solution of 5 g. of yamogenin acetate in 500 cc. of ethanol was added a solution of 150 cc. of concentrated hydrochloric acid in 335 cc. of ethanol. The mixture was refluxed on the steam-bath for eighteen hours and then poured into water. The precipitate was filtered, dried and acetylated with 14 cc. of boiling acetic anhydride. After cooling the acetylation mixture to room temperature, it was filtered. The filtrate was concentrated to three-fourths volume and cooled to give crystals which were washed with cold acetic anhydride and methanol, m. p. 158–172°. Recrystallization from methanol gave material melting 185–189°. After standing overnight, the acetic anhydride mother liquor yielded a second crop of crystals, m. p. 170–180°. Recrystallization from methanol gave material melting 185–189°. The two fractions, wt. 0.5 g., were combined and further crystallized from acetone and then ethyl acetate to give material, m. p. and mixed m. p. with diosgenin acetate, 192–195°. A mixture with yamogenin acetate melted 164–174°.

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

Hydrolysis of the acetate with 2% alcoholic potassium hydroxide solution for twenty minutes at steam-bath temperature gave diosgenin which was crystallized from ether as needles, m. p. 205-206°; a mixture with diosgenin (212°) melted 206-210°. A mixture with yamogenin (200°) melted 187–195°.

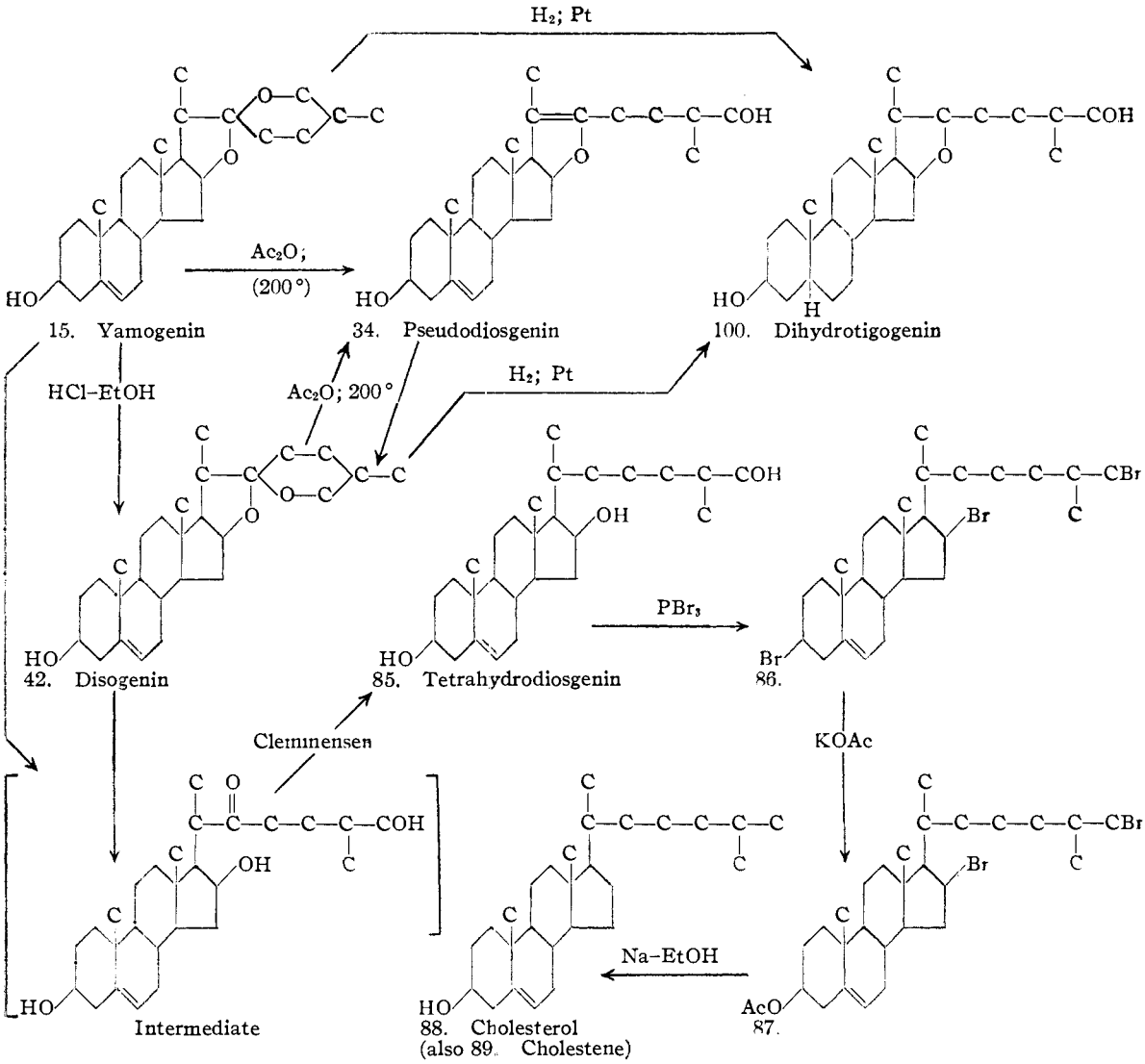
Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.3; H, 10.2.

In fact, since nearly all of the sapogenins have been chemically related to diosgenin, their steroidal nature has been established. This follows from the fact that diosgenin has been converted to cholesterol.[80] Thus, the first definite proof that the steroidal sapogenins have the same carbon skeleton as cholesterol and that they have twenty-seven carbon atoms is provided by the series of reactions, 42 → 85 → 86 → 87 → 88 + 89, whereby diosgenin is converted to cholesterol (88) and 5-cholestene (89). This conversion also provides convincing evidence for the C_5-unsaturation and 3-hydroxyl group in 42.

Conversion to Sarsasapogenin, Neochlorogenin and β-Neochlorogenin.—Numerous nuclear reactions of diosgenin (42) have been applied successfully to yamogenin (15). Oppenauer oxidation gives 4-dehydroneotigogenone (90) which is reduced with hydrogen and palladium–barium sulfate catalyst to sarsasapogenone (91).

4-Dehydroneotigogenone.—A solution of 5 g. of yamogenin and 5 g. of aluminum *t*-butylate in 25 cc. of dry acetone and 250 cc. of dry toluene was refluxed for sixteen hours, proper precautions being taken to keep moisture out of the system. The toluene was distilled *in vacuo*. The gelatinous residue was dissolved in dilute hydrochloric acid and extracted with ether. The ethereal solution was washed with dilute alkali, water and then evaporated. A suspension of the residue in hot water was steam distilled for one hour to remove the volatile condensation products. The mixture was cooled and ether extracted. 4-Dehydroneotigogenone crystallized as thick prisms, m. p. 196–198°; yield, 3 g. A mixture with 4-dehydrotigogenone (180°) melted 170–172°.

(80) Marker and Turner, THIS JOURNAL, **63**, 767 (1941).



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

Sarsasapogenone from 4-Dehydroneotigogenone.—A solution of 0.5 g. of 4-dehydroneotigogenone in 150 cc. of ether was shaken with 0.5 g. of 3% palladium-barium sulfate catalyst and hydrogen at room temperature and 3 atm. for five hours. The mixture was filtered through kieselguhr and the filtrate was evaporated. The residue was crystallized from ethanol to give sarsasapogenone, m. p. and mixed m. p., 225-228°; yield, 0.25 g.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.2; H, 10.4.

Since sarsasapogenone (91) has been reduced to sarsasapogenin (22), it follows that yamogenin has been converted to sarsasapogenin (22). This series of reactions parallels the conversion of diosgenin (42) to smilagenin (23). It specifically illustrates the presence of the normal side-chain in yamogenin (15). Mild oxidation of yamogenin by the method of Windaus[81,82] gives the enedione (92) which has been reduced to neochlorogenone (93). Further reduction of this product (93) with sodium and alcohol gives neochlorogenin (94) in analogy to the reduction of chlorogenone (52) to chlorogenin (53).[51]

4-Dehydroneochlorogenone.—To a solution of 5 g. of yamogenin in 250 cc. of acetic acid was added 5 g. of chromic anhydride dissolved in 100 cc. of 80% acetic acid at 16°. After maintaining the temperature at 18–20° for one hour, the excess chromic acid was destroyed with ethanol. Water (500 cc.) and ether (3 liters) was added to the reaction mixture. The ethereal extract was washed with water, dilute alkali and water. The ether was evaporated to give 4-dehydroneochlorogenone, m. p. 227–228°; yield 1.4 g.

Anal. Calcd. for $C_{27}H_{38}O_4$: C, 76.0; H, 9.0. Found: C, 75.6; H, 9.2.

Neochlorogenone.—A solution of 200 mg. of the above enedione in 25 cc. of acetic acid and 2 cc. of water was refluxed with 1 g. of zinc dust for two hours. Another gram of zinc dust was added and the refluxing was continued an additional two hours. The mixture was cooled and the supernatant liquid was decanted. The residue

(81) Windaus, *Ber.*, **39**, 2249 (1906).
(82) Windaus, *ibid.*, **40**, 257 (1907).

was washed with 10 cc. of acetic acid. The combined acetic acid solutions were diluted with 50 cc. of water and extracted with one liter of ether. The ethereal solution was washed with water, dilute potassium hydroxide and water and then evaporated to a small volume and cooled to give neochlorogenone as small plates, m. p. 240-241°. A mixture with chlorogenone (237°) melted 227–232°.

In a second run, 13.5 g. of yamogenin in 200 cc. of glacial acetic acid was oxidized at 18–20° for one hour with 13.5 g. of chromic anhydride dissolved in 250 cc. of 80% acetic acid. The reaction mixture was then heated under reflux for five hours with 30 g. of zinc dust. The mixture was worked up as described above and neochlorogenone was crystallized from ether as small plates, m. p. 235–237°, wt. 4 g. Recrystallizations from ether raised the melting point to 246–248°; yield 2 g.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.3; H, 9.5.

Neochlorogenin.—To a refluxing solution of 0.9 g. of neochlorogenone in 250 cc. of absolute ethanol was added 20 g. of sodium in small strips. After the sodium had reacted the solution was diluted with 500 cc. of water and the precipitate was extracted with 1.5 liters of ether. The ethereal solution was washed with water and evaporated. The residue after treatment with Norite in acetone was crystallized to give neochlorogenin as needles, m. p. 269–270°; yield 0.1 g. A mixture with chlorogenin (274°) melted 247–250°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.9; H, 10.2.

Neochlorogenin diacetate was prepared and was crystallized from methanol at ice–salt temperature, m. p. 196–198°.

On the other hand, catalytic hydrogenation (Adams catalyst) gives β-neochlorogenin (95), analogous to the reduction of 52 to β-chlorogenin (54).[51] In this manner two new sapogenins have been prepared identical with chlorogenin and β-chlorogenin, except that they have the normal instead of the *iso* side-chain.

β-Neochlorogenin.—A solution of 1 g. of neochlorogenone in 250 cc. of 95% ethanol was shaken for four hours with 0.3 g. of Adams catalyst and hydrogen at room temperature and 3 atm. The mixture was filtered and the filtrate was evaporated to dryness. The solid residue was crystallized from a small volume of acetone as needles, m. p. 249–251°; yield, 0.6 g. This substance is β-neochlorogenin. A mixture with β-chlorogenin (248°) melted 231–233°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.6; H, 10.3.

A solution of β-neochlorogenin, 0.4 g., in 1.2 cc. of acetic anhydride was refluxed for twenty minutes and then cooled to −5° for several hours. The fine needles of β-neochlorogenin diacetate were filtered, washed with cold methanol, and dried, m. p. 200–202°. Recrystallization from acetone gave square plates, m. p. 205–207°.

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

Conversion to Neotigogenin.—When yamogenin is reduced catalytically it is converted to neotigogenin (96), which can be oxidized to neotigogenone.

Neotigogenin from Yamogenin.—A solution of yamogenin acetate in 400 cc. of ether acidified with a few drops of acetic acid was shaken with 0.5 g. of Adams catalyst and hydrogen at room temperature and 3 atm. for four hours. The mixture was filtered and the filtrate was evaporated. The residue was dissolved in ether, treated with Norite and crystallized to give the acetate of neotigogenin as prisms, m. p. and mixed m. p., 179–180°; yield, 0.3 g. A mixture with starting material (180°) melted 171–173°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.0; H, 10.3.

Hydrolysis of the acetate with alcoholic potash and crystallization from ether gave needles, m. p. and mixed m. p. with neotigogenin, 202–203°.

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

Neotigogenone.—To a solution of 8.5 g. of neotigogenin (from yamogenin) in 340 cc. of acetic acid was added at 20° 8.5 g. of chromic anhydride in 170 cc. of 80% acetic acid. The mixture after standing at 25–30° for two hours was ether extracted. The ethereal solution was washed with water, dilute alkali and water and then evaporated. Neotigogenone crystallized from acetone, m. p. 216–218°; yield, 3.5 g.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 77.9; H, 10.0.

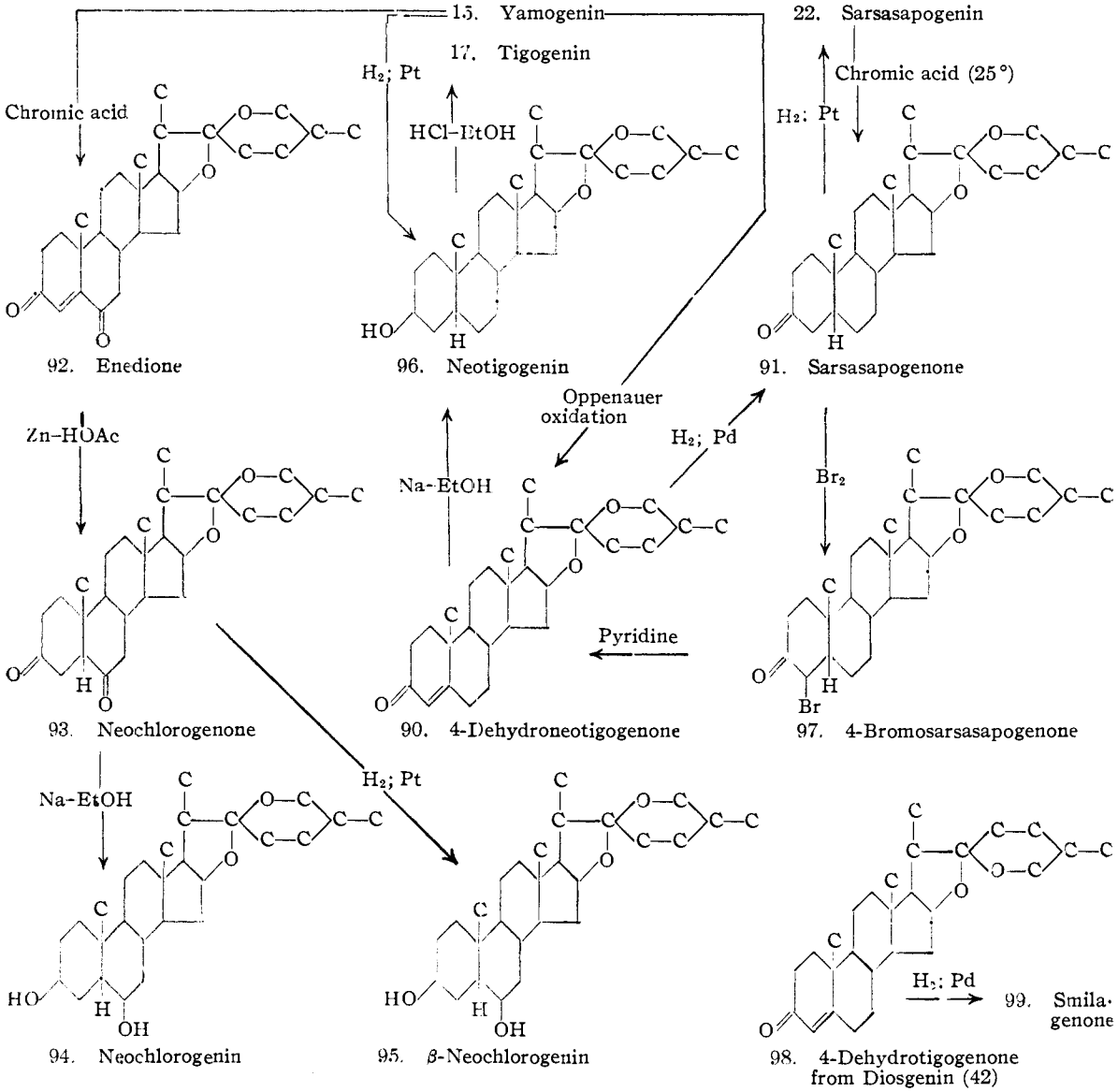
Neotigogenin (96) differs from tigogenin (17) in the same manner that sarsasapogenin (22) differs from smilagenin (23), that is, in the configuration at C-22. This is shown[83] by the preparation of neotigogenin (96) from sarsasapogenin (22) in the same manner that tigogenin (17) is prepared from smilagenin (23); whereby, the configuration of the C-5 hydrogen is reversed. This is illustrated by the route 22 → 91 → 97 → 90 → 96.

These experiments, together with other interconversions, show that tigogenin (17) and therefore, gitogenin (55), digitogenin (74), chlorogenin (53), diosgenin (42), hecogenin (3), manogenin (8), rockogenin (12), agavogenin (1), kammogenin (4), yuccagenin (16), samogenin (13), and mexogenin (9) have the same *iso* side-chain configuration as smilagenin (23), while sarsasapogenin (22), neotigogenin (96), yamogenin (15) and lilagenin (7) possess the normal configuration. The transformation of 4-dehydrotigogenone (98) to smilagenone (99) further supports this relationship. The *iso* and normal side-chains are arbitrarily but consistently represented as in 42 and 15, respectively. Just as yamogenin can be isomerized by acid treatment to diosgenin, so neotigogenin (96) is converted to tigogenin (17). It is interesting that sapogenins having the *iso* side-chain are little affected by this treatment, indicating that they have the stable configuration around C-22.

Side-Chain Derivatives.—The fact that the difference between diosgenin and yamogenin is limited to the side-chain is further illustrated by their formation of identical side-chain derivatives, namely, pseudodiosgenin (34), dihydrotigogenin (100) and tetrahydrodiosgenin (85). It is noted that in each of these experiments the asymmetry of the 22-carbon atom is destroyed.

Tetrahydrodiosgenin from Yamogenin.—To a refluxing solution of 1 g. of yamogenin in 100 cc. of ethanol containing 30 g. of amalgamated zinc was added 30 cc. of concentrated hydrochloric acid during two and one-half hours (5-cc. portions every thirty minutes). After refluxing for an additional thirty minutes, the solution was decanted, cooled and diluted with 2 liters of ether. The ethereal solution was washed with water and evaporated. The residue was crystallized from ether–pentane and then from

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



acetone as plates, m. p. 175–177°. A mixture with tetrahydrodiosgenin (179°) melted 177–179°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.1; H, 10.9.

The triacetate was prepared and was crystallized from acetic anhydride, m. p. 109–111°. Recrystallization from pentane gave material, m. p. 114°; a mixture with tetrahydrodiosgenin triacetate (119°) melted 116–118°.

Anal. Calcd. for $C_{33}H_{52}O_6$: C, 72.8; H, 9.6. Found: C, 72.7; H, 9.5.

Dihydrotigogenin from **Yamogenin.**—A solution of 1 g. of yamogenin acetate in 100 cc. of acetic acid was shaken for eight hours with 0.5 g. of Adams catalyst and hydrogen at 70° and 3 atm. The solution was filtered and the filtrate was evaporated *in vacuo* on the steam-bath. The oily residue was hydrolyzed with 100 cc. of 10% alcoholic potash at steam-bath temperature for twenty minutes. The mixture was cooled, diluted with water and extracted with one liter of ether. The ether layer was washed with water and then evaporated. Crystallization of the residue from acetone and then ethyl acetate gave small plates, m. p. and mixed m. p. with dihydrotigogenin, 168–170°; yield, 0.4 g.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.4; H, 11.2.

Pseudodiosgenin from **Yamogenin.**—Yamogenin acetate, 1.5 g., was heated in a sealed tube with 5 cc. of acetic anhydride for ten hours at 200°. The solvent was removed *in vacuo* on the steam-bath. The residue was dissolved in methanol, treated with Norite and crystallized. Recrystallization from methanol gave pseudodiosgenin diacetate, m. p. and mixed m. p., 101–102°; yield 0.4 g.

Anal. Calcd. for $C_{31}H_{46}O_5$: C, 74.7; H, 9.3. Found: C, 74.7; H, 9.4.

Bromination of yamogenin acetate with two moles of bromine in acetic acid gives a good yield of the 5,6,23-tribromo compound. Debromination of this with zinc in acetic acid gives yamogenin acetate. On the other hand, when the tribromo compound is debrominated with potassium

iodide in ethanol,[84] the bromine in the side-chain is not affected and 23-bromoyamogenin acetate results. Further treatment of the latter with zinc in acetic acid gives yamogenin acetate.

23-Bromoyamogenin Acetate.—To a solution of 5 g. of yamogenin acetate in 250 cc. of acetic acid was added 11.0 cc. of a 1.05 *M* bromine–acetic acid solution during ten minutes at room temperature; then 5 drops of 48% hydrobromic acid followed by an additional 11.0 cc. of bromine–acetic acid solution during ten minutes at room temperature. After standing an additional thirty minutes, the reaction mixture was poured into 250 cc. of water and the precipitate of 5,6,23-tribromoyamogenin acetate was filtered, washed with water and dried; yield 7.5 g.

Debromination of this material with zinc in acetic acid, for fifteen hours, as described before gave yamogenin acetate, m. p. and mixed m. p. 179–180°.

A solution of 5 g. of the tribromo acetate from above in 1250 cc. of ethanol containing 2.2 g. of potassium iodide was refluxed for two hours. The reaction mixture was decolorized with sodium bisulfite, concentrated *in vacuo* on the steam-bath, cooled and ether extracted. The ethereal solution was washed with water and evaporated to give 23-bromoyamogenin acetate as crystals, m. p. 193–194° dec.

Anal. Calcd. for $C_{29}H_{43}O_4Br$: C, 65.0; H, 8.1. Found: C, 65.0; H, 8.2.

Hydrolysis of a 0.1-g. portion of 23-bromoyamogenin acetate with 100 cc. of 1% potassium hydroxide gave the bromogenin which was crystallized twice from ether, m. p. 193° dec.

Anal. Calcd. for $C_{27}H_{41}O_4Br$: C, 65.7; H, 8.4. Found: C, 65.4; H, 8.4.

Debromination of the 23-bromoacetate with zinc and acetic acid for ten hours at 90° gave yamogenin acetate, m. p. and mixed m. p., 176–179°.

Yuccagenin

During the course of our investigation of the *Yucca* for steroidal sapogenins, we have isolated yuccagenin (16) from *Yucca Schottii* Engelm., *Yucca elata* Engelm. and *Yucca flaccida* Haw.

By catalytic hydrogenation yuccagenin can be converted to gitogenin (55).

Gitogenin from Yuccagenin.—Yuccagenin, 0.2 g., in 500 cc. of ether containing a few drops of acetic acid was catalytically hydrogenated (Adams catalyst) at room temperature and 3 atm. pressure for two hours. The mixture was filtered and gitogenin was crystallized from ether as needles, m. p. and mixed m. p., 264–268°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 75.2; H, 10.2.

The diacetate was crystallized from methanol as fine needles, m. p. and mixed m. p. with gitogenin diacetate, 240–242°.

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

This fact shows that it differs from the latter (55) only in possessing a double bond.

Conversion to Chlorogenonic Acid.—The location of the double bond is established by the formation of chlorogenonic acid (58) from yuccagenin by a method somewhat like that employed for diosgenin (42) and cholesterol (88).[81-87] Our conversion is illustrated by the series of reactions, 16 → 101 → 102 → 58. The reaction of yuccagenin (16) with hydrogen peroxide in acetic acid forms the 2,3,5,6-tetrol (101). The latter with boiling acetic anhydride forms a triacetate. This might be expected if the double bond is at carbons 5–6 since the 5-hydroxyl is tertiary and does not react. By analogy, diosgenin upon like treatment forms the 3,5,6-triol which forms a diacetate.[46] Mild oxidation of the tetrol (101) gives a monobasic acid (102) having the composition $C_{27}H_{38}O_7$. The 3-carboxyl group has probably lactonized with the 5-hydroxyl since the usual dehydration treatment employed for a 3,6-diketo-5-ol gives unchanged material. Treatment of this product (102) with zinc in acetic acid gives a keto-diacid identical with chlorogenonic acid (58).

Reaction of Yuccagenin with Hydrogen Peroxide.—To a hot suspension of 3 g. of yuccagenin in 30 cc. of glacial acetic acid was added 3 cc. of 30% hydrogen peroxide (Merck Superoxol) after which the mixture was heated for a total of two hours on the steam-bath. The solid material dissolved completely in the first fifteen minutes, but after heating an additional thirty minutes, a crystalline precipitate formed. The reaction mixture was cooled and poured into 200 cc. of water. The solid was filtered and washed with water. It was then hydrolyzed with a solution of 6 g. of potassium hydroxide in 350 cc. of ethanol for thirty minutes. The solid material did not completely dissolve during this hydrolysis. The mixture was diluted with an equal volume of water, neutralized with dilute acid and evaporated *in vacuo* on the steam-bath to a small volume. The crude tetrol was filtered and dried, wt. 1.9 g. Crystallization from methanol gave small plates, m. p. 356°, wt. 0.8 g.

Anal. Calcd. for $C_{27}H_{44}O_6$: C, 69.8; H, 9.6. Found: C, 69.2; H, 9.6.

The triacetate was prepared with boiling acetic anhydride and was crystallized from an ether–pentane mixture as plates, m. p. 254–256°.

Anal. Calcd. for $C_{33}H_{50}O_9$: C, 67.1; H, 8.5. Found: C, 66.9; H, 8.7.

Oxidation of the Tetrol from Yuccagenin.—To a solution of 1.7 g. of the tetrol from yuccagenin in 500 cc. of acetic acid was added at 25°, 1.7 g. of chromic anhydride in 50 cc. of 80% acetic acid. After standing one hour at room temperature, the mixture was diluted with 20 cc. of ethanol to destroy the excess chromic acid and then was evaporated *in vacuo* on the steam-bath to a small volume. The residue was taken up in ether and the ethereal solution was washed thoroughly with water and then with dilute sodium carbonate. The alkaline wash was acidified and re-extracted with ether. The acid product was crystallized from methanol, m. p. 225–226°; yield, 0.8 g. This material did not readily absorb bromine in acetic acid.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.3; H, 8.1; neut. equiv. for monoacid, 474. Found: C, 68.5; H, 8.2; neut. equiv., 475.

Through a solution of 0.3 g. of this substance in 100 cc. of dry chloroform was bubbled dry hydrogen chloride for three hours at ice-bath temperature. The reaction mixture was diluted with ether and washed with water. The product crystallized from methanol, m. p. and mixed m. p. with starting material 224–226°; wt. 0.2 g.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.3; H, 8.1; neut. equiv. for monoacid, 474. Found: C, 68.5; H, 8.1; neut. equiv., 478.

Chlorogenonic Acid.—A mixture of 200 mg. of the above product in 15 cc. of acetic acid, 0.5 cc. of water and 0.4 g. of zinc dust was heated under reflux for four hours. The mixture was concentrated and then ether extracted.

(84) Linneman and von Zotta, *Ann.*, **192**, 102 (1878).
(85) Criegee, *Ber.*, **65**, 1720 (1932).
(86) Pichard and Yates, *J. Chem. Soc.*, **93**, 1678 (1908).
(87) Westphalen, *Ber.*, **48**, 1064 (1915).

The ethereal solution was washed thoroughly with water and then evaporated. The residue was crystallized from aqueous acetic acid to give chlorogenonic acid, m. p. and mixed m. p. with an authentic sample, 233–234°; yield, 60 mg.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.5. Found: C, 68.1; H, 8.5.

The dimethyl ester was prepared with an ethereal solution of diazomethane and crystallized from methanol, m. p. and mixed m. p. with an authentic sample of the dimethyl ester of chlorogenonic acid, 162–163°.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.0; H, 8.8. Found: C, 69.3; H, 8.9.

Conversion of 7-Ketogitogenic Acid.—Yuccagenin has likewise been converted to 7-ketogitogenic acid (81). This series of reactions, *viz.*, 16 → 103 → 104 → 105 → 106 → 107 → 81, follows essentially that used for converting diosgenin (42) to 7-ketotigogenin acetate (59). Thus, yuccagenin diacetate is converted *via* 5,6,23-tribromoyuccagenin diacetate (103) to 23-bromoyuccagenin diacetate (104) and the latter is oxidized to 7-keto-23-bromoyuccagenin diacetate (105) by the method of Windaus, Lettré and Schenck.[59] The double bond in the latter (105) is selectively hydrogenated to give 7-keto-23-bromogitogenin diacetate (106). Debromination of this substance (106) gives 7-ketogitogenin diacetate (107). The presence of the carbonyl group in 107 has been ascertained by its formation of a 2,4-dinitrophenylhydrazone. Its removal by the Wolff–Kishner method gives gitogenin (55), proving that the basic structure of 107 has not been altered. Hydrolysis of the keto-diacetate (107) followed by mild oxidation gives 7-ketogitogenic acid (81). This compound is entirely different from digitogenic acid (75) and digitoic acid (76). The non-identity is further established by a comparison of their dimethyl esters. The significance of these experiments has been discussed elsewhere.

5,6,23-Tribromoyuccagenin Diacetate.—To a solution of 5.0 g. of yuccagenin diacetate in 250 cc. of glacial acetic acid at 20° was added during ten minutes, 9.8 cc. of a 1.05 *M* bromine–acetic acid solution. After standing for ten minutes, a few drops of 48% hydrobromic acid was added followed by an additional 9.8 cc. of bromine solution during fifteen minutes. The reaction mixture was allowed to stand at room temperature for thirty minutes and then diluted with four volumes of water. The precipitate was filtered and washed with water. Crystallization from acetone gave plates, m. p. 215° dec. Yield was 2.5 g.

Anal. Calcd. for $C_{31}H_{45}O_6Br_3$: C, 49.6; H, 5.8. Found: C, 49.8; H, 6.0.

In a like manner, 5-g. runs were made in which a total of 74 g. of precipitated and air dried product was obtained from 50 g. of yuccagenin diacetate.

Debromination of the tribromide gave yuccagenin diacetate. For this purpose, a solution of 0.5 g. of the tribromide in 75 cc. of acetic acid was heated on the steam-bath for nine hours with 5 g. of zinc dust. After the removal of the zinc, the solution was diluted with ether. The ethereal solution was washed with water, sodium carbonate and water and then evaporated. The residue was crystallized from methanol to give needles, m. p. and mixed m. p. with yuccagenin diacetate, 178–179°.

23-Bromoyuccagenin Diacetate.—A suspension of 2 g. of the tribromide and 1 g. of potassium iodide in 450 cc. of ethanol was refluxed for two hours. The suspended solid slowly dissolved and the reaction mixture darkened with the liberated iodine. The solution was decolorized by the addition of a saturated solution of sodium bisulfite. Water was added and the precipitate was dissolved in ether. After washing with water, the ethereal solution was evaporated and the monobromide was crystallized as needles, m. p. 229° dec.

Anal. Calcd. for $C_{31}H_{45}O_6Br$: C, 62.7; H, 7.6. Found: C, 62.9; H, 7.6.

In another run, the precipitated tribromide from above, wt. 37 g., was reacted to give the precipitated monobromide, m. p. 202° dec.; wt. (after air drying overnight and drying *in vacuo* at 40° for five hours) 37 g. Crystallization from acetone gave plates, m. p. 205° dec., wt. 22 g.

Debromination of 23-Bromoyuccagenin Diacetate.—A solution of 0.5 g. of 23-bromoyuccagenin diacetate in 50 cc. of acetic acid was heated on the steam-bath for nine hours with 4 g. of zinc dust. After decantation from the zinc dust, water and ether were added. The ethereal extract was washed with water, sodium carbonate and water. The ether was removed and the residue was crystallized from methanol as needles, m. p. and mixed m. p. with yuccagenin diacetate, 178–179°.

7-Keto-23-bromoyuccagenin Diacetate.—To a solution of 15 g. of 23-bromoyuccagenin diacetate in 750 cc. of glacial acetic acid at 50–55° was added with continuous stirring a solution of 12 g. of chromic anhydride in 200 cc. of 50% acetic acid during five hours (40 cc. added dropwise per hour). The temperature was maintained at 50–55° for an additional five hours. The excess chromic acid was destroyed with 10 g. of zinc dust. The mixture was filtered and the filtrate was diluted with water to 5 liters and extracted with 5 liters of ether. The water layer was re-extracted with 5 liters of ether. The total ethereal solution was washed thoroughly with water, potassium bicarbonate and water. The ether was removed and the oily residue, 10 g., was crystallized from 75 cc. of ether at −5° to give 7-keto-23-bromoyuccagenin diacetate as needles, m. p. 224° dec., yield 1.5 g. A mixture with starting material (229°) melted 215° dec.

Anal. Calcd. for $C_{31}H_{43}O_7Br$: C, 61.3; H, 7.1. Found: C, 61.6; H, 7.2.

The mother liquors from the crystallization of this material combined with those from a second run were hydrogenated (10 g. palladium–barium sulfate catalyst) for four hours. The product in 500 cc. of acetic acid was then debrominated in four portions with 30 g. of zinc dust as described below. Treatment of the debrominated product with Girard reagent T gave a negligible amount of ketone fraction. The Girard "non-ketone" fraction was rehydrogenated (palladium–barium sulfate) for two hours and then crystallized from hot methanol as needles, 268–271°; wt. 1.1 g. Recrystallization from ether gave needles, m. p. and mixed m. p. with 7-ketogitogenin diacetate from below, 279-282°.

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

Hydrolysis of this substance gave 7-ketogitogenin, m. p. and mixed m. p., 257–262°.

7-Ketoyuccagenin Diacetate.—A solution of 0.2 g. of 7-keto-23-bromoyuccagenin diacetate in 20 cc. of acetic acid was treated with 1.2 g. of zinc dust for five hours at steam-bath temperature. The product was ether extracted and finally crystallized from methanol to give 7-ketoyuccagenin diacetate as needles, m. p. 237–239°; wt. 0.1 g. This material does not melt with decomposition and gives a negative Beilstein test for halogen. A mixture with kammogenin diacetate (243°) melted twenty degrees low.

Anal. Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.1; H, 8.7.

7-Keto-23-bromogitogenin Diacetate.—A solution of 2 g. of 7-keto-23-bromoyuccagenin diacetate in 400 cc. of ether was shaken with 4 g. of 3% palladium-barium sulfate catalyst and hydrogen at room temperature and 3 atm. for two hours. After filtering through kieselguhr,

the reaction mixture was concentrated and cooled to give 7-keto-23-bromogitogenin diacetate, m. p. 230–231° dec.; yield 0.5 g.

Anal. Calcd. for $C_{31}H_{45}O_7Br$: C, 61.1; H, 7.4. Found: C, 61.1; H, 7.0.

7-Ketogitogenin Diacetate.—To a solution of 0.45 g. of 7-keto-23-bromogitogenin diacetate in 50 cc. of acetic acid at steam-bath temperature was added 3 g. of zinc dust. The mixture was heated for fifteen hours, cooled and filtered. The solid was washed with ether. The ether wash and filtrate was diluted to 500 cc. with ether and washed thoroughly with water, sodium bicarbonate and water. The ether was evaporated to a small volume (5 cc.) to give fine needles of 7-ketogitogenin diacetate, m. p. 269–272°; yield 0.2 g. Recrystallization from ether gave needles, m. p. 279–282°. This material gave a negative Beilstein test for halogen.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 69.9; H, 8.6.

The mother liquors from the crystallization of 7-keto-23-bromogitogenin diacetate were evaporated to dryness. The residue dissolved in 150 cc. of acetic acid was debrominated in three portions. The product combined with the ether mother liquor from the crystallization of 7-ketogitogenin diacetate was rehydrogenated (palladium–barium sulfate) for three hours to give an additional 0.8 g.

The **2,4-dinitrophenylhydrazone** of 7-ketogitogenin diacetate was prepared in a like manner as described for hecogenin acetate. The derivative was crystallized from methanol as orange needles, m. p. 267–270°.

Anal. Calcd. for $C_{37}H_{40}O_{10}N_4$: C, 62.5; H, 7.1. Found: C, 62.2; H, 7.1.

Gitogenin from 7-Ketogitogenin Diacetate.—The procedure duplicated that described for the Wolff–Kishner reduction of hecogenin acetate using 0.67 g. of 7-ketogitogenin diacetate. The product crystallized from ether as needles, m. p. and mixed m. p. with gitogenin, 270–272°; yield, 0.3 g.

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

The diacetate was prepared and was crystallized from methanol as needles, m. p. 246–247°. A mixture with gitogenin diacetate (242°) melted 242–245°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.3; H, 9.7.

7-Ketogitogenin.—A solution of 0.45 g. of 7-ketogitogenin diacetate in 30 cc. of 2% alcoholic potassium hydroxide was heated on the steam-bath for twenty minutes. The solution turned pale green and finally dark green. The 7-ketogitogenin was crystallized from ether as fine needles, m. p. 257–261°; yield, 185 mg.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.1; H, 9.6.

7-Ketogitogenic Acid.—To a solution of 0.36 g. of 7-ketogitogenin from above in 35 cc. of glacial acetic acid was added a solution of 0.35 g. of chromic anhydride in 5 cc. of 80% acetic acid. The reaction mixture was allowed to stand at room temperature for one hour and then diluted with ether. The ethereal solution was washed with water and the water layer was reextracted. The combined ethereal solution was washed well with water and then with potassium carbonate. The alkaline wash was acidified and the precipitated 7-ketogitogenic acid was crystallized from ether, m. p. 290–293° dec.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.5; neut. equiv., 238. Found: C, 67.7; H, 8.7; neut. equiv., 232.

The **dimethyl ester** prepared with an ethereal solution of diazomethane was crystallized from aqueous methanol as white plates, m. p. 187–189°. A mixture with the dimethyl ester of hecogenic acid (187°) melted twenty degrees low.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.0; H, 8.8. Found: C, 68.7; H, 9.0.

Pseudoyuccagenin and Pregnane Derivatives.—Yuccagenin can be converted to a pseudo sapogenin which is reconverted to the original sapogenin when treated with acid. The pseudo-compound after oxidation and subsequent hydrolysis is converted to 5,16-pregnadien-2,3(β)-diol-20-one. Other than having a C-2 hydroxyl group, this pregnene compound is identical with 5,16-pregnadien-3(β)-ol-20-one (37). The absorption curves (Fig. 2) bear out this close relationship. Selective hydrogenation of the 16,17-double bond using 3% palladium–barium sulfate catalyst gives 5-pregnen-2,3(β)-diol-20-one. Additional support for the structure of pseudoyuccagenin triacetate is given by its conversion to the triacetate of dihydro-pseudogitogenin.

Pseudoyuccagenin.—A mixture of 10 g. of yuccagenin diacetate and 15 cc. of acetic anhydride was heated at 200° in a sealed tube for ten hours. After removal of the acetic anhydride and treatment with Norite, the product was crystallized from methanol as white needles, m. p. 141–145°; yield 6 g. Recrystallization from methanol raised the melting point to 145–147°.

Anal. Calcd. for $C_{33}H_{48}O_7$: C, 71.2; H, 8.7. Found: C, 71.5; H, 9.0.

Hydrolysis of the triacetate with dilute alkali gave pseudoyuccagenin which was crystallized from ether as needles, m. p. 181–182°.

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

This product was readily converted to yuccagenin with alcoholic hydrochloric acid treatment, m. p. and mixed m. p., 246–248°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 74.8; H, 9.8.

Oxidation of Pseudoyuccagenin Triacetate.—To a solution of 3 g. of pseudoyuccagenin triacetate in 150 cc. of glacial acetic acid at 25° was added during ten minutes a solution of 1.8 g. chromic anhydride in 15 cc. of 80% acetic acid. The reaction mixture was worked up as described for the oxidation of pseudohecogenin. The acetylated product, 5,16-pregnadien-2,3(β)-diol-20-one diacetate, crystallized from methanol as needles, m. p. 178–181°; yield 0.8 g.

Anal. Calcd. for $C_{25}H_{34}O_5$: C, 72.4; H, 8.3. Found: C, 72.1; H, 8.4.

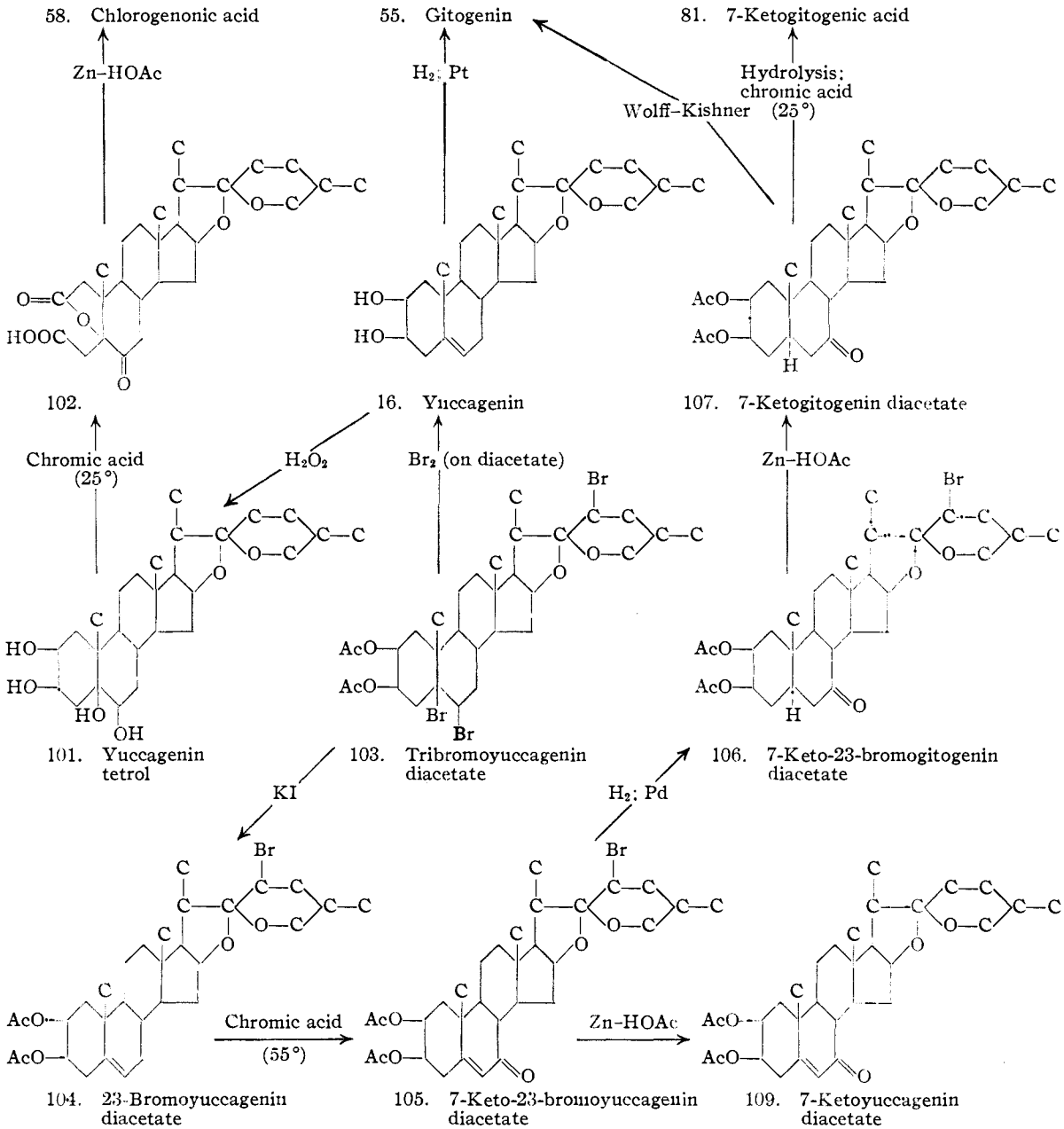
5-Pregnen-2,3(β)-diol-20-one Diacetate.—A solution of 0.1 g. of 5,16-pregnadien-2,3(β)-diol-20-one diacetate in 300 cc. of ether was shaken with 0.2 g. of 3% palladium–barium sulfate catalyst and hydrogen at room temperature and 3 atm. for two hours. After filtering through kieselguhr the ethereal solution was concentrated and cooled to give 5-pregnen-2,3(β)-diol-20-one diacetate as needles, m. p. 189–192°. A mixture with starting material melted 167–170°.

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.1; H, 8.7. Found: C, 72.1; H, 8.8.

Triacetate of Dihydropseudogitogenin from Pseudoyuccagenin Triacetate.—A solution of 1 g. of pseudoyuccagenin triacetate in 100 cc. of glacial acetic acid was shaken with Adams catalyst and hydrogen at room temperature and 3 atm. for six hours. After filtering, the filtrate was concentrated *in vacuo* on the steam-bath and dihydropseudogitogenin triacetate crystallized from aqueous acetic acid as needles, m. p. and mixed m. p., 154–156°; yield 0.6 g.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.7; H, 9.4. Found: C, 70.7; H, 9.5.

A solution of 1 g. of pseudogitogenin triacetate was catalytically hydrogenated in a manner described for pseudoyuccagenin triacetate. The product was crystal-



lized from aqueous acetic acid as needles, m. p. and mixed m. p. with the product from the hydrogenation of pseudoyuccagenin triacetate, 154–156°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.7; H, 9.4. Found: C, 70.6; H, 9.6.

Catalytic hydrogenation of yuccagenin (16) under the conditions used for the formation of the dihydrosapogenins gives dihydrogitogenin.

Dihydrogitogenin from Yuccagenin.—A solution of 2 g. of yuccagenin diacetate in 100 cc. of acetic acid was shaken with hydrogen and 1 g. of Adams catalyst for eight hours at 70° and three atm. The product obtained from acetic acid was hydrolyzed with 10% alcoholic potash for twenty minutes on the steam-bath and finally crystallized from acetone, m. p. and mixed m. p. with dihydrogitogenin, 195–197°; yield 1.5 g.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.3; H, 10.8.

The triacetate crystallized from methanol, m. p. 117–118°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.7; H, 9.4. Found: C, 70.5; H, 9.3.

Kammogenin

Kammogenin (4) was first isolated from the steroidal fractions obtained from *Yucca Harrimaniae* Trel. and *Yucca brevifolia* Engelm. It occurred in the mother liquors of each after the separation of sarsasapogenin and smilagenin, respectively. It was also obtained from *Yucca Schottii* Engelm. and *Samuela carnerosana* Trel.

The proof for structure (4) has been accumulated by relating kammogenin to other sapogenins by characteristic reduction reactions. Thus, reduction by the Wolff–Kishner method removes the carbonyl group, giving yuccagenin (16) which fixes the position of unsaturation at carbons 5–6.

Yuccagenin from Kammogenin.—The procedure and quantities of reactants were identical with those for the Wolff–Kishner reduction of hecogenin. Yuccagenin was crystallized from methanol as needles, 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 diacetate was crystallized from methanol as needles, m. p. and mixed m. p. with yuccagenin diacetate, 176–178°.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.1; H, 9.1.

Mild catalytic hydrogenation of kammogenin diacetate yields the diacetate of manogenin (8). Consequently, these two compounds differ only in that the former (4) possesses a double bond. Prolonged hydrogenation of 4 introduces a third hydroxyl group to give agavogenin (1). Reduction with sodium in ethanol attacks the 12-carbonyl group but not the double bond to give 12-dihydrokammogenin (108). This new substance (108) forms a triacetate. Although the latter could not be obtained crystalline, the presence of three acetyl groups is demonstrated by its conversion to the crystalline triacetate of agavogenin (1) by means of catalytic hydrogenation. Kammogenin diacetate is unaffected by the usual conditions of hydrogenation (3% palladium–barium sulfate) employed for the saturation of a double bond in a conjugated ketone system; however, a similar hydrogenation using a large quantity of catalyst gives the diacetate of manogenin (8).

Reduction of Kammogenin. (1) **Hydrogen–Adams Catalyst.**—A solution of 0.2 g. of kammogenin diacetate in 200 cc. of ether containing a few drops of acetic acid was shaken with 0.2 g. of Adams catalyst and hydrogen at room temperature and 3 atm. for fifteen minutes. The mixture was filtered and the product was crystallized from ether, m. p. and mixed m. p. with the diacetate of manogenin, 241–242°. A mixture with starting material melted twenty degrees low.

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

(2) **Hydrogen-Palladium Catalyst.**—A solution of 0.2 g. of kammogenin diacetate in 200 cc. of ether was shaken with 1 g. of 3% palladium–barium sulfate catalyst and hydrogen for two hours at room temperature and 3 atm. The product was crystallized from methanol as needles, m. p. and mixed m. p. with manogenin diacetate, 243°.

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 diacetate gave manogenin which was crystallized from ether, m. p. and mixed m. p., 240–242°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.8; H, 9.6.

Hydrogenation under similar conditions using only 0.2 g. of catalyst gave material which did not depress the melting point of the starting material. A mixture with manogenin diacetate melted twenty degrees low.

(3) **Sodium and Alcohol.**—To a hot solution of 2 g. of kammogenin diacetate in 500 cc. of absolute ethanol was added during one hour 45 g. of sodium in small strips. The reaction was cooled and ether extracted. The ethereal solution was washed well with water, concentrated and cooled to give 12-dihydrokammogenin which was recrystallized from ether and then methanol as needles, m. p. 216–218°; yield, 0.2 g.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.3; H, 9.5.

The triacetate prepared with boiling acetic anhydride (refluxed one hour) could not be obtained crystalline. Upon hydrogenation (Adams catalyst) in ether containing a few drops of acetic acid for two hours at room temperature and 3 atm., however, it gave the triacetate of agavogenin. The latter was crystallized from methanol, m. p. and mixed m. p., 225–228°.

The absorption spectrum (Fig. 1) for kammogenin (4) agrees with its structure in showing a distinct peak at λ 286 mμ, ϵ = 30.

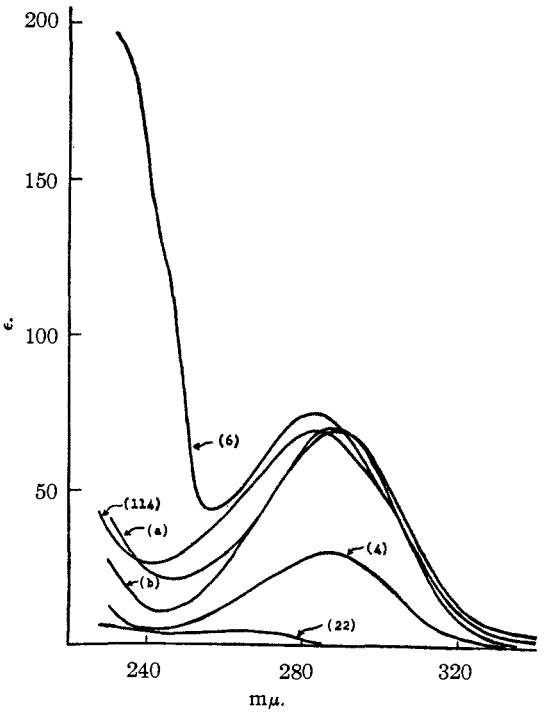
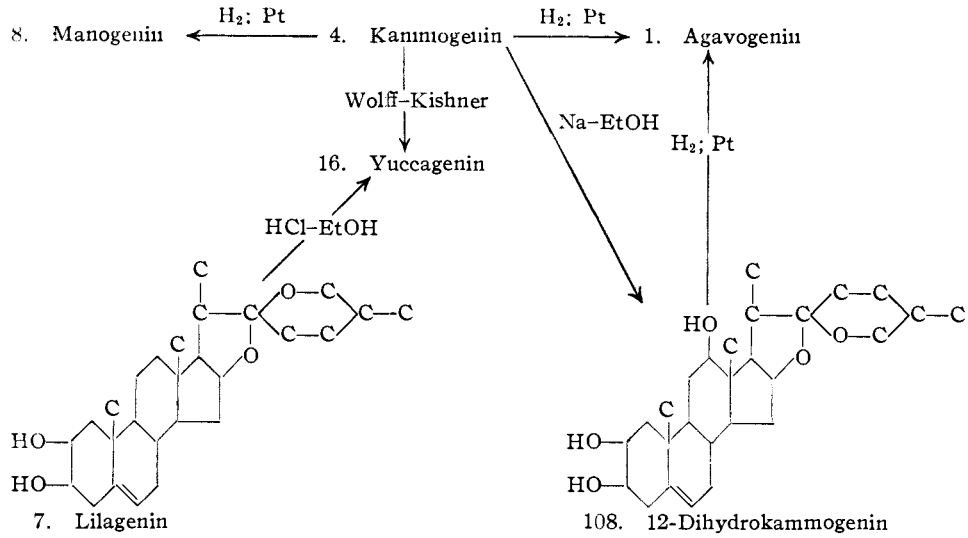


Fig. 1.—Absorption curves taken in absolute ethanol of: kryptogenin (6), 5,6-dihydrokryptogenin (114), 12-ketocholanic acid (a), 3,12-diketocholanic acid (b), kammogenin (4), sarsasapogenin (22).

Although kammogenin (4) forms a monosemicarbazone, it is unaffected by the conditions of a mild Clemmensen reaction, indicating once again the inertness of the carbonyl group.

Semicarbazone.—An alcoholic solution of semicarbazide acetate was prepared by adding a solution of 0.2 g. of potassium acetate in 2 cc. of 95% ethanol to a solution of 0.2 g. of semicarbazide hydrochloride in 0.6 cc. of water. The potassium chloride which precipitated was filtered. The filtrate was then added to a solution of 0.2 g. of the diacetate of kammogenin in 2 cc. of dry pyridine and 2 cc. of absolute ethanol. After the addition of 4 cc. of water, the mixture was allowed to stand at room temperature for seven days. It was then poured into water. The precipitate was filtered, washed with water, and dried. It was then washed well with ether to give crystals, m. p. 279° dec. This material is very soluble in ethanol.

Anal. Calcd. for $C_{32}H_{47}O_7N_3$: C, 65.6; H, 8.1. Found: C, 65.4; H, 8.4.



7. Lilagenin 108. 12-Dihydrokammogenin

Additional evidence for the elimination of the 7-position for the carbonyl group in the ketosapogenins has been found in the non-identity of kammogenin diacetate with 7-ketoyuccagenin diacetate (109). The latter is prepared by the zinc–acetic acid reduction of the corresponding 23-bromo compound (105).

Kammogenin readily undergoes the pseudo-reaction yielding crystalline pseudokammogenin triacetate. Hydrolysis of this forms pseudokammogenin which upon treatment with mineral acid reverts to the original sapogenin.

Pseudokammogenin.—Kammogenin diacetate, 4 g., was heated with 15 cc. of acetic anhydride at 200° for ten hours. The reaction mixture was evaporated to dryness *in vacuo* on the steam-bath and the residue after treatment with Norite, was crystallized from methanol, m. p. 147–148°; yield 3.2 g.

Anal. Calcd. for $C_{33}H_{46}O_8$: C, 69.5; H, 8.1. Found: C, 69.2; H, 8.0.

This material after hydrolysis with boiling 5% potassium hydroxide and crystallization from acetone gave pseudokammogenin, m. p. 188–189°.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 72.9; H, 9.1. Found: C, 72.6; H, 9.0.

Pseudokammogenin, 0.2 g., in 50 cc. of ethanol was treated with 5 cc. of concentrated hydrochloric acid in 50 cc. of ethanol at steam-bath temperature for one hour. The product was ether extracted and crystallized from ether to give kammogenin, m. p. and mixed m. p., 240–242°; wt. 0.1 g.

Lilagenin

Previously, the isolation of lilagenin (7) from *Lilium rubrum magnificum* was reported.[88] In the present work, we have found a second and better source in *Lilium Humboldtii* Roezl and Leichtl. This new sapogenin is isomeric with yuccagenin (16) and is related to the latter in exactly the same manner as sarsasapogenin (22) is to smilagenin (23), differing only in the configuration of the side-chain. Thus, acid treatment of lilagenin causes inversion at C-22 to give yuccagenin (16).[6]

(88) Marker, Turner, Shabica, Jones, Krueger and Surmatis, THIS JOURNAL, **62**, 2620 (1940).

Yuccagenin from Lilagenin.—To a boiling solution of 275 mg. of lilagenin in 27 cc. of 95% ethanol was added a solution of 8.2 cc. of concentrated hydrochloric acid in 18 cc. of 95% ethanol. The resulting solution was heated under reflux on the steam-bath for twenty-three hours, during which time needle-like crystals formed on the sides of the flask. The mixture was taken up in ether and the ethereal solution was washed thoroughly with water and evaporated to give yuccagenin, m. p. and mixed m. p., 242–243°; yield 150 mg. A mixture with lilagenin (246°) melted 215–225°.

Anal. Calcd. for $C_{27}H_{42}O_4$; C, 75.3; H, 9.8. Found: C, 75.2; H, 10.0.

This material after acetylation and crystallization from acetic anhydride gave yuccagenin diacetate, m. p. and mixed m. p., 176–178°.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.4; H, 9.2.

It has been shown how other pairs of isomeric sapogenins are similarly related, namely, yamogenin and diosgenin; neotigogenin and tigogenin; texogenin and samogenin. The first of each pair is converted to the other by treatment with ethanolic hydrochloric acid.

Mexogenin and Samogenin

Mexogenin (9) and samogenin (13) were obtained principally from *Samuela carnerosana* Trel. and *Yucca Schottii* Engelm. Although they may not be obtained at all times from the latter, particularly in plants which have just finished flowering, they are obtained in appreciable quantities (in addition to other sapogenins) from plants which are ready to flower. *Samuela carnerosana* Trel. is the better source since these two sapogenins usually predominate and can be isolated more easily.

Relationship between Mexogenin and Samogenin.—Mexogenin can be converted readily to samogenin by the Wolff–Kishner method.

Samogenin from Mexogenin.—The procedure (Wolff-Kishner reduction) and quantities of reactants were identical with those used for the preparation of tigogenin from hecogenin. Samogenin crystallized from methanol, m. p. and mixed m. p., 205–207°; yield 0.3 g.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.9; H, 10.3.

The acetate of this material crystallized from pentane, m. p. and mixed m. p. with samogenin diacetate, 195–198°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.0; H, 9.4.

The presence of a keto group in mexogenin suggests that it may be related to the 12-ketosapogenins, namely, manogenin and hecogenin. This is further indicated by the fact that mexogenin (9) and its reduction product, samogenin (13), are isomeric with manogenin (8) and its reduction product, gitogenin (55), respectively. Furthermore, the non-reactivity of the carbonyl group in mexogenin resembles that in manogenin, especially in the fact that it is unchanged by the mild Clemmensen treatment. Since a keto group at C-7 or C-6 is reactive under these conditions, these positions are unlikely for the carbonyl group in 9. Mexogenin reacts with semicarbazide acetate under the conditions employed for the formation of hecogenin semicarbazone; it also forms a 2,4-dinitrophenylhydrazone.

Semicarbazone.—An alcoholic solution of semicarbazide acetate was prepared by adding a solution of 0.5 g. of semicarbazide hydrochloride in 1.5 cc. of water to a solution of 0.5 g. of potassium acetate in 5 cc. of absolute ethanol. The precipitated potassium chloride was filtered. To a solution of 0.5 g. of mexogenin diacetate in 5 cc. of absolute ethanol and 5 cc. of dry pyridine was added the ketone reagent and 10 cc. of water. The mixture was allowed to stand at room temperature for four days. It was then poured into water and the precipitated solid was filtered, washed with water and dried. The crude semicarbazone was digested with 100 cc. of boiling ether and dried, m. p. 213–214° dec.

Anal. Calcd. for $C_{32}H_{49}O_7N_3$: C, 65.4; H, 8.4. Found: C, 64.5; H, 8.7.

2,4-Dinitrophenylhydrazone.—This was prepared in like manner as described for the derivative of hecogenin. It was crystallized from ethanol as needles, m. p. 290–291° dec.

Anal. Calcd. for $C_{37}H_{40}O_{10}N_4$: C, 62.5; H, 7.1. Found: C, 62.1; H, 7.1.

Catalytic hydrogenation or reduction of mexogenin using sodium in ethanol introduces a third hydroxyl group which can be acetylated as in the case of 12-dihydrosapogenins.

Reduction of Mexogenin.—1. A solution of 0.3 g. of mexogenin diacetate in ether containing a few drops of acetic acid was hydrogenated for two hours at room temperature and 3 atm. over Adams catalyst. The product was hydrolyzed and crystallized from ether to give 12-dihydromexogenin, m. p. 209–211°.

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

The acetate crystallized from methanol, m. p. 127–129°.

Anal. Calcd. for $C_{33}H_{50}O_8$: C, 68.9; H, 8.8. Found: C, 68.8; H, 8.9.

2. A mixture of 1 g. of mexogenin, 0.5 g. of Adams catalyst and 200 cc. of acetic acid was shaken with hydrogen at 70° and 3 atm. for twelve hours. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was hydrolyzed with dilute alcoholic potash and then crystallized from acetone to give the tetrahydrosapogenin (open side-chain), m. p. 204–206°.

Anal. Calcd. for $C_{27}H_{46}O_5$: C, 72.0; H, 10.3. Found: C, 71.7; H, 10.2.

3. To a solution of 1 g. of mexogenin diacetate in 300 cc. of hot absolute ethanol was added 23 g. of sodium strips during thirty minutes. After the sodium had dissolved, the mixture was cooled and ether extracted. The ethereal solution was washed with water, dilute hydrochloric acid and water, and then evaporated to give 12-dihydromexogenin, m. p. 212–213°. A mixture with the product from the cold catalytic hydrogenation of mexogenin melted 212–213°.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.3; H, 9.9. Found: C, 71.8; H, 10.1.

The triacetate was prepared and was crystallized from methanol as needles, m. p. and mixed m. p. with 12-dihydromexogenin triacetate from above, 129°.

Anal. Calcd. for $C_{33}H_{50}O_8$: C, 68.9; H, 8.8. Found: C, 68.5; H, 9.0.

These and subsequent reactions show that mexogenin and samogenin probably differ from manogenin and gitogenin, respectively, only in having the coprostane configuration of the hydrogen atom at C-5. All four sapogenins are unaffected by treatment with hydrochloric acid in ethanol at the boiling point indicating that they all have the *iso*-configuration of the side-chain.[83]

Ethanolic Hydrochloric Acid Treatment of Samogenin.—To a solution of 1 g. of samogenin in 170 cc. of ethanol was added 30 cc. of concentrated hydrochloric acid. The mixture was heated under reflux for four days. The product was precipitated with water and filtered. Crystallization from methanol gave material melting 205–208° which did not depress the starting material.

The acetate crystallized from methanol as needles, m. p. and mixed m. p. with samogenin diacetate, 195–197°.

Although mexogenin and samogenin form precipitates with alcoholic digitonin solutions, indicating 3(β)-hydroxyl groups, their formation requires an alcoholic medium diluted with more water than that required for manogenin and gitogenin. This fact may be attributed to the difference of configuration of the hydrogen atom at C-5. Thus, Schoenheimer and Dam[89] found that the digitonide of coprostanol is more soluble in alcohol than that for cholestanol, and in like manner we have found that the digitonide of smilagenin is more soluble than the digitonide of tigogenin.

As is characteristic of steroids having the copro-hydrogen at C-5, the epimer (112) of samogenin is formed when the latter is heated with sodium ethoxide in a sealed tube at 200°. *epi*-Samogenin (112) like other steroids having the *alpha* configuration of the hydroxyl group at C-3 is not precipitated by digitonin in aqueous alcohol.

***epi*-Samogenin.**—A solution of 3 g. of sodium metal in 60 cc. of absolute ethanol and 1 g. of samogenin were placed in a sealed tube and heated for twelve hours at 200°. The product was extracted with ether and the ethereal solution was washed free of alkali. The solvent was removed and *epi*-samogenin was crystallized from methanol at 40°, m. p. 235–237°; yield 0.4 g.

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

The diacetate crystallized from methanol as white plates, m. p. 212–215°; a mixture with samogenin diacetate (198°) melted 175°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.8; H, 9.2.

(89) Schoenheimer and Dam, *Z. physiol. Chem.*, **215**, 59 (1933).

Oxidation of samogenin (or mexogenin) gives only an acidic product, thus indicating two adjacent hydroxyl groups. 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.

Mexogenic Acid.—Mexogenin, 0.5 g., in 50 cc. of acetic acid was oxidized with 0.5 g. of chromic anhydride dissolved in 5 cc. of 80% acetic acid at 20–24° during thirty minutes. The acidic fraction was isolated and crystallized from acetone, m. p. 304–306° dec. A mixture with 7-ketogitogenic acid (293°) melted ten degrees low.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.0; H, 8.5. Found: C, 67.7; H, 8.8.

The **dimethyl ester** was prepared with diazomethane in ether and was crystallized from methanol, m. p. 148–151°.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.0; H, 8.8. Found: C, 69.0; H, 8.7.

Oxidation of Samogenin to Samogenic Acid.—A solution of 0.2 g. of samogenin in 50 cc. of acetic acid was treated at room temperature with 0.2 g. of chromic anhydride dissolved in 10 cc. of 80% acetic acid for thirty minutes. The reaction mixture was extracted with ether and the ethereal solution was washed with water and 10% sodium carbonate. The alkali wash was acidified and the precipitated solid was crystallized from acetic acid, m. p. 264° dec. Recrystallization from acetone gave needles, m. p. 270–271° dec.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.1; H, 9.2. Found: C, 70.2; H, 9.2.

The **dimethyl ester** was prepared with diazomethane in ether and was crystallized from methanol, m. p. 159–160°.

Anal. Calcd. for $C_{29}H_{46}O_6$: C, 71.0; H, 9.5. Found: C, 71.3; H, 9.5.

Side-chain Reactions of Samogenin and Mexogenin.—Samogenin and mexogenin give the usual sapogenin reactions. Thus, both form pseudosapogenins which are reconverted with acid to the original sapogenin, both hydrogenate to the dihydrosapogenins, and mexogenin brominates at room temperature to a monobromo compound. By analogy to other sapogenins, this latter substance should be the 23-bromo derivative. It is unaffected by boiling pyridine, but it can readily be debrominated with zinc in acetic acid to the original sapogenin.

Pseudosamogenin.—Samogenin diacetate, 5 g., and 15 cc. of acetic anhydride were heated in a sealed tube for ten hours at 200°. The acetic anhydride was removed *in vacuo* and the residue was hydrolyzed with alcoholic potassium hydroxide for thirty minutes. The product was extracted with ether and crystallized from methanol as needles, m. p. 178–181°. A mixture with samogenin melted thirty degrees low. A mixture with the pseudosapogenin (184°) from texogenin melted 179–184°.

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

This product after alkaline hydrolysis and treatment with alcoholic hydrochloric acid gave samogenin, m. p. and mixed m. p., 210°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.8; H, 10.1.

The *diacetate* was prepared and was crystallized from methanol as needles, m. p. and mixed m. p. with samogenin diacetate, 195–198°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.9; H, 9.3.

Dihydrosamogenin.—A solution of 1 g. of samogenin in 250 cc. of acetic acid was shaken with 1 g. of Adams catalyst and hydrogen at 70° and 3 atm. for seven hours. The product which crystallized from acetic acid was hydrolyzed with alcoholic potash and then crystallized from acetone, m. p. 214–215°; yield 0.5 g. A mixture with starting material (210°) melted 190–200°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.6; H, 10.5.

Pseudomexogenin.—A solution of 5 g. of mexogenin in 15 cc. of acetic anhydride was heated at 200° for twelve hours in a sealed tube. The solvent was removed and the residue was hydrolyzed with 200 cc. of boiling 5% alcoholic potassium hydroxide for thirty minutes. The hydrolysis mixture was extracted with ether and the ethereal solution was washed and evaporated until crystallization started and then cooled to give crystals which were recrystallized from acetone, m. p. 143–145°; yield, 3 g.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.4; H, 9.4.

A solution of 0.5 g. of this material in 100 cc. of ethanol and 5 cc. of conc. hydrochloric acid was warmed for thirty minutes and then allowed to stand overnight at room temperature. The product was ether extracted and crystallized from ether, m. p. and mixed m. p., with mexogenin, 246–248°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.2; H, 9.4.

23-Bromomexogenin Diacetate.—Mexogenin diacetate, wt. 1.0 g., in 10 cc. of chloroform was brominated with 0.75 g. of bromine in 10 cc. of chloroform at room temperature. After standing twenty minutes, the mixture was evaporated *in vacuo* at room temperature and the residue was crystallized from an ether–pentane mixture, m. p. 216° dec.

Anal. Calcd. for $C_{31}H_{45}O_7Br$: Br, 13.1. Found: Br, 13.4.

Treatment of this material with 35 cc. of pyridine at the boiling point for eight hours did not change it.

Anal. Calcd. for $C_{31}H_{45}O_7Br$: C, 61.1; H, 7.4. Found: C, 61.1; H, 7.5.

Further treatment of the recovered material with 6 g. of zinc dust in 100 cc. of acetic acid for five hours on the steam-bath gave material which was hydrolyzed and crystallized from ether to give mexogenin, m. p. and mixed m. p., 245–246°; yield 0.3 g. from 1 g. of mexogenin diacetate. This was further identified as mexogenin diacetate, m. p. and mixed m. p., 206–208°. Kammogenin could not be detected in the mother liquor.

Texogenin

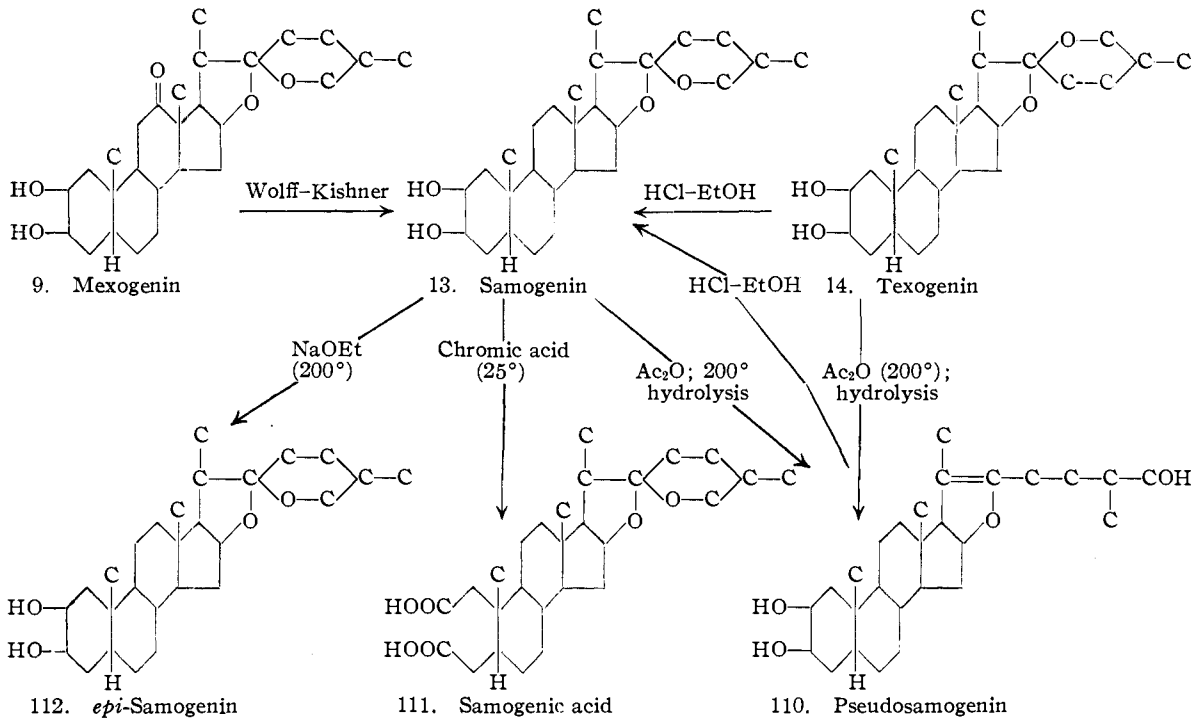
Texogenin (14) has been obtained from the sapogenin fraction of *Yucca Schottii* Engelm. in relatively small amounts.

Acid isomerization of texogenin gives samogenin (13).

Samogenin from Texogenin.—A solution of 0.5 g. of texogenin in 85 cc. of ethanol containing 15 cc. of concentrated hydrochloric acid was refluxed for four days. Water was added and the solid was extracted with ether. Crystallization from ether gave platelets, m. p. and mixed m. p. with samogenin 203–204°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.8; H, 10.1.

Texogenin, therefore, differs from samogenin (13) only in the configuration of the side-chain. Since samogenin is unaffected by this treatment, it has the *iso* side-chain and texogenin has the normal side-chain, by analogy to other C-22 isomeric sapogenins. The fact that the difference in samogenin and texogenin is limited to the side-chain is shown by the formation of the same pseudosapogenin. Thus, treatment of either



texogenin or samogenin in a sealed tube at 200° for ten hours followed by hydrolysis gives pseudosamogenin (110).

Pseudosamogenin from Texogenin.—Texogenin, wt. 1.5 g., was heated with 5 cc. of acetic anhydride in a sealed tube at 200° for ten hours. The acetic anhydride was removed *in vacuo*. The residual sirup was hydrolyzed with alcoholic potash and extracted with ether. The ether was removed and the residue after treatment with Norite was crystallized from methanol, m. p. 184°. A mixture with pseudosamogenin melted 179–184°.

Pseudosamogenin (110) unlike pseudosarsasapogenin (24) reverts to the sapogenin with the *iso* side-chain upon treatment with mineral acid. This fact is significant for this reason. Texogenin in its isolation from *Yucca Schottii* Engelm. is obtained from a fraction which has undergone the pseudo reaction and the subsequent acid isomerization reaction. Since it has been shown that pseudosamogenin (or pseudotexogenin) reverts to samogenin, it is apparent that texogenin in this fraction was not completely converted to pseudosamogenin; consequently a portion was fortunately isolated.

Acid Isomerization of Pseudosamogenin from Texogenin.—A solution of the pseudosapogenin from texogenin, wt. 0.8 g., in 40 cc. of ethanol and 5 cc. of concd. hydrochloric acid was heated under reflux for one hour. The reaction mixture was ether extracted and the ethereal solution was washed with water and evaporated to give samogenin, m. p. and mixed m. p., 207–208°; yield 0.5 g. From the mother liquor was isolated an additional 0.15 g. of samogenin. No texogenin could be found.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.9; H, 10.1.

Samogenin diacetate was prepared and was crystallized from methanol as fine needles, m. p. and mixed m. p., 199–200°,

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.2; H, 9.4.

Oxidation of texogenin (14) under mild conditions with chromic acid in acetic acid yields texogenic acid (side-chain isomer of 111).

Oxidation of Texogenin.—To a solution of 0.5 g. of texogenin in 40 cc. of glacial acetic acid at 20° was added 10 cc. of 90% acetic acid containing 0.45 g. of chromic anhydride. The temperature was maintained at 25° for one hour after which water and ether were added. The ethereal solution, after washing with water, was extracted with dilute potassium hydroxide solution. The alkaline extract was acidified and extracted with ether. After washing with water the ether was evaporated and the residue crystallized from acetone to give small plates of texogenic acid, m. p. 268–269° dec.; yield 50 mg.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.1; H, 9.2. Found: C, 70.1; H, 9.2.

Kryptogenin

Kryptogenin was first isolated from the sapogenin fraction of *Beth* root,[2,3] in which it accounted for 20–50% of the total crystalline steroidal fraction. It was later found in numerous species of the *Dioscoreas*. In our present study, we have indicated its structure to be 6. This has been done: (a) by relating it to previously characterized sapogenins and their derivatives, namely, diosgenin (42), tigogenin (17), dihydrotigogenin (100), and dihydro-pseudotigogenin (33); (b) by the formation of its 5,6-dihydro derivative from the diacetate of dihydrotigogenin (100); and (c) by the establishment and study of its 1,4-diketone system.

Kryptogenin forms a diacetate and readily absorbs two atoms of bromine in cold acetic acid solution without liberation of hydrogen bromide.

The dihalide (113) reverts to the original olefin upon treatment with zinc and acetic acid or with pyridine.

Kryptogenin Diacetate.—This material was prepared by the reaction of kryptogenin with pyridine–acetic anhydride mixture or with boiling acetic anhydride. It crystallized from acetone as needles, m. p. 152–153°.

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

The free genin was prepared by refluxing a solution of 10 g. of kryptogenin diacetate in 2 liters of methanol and 1.2 liters of 5% potassium bicarbonate for five hours. The product was ether extracted and crystallized from acetone, m. p. 187–189°; wt. 6 g.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.1; H, 9.7.

Hydrolysis of the diacetate was also accomplished by refluxing a solution of kryptogenin diacetate, 0.5 g., in 100 cc. of ethanol containing 10 cc. of concentrated hydrochloric acid for ninety minutes and then allowing it to stand at room temperature for three days. The product was ether extracted and crystallized from acetone as plates, m. p. and mixed m. p. with kryptogenin from above, 187–189°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.1; H, 9.7.

Diacetate of 5,6-Dibromokryptogenin.—A solution of 10 g. of kryptogenin diacetate in 150 cc. of acetic acid was treated with excess bromine–acetic acid solution (1 g. of bromine per 10 cc. of acetic acid) at room temperature. After the addition of 40 cc., the bromine solution was no longer decolorized. An additional 70 cc. was added. No hydrogen bromide was evolved. After standing twenty minutes, the mixture was diluted with water and filtered. The solid was washed with water and crystallized from acetone, m. p. 138–140°; yield 11.3 g.

Anal. Calcd. for $C_{31}H_{46}O_6Br_2$: Br, 23.7. Found: Br, 24.2.

The addition of hydrobromic acid to the bromination mixture did not result in any side-chain substitution.

Debromination of 5,6-Dibromokryptogenin Diacetate. (a) **With Pyridine.**—A solution of 0.2 g. of material in 10 cc. of pyridine was refluxed for seven hours. The product was ether extracted and crystallized from acetone as kryptogenin diacetate, m. p. and mixed m. p., 150°. This was hydrolyzed with potassium bicarbonate to give kryptogenin, m. p. and mixed m. p., 189°.

(b) **With Zinc–Acetic Acid.**—A solution of 0.5 g. of the dibromo-compound in 15 cc. of acetic acid heated on the steam-bath was treated with 0.5 g. of zinc dust for ten minutes. The product was ether extracted and crystallized from acetone, m. p. and mixed m. p. with kryptogenin diacetate, 150°. Hydrolysis gave the free genin, m. p. and mixed m. p., 189°.

In another run, 0.5 g. of the diacetate of the dibromo compound in 300 cc. of acetic acid was treated at steam-bath temperature with 18 g. of zinc dust during twenty minutes. The product crystallized from acetone, m. p. 228–230°; yield 0.2 g. This material was not further identified.

Diosgenin and Tigogenin from Kryptogenin.—The assignment of the double bond to the 5,6-position follows from the fact that kryptogenin is reduced with sodium in isopropyl alcohol or by the Meerwein method to diosgenin (42).

Diosgenin from Kryptogenin.—a. To a refluxing solution of 5 g. of kryptogenin diacetate in 1 liter of isopropyl alcohol (98–99% pure) was added 100 g. of sodium strips during one hour. An additional liter of isopropyl alcohol was added at the end of the sodium addition. The reaction mixture was cooled and the excess sodium (20 g. or less) was dissolved in ethanol and the mixture was extracted with 4 liters of ether. The ethereal solution was washed with water and evaporated. The residue was taken up in 300 cc. of acetone and the insoluble material was filtered, m. p. 229–237°. From the acetone-soluble fraction was obtained a second and third crop of crystals, m. p. 228–237°; total weight of high melting material, 2 g. Recrystallization of this material from acetone gave 16,23,22-tetrahydrofesogenin described below, m. p. and mixed m. p., 238–240°. The acetone mother liquor from the third crop was evaporated and the residue was acetylated with boiling acetic anhydride and crystallized from the acetylation mixture, m. p. 185–195°; wt. 2.5 g. Recrystallization from acetone gave diosgenin 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, 76.2; H, 9.7.

Hydrolysis of the acetate and crystallization from methanol gave diosgenin as needles, m. p. 212–214°. A mixture with diosgenin (208°) melted 209–212°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 77.9; H, 10.6.

b. A mixture of 1 g. of kryptogenin, 3 g. of aluminum isopropylate and 100 cc. of dry isopropyl alcohol was heated under reflux for six hours and then concentrated by distillation to 50 cc. during six hours. The residue was taken up in ether and the ethereal solution was washed with dilute sulfuric acid and water and evaporated to give diosgenin, m. p. and mixed m. p., 207–209°; wt. 0.1 g. The acetate was prepared and crystallized from acetone, m. p. and mixed m. p. with diosgenin acetate, 195–197°.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 75.9; H, 10.1.

In a second run, the same conditions were employed with the exception that wet isopropyl alcohol (80 cc. of dry isopropyl alcohol containing five drops of water) was used. The product was diosgenin, m. p. and mixed m. p., 202–204°.

In a third run using anhydrous conditions, a product melting 191–192°, wt. 0.5 g., was obtained. This material shows no absorption in the ultraviolet region.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.1; H, 10.1.

The acetate of the latter was prepared with boiling acetic anhydride and crystallized from methanol, m. p. 216–219°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.9; H, 9.6.

Catalytic hydrogenation (Adams catalyst) of the free material from this run in ether containing a few drops of acetic acid for two hours at room temperature and 3 atm. gave material which was crystallized from dilute alcoholic potash, m. p. 210–212°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 75.1; H, 10.6.

This material was not further studied.

In still another run under anhydrous conditions using *kryptogenin diacetate* instead of the free genin, the major product was starting material.

Under these conditions the carbonyl group at C-16 is changed to a hydroxyl group, resulting in the formation of the spiro-ketal side-chain. The latter is stable to further reduction in the presence of sodium and alcohol. The simultaneous reduction of the 16-keto group and the 5,6-double bond in kryptogenin can be accomplished. Thus, catalytic hydrogenation (Adams catalyst) for several hours in ether containing several ml. of acetic acid gives tigogenin (17). On the other hand, similar hydrogenation in acetic acid for a few minutes or in ether using only a few drops of acetic acid for two hours gives 5,6-dihydrokryptogenin (114),

which itself can be converted to tigogenin by sodium in isopropyl alcohol or by the Meerwein reduction.

Tigogenin from Kryptogenin.—A solution of 1 g. of kryptogenin in 500 cc. of ether containing 2 cc. of acetic acid was shaken with Adams catalyst and hydrogen at room temperature and 3 atm. for two and one-half hours. The product was crystallized from acetone to give tigogenin, m. p. and mixed m. p., 203–205°; wt. 0.7 g. A portion was acetylated and was crystallized from methanol as plates, m. p. and mixed m. p. with tigogenin acetate, 206–208°.

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

Cold oxidation of the free genin with chromic acid in acetic acid gave tigogenone which was crystallized from ether, m. p. and mixed m. p., 205–207°.

5,6-Dihydrokryptogenin. a. Hydrogenation in Ether–Acetic Acid.—A solution of 8 g. of kryptogenin diacetate in 800 cc. of ether containing five drops of acetic acid was shaken with 3 g. of Adams catalyst and hydrogen at 3 atm. and room temperature for three hours. The product was crystallized from methanol, m. p. 121–123°; yield 6.5 g. This material could not be further acetylated. A mixture with the diacetate of dihydrotigogenin, 123°, melted 90–95°.

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

The free genin was prepared by refluxing a solution of the diacetate, 3 g., in 600 cc. of methanol and 360 cc. of 5% potassium bicarbonate for five hours. The mixture was ether extracted and 5,6-dihydrokryptogenin was crystallized from ether, m. p. 169–171°; wt. 2.4 g.

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

The hydrolysis can also be accomplished by heating a solution of the diacetate, 14 g., in 840 cc. of methanol with 9 g. of potassium hydroxide in 365 cc. of water for twenty minutes at steam-bath temperature, yield 5 g.

5,6-Dihydrokryptogenin was also prepared by the catalytic hydrogenation of the unacetylated genin, 0.5 g., in ether (500 cc.) containing five drops of acetic acid for two hours at 3 atm. and room temperature; m. p. and mixed m. p. with above, 169–171°; wt. 0.4 g. A mixture of the diacetate of the latter and 5,6-dihydrokryptogenin diacetate melted 121–123°.

b. Hydrogenation in Acetic Acid.—Catalytic hydrogenation of kryptogenin diacetate, 2 g., in 150 cc. of acetic acid using 0.5 g. of Adams catalyst at room temperature and *two pounds pressure* for fifteen minutes gives 5,6-dihydrokryptogenin diacetate crystallized from methanol as plates, m. p. and mixed m. p., 121–123°; yield 1.1 g.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.9; H, 9.3.

A larger run was made in which 20 g. of kryptogenin diacetate in 750 cc. of acetic acid was shaken with 3 g. of Adams catalyst and hydrogen at 27° and ten pounds pressure for fifteen–seventeen minutes. The pressure dropped to four pounds during this time; yield 15 g.

Tigogenin from 5,6-Dihydrokryptogenin. a. Sodium and Isopropyl Alcohol Reduction.—To a boiling solution of 5 g. of the diacetate of 5,6-dihydrokryptogenin in 1500 cc. of dry isopropyl alcohol was added 100 g. of sodium strips during one hour. The reaction mixture was worked up as described for the sodium–isopropyl alcohol reduction of kryptogenin, to give tigogenin directly from the ether extract, m. p. and mixed m. p., 203–207°; yield 2.3 g. No hexahydrofesogenin could be isolated.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 77.7; H, 10.9.

Tigogenin acetate was prepared and was crystallized from acetic anhydride as needles, m. p. and mixed m. p., 207–210°.

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

b. By the Meerwein Reduction.—5,6-Dihydrokryptogenin, wt. 1 g., was reduced under the anhydrous conditions described for the similar reduction of kryptogenin. The product was crystallized from acetone to give tigogenin, m. p. and mixed m. p., 200–203°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 77.7; H, 10.9.

The acetate was prepared and was crystallized from acetone, m. p. 196–198°. A mixture with tigogenin acetate (208°) melted 204–208°.

It is noteworthy that the conversion 6 → 42 or 17 gives the strongest support to a spiro-ketal structure for the sapogenin side-chain.

Dihydrotigogenin and Dihydro-pseudotigogenin from Kryptogenin.—When the acetate of tigogenin (17) is catalytically reduced in hot acetic acid using Adams catalyst, it takes up one mole of hydrogen to give dihydrotigogenin (100). Reduction of kryptogenin or its diacetate or dihydrokryptogenin diacetate in a like manner gives the same product (100). Under these conditions, dehydration of an intermediate dihydroxy compound probably occurs, forming the tetrahydrofuran ring. By analogy, the diphenylcarbinol (115) of desoxytigogenin lactone (116) loses water between the two hydroxyl groups with the formation of a compound having the properties of a tetrahydrofuran derivative.[22] It could be possible, however, that the reduction of the unacetylated kryptogenin proceeds through the formation of a 16-hydroxy compound forming tigogenin which then undergoes further reduction to the dihydro-compound (100). Although hydrogenation (Adams catalyst) of the diacetate of kryptogenin (6) in acetic acid at room temperature for fifteen minutes gives the diacetate of 5,6-dihydrokryptogenin (114), prolonged hydrogenation gives the diacetate of dihydrotigogenin (100).

Dihydrotigogenin from Kryptogenin.—**a.** The catalytic hydrogenation of kryptogenin diacetate in acetic acid for fifteen minutes has been described above. Prolonged hydrogenation (two hours) at a higher pressure (3 atm.) gave dihydrotigogenin diacetate which was crystallized from methanol, m. p. and mixed m. p., 114–116°; yield, 1.2 g. from 2 g. of kryptogenin diacetate.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.0; H, 10.0. Found: C, 74.1; H, 10.0.

The free genin was prepared by the hydrolysis of the diacetate with alcoholic potash and was crystallized from ether, m. p. and mixed m. p. with dihydrotigogenin, 170°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.2; H, 11.1.

b. Kryptogenin diacetate, 1 g., in 200 cc. of acetic acid was shaken with 0.5 g. of Adams catalyst and hydrogen at 70° and 3 atm. for eight hours. The residue remaining after the removal of the acetic acid was dissolved in 140 cc. of ethanol and hydrolyzed with 4 g. of potassium bicarbonate in 60 cc. of water for ninety minutes at steam-bath temperature. The product was ether extracted and crystallized from ether as plates, m. p. and mixed m. p. with dihydrotigogenin, 167–170°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.1; H, 10.7.

In another run, the unacetylated genin was hydrogenated in like manner and the product after acetylation was crystallized from methanol to give dihydrotigogenin diacetate, m. p. and mixed m. p., 115–116°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.0; H, 10.0. Found: C, 73.8; H, 9.9.

Hydrolysis of the diacetate gave dihydrotigogenin, m. p. and mixed m. p., 170°.

Dihydrotigogenin from Dihydrokryptogenin.—Catalytic hydrogenation of 5,6-dihydrokryptogenin in hot acetic acid as described above for kryptogenin followed by acetylation gave dihydrotigogenin diacetate which was crystallized from methanol, m. p. and mixed m. p., 114–116°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.0; H, 10.0. Found: C, 74.1; H, 9.9.

Hydrolysis of the diacetate gave dihydrotigogenin which was crystallized from acetone, m. p. and mixed m. p., 166–168°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.4; H, 11.1.

On the basis of two color tests with Schiff reagent and 1,4-dihydroxynaphthalene,[90] Noller and Barusch[91] suggested kryptogenin to be a ketoaldehyde. The diketo compounds, namely, tigogenoic acid (117), chlorogenoic acid (analog of 117 prepared from chlorogenin) and methyl chlorogenoate diacetate, all give negative results with these reagents.[91]

Our full experimental data on kryptogenin readily show that a ketoaldehyde structure could not account for its reactions. Thus, the conversion of kryptogenin diacetate to dihydrotigogenin diacetate by catalytic reduction (Adams catalyst in acetic acid) eliminates the presence of an aldehyde group at C-27. Other reactions shown by kryptogenin that are inconsistent with a ketoaldehyde structure are pointed out later. Moreover, we have repeated the color experiments with pure krytogenin and its diacetate from many species of *Trillia* as well as from bethogenin and obtained negative tests. These results are in agreement with the results obtained with the other diketo compounds, tigogenoic acid (117), etc., as shown by Noller.[91]

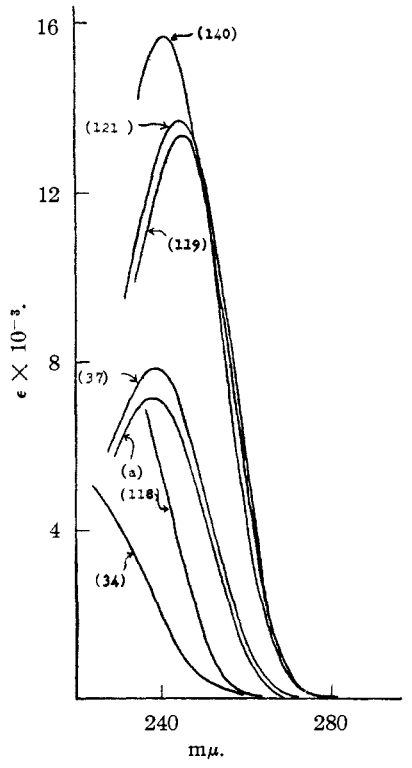


Fig. 2.—Absorption curves taken in absolute ethanol of: pennogenone (140); fesogenin (121); 5,6-dihydrofesogenin (119); 5,16-pregnadien-3(β)-ol-20-one (37); 5,16-pregnadien-2,3(β)-diol-20-one (a); pseudokryptogenin (pseudopennogenin) (118). Absorption curve taken in isoöctane of pseudodiosgenin diacetate (34).

(90) Raudnitz and Puluj, *Ber.*, **64**, 2212 (1931).

(91) Noller and Barusch, THIS JOURNAL, **65**, 1786 (1943).

Pseudokryptogenin.—When kryptogenin (6) or its 5,6-dihydro compound (114) is heated with acetic anhydride at 200°, it is converted to pseudokryptogenin (118) or pseudo-5,6-dihydrokryptogenin. These pseudo compounds are reconverted to the original compounds by acid treatment.

Pseudokryptogenin.—A solution of 5 g. of kryptogenin diacetate in 15 cc. of acetic anhydride was heated at 200° in a sealed tube for ten hours. The solvent was removed *in vacuo* and the residue was hydrolyzed with excess potassium bicarbonate (or alcoholic potash) in aqueous ethanol. The product was ether extracted, crystallized from acetone and then recrystallized from ether to give pseudokryptogenin, m. p. 189–192°; yield 1 g. A mixture with kryptogenin melted 162–167°.

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.6; H, 9.8. Found: C, 78.5; H, 9.8.

Treatment of this product, 0.2 g., with 50 cc. of ethanol containing two and one-half cc. of concentrated hydrochloric acid for thirty minutes on the steam-bath isomerized it to kryptogenin, m. p. and mixed m. p., 182–184°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.1; H, 9.7.

Pseudo-5,6-dihydrokryptogenin.—A solution of 25 g. of 5,6-dihydrokryptogenin diacetate in 30 cc. of acetic anhydride was heated in a sealed tube for twelve hours at 200°. The solvent was removed *in vacuo* at steam-bath temperature and the residue was crystallized from 100 cc. of ethanol, m. p. 96–98°; yield, 15 g.

Anal. Calcd. for $C_{31}H_{46}O_5$: C, 74.7; H, 9.3. Found: C, 74.5; H, 9.4.

Hydrolysis of this material with alcoholic potash followed by isomerization with alcoholic hydrochloric acid gave 5,6-dihydrokryptogenin, m. p. and mixed m. p., 169–171°.

The formation of these pseudo compounds may be regarded as simple dehydrations of the dienols. The double bond at carbons 20–22 in the pseudosapogenins has been definitely established by its oxidative cleavage, forming an ester of a β-hydroxyketone as in 35. Pseudokryptogenin and pseudo-5,6-dihydrokryptogenin diacetate probably differ from the other pseudosapogenins in possessing a double bond at carbons 16–17 as well as at carbons 20–22. Since pseudokryptogenin readily forms a diacetate and does not show the typical absorption for a carbonyl group (Fig. 2) the possibility of an aldehyde group in 6 is eliminated. Catalytic hydrogenation of pseudokryptogenin (118) in acetic acid gives dihydropseudotigogenin (33), obtained by like reduction of pseudotigogenin (29).

Dihydropseudotigogenin from Pseudokryptogenin.—An acetic acid solution of 1 g. of pseudokryptogenin was shaken with 0.5 g. of Adams catalyst and hydrogen at room

temperature and 3 atm. for five hours. The product was crystallized from acetic acid, washed with ether and dried, m. p. and mixed m. p. with dihydropseudotigogenin, 202–204°.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.0; H, 11.2.

The diacetate was prepared by boiling a solution of 0.2 g. of the above material in 10 cc. of acetic anhydride for thirty minutes. The acetylation mixture was cooled to −5° and the needle-like crystals were filtered, m. p. and mixed m. p. with dihydropseudotigogenin diacetate, 122–124°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.0; H, 10.0. Found: C, 73.9; H, 9.9.

Kryptogenin Derivatives from the Dihydrosapogenins.—The synthesis of the diacetate of 5,6-dihydrokryptogenin (114) from the diacetate of dihydrotigogenin (100) is accomplished in good yields by the oxidation of the latter. Its identity is established by a direct comparison of the free and acetylated genins, the anhydro compound (119), and the 3-keto acid (120) and its methyl ester. In this manner, a hydroxyl group at C-27 is definitely established and the possibility of an aldehyde group at C-27 definitely eliminated.

5,6-Dihydrokryptogenin Diacetate from Dihydrotigogenin Diacetate.—Dihydrotigogenin diacetate was prepared by the catalytic hydrogenation (Adams catalyst) of 20 g. of diosgenin acetate in acetic acid at 70° and 3 atm. for eight hours followed by acetylation. The theoretical amount of hydrogen was absorbed. To a stirred solution of this material in 350 cc. of acetic acid at steam-bath temperature was dropped during two hours a solution of 10 g. of chromic anhydride in 100 cc. of 80% acetic acid. The reaction mixture was heated for two additional hours, cooled, treated with 50 cc. of methanol to destroy the excess chromic acid, concentrated *in vacuo* on the steam-bath, and finally ether extracted. The ethereal solution was washed with water, 3% alkali and water and evaporated. The residue was crystallized at −5° from 150 cc. of ethanol to give 5,6-dihydrokryptogenin diacetate, m. p. and mixed m. p., 121–123°, yield 10 g. A mixture with dihydrotigogenin diacetate (116°) melted 99–102°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.0; H, 9.4.

The diacetate, 2 g., in 400 cc. of methanol was hydrolyzed with 240 cc. of 5% potassium bicarbonate at reflux temperature for five hours. Dihydrokryptogenin crystallized from ether, m. p. and mixed m. p., 169–171°; wt. 1.6 g.

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

The character of this oxidation product was further established by converting 0.9 g. to 5,6-dihydrofesogenin as described below, m. p. and mixed m. p., 213–215°; wt. 0.5 g.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 77.9; H, 10.2.

Further oxidation of the hydrolyzed oxidation product, wt. 1 g., with cold chromic–acetic acid mixture as described previously gave 3-dehydrotigogenoic acid, m. p. and mixed m. p., 186–188°; wt. 0.4 g.

Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.9; H, 9.1. Found: C, 72.7; H, 9.1.

The methyl ester was prepared with diazomethane in ether and was crystallized from ether, m. p. and mixed m. p. with 3-dehydrotigogenoic acid methyl ester, 142–143°.

Anal. Calcd. for $C_{28}H_{42}O_5$: C, 73.3; H, 9.2. Found: C, 73.4; H, 9.2.

Oxidation of dihydrogitogenin triacetate under these conditions takes a similar course, giving the analog of 5,6-dihydrokryptogenin diacetate. The product is isolated as the triol.

Oxidation of Dihydrogitogenin Triacetate.—Dihydrogitogenin prepared by an eight-hour hydrogenation (Adams catalyst) at 70° and 3 atm. of 5 g. of gitogenin was converted to the triacetate with boiling acetic anhydride. The triacetate in 90 cc. of acetic acid was oxidized at 90° with a solution of 2.5 g. of chromic anhydride in 25 cc. of 80% acetic acid added during two hours. The reaction mixture was heated an additional hour and cooled. The excess chromic acid was destroyed with 15 cc. of methanol. The solution was concentrated *in vacuo* on the steam-bath and then ether extracted. The ethereal solution was washed with water, dilute alkali and water. The residue which crystallized from ether was refluxed with 30 g. of potassium bicarbonate in one liter of ethanol and 600 cc. of water for four hours. The product was ether extracted and crystallized from ether, m. p. 193–195°. A mixture with dihydrogitogenin (195°) melted 184–186°, yield, 0.5 g. This substance is the gitogenin analog of kryptogenin.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.3; H, 9.9. Found: C, 72.2; H, 10.1.

Reactions of the 1,4-Diketone System.—The absorption spectra (Fig. 1) of kryptogenin and of 5,6-dihydrokryptogenin in absolute ethanol, $\lambda_{max.}$ = 243 mμ (ϵ = 70) and 284 mμ (ϵ = 75), respectively, are characteristic of carbonyl groups. The absorption spectrum evidence for a monoketone or diketone is limited, however, since both systems give almost identical absorption. For example, both 3,12-diketocholanic acid and 12-ketocholanic acid show absorption at 288 mμ (ϵ = 70). Convincing evidence of the presence of two carbonyl groups in kryptogenin or dihydrokryptogenin is provided by formation of dioximes under mild conditions.

Kryptogenin Dioxime.—A solution of 0.5 g. of kryptogenin and 0.4 g. of hydroxylamine hydrochloride in 4 cc. of pyridine and 4 cc. of absolute ethanol was heated on the steam-bath for one hour. Crystals formed during the first fifteen minutes. The reaction mixture was filtered and the crystals were washed with water, dried and recrystallized from ethanol, m. p. 251° dec.

Anal. Calcd. for $C_{27}H_{44}O_4N_2$: C, 70.4; H, 9.6; N, 6.1. Found: C, 70.6; H, 9.8; N, 6.0.

The dioxime of kryptogenin diacetate was prepared in a like manner. The reaction mixture was poured into water and ether extracted. The ethereal solution evaporated slowly at room temperature to give needles, m. p. 200–203°, which were recrystallized from ethanol, m. p. 202–205°. This material gave a positive test for nitrogen.

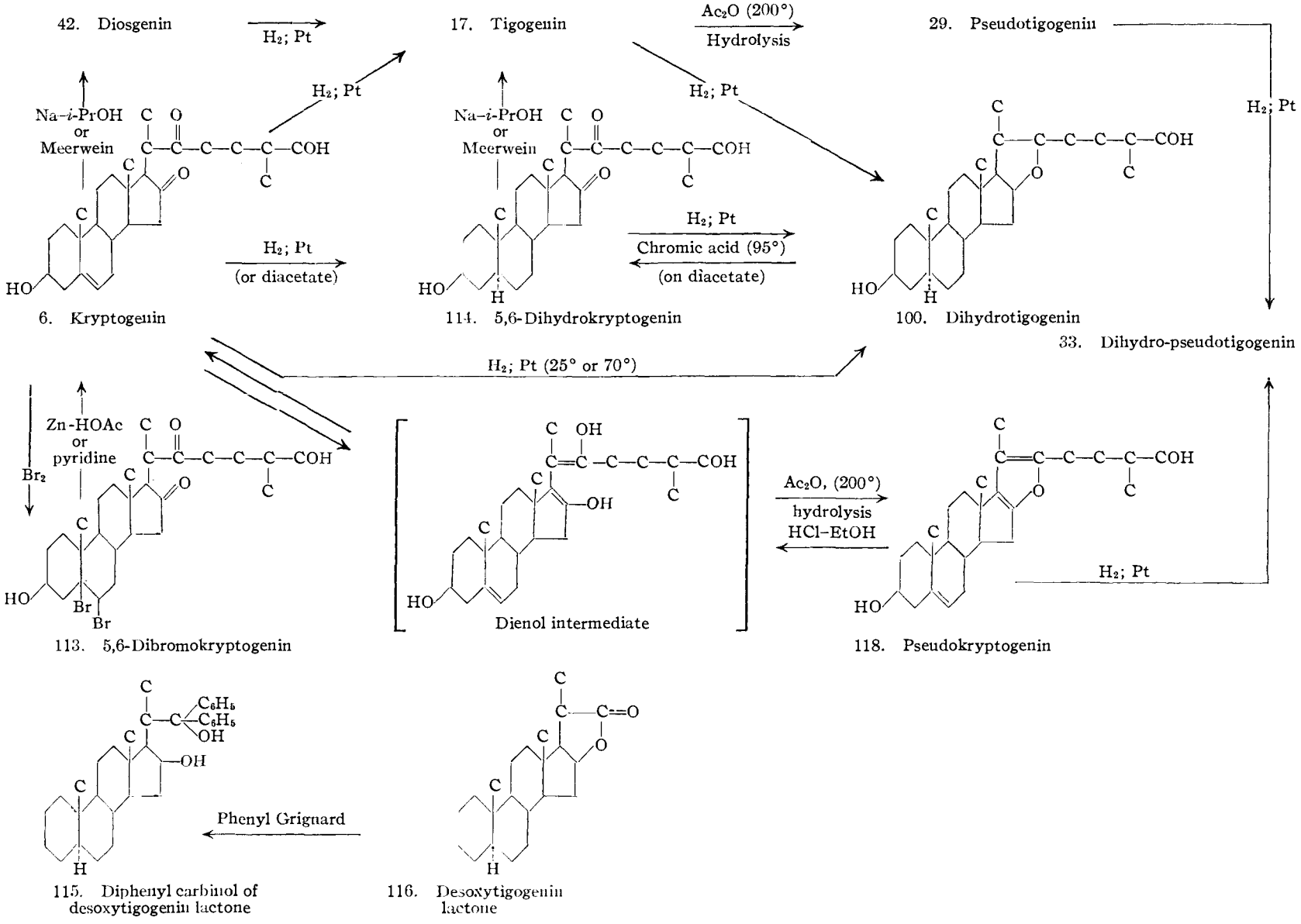
Anal. Calcd. for $C_{31}H_{48}O_6N_2$: C, 68.4; H, 8.9. Found: C, 68.1; H, 8.6.

5,6-Dihydrokryptogenin Dioxime.—Treatment of 5,6-dihydrokryptogenin, 0.5 g., with 0.4 g. of hydroxylamine hydrochloride in 4 cc. of pyridine and 4 cc. of absolute ethanol, for one hour at steam-bath temperature gave the dioxime, m. p. 258° dec.

Anal. Calcd. for $C_{27}H_{46}O_4N_2$: C, 70.1; H, 10.0. Found: C, 70.1; H, 9.9.

Kryptogenin shows several reactions characteristic of the 1,4-diketone system. On treatment with strong alcoholic potash it loses water to give fesogenin (121).

Fesogenin.—A solution of 10 g. of kryptogenin diacetate in 750 cc. of methanol containing 150 g. of potassium hydroxide and 750 cc. of water was refluxed on the steam


42. Diosgenin
H₂; Pt
17. Tigogenin
Ac₂O (200°)
Hydrolysis
29. Pseudotigogenin
H₂; Pt
Na-i-PrOH
or
Meerwein
H₂; Pt
H₂; Pt
H₂; Pt
(or diacetate)
6. Kryptogenin
114. 5,6-Dihydrokryptogenin
H₂; Pt
Chromic acid (95°)
(on diacetate)
100. Dihydrotigogenin
33. Dihydro-pseudotigogenin
H₂; Pt (25° or 70°)
Zn-HOAc
or
pyridine
Br₂
113. 5,6-Dibromokryptogenin
Dienol intermediate
Ac₂O, (200°)
hydrolysis
HCl-EtOH
118. Pseudokryptogenin
H₂; Pt
Phenyl Grignard
115. Diphenyl carbinol of
desoxytigogenin lactone
116. Desoxytigogenin
lactone

bath for three hours. The reaction mixture was ether extracted and the product was crystallized from acetone to give fesogenin, m. p. 179–180°; yield 6.3 g.

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

Acetylation of this substance either with boiling acetic anhydride or with pyridine–acetic anhydride mixture gave non-crystalline material which upon hydrolysis reverted to fesogenin.

This conversion is the typical formation of a 5-membered ring by an internal aldol condensation of a 1,4-diketone. Striking and pertinent examples of this change are given in the works of Blaise[92] on the cyclization of 3,6-octanedione and of Borsche, Fels and Menz[93, 94] on α-acetylpropiophenone. This type of reaction is also the basis of the Robinson[95] synthesis of cyclopentenophenanthrenes. The reduction of the double bond at the 16–23 position in 121 without reduction of the carbonyl group can be achieved with hydrogen in the presence of palladium-in-ether. Treatment of this 16,23-dihydrofesogenin (122) with sodium in isopropyl alcohol forms a third hydroxyl group, giving 16,23,22-tetrahydrofesogenin (123). The latter can be acetylated with boiling acetic anhydride to a triacetate.

16,23-Dihydrofesogenin.—A solution of 0.2 g. of fesogenin in 300 cc. of ether was shaken with 0.2 g. of 3% palladium–barium sulfate catalyst and hydrogen at room temperature and 3 atm. for two hours. 16,23-Dihydrofesogenin crystallized from acetone as needles, m. p. 213°; wt. 0.1 g.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 77.8; H, 10.2.

Thus, the conversions 121 → 122 → 123 clearly show the presence of an α,β-unsaturated ketone system in fesogenin (121). Further proof of this fact is effected by the direct reduction of 121 with sodium in isopropyl alcohol to the tetrahydro compound (123).

16,23,22-Tetrahydrofesogenin from Fesogenin.—To a solution of 0.5 g. of fesogenin in 150 cc. of hot isopropyl alcohol was added 10 g. of sodium strips. The reaction mixture was heated on the steam-bath until the sodium dissolved, then cooled and ether extracted. The product crystallized from acetone to give 16,23,22-tetrahydrofesogenin in 70% yield, m. p. 238–240°.

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

The triacetate was prepared with boiling acetic anhydride and was crystallized from methanol, m. p. 157–160°.

Anal. Calcd. for $C_{33}H_{50}O_6$: C, 73.0; H, 9.3. Found: C, 73.2; H, 9.1.

16,23,22-Tetrahydrofesogenin from Kryptogenin Diacetate.—To a boiling solution of 5 g. of kryptogenin diacetate in 1.5 liters of isopropyl alcohol was added 100 g. of sodium strips. The reaction mixture was worked up as described above for the preparation from fesogenin to give 16,23,22-tetrahydrofesogenin which was crystallized from ethyl acetate, m. p. and mixed m. p. with product from fesogenin, 237–240°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 77.6; H, 10.6.

The triacetate was identical with the triacetate from the material prepared from fesogenin, m. p. and mixed m. p., 158–160°.

From the mother liquor after the removal of 16,23,22-tetrahydrofesogenin was obtained 0.4 g. of crude material melting 225–230°. This was further purified as the diacetate by acetylation and crystallization from methanol, m. p. 217–221°.

Anal. Calcd. for $C_{31}H_{48}O_5$: C, 74.4; H, 9.7. Found: C, 74.7; H, 9.4.

Catalytic hydrogenation of this diacetate in ether containing several drops of acetic acid gave an oily product.

Hydrolysis of this diacetate with excess 10% alcoholic potassium hydroxide for thirty minutes gave an oil which was further treated with alcoholic potash for three and one-half hours. The product was ether extracted and then crystallized from ether–pentane to give material melting 137–147°, which resolidified and melted at 178–181°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 77.4; H, 10.3.

This material was not further characterized.

The complete reduction with sodium and alcohol of a system containing a double bond and a carbonyl group can be accomplished only when these groups are in a position of conjugation. In most cases the sodium–isopropyl alcohol reduction of kryptogenin yields this tetrahydro compound (123).

It is probable that the alkalinity of the reaction mixture causes an internal aldol condensation, giving fesogenin (121), which is then reduced to 123. On the other hand, if reduction of the 16-keto group should occur first, the condensation would be prevented and the spiro-ketal side-chain would form, thus giving diosgenin (42). Actually, diosgenin (42) has been obtained by this reduction. So far we have found no way of controlling the reaction to give only one of these products (42) or (123).

The conversions 6 → 121 → 122 → 123 are duplicated when 5,6-dihydrokryptogenin and its subsequent derivatives are treated as described above. Thus, alkaline treatment of 5,6-dihydrokryptogenin (114) gives 5,6-dihydrofesogenin (119). The latter can be reduced either to 5,6,16,-23-tetrahydrofesogenin (124) or 5,6,16,23,22-hexahydrofesogenin (125). This product is also obtained by the catalytic or chemical reduction of many of the various reduction products obtained in this work as shown in the following experimental section. In the formation of this hexahydro compound by catalytic reduction, a product melting 224° is sometimes obtained. This is apparently a polymorphic form.

5,6-Dihydrofesogenin.—5,6-Dihydrokryptogenin diacetate, 5 g., refluxed with 75 g. of potassium hydroxide in 75 cc. of water and one liter of methanol for four hours gave 5,6-dihydrofesogenin which was crystallized from ether, m. p. 214–215°; yield 2.2 g.

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

This material forms a non-crystalline acetate.

5,6,16,23-Tetrahydrofesogenin.—Hydrogenation of 5,6-dihydrofesogenin, 1 g., in ether for two hours at room temperature and 3 atm. with 0.5 g. of 3% palladium–barium sulfate catalyst gave 5,6,16,23-tetrahydrofesogenin which crystallized from acetone as needles, m. p. 217–

(92) Blaise, *Compt. rend.*, **158**, 708 (1914).
(93) Borsche and Fels, *Ber.*, **39**, 1922 (1906).
(94) Borsche and Menz, *ibid.*, **41**, 90 (1908).
(95) Robinson, *J. Chem. Soc.*, 1391 (1938).

219°; wt. 0.4 g. A mixture with starting material (215°) melted 208–213°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.7. Found: C, 78.1; H, 10.3.

5,6,16,23,22-Hexahydrofesogenin. a. From Fesogenin.—A solution of 1 g. of fesogenin in 500 cc. of ether containing three drops of acetic acid was shaken with 0.3 g. of Adams catalyst and hydrogen at room temperature and 3 atm. for two hours. The product crystallized during the hydrogenation. It was dissolved in hot ether, filtered free of catalyst and then crystallized from acetone, m. p. 224°; yield 0.5 g.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.5; H, 11.1. Found: C, 77.5; H, 11.2.

In another run material melting 245–247° was obtained. This is probably a polymorphic form.

This material forms a non-crystalline acetate and benzoate.

b. From **5,6-Dihydrofesogenin.** 1. **By Catalytic Hydrogenation.**—In a like manner as described in (a), the hexahydro compound was prepared from 5,6-dihydrofesogenin and was crystallized from ether, m. p. and mixed m. p. with the low melting form, 222–224°.

Anal. Found: C, 77.1; H, 11.4.

2. By **Sodium and Isopropyl Alcohol Reduction.**—A solution of 1.5 g. in 600 cc. of hot isopropyl alcohol was treated with 40 g. of sodium strips. The product was ether extracted and crystallized, m. p. and mixed m. p. with the high melting form, 244°; yield 0.8 g.

Anal. Found: C, 77.8; H, 11.0.

c. From **16,23-Dihydrofesogenin.**—This compound, 0.2 g., was catalytically hydrogenated as described under (a) to give material melting 224–230°. A mixture with the low melting form of the hexahydro-compound melted 224–229°, indicating a mixture of the two polymorphic forms.

Anal. Found: C, 77.0; H, 10.9.

d. From **5,6,16,23-Tetrahydrofesogenin.**—This compound, 0.1 g., was catalytically hydrogenated as described under (a) to give the low melting form of the hexahydro-compound, m. p. and mixed m. p., 222–224°.

Anal. Found: C, 77.2; H, 11.1.

e. From **16,23,22-Tetrahydrofesogenin.**—This compound 0.18 g., was catalytically hydrogenated as described under (a) to give a high melting hexahydro compound from ethyl acetate, m. p. and mixed m. p., 246°.

Anal. Found: C, 77.5; H, 11.0.

Catalytic hydrogenation in acetic acid at 70° for eight hours using Adams catalyst also gave the high form, m. p. and mixed m. p., 247°.

Anal. Found: C, 77.5; H, 10.9.

f. From **5,6-Dihydrokryptogenin.**—To a solution of 0.5 g. in 150 cc. of hot isopropyl alcohol was added 10 g. of sodium strips. The product was crystallized from acetone, m. p. and mixed m. p. with the high form, 245–247°; yield 0.3 g.

Anal. Found: C, 77.6; H, 11.0.

5,6-Dihydrokryptogenin diacetate treated in a similar manner gave the same product, m. p. and mixed m. p., 245–247°; yield, 1.7 g. from 4 g. of the diacetate.

Anal. Found: C, 77.1; H, 11.2.

Mild oxidation of the hexahydro compound (125) with chromic acid in acetic acid gives largely a ketolactone (126), formed by the oxidation of the C-27 hydroxyl group followed by lactonization.

Oxidation of 5,6,16,23,22-Hexahydrofesogenin.—To a solution of 0.5 g. of the hexahydro compound in 100 cc. of acetic acid was added 0.6 g. of chromic anhydride in 80% acetic acid. The mixture after standing thirty minutes at room temperature was ether extracted. The ethereal solution was washed with water, 10% potash and water. Acidification of the alkali wash gave a very small acid fraction. The ethereal solution was evaporated and the residue was boiled with alcoholic potash for fifteen minutes. Ether extraction of the hydrolysis mixture gave very little neutral material. The alkaline water layer from the hydrolysis mixture was acidified and the solid lactonic material was dissolved in ether and crystallized from acetone, m. p. 286–290°.

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.6; H, 9.8. Found: C, 78.1; H, 9.8.

Catalytic hydrogenation (Adams catalyst) of fesogenin (121) in hot acetic acid apparently causes the loss of water between the C-22 and C-27 hydroxyl groups, forming a cyclic ether (127). Whereas fesogenin (121) has three acetylatable hydroxyl groups, this product forms only a monoacetate, indicating that two hydroxyl groups have been removed from the former in the formation of the latter. Mild oxidation gives a neutral 3-keto-compound, further showing the absence of a primary hydroxyl group at C-27. Catalytic hydrogenation of hexahydrofesogenin (125) in a like manner, followed by acetylation, also results in the formation of the monoacetate of this product.

Catalytic Hydrogenation of Fesogenin at 70°.—A solution of 0.7 g. of fesogenin in acetic acid was shaken with Adams catalyst and hydrogen at 70° and 3 atm. for seven hours. The product was acetylated and was crystallized from methanol, m. p. 166–168°; wt. 0.1 g.

Anal. Calcd. for $C_{29}H_{46}O_3$: C, 78.7; H, 10.5. Found: C, 78.7; H, 10.4.

Hydrolysis of the monoacetate with alcoholic potash gave material which was crystallized from acetone, m. p. 183–184°.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.9; H, 11.1. Found: C, 80.6; H, 11.1.

Cold oxidation of the unacetylated material with chromic-acetic acid mixture for fifty-five minutes gave mostly neutral material which was crystallized from methanol, m. p. 223–225°. This depresses the melting point of 5,6,16,23,22-hexahydrofesogenin.

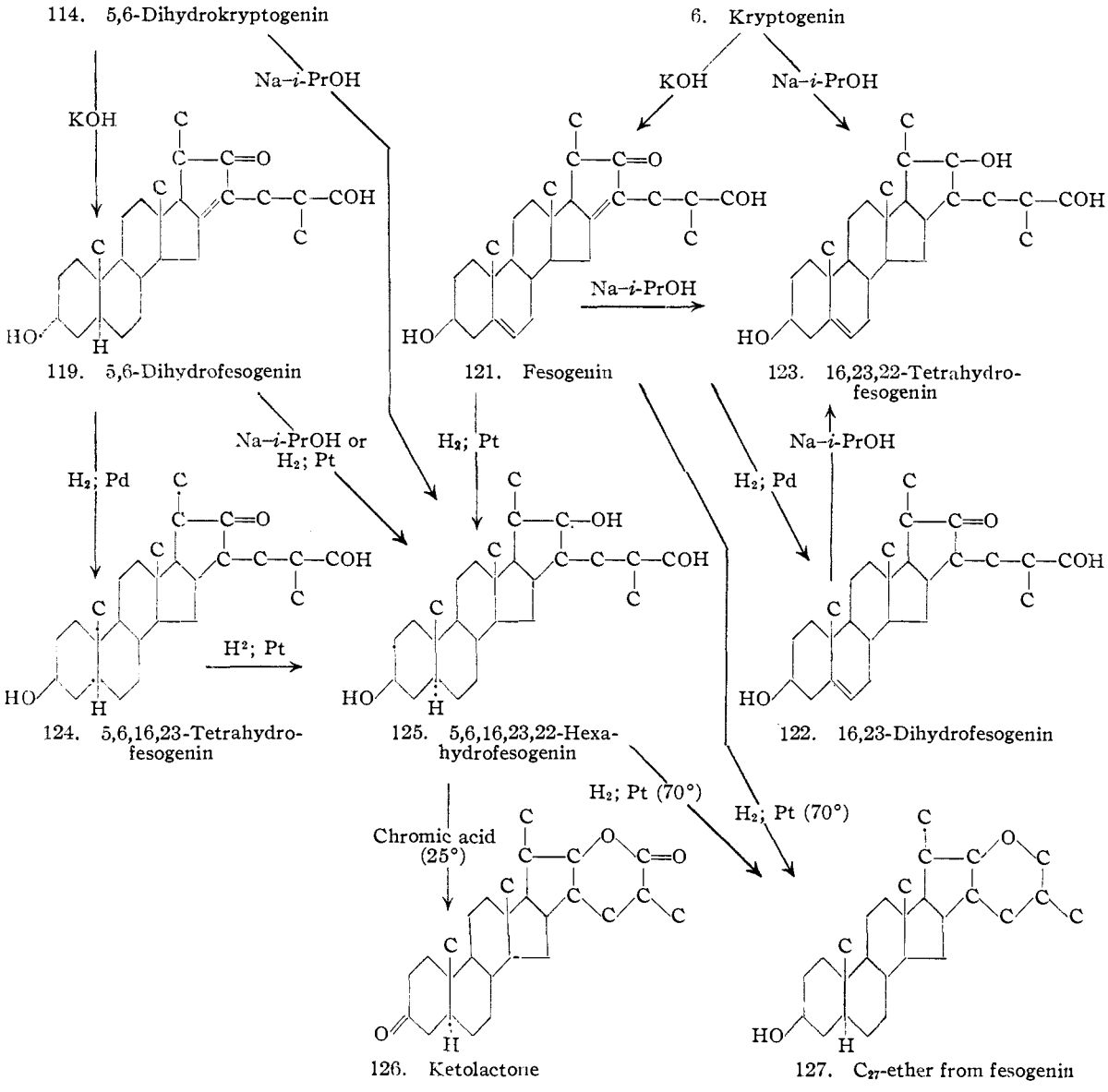
Anal. Calcd. for $C_{27}H_{42}O_2$: C, 80.9; H, 11.1. Found: C, 81.2; H, 10.8.

Catalytic hydrogenation of hexahydrofesogenin in like manner followed by acetylation gave material, m. p. and mixed m. p. with acetate from above, 166–167°.

The spectrographic data are in complete agreement with the assignment of an α,β-unsaturated carbonyl system to fesogenin. Thus, the absorption bands (Fig. 2) at 245 mμ ($\epsilon \times 10^{-3} = 13.7$) for 121 and the 5,6-dihydro compound (119) correspond in position and intensity with those given by α,β-unsaturated ketones. The reduction products, namely, 123 and 125, show no such selective absorption in the ultraviolet region.

In addition to the absorption data and the internal aldol condensation on treatment with alkali, the 1,4-diketone system in kryptogenin is indicated by the catalytic, Meerwein and sodium alcohol reductions of kryptogenin (6) and 5,6-dihydrokryptogenin (114) to diosgenin (42) and tiogogenin (17), respectively, and by the catalytic reduction of kryptogenin diacetate to the diacetate of dihydrotigogenin (100), all previously discussed. This latter reaction is analogous to the reduction of acetonylacetone to 2,5-dimethyltetrahydrofuran by Sabatier and Mailhe.[96]

(96) Sabatier and Mailhe, *Ann. Chim.*, (8) **16**, 70 (1909).

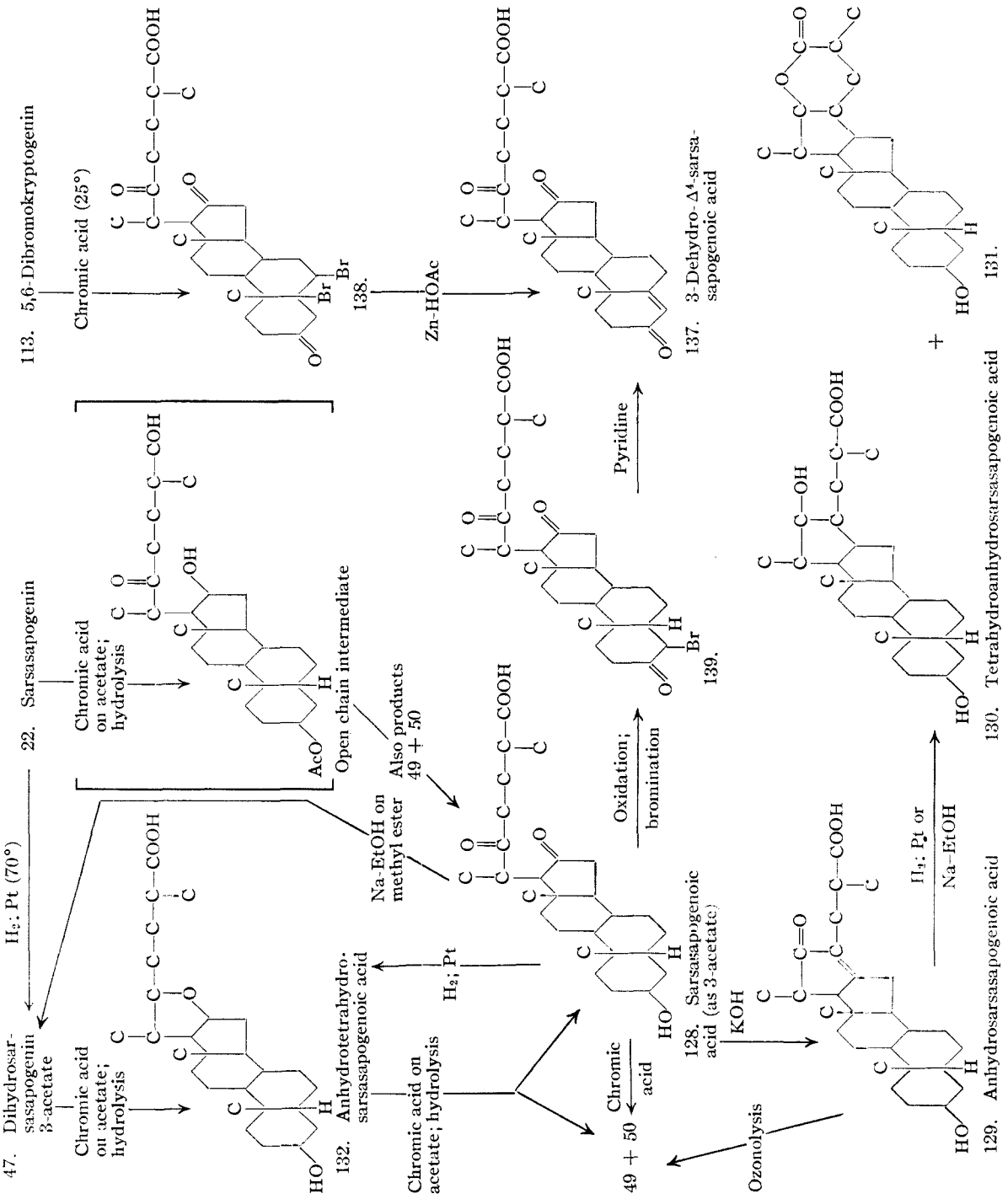


Kryptogenin and Sarsasapogenoic Acid.—The similarity between the structure of kryptogenin and the structure of sarsasapogenoic acid (128) has made it desirable to interrelate them, thus giving a more secure foundation for the former.

Sarsasapogenoic acid is obtained by the energetic oxidation of sarsasapogenin acetate.[97,98] Upon treatment with alkali it loses water to give anhydrosarsasapogenoic acid (129) which can be reduced either with sodium in ethanol or catalytically to tetrahydroanhydrosarsasapogenoic acid (130). Oxidation of sarsasapogenoic acid acetate with hot chromic acid in acetic acid[23b,99] gives only acids consisting of either the C_{22}-16-keto acid (49) or the acetate of 3-(β)-hydroxy-*etio*-bilianic acid (50) with no neutral or lactone material.[100] These acids are also obtained by the oxidation of sarsasapogenin acetate.[23b,101] Permanganate[102] and nitric acid[103] oxidations of the sapogenoic acid give only the 16-keto-acid (49) although these oxidizing agents are known to oxidize sarsasapogenin acetate to the desired lactone (48).

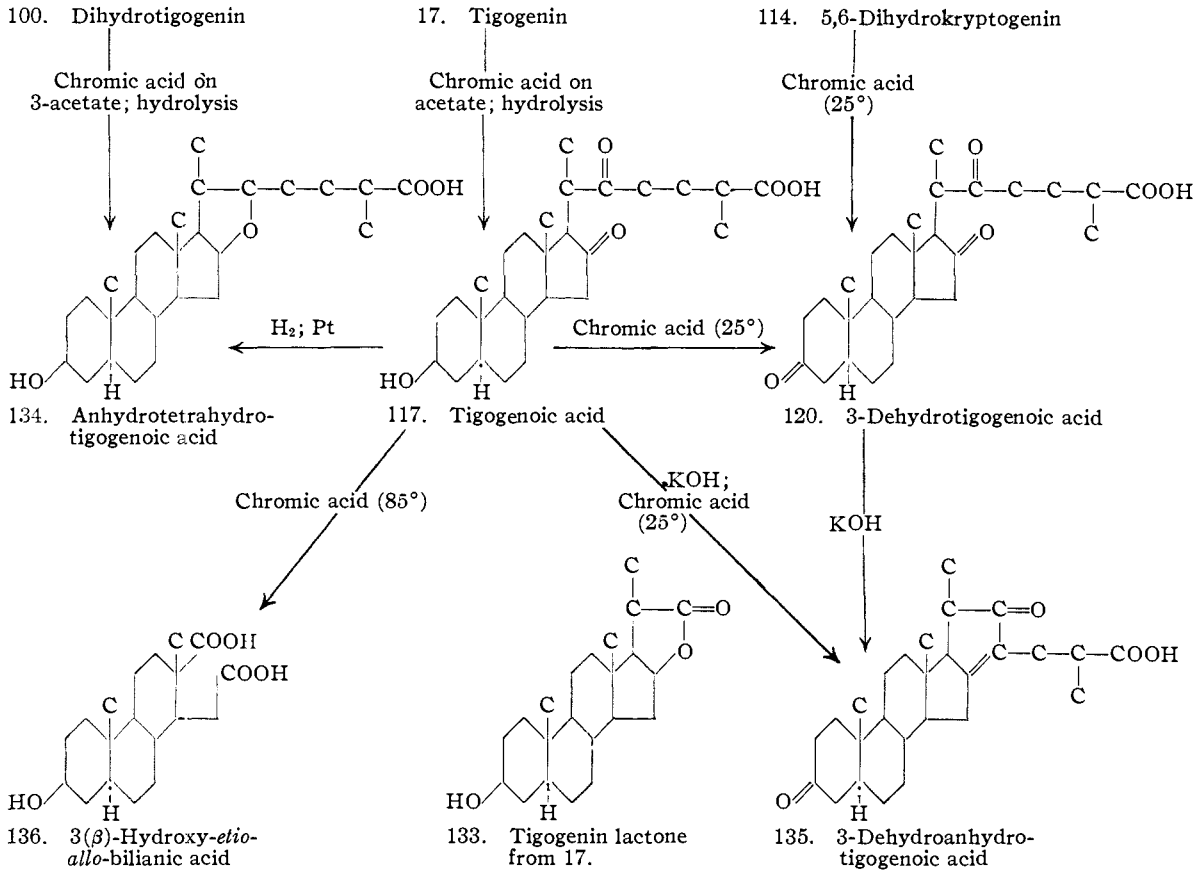
Marker and Rohrmann[99] showed anhydrosarsasapogenoic acid (129) to be an α,β-unsaturated ketone of structure 129 which also accounts for the properties of tetrahydroanhydrosarsasapogenoic acid (130) and its lactone (131). The

(97) Fieser and Jacobsen, THIS JOURNAL, **60**, 28 (1938).
(98) Fieser and Jacobsen, *ibid.*, **60**, 2753 (1938).
(99) Marker and Rohrmann, *ibid.*, **61**, 2072 (1939).
(100) Marker and Shabica, *ibid.*, **64**, 813 (1942).
(101) Marker and Rohrmann, *ibid.*, **61**, 1285 (1939).
(102) Marker and Rohrmann, *ibid.*, **62**, 222 (1940).
(103) Marker, Turner and Ulshafer, *ibid.*, **63**, 763 (1941).



structure (129) is confirmed by ozonolysis, giving in good yields the C_{22}-keto-acid (49), 3(β)-hydroxy-16-keto-bisnorcholanic acid, which is then reduced either with sodium and ethanol or catalytically to sarsasapogenin lactone (48), further identified as the acetate.[101,104] The derivation of 129 from sarsasapogenoic acid (128) simply involves an internal aldol condensation. The structure of 128 as a diketone, indicated by its formation of a dioxime, is well established by its catalytic reduction to anhydrotetrahydrosarsasapogenoic acid (132).[99] This product (132) is also obtained by the oxidation of the 3-monoacetate of dihydrosarsasapogenin (47). The identity is established by direct comparison of the free acids, the methyl esters, the methyl ester benzoates and the 3-dehydro-acids. As additional

(104) Marker, Shabica and Turner, THIS JOURNAL, 63, 2274 (1941).



proof of the identity of the two acids, the methyl ester of sarsasapogenoic acid (128) is reduced with sodium and alcohol to give dihydrosarsasapogenin (47), further identified as the diacetate.[105]

Marker and Rohrmann[43] also studied the acids from the oxidation of the diacetate of dihydrosarsasapogenin (47). Oxidation of the latter with hot chromic acid gives the C_{22}-16-keto-acid (49), 3(β)-hydroxy-*etio*-bilianic acid acetate (50) and sarsasapogenin lactone acetate (48). In much the same manner, oxidation of the methyl ester acetate of the acid (132) from either dihydrosarsasapogenin 3-acetate (47) or sarsasapogenoic acid (128) gives the same C_{22}-16-keto-acid (49), C_{19}-dibasic acid (50) and sarsasapogenoic acid (128) isolated as the anhydro-acid (129).

Tigogenoic acid (117) was first obtained by Tschesche and Hagedorn[22] along with tigogenin lactone (133) from the energetic oxidation of tigogenin (17). As would be expected, this acid (117) undergoes the reactions characteristic of its analog, sarsasapogenoic acid. Most important of these[103] are the catalytic reduction, aldol condensation and oxidation, whereby, anhydrotetrahydrotigogenoic acid (134), 3-dehydroanhydrotigogenoic acid (135) and 3(β)-hydroxy-*etio-allo*-bilianic acid (136) are obtained, respectively. The acid (134) from the catalytic reduction of tigogenoic acid (117) is shown to be identical with the acid from the mild oxidation of dihydrotigogenin (100) 3-acetate. Cold oxidation of 5,6-dihydrokryptogenin (114) gives 3-dehydrotigogenoic acid (120) also obtained from tigogenoic acid (117).

3-Dehydrotigogenoic Acid from 5,6-Dihydrokryptogenin.—To a solution of 1 g. of 5,6-dihydrokryptogenin in 25 cc. of acetic acid was added 0.7 g. of chromic anhydride in 15 cc. of 80% acetic acid. The mixture was allowed to stand for ninety minutes at room temperature and then it was ether extracted. After washing the ethereal solution with water to remove the acetic acid, it was given a potassium carbonate wash. The latter was acidified and the precipitated solid was crystallized from ether, m. p. and mixed m. p. with 3-dehydrotigogenoic acid (120) from the oxidation of dihydrotigogenin, 185–187°; yield 0.4 g.

Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.9; H, 9.1. Found: C, 73.0; H, 9.1.

The methyl **ester** was prepared with diazomethane in ether and was crystallized from acetone and then ether, m. p. and mixed m. p. with the methyl ester of 3-dehydrotigogenoic acid, 141–143°.

Anal. Calcd. for $C_{28}H_{42}O_5$: C, 73.3; H, 9.2. Found: C, 73.4; H, 9.2.

It can be seen therefore that the reactions of sarsasapogenoic acid (128), tigogenoic acid (117), and kryptogenin (6) are entirely analogous. These compounds undergo catalytic hydrogenation to give new 5-membered rings. All three lose the elements of water by an internal aldol condensation. The anhydro compounds are α,β-

(105) Marker and Shabica, THIS JOURNAL, **64**, 721 (1942).

unsaturated ketones, which give similar reduction products.

Alkali Treatment of 3-Dehydrotigogenoic Acid.—A solution of 1 g. of 3-dehydrotigogenoic acid in 150 cc. of 95% ethanol was treated at steam-bath temperature with a solution of 30 g. of potassium hydroxide in 30 cc. of water for five hours. The reaction mixture was cooled, acidified with hydrochloric acid and ether extracted. The product was crystallized from acetone, m. p. 232–234° dec.; wt. 0.23 g. This material is 3-dehydroanhydrotigogenoic acid.

Anal. Calcd. for $C_{27}H_{38}O_4$: C, 76.0; H, 9.0. Found: C, 75.4; H, 9.5.

Sarsasapogenoic acid and kryptogenin have been related by making the triketoacid (137) from each. Thus, kryptogenin (6) after bromination to the 5,6-dibromo compound (113) on mild oxidation at room temperature has its C-27 hydroxyl and its 3-hydroxyl converted, respectively, to a carboxyl and a carbonyl group (138). Debromination gives 137, the triketoacid, 3-dehydro-Δ^4-sarsasapogenoic acid. This key substance can also be prepared[99] from sarsasapogenoic acid (128) by oxidation and bromination to 139 which is then debrominated with pyridine.

3-Dehydro-Δ^4-sarsasapogenoic Acid from Kryptogenin.—To a solution of 6 g. of kryptogenin in 150 cc. of acetic acid at 20° was added 14.1 cc. of molar bromine–acetic acid solution during ten minutes. After standing for twenty minutes, the solution was treated with a solution of 4.2 g. of chromic anhydride in 50 cc. of 80% acetic acid at room temperature and allowed to stand for ninety minutes more. The reaction mixture was then heated on the steam-bath with 12 g. of zinc dust for fifteen minutes. After filtering, the filtrate was concentrated *in vacuo* on the steam-bath to a small volume and then taken up in ether. The ethereal solution was washed with water and then 5% potassium hydroxide to remove the acid fraction. The acids were regenerated with dilute hydrochloric acid, ether extracted, treated with Norite in acetone and crystallized slowly from ether at room temperature and then acetone, m. p. 208–210° dec. This material did not depress the melting point of 3-dehydro-Δ^4-sarsasapogenoic acid obtained from sarsasapogenoic acid.

Anal. Calcd. for $C_{27}H_{38}O_5$: C, 73.3; H, 8.7. Found: C, 72.9; H, 9.0.

The **methyl ester** was prepared with diazomethane in ether and was crystallized from aqueous methanol as plates, m. p. 105°. This did not depress the melting point of an authentic sample of the methyl ester of 3-dehydro-Δ^4-sarsasapogenoic acid prepared from sarsasapogenoic acid.

Anal. Calcd. for $C_{28}H_{40}O_5$: C, 73.7; H, 8.8. Found: C, 73.6; H, 9.2.

Pennogenin and Nologenin

Small amounts of pennogenin and nologenin have been isolated from *Beth* root along with large quantities of diosgenin and kryptogenin. It is interesting to find that the structural formulas of the four sapogenins are closely related. The similarity, relationship, and evidence for the structures of diosgenin and kryptogenin have been discussed. The interrelationship of these with pennogenin and nologenin gives strong evidence for the structural formulations of the latter as 11 and 10, respectively.

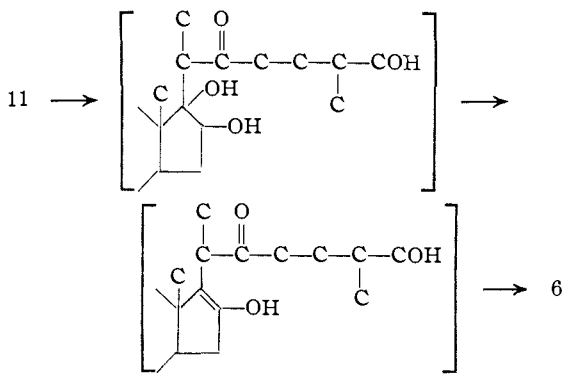
Pennogenin.—Two of the oxygen atoms in pennogenin (11) ($C_{27}H_{42}O_4$) are present as hydroxyl groups, one of which reacts with boiling acetic anhydride to form a monoacetate ($C_{29}H_{44}O_6$). The others are present as inert oxygen atoms in the characteristic sapogenin side-chain. The presence of a free carbonyl group is eliminated on the grounds that pennogenin shows no ultraviolet absorption for this group and does not react with the usual ketone reagents. Treatment of pennogenin (11) with boiling 2 *N* ethanolic hydrochloric acid for two hours gives kryptogenin (6), further identified as the diacetate.

Kryptogenin from Pennogenin.—A solution of 1 g. of pennogenin in 100 cc. of 95% ethanol containing 5 cc. of concentrated hydrochloric acid was heated on the steam-bath for two hours. The product was ether extracted and crystallized from acetone to give kryptogenin, m. p. and mixed m. p., 184–186°; yield 0.4 g.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.0; H, 10.0.

The diacetate was prepared and was crystallized from acetone, m. p. 144–146°. A mixture with kryptogenin diacetate (153°) melted 146–153°. The benzoate was prepared from pennogenin with benzoyl chloride in pyridine at room temperature. It was crystallized from benzene–methanol mixture (1:4) as white plates, m. p. 250–251°.

This reaction probably proceeds by the opening of the spiro-ketal side-chain followed by the dehydration of the tertiary hydroxyl group in the subsequent β-hydroxy-ketone system, *viz.*



The tertiary hydroxyl group in pennogenin is also removed by the conditions employed for the formation of the pseudosapogenins. Thus, when pennogenin is heated with acetic anhydride it yields pseudokryptogenin (118), identified by its conversion to dihydropseudotigogenin (33) and its diacetate.

Pseudokryptogenin from Pennogenin.—A mixture of 6 g. of pennogenin acetate and 18 cc. of acetic anhydride was heated at 200° for ten hours. The mixture was evaporated *in vacuo* on the steam-bath and then re-evaporated with ethanol to remove the remaining acetic anhydride. After decolorizing with Norite the product was crystallized from methanol, m. p. 68–73°. This was further purified by hydrolysis with 5% ethanolic potash and crystallization from ether and then methanol, m. p. and mixed m. p. with pseudokryptogenin, 192–193°; yield 1.3 g.

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.6; H, 9.8. Found: C, 77.9; H, 9.6.

The diacetate was prepared and crystallized from methanol, m. p. 120–125°. Recrystallizations from methanol gave material melting 115–125° and 124–126°.

In a second run, the product from 20 g. of pennogenin

acetate and 25 cc. of acetic anhydride was hydrolyzed directly and then crystallized from acetone to give 11 g. of pseudokryptogenin.

For the isomerization of this material to kryptogenin, a solution of 3 g. in 100 cc. of ethanol containing 15 cc. of concentrated hydrochloric acid was refluxed for one hour. The product was ether extracted and crystallized from acetone, m. p. and mixed m. p. with kryptogenin, 185–186°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 74.9; H, 9.9.

The diacetate was prepared and crystallized from methanol, m. p. and mixed m. p. with kryptogenin diacetate, 145–147°.

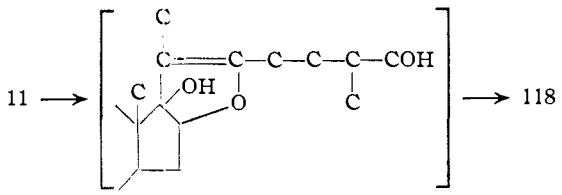
Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.4; H, 9.1.

For the catalytic hydrogenation of the pseudosapogenin from pennogenin to dihydropseudotigogenin, 1 g. in ether containing a few drops of acetic acid was shaken with hydrogen and Adams catalyst for three hours at 3 atm. and room temperature. The product crystallized from ether, m. p. and mixed m. p., 199–202°; wt. 0.7 g.

The diacetate was prepared and crystallized from acetic anhydride, m. p. and mixed m. p. with dihydropseudotigogenin diacetate, 124–126°. A mixture with pseudopennogenin diacetate (124°) melted 95–100°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.0; H, 10.0. Found: C, 73.6; H, 10.3.

It is likely that kryptogenin undergoes this reaction through its dienol. On the other hand, pennogenin probably undergoes the usual pseudosapogenin formation shown by the other sapogenins along with simultaneous dehydration of its tertiary hydroxyl group at C-17, *viz.*



Pseudokryptogenin from pennogenin shows strong absorption in the 240 mμ region (Fig. 2) and gives a curve very much like that shown by its analog, pseudodiosgenin (34).

Although pennogenin loses a hydroxyl group in the above reactions, it resists other dehydration treatments such as heating with phosphorus oxychloride or dry hydrogen chloride in chloroform. It is likely that under these conditions the spiroketal side-chain does not open, thus preventing the formation of the intermediate unstable β-hydroxyketone.

The conversion of pennogenin to kryptogenin by the above methods fixes the location of its double bond and one of its hydroxyl groups as carbons 5–6 and 3-β, respectively. In analogy to other steroids having a proximity of these functional groups, pennogenin undergoes the Oppenauer oxidation reaction to give an α,β-unsaturated ketone.

Oppenauer Oxidation of **Pennogenin**.—A mixture of 1 g. of pennogenin, 1 g. of aluminum *t*-butylate, 5 cc. of dry acetone and 50 cc. of dry toluene was refluxed for ten hours. To the reaction mixture after cooling was added dilute hydrochloric acid. The mixture was ether extracted and the ethereal solution was washed with water and evaporated. The residue was crystallized from an ether–pentane solution to give pennogenone, m. p. 186–189°; yield 0.7 g.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.4; H, 9.6.

This unsaturated ketone reacted with semicarbazide acetate to give a monosemicarbazone which was crystallized from methanol, m. p. 233–236° dec.

Catalytic hydrogenation of the unsaturated ketone in ether containing several drops of acetic acid using Adams catalyst gave a product which was crystallized from an ether–pentane solution, m. p. 198–201°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.9; H, 10.3.

The absorption data (Fig. 2) for this new substance (140) show the characteristic α,β-unsaturated ketone peak at $\lambda_{max.}$ = 241 mμ ($\epsilon \times 10^{-3}$ = 15.8) and is similar to that given by progesterone (43) and cholestenone, both of which have peaks at this wave length. All three compounds have molar absorption maxima of 15,800.

The double bond in pennogenin readily reacts with bromine in acetic acid solution and hydrogenates (Adams catalyst) at room temperature to 5,6-dihydropennogenin.

5,6-Dihydropennogenin.—Pennogenin acetate, 1 g., in 400 cc. of ether containing several drops of acetic acid was shaken with Adams catalyst and hydrogen for two hours at 3 atm. and room temperature. The product was crystallized from ether and then methanol, m. p. 176–178°; yield 0.8 g. Hydrolysis of this material with 5% alcoholic potash gave the free genin which was crystallized from methanol, m. p. 224–226°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.8; H, 10.1.

5,6-Dihydropennogenin acetate was prepared from this material and was crystallized from acetone as needles, m. p. 178–180°.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.4; H, 9.8. Found: C, 73.2; H, 9.6.

Prolonged hydrogenation at a higher temperature, however, causes the absorption of an additional mole of hydrogen. By analogy to other dihydrosapogenins formed under like conditions, this hydrogenation product should be the open side-chain derivative.

Hot **Catalytic Hydrogenation** of **Pennogenin Acetate**.—A solution of 5 g. of pennogenin acetate in 200 cc. of acetic acid was shaken with 1 g. of Adams catalyst and hydrogen at 70° and 3 atm. for eight hours. The product after the removal of the solvent and spent catalyst was hydrolyzed with 5% alcoholic potash and crystallized from acetone, m. p. 168–170°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.9; H, 10.9.

This material formed a non-crystalline acetate.

Nologenin,—Four of the five oxygens in nologenin ($C_{27}H_{44}O_5$) are present as hydroxyl groups, two of which are acetylated with boiling acetic anhydride forming a diacetate ($C_{31}H_{48}O_7$). Mild treatment of nologenin (10) with boiling 2 *N* ethanolic hydrochloric acid for one hour gives pennogenin (11), isolated as the monoacetate.

Pennogenin from **Nologenin**.—To a solution of 250 mg. of nologenin in 30 cc. of 95% ethanol was added 6 cc. of concentrated hydrochloric acid. After refluxing for one hour the solution was diluted with water and extracted with ether. The ethereal solution was washed with water and evaporated. The oily residue was refluxed for

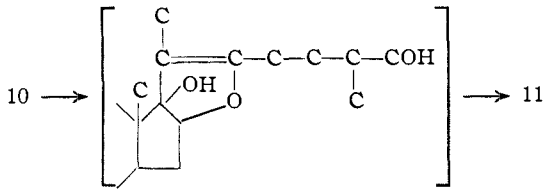
twenty minutes with 1 cc. of acetic anhydride. After cooling the mixture was filtered and the crystals were washed with cold methanol and recrystallized from acetone to give pennogenin acetate as needles, m. p. and mixed m. p., 198–199°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.8; H, 9.4.

Hydrolysis of the acetate with alcoholic potassium hydroxide gave pennogenin, m. p. and mixed m. p., 237–239°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.6; H, 10.2.

This reaction probably proceeds by the dehydration of the tertiary hydroxyl group at C-20 to give an intermediate compound, analogous to the pseudosapogenins. The side-chain in the latter would then immediately close in the acid medium (by analogy to other pseudosapogenins) to give the side-chain of pennogenin (11), *viz.*



As would be expected, prolonged treatment of nologenin under these conditions gives kryptogenin (6).

Bethogenin

Noller and co-workers[106] reported the isolation of diosgenin and a new steroidal sapogenin, bethogenin, in equal quantities from *Beth* root (obtained from S. B. Penick and Co.). For this new substance they proposed the formula $C_{28}H_{44}O_4$. Two of the oxygen atoms are present as hydroxyl and methoxyl groups, while the other two inert oxygens are assigned to the side-chain characteristic of the steroidal sapogenins.[107]

In the course of our investigation of plant sources for sapogenins, we have processed over ten thousand pounds of *Beth* root from twenty sources (including several lots from S. B. Penick and Co.) and have failed to find this substance. In all cases diosgenin (42), kryptogenin (6), pennogenin (11), nologenin (10) and fesogenin (121) accounted for over 85% of the total crystalline products.

On the basis of these plant studies, we long suspected that bethogenin (141) was formed from kryptogenin.[108] That this assumption is correct is shown by the fact that kryptogenin (6) readily gives bethogenin.[7] Thus, treatment of the former (6) with methanol containing a small amount of hydrochloric acid, followed by crystallization of the product from 2% methanolic potassium hydroxide gives the latter (141) in almost quantitative yields. On the other hand, similar treatment of kryptogenin with ethanol free from methanol gives only unchanged material even under more vigorous conditions than above.

Bethogenin from Kryptogenin.—To a boiling solution of 1 g. of kryptogenin in 500 cc. of methanol was added 5 cc. of concentrated hydrochloric acid. The solution was refluxed for one hour and then allowed to stand for three days at room temperature. During this time long needles formed, m. p. 110°; wt. 1 g. Recrystallization from 2% methanolic potash gave bethogenin, m. p. 191–193°. A mixture with kryptogenin (189°) melted 160°.

Anal. Calcd. for $C_{28}H_{44}O_4$: C, 75.6; H, 10.0. Found: C, 75.6; H, 10.1.

In a larger run, 26.5 g. of kryptogenin in 1300 cc. of boiling methanol was treated with 130 cc. of conc. hydrochloric acid for two hours. The reaction mixture was concentrated to one-third volume by a stream of air and then seeded with bethogenin. The crystalline solid was recrystallized from 1200 cc. of hot methanol containing 28 g. of potassium hydroxide dissolved in 200 cc. of methanol. After refluxing for fifteen minutes, the solution was air-blown until crystals appeared, cooled, filtered and dried, m. p. 190–192°; yield 19.7 g. A sample of bethogenin upon standing several weeks melted thirty degrees lower.

The **acetate** was prepared by treating 0.2 g. of bethogenin in 2 cc. of dry pyridine with 1.2 cc. of acetic anhydride. Within fifteen minutes crystals formed which were filtered and washed with water, m. p. 230°.

Anal. Calcd. for $C_{30}H_{46}O_5$: C, 74.0; H, 9.5. Found: C, 73.9; H, 9.5.

The **benzoate** was prepared by treating 0.2 g. of bethogenin in 10 cc. of dry pyridine with 3 cc. of benzoyl chloride. After standing four hours, the mixture was worked up to give a product which was repeatedly crystallized from a benzene–methanol mixture (1:4), m. p. 220°.

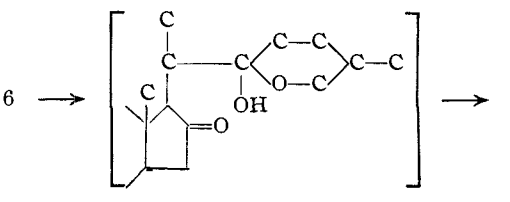
Anal. Calcd. for $C_{35}H_{48}O_5$: C, 76.6; H, 8.8. Found: 76.8; H, 8.7.

Bethogenin acetate, 0.2 g., was hydrolyzed with 0.2 g. of potassium hydroxide in 40 cc. of methanol at steam-bath temperature for twenty minutes to give bethogenin, m. p. and mixed m. p., 192–193°.

Treatment of bethogenin, 1 g., under stronger alkali conditions such as 30 g. of potassium hydroxide in 150 cc. of ethanol and 30 cc. of water for twelve hours at steam-bath temperature gave a product which was crystallized from acetone, m. p. 187–189°. A mixture with fesogenin (180°) melted 160–165°. This product was not further studied.

Kryptogenin (6) shows strong absorption in the ultraviolet region characteristic of the ketone grouping, $\lambda_{max.} = 2850$ mμ, $\log \epsilon = 1.81$. Bethogenin does not show this typical carbonyl absorption. Noller reports,[107] however, that bethogenin after treatment with hydrogen bromide in acetic acid gives a product showing almost identical absorption as above, $\lambda_{max.} = 2850$ mμ, $\log \epsilon = 1.77$.

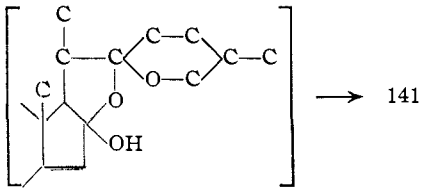
In view of the method of formation of bethogenin from kryptogenin, we suggest structure 141 for the former. This reaction probably proceeds through two hemi-ketalization products followed by methylation, *viz.*



(106) Lieberman, Chang, Barusch and Noller, THIS JOURNAL, **64**, 2581 (1942).

(107) Noller and Barusch, *ibid.*, **65**, 1435 (1943).

(108) The formula assigned by Noller and Barusch[91] to bethogenin was based on the assumption that kryptogenin was a ketoaldehyde; however, the present evidence for kryptogenin as a 1,4-diketone eliminates their formulation.



A pertinent illustration of the ketal formation is the conversion of kryptogenin (6) to diosgenin (42). The subsequent methylation by methanolic hydrochloric acid is completely analogous to the transformation of glucose to α-methyl-glucoside under like treatment. In addition, the formula is in complete accord with the facts in having a reactive hydroxyl group, a methoxyl group, and two inert oxygens. Furthermore, formulation of bethogenin as 141 accounts for the lack of typical carbonyl absorption. Treatment of bethogenin (141) with hydrobromic acid in acetic acid solution regenerates the carbonyl groups of kryptogenin, giving the latter in almost quantitative yields.

Kryptogenin from Bethogenin.—A solution of 1 g. of bethogenin in 125 cc. of acetic acid and 12.5 cc. of 48% hydrobromic acid was allowed to stand at room temperature for one hour, then poured into water and ether extracted. The ethereal solution was washed well with water, 10% potassium carbonate and water. The residue from ether was dried and acetylated with acetic anhydride–pyridine mixture (6 cc.; 10 cc.) for two and one-half hours. The small amount of precipitate was filtered off. The filtrate was diluted with water, cooled and ether extracted. The ethereal solution was washed successively with water, 10% hydrochloric acid, water, 10% potassium carbonate and water. The residue from ether was crystallized from acetone, m. p. and mixed m. p. with kryptogenin diacetate, 149–151°; yield 0.7 g.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.1; H, 8.9.

The diacetate was hydrolyzed in methanol with 5% potassium bicarbonate solution and kryptogenin was crystallized from ether, m. p. and mixed m. p., 185–186°. A mixture with bethogenin melted 160–165°.

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

A similar reaction occurs when bethogenin is treated with hydroxylamine in pyridine. In this case the methoxyl group is lost and a dioxime is formed, melting 250–253° dec., which is the dioxime prepared from kryptogenin.

Kryptogenin Dioxime from Bethogenin.—Treatment of 0.5 g. of bethogenin with 0.4 g. of hydroxylamine hydrochloride in 4 cc. of pyridine and 4 cc. of absolute ethanol at steam-bath temperature for one hour gave the dioxime of kryptogenin which was crystallized from methanol, m. p. and mixed m. p., 253–255° dec.

Anal. Calcd. for $C_{27}H_{44}O_4N_2$: C, 70.4; H, 9.6; N, 6.1. Found: C, 70.6; H, 9.7; N, 6.0.

Additional evidence for the structural formula of bethogenin (141) has been found in the formation of 5,6-dihydrobethogenin from 5,6-dihydrokryptogenin (114). This 5,6-dihydrobethogenin gives a dioxime identical to the one prepared from 5,6-dihydrokryptogenin and reverts with acid to 5,6-dihydrokryptogenin. These reactions are completely analogous to those shown by bethogenin.

5,6-Dihydrobethogenin.—This was prepared in the same manner as described for bethogenin using 5,6-dihydrokryptogenin. Recrystallization of the product from 2% alcoholic potash gave 5,6-dihydrobethogenin, m. p. 210–211°; yield 0.4 g. from 0.5 g. of 5,6-dihydrokryptogenin.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 75.3; H, 10.4. Found: C, 74.9; H, 10.4.

The **acetate** was prepared with acetic anhydride and pyridine and was crystallized from methanol, m. p. 195–196°.

Anal. Calcd. for $C_{30}H_{48}O_5$: C, 73.7; H, 9.9. Found: C, 73.5; H, 9.7.

The **benzoate** was prepared with benzoyl chloride and pyridine and was crystallized from a benzene–methanol mixture (1:4), m. p. 206–208°.

Anal. Calcd. for $C_{35}H_{50}O_5$: C, 76.3; H, 9.2. Found: C, 76.5; H, 9.4.

Treatment of 5,6-dihydrobethogenin, 0.5 g., with 0.4 g. of hydroxylamine hydrochloride in 4 cc. of pyridine and 4 cc. of absolute ethanol for one hour at steam-bath temperature gave 5,6-dihydrokryptogenin dioxime, m. p. and mixed m. p., 258° dec.

Anal. Calcd. for $C_{27}H_{46}O_4N_2$: N, 6.1. Found: N, 6.2.

Treatment of 5,6-dihydrobethogenin with hydrobromic acid in acetic acid duplicated the procedure described for bethogenin. The product after acetylation was crystallized from methanol, m. p. and mixed m. p., with 5,6-dihydrokryptogenin diacetate, 119–121°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.8; H, 9.4.

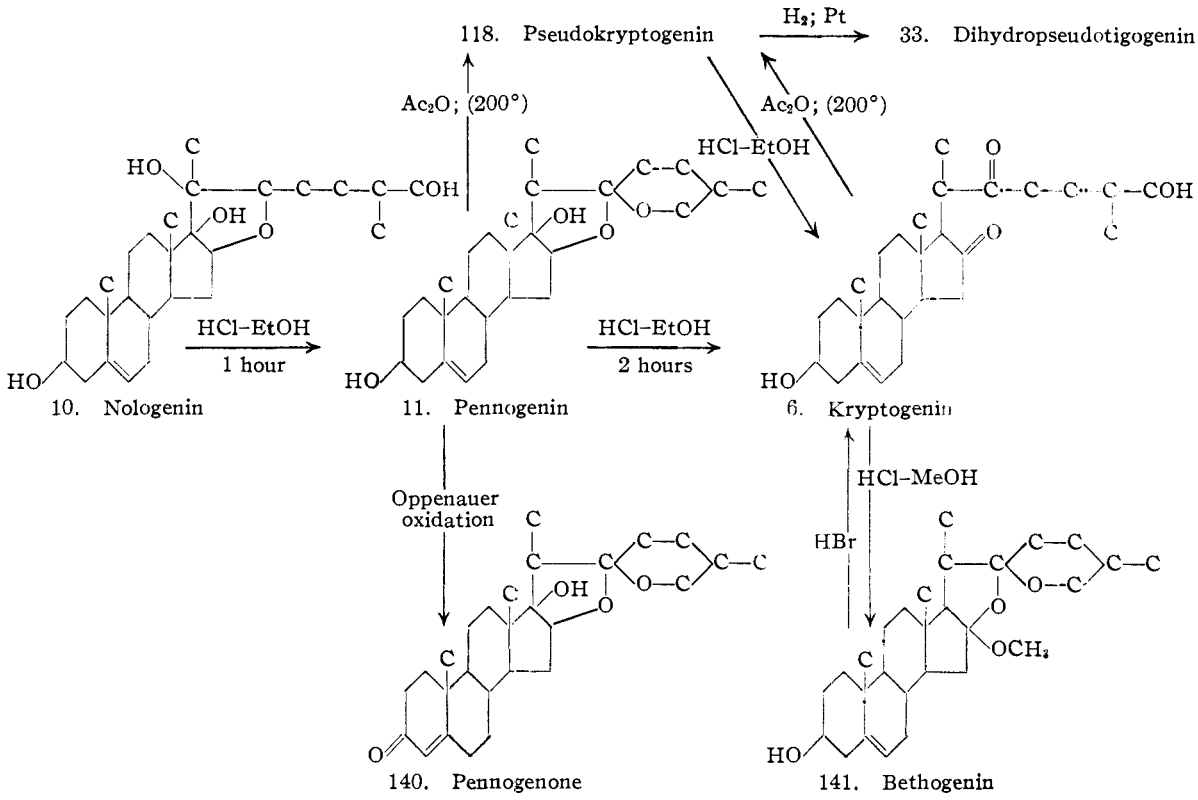
The diacetate was hydrolyzed in methanol with 5% potassium bicarbonate and the product was crystallized from ether, m. p. and mixed m. p. with 5,6-dihydrokryptogenin, 168–170°.

BIOGENESIS OF THE STEROIDAL SAPOGENINS

Earlier work on the biogenesis of steroidal hormones and related compounds[109] has focused our attention on this problem both in animals and plants. In the present work, it is particularly interesting to find so many different steroidal substances occurring in the same general classification of plants. Furthermore, it is noteworthy that the twenty-two sapogenins encountered in these plant investigations are all chemically interrelated by simple laboratory processes. Because of their joint occurrence in plants and their closely related structures, it is entirely possible that the steroidal sapogenins are biologically interrelated as well. Indeed this seemed to be so much the case that long before several of the new sapogenins were isolated, we were able to predict their possible isolation. Moreover, such a biological interpretation explains many experimental results which otherwise seem entirely ambiguous and meaningless.

Before considering the nature of this interrelationship it is desirable to correlate the structures of the various isolated steroidal sapogenins with the age of the plants and the time of collection. With this purpose in mind, two types of plants, namely, *Yucca Schottii* Engelm, and *Agave huachucensis* Baker were extensively studied. The results are summarized in Table II.

(109) Marker, THIS JOURNAL, **60**, 1725 (1938).



The four collections of *Yucca Schottii* Engelm. consisted of mature plants of approximately the same age. These were collected from a single locality. As a matter of fact the September and December collections consisted of branched cortices from the same plant. It is noted that in Table II the sapogenins isolated from *Yucca Schottii* Engelm. are listed with respect to the flowering period of the plant. This is significant as will be shown later. It also is noted that soon after flowering (September collection) the plant contains predominantly the saponins of yuccagenin, whereas three months *before flowering* (March collection), it contains relatively large quantities of the saponins of three sapogenins, namely, yuccagenin, smilagenin and kammogenin, as well as smaller quantities of the saponins of four other sapogenins. Furthermore, at the half-way interval, that is, the December collection, the sapogenin fraction is not nearly so complex as that from the later collection (March) but it is still definitely more so than that from the earlier collection (September). Moreover, at the time of inflorescence, the flowers and flower stalk, consisting of the major portion of the plant, give a mixture of steroidal sapogenins entirely different from those previously encountered. This striking difference is further illustrated in the steroidal content of the fruit which contains predominantly sarsasapogenin.

In the same manner, collections of *Agave huachucensis* Baker (and other plants listed in Table II), consisting of different age plants, give steroidal fractions with varying complexity. Unless certain other factors are considered, these facts, indeed, seem very confusing.

The first consideration of outstanding importance is a correlation between the steroidal changes (Table II) in the plant and the structures of the sapogenins. Thus, considering *Yucca Schottii* Engelm., three outstanding facts are noted. First, the relative quantity of yuccagenin isolated is progressively smaller from September to July. Second, other sapogenins appear in the yuccagenin fraction, starting in December and becoming progressively more abundant in March and July. In processing the different collections, this fact was very obvious. Third, the structures of the major occurring sapogenins isolated from the December, March and July collections have successively fewer oxygenated groups. Thus, the relatively large quantity of gitogenin ($C_{27}H_{44}O_4$) in the December collection is replaced by smilagenin ($C_{27}H_{44}O_3$) in the March collection. Also the kammogenin ($C_{27}H_{42}O_5$) fraction in the March collection is replaced by gitogenin ($C_{27}H_{44}O_4$) in the July collection. Moreover, the unsaturated sapogenins, namely, yuccagenin and kammogenin, gradually give way to the saturated sapogenins.

In considering *Agave huachucensis* Baker, similar facts are outstanding. First, the quantity of manogenin is progressively smaller from the young plant to the flowering plant; second, with the medium age plant other sapogenins start to

TABLE II

A COMPARISON OF THE SAPOGENINS ISOLATED FROM DIFFERENT AGE PLANTS

(1) *Yucca Schottii* Engelm.

3 mos. (Sept.) after flowering	6 mos. (Dec.) after flowering	3 mos. (March) before flowering season	Flowering period: Flowers	Flowering period: Fruit (July)
Yuccagenin, 100%[a]	Yuccagenin, 76% Kammogenin, 16% Gitogenin, 8%	Yuccagenin, 64% Kammogenin, 12% Smilagenin, 15% traces, samogenin, gitogenin, mexogenin, texogenin	Yuccagenin, 40% Gitogenin, 40% Smilagenin, 20%	Sarsasapogenin, 100%

(2) *Agave huachucensis* Baker

Young	Medium	Old 3 mos. before flowering	Flowering period (old): Flowers	Flowering period (old): Fruit
Manogenin, 100%	Manogenin, 90% Hecogenin, 10%	Gitogenin, 52% Manogenin, 21% Hecogenin, 23% trace, agavogenin	Hecogenin, 100%	Hecogenin, 100%

(3) *Agave gracilipes* Trel.

Medium and old	Old
Manogenin, 63% Hecogenin, 18% Gitogenin, 13% traces, rockogenin, tigogenin	Hecogenin, 100%

(4) *Agave Schottii* Engelm.

Medium	Old
Gitogenin, 100%	Gitogenin, 30% Tigogenin, 70%

(5) *Manfreda maculosa* Hook. (Agave[b])

Medium	Flowering period: Flowers	Flowering period: Roots
Manogenin, 100%	Hecogenin, 100%	Manogenin, 100%

(6) *Yucca flaccida* Haw.

Young (April) before flowering season	Old (Aug.) after flowering
Smilagenin, 90% Yuccagenin, trace	Smilagenin, 100%

(7) *Yucca elata* Engelm.

Immature (Dec.)	Old (Sept.) after flowering
Sarsasapogenin, 38% Yuccagenin, 62%	Sarsasapogenin, 100%

(8) *Samuela carnerosana* Trel.

Young (March) 3 mos. before flowering season	Old (Nov.) after flowering
Samogenin, 39% Smilagenin, 38% Mexogenin, 26% trace, kammogenin	Samogenin, 100%

[a] The percentage of the total isolated sapogenins is indicated. [b] The *Manfreda* have also been classified as *Agave*.

contaminate the sapogenin fraction; third, the structures of the major occurring sapogenins isolated from young, medium age, old and flowering plants, successively, have fewer oxygenated groups. This fact is particularly noticeable in that manogenin ($C_{27}H_{42}O_5$), dominating in the young plants, is largely replaced by gitogenin ($C_{27}H_{44}O_4$) in old plants. In the flower stalks and fruit, manogenin is missing altogether and hecogenin ($C_{27}H_{42}O_4$) predominates.

In a similar manner, a correlation between the occurrence of the sapogenins and the structures of the sapogenins may be drawn up for other plants listed in Table II. For example, the replacement of manogenin ($C_{27}H_{42}O_5$) in young *Agave gracilipes* Trel. by hecogenin ($C_{27}H_{42}O_4$) in the old plant is particularly noticeable; also manogenin by hecogenin in *Manfreda maculosa* Hook. and gitogenin ($C_{27}H_{44}O_4$) by tigogenin ($C_{27}H_{44}O_3$) in *Agave Schottii* Engelm.

A second consideration of outstanding importance is the correlation between the pronounced steroidal change discussed above and the approach of the flowering season of the plant. Many of the mature *Yucca*, including *Yucca Schottii* Engelm. flower almost annually. After the flowering period is over, the flower stalk dies and drops off. The relationship of this fact with the steroidal change of the plant is significant, for it may explain why the steroidal fraction of the plant *soon after flowering* (September collection of *Yucca Schottii* Engelm.) is predominantly yuccagenin while that from the *flowering* plant (July collection) is a mixture of considerable complexity, having at least three sapogenins. In a simple manner, the flower stalk dies and drops off, leaving the remainder of the plant with a "purified" steroidal fraction, namely, the saponins of yuccagenin. This seemingly novel distribution of the saponins in the flowering plant is clearly illustrated by a study of *Manfreda maculosa* Hook. It has been shown there that the flower stalks contain largely the saponins of hecogenin while the roots of the same plant contain saponins of manogenin.

Furthermore, it is noteworthy that the discarded flower stalk and fruit of *Yucca Schottii* Engelm. carries with it relatively large quantities of "simplified" sapogenins (present as saponins). This interesting change in the steroidal content after flowering season has also been observed with *Samuela carnerosana* Trel. The case of *Yucca elata* Engelm. is considered later.

The *Yucca* does not die after flowering but proceeds to rebuild itself for another flowering season. During this time and starting soon after the flowering season, the steroidal changes occur as discussed above. Thus, along with the flowering cycle of the *Yucca*, the steroidal content also undergoes a cycle. By way of a summary, the steroidal content of the *Yucca* soon after flowering is predominantly the saponins of one sapogenin; then saponins of other sapogenins are formed; these gradually undergo a change to give saponins of sapogenins with fewer oxygenated groups; and, finally, the latter are discarded in the flower stalks.

On the other hand, unlike the *Yucca*, the *Agave* dies after its flowering period. Nevertheless, as with the *Yucca* there is a definite correlation between the steroidal change and the approach of the flowering season. Thus, it is readily seen that as *Agave huachucensis* Baker ages, its steroidal content changes. This change has been discussed. At the flowering period, the "simplified" saponins, namely, those of hecogenin, are discarded with the flowers. This fact is further illustrated by the study of *Manfreda maculosa* Hook.

In the study of *Yucca elata* Engelm., young immature plants were first investigated. These plants were under cultivation at the University Farms, Tucson, Arizona, and had not as yet flowered. Thus, the steroidal change which occurs with the approaching flowering period was taking place. This is illustrated by the rather complex mixtures of sapogenins obtained.

However, after the flowering period occurs, only one sapogenin predominates as shown by the September collection.

These correlations strongly suggest a biogenetic interrelationship between the saponins of the steroidal sapogenins. The nature of this is now discussed in two parts: first, that of the nucleus and, second, that of the side-chain.

For convenience of discussion, the saponins of the known steroidal sapogenins are divided into two groups: group I—those which are oxygenated in the third ring (ring C), namely, the saponins of hecogenin, kammogenin, manogenin, mexogenin, agavogenin and rockogenin, and group II—those which are not oxygenated in the third ring (the saponins of the remaining sapogenins are in this group). Although the saponin of digitogenin falls into the second category, we have been unable to isolate this sapogenin in our present plant studies and for this reason we have not attempted to place it in our present scheme. Both groups of compounds have in common (1) a hydroxyl group at C-3 having the *beta* configuration and (2) two series of compounds having either the cholestane or coprostane configuration at C-5.

The saponins of Group I are discussed first.

All the naturally occurring steroidal sapogenins isolated before the present work have been hydroxy compounds, and the isolation of sapogenins with 12-carbonyl groups was surprising. It is very likely, however, that the vigorous acid hydrolysis of the parent saponins altered the original structure of the sapogenin. Such could be the case if originally there had been hydroxyl groups at C-11 and C-12, for it is conceivable that, under the conditions of the acid hydrolysis, dehydration could occur at C-11 with the formation of a carbonyl group at C-12 by rearrangement, thus forming the saturated 12-keto-sapogenins, such as manogenin and hecogenin. This dehydration might take place during the glucosidic cleavage in the presence of acid or directly by the splitting out of the sugar group.

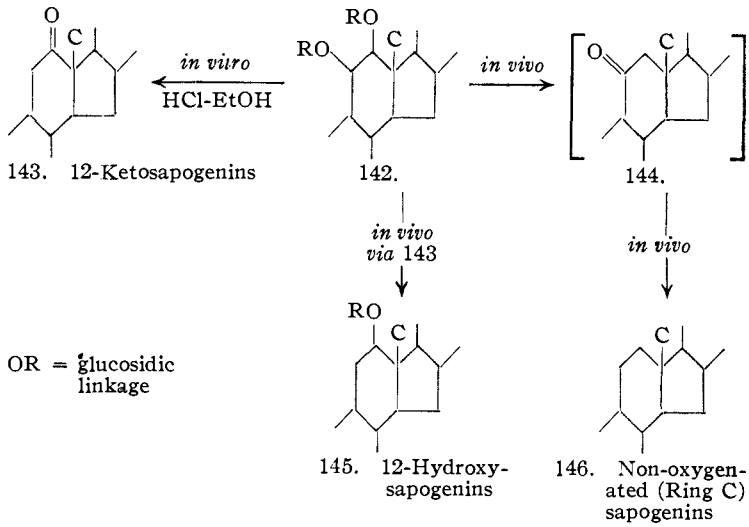
The possibility that the 12-keto groups are formed during the acid hydrolysis of the parent saponin is supported by studies on the latter. Thus, the parent saponin of manogenin from *Agave huachucensis* Baker has been treated under the conditions of two reduction reactions successfully used for the carbonyl group in its derived ketosapogenin; these are the Wolff–Kishner reaction and the reduction with sodium and alcohol. Under the conditions of the former, it might be expected that a 12-keto group if present in the saponin would be reduced to the methylene and in the latter case, it would be converted to a hydroxyl group. However, the reaction product in each case after hydrolysis is manogenin and not the expected product, gitogenin or agavogenin, respectively. Consequently, it is supposed that the 12-ketosapogenins are formed from dihydroxylated compounds.

It is likely that this course of hydrolysis and dehydration also occurs enzymatically in the plant. However, the resulting 12-keto compounds might be rapidly reduced by enzyme systems to 12-hydroxy compounds. Such a reduction of the 12-keto-group takes place in the laboratory. For example, agavogenin and rockogenin are formed either by catalytic hydrogenation or sodium–ethanol reduction of the corresponding ketone compounds. Moreover, the bio-reduction of ketosapogenins to hydroxy compounds has been observed *in vivo*. Thus, tigogenone (18) and sarsasapogenone (91) administered to a dog fed on a meat diet gave the corresponding 3-hydroxy compounds.[110] The occurrence of the 12-hydroxysapogenins, rockogenin and agavogenin, in the plant in such small amounts indicates that this course of dehydration and reduction takes place *in vivo* only to a small extent.

It is also possible that the dehydration of the 11,12-dihydroxy compounds *in vivo* might take a

(110) Marker, Wagner and Ulshafer, THIS JOURNAL, **64**, 1653 (1942).

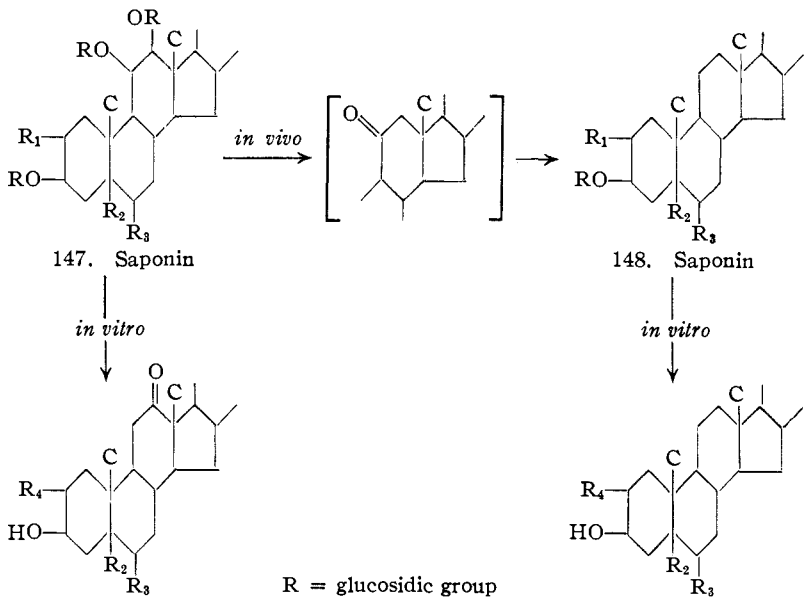
second course, forming 11-keto compounds. Since the C_{11}-oxygenated sapogenins have not been isolated in our plant experiments, it is likely that the C-11 ketones if formed are rapidly reduced to methylene groups. This type of reaction readily occurs in the laboratory. Thus, it has been shown[111] that the catalytic reduction of urane-3,11,20-trione proceeds with the elimination of the carbonyl group at C-11 to give pregnan-3,20-dione. Similarly, when 7-keto-cholesteryl chloride is hydrogenated catalytically, α-cholestyl chloride is the major product.[112] Furthermore, an 11-ketone may be removed via the dehydration of the 11-carbinol[72] followed by reduction. In a similar manner, it may be supposed that the adrenal compounds (C_{11}-oxygenated) are formed by this second course, whereas the bile acids (C_{12}-oxygenated) are formed from the 12-keto compounds.



143. 12-Ketosapogenins
142.
144.
145. 12-Hydroxy-sapogenins
146. Non-oxygenated (Ring C) sapogenins

The formation of the 12-keto sapogenins, the 12-hydroxy sapogenins, and the C-12-non-oxygenated sapogenins are summarized in formulas 142–146.

These postulations are in accord with the results of the plant studies (Table II). Thus, the replacement of the Group I sapogenins (ring C oxygenated sapogenins) isolated in the March collection of *Yucca Schottii* Engelm. by the Group II sapogenins in the July collection is explained. The saponin (147) of kammogenin (4) is converted by the loss of two sugar groups to the saponin (148) of yuccagenin (16). Similarly, the saponin (147) of mexogenin (9) is converted to the saponin (148) of samogenin (13). The latter undergoes a further loss of sugar groups (discussed later) in the lower ring system, giving finally the saponin of smilagenin. It is noted that these changes occur during the three months before the flowering period.



147. Saponin
148. Saponin
149. Sapogenin
150. Sapogenin

Cycle	R_1	R_2	R_3	R_4
Kammogenin—Yuccagenin	OR	Double bond		OH
Mexogenin—Samogenin	OR	*copro* H	H	OH
Manogenin—Gitogenin	OR	*allo* H	H	OH
Hecogenin—Tigogenin	H	*allo* H	H	H

Other examples of this change are found in the plants listed in Table II. In *Samuela carnerosana* Trel. samogenin saponin (148) is formed from the mexogenin saponin (147). In *Agave huachucensis* Baker, the saponin (147) of manogenin (8) in medium age plants is largely converted to the saponin (148) of gitogenin (55).

The joint occurrence of gitogenin (55) and manogenin (8) in *Agave gracilipes* Trel. indicates that the saponin of the former arises from the saponin of the latter in like manner. Furthermore, the saponin (148) of tigogenin (17) may arise from the saponin (147) of hecogenin (3).

In order to account for the saponins of sapogenins having either the cholestane (*allo*) or coprostane (regular) configuration of the hydrogen atom at C-5, it is supposed that both series are derived from a 5,6-unsaturated compound.

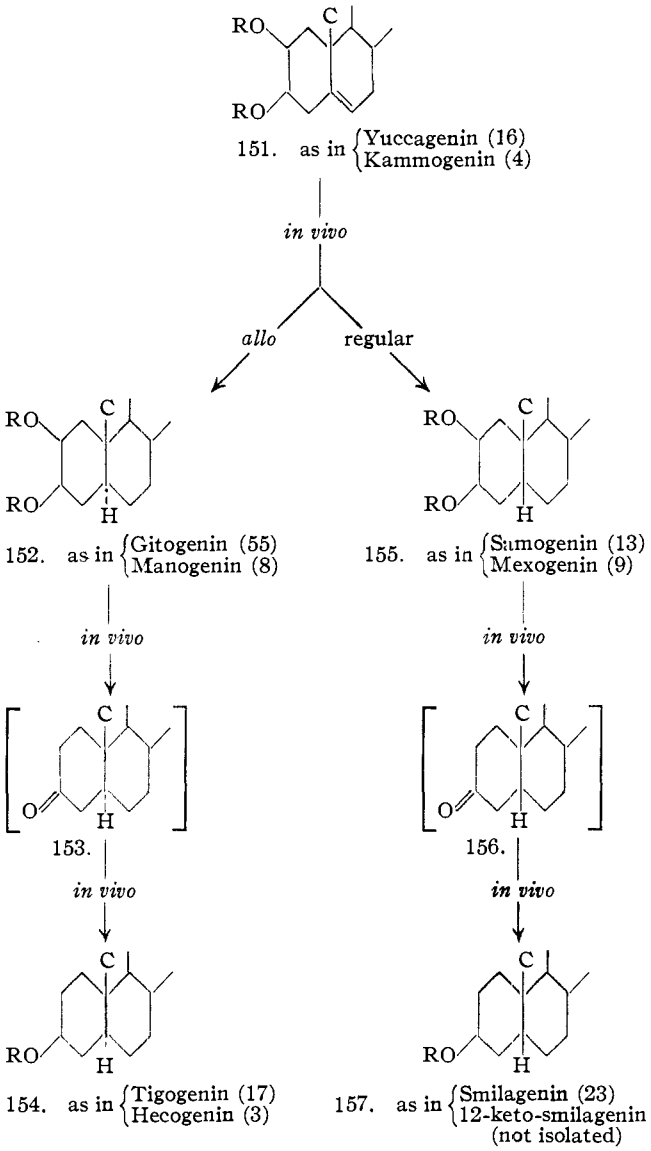
(111) Marker, Kamm, Wittle, Oakwood and Lawson, THIS JOURNAL, **60**, 1061 (1938).

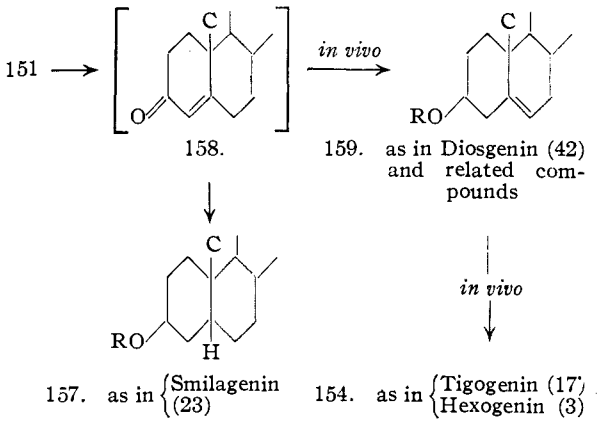
(112) Marker, Kamm, Fleming, Popkin and Wittle, *ibid.*, **59**, 619 (1937).

There are three of these compounds, namely, yuccagenin (16), kammogenin (4) and diosgenin (42). The scheme by which these give rise to the isomeric monohydroxy- and dihydroxy- sapogenins is summarized in formulas 151 to 154 and 151 to 157.

Reduction of the 5,6-double bond in 151 by enzymes in the plant could probably take both stereochemical courses forming compounds with the cholestane and coprostane configuration at C-5. Representatives of both series are indicated as 152 and 155, respectively. Thus far, such a reduction in the laboratory of 5-unsaturates is known to give mostly cholestane compounds; however, bio-reduction probably takes both courses equally well.

Enzymatic hydrolysis and dehydration of the isomeric 2,3-dihydroxylated compounds would form the intermediate ketones, 153 and 156. Since none of the latter compounds have been isolated, they must be rapidly reduced to the corresponding monohydroxy sapogenins. This change from the dihydroxylated compound involves an over-all loss of one sugar group.





Enzymatic hydrolysis and dehydration of the saponins (151) of the dihydroxy-5,6-unsaturated sapogenins, yuccagenin or kammogenin, may lead to the formation of the saponin (159) of diosgenin (42). It may be supposed that a Δ^4-3-keto compound such as 158 is the intermediate. The postulation of the shift of the double bond into conjugation with the 3-keto group in 158 is in accordance with experimental facts. Bio-reduction of this unsaturated ketone (158) could yield the saponin (159) of diosgenin or the saponin (157) of smilagenin. A striking example of this type of reduction is found in the biological transformation of 4-dehydrotigogenone (98) in the animal to diosgenin (42), smilagenin (23) and *epi*-smilagenin.[113,114] The conversion of cholestenone, the analog of 4-dehydrotigogenone (98), to cholesterol (88), analog of diosgenin (42), by chemical methods has also been reported.[115,116]

These postulated biogenetic routes are also illustrated by the plant studies (Table II). In *Yucca Schottii* Engelm., the saponin of yuccagenin (16) (September collection) is replaced to a small extent by the saponin of gitogenin (55) (December collection). Larger quantities of the latter are formed in the period preceding the flowering season. Since no tigogenin (17) was isolated from this plant, the saponin of gitogenin apparently does not undergo any further change. Instead it is stored and finally discarded in the

(113) Marker, Wagner, Turner and Wittbecker, THIS JOURNAL, **63**, 1769 (1941).

(114) Marker, Wittbecker, Wagner and Turner, *ibid.*, **64**, 818 (1942).

(115) Wagner-Jauregg and Werner, *Z. physiol. Chem.*, **208**, 72 (1932).

(116) Lettré, *ibid.*, **221**, 73 (1933).

flower stalks. Bio-reduction of yuccagenin saponin by the alternate route is shown by the isolation of small amounts of samogenin from the March collection. Since only a relatively small amount of the latter is isolated, its saponin probably undergoes a second change to form the saponin of smilagenin. The latter is stored and discarded with the flower stalk. Other possible routes of biogenesis of samogenin and gitogenin saponins may occur. Thus, the formation of the saponins of samogenin and gitogenin from the saponins of mexogenin and manogenin, respectively, has been discussed. The saponins of these isomeric ketosapogenins in turn may equally well arise from the saponin of kammogenin by the two-way bio-reduction of its double bond. That the saponin of yuccagenin may also arise from the kammogenin saponin has been pointed out. The isolation of texogenin and sarsasapogenin from this plant is discussed later.

The study of *Samuela carnerosana* Trel. further illustrates the biogenetic relationship of those sapogenins found in *Yucca Schottii* Engelm. Thus, the route, kammogenin saponin → mexogenin saponin → samogenin saponin → smilagenin saponin is very well indicated since all of the corresponding aglycones have been isolated. It is very likely that the saponin of smilagenin is discarded with the flower stalk since plants soon after flowering contain only the saponin of samogenin. Like the *Yucca* this species rebuilds itself for another flowering season.

In the study of *Agave huachucensis* Baker, these biogenetic routes are indicated. Thus, the saponin of manogenin in young plants undergoes the loss of a sugar group to give the saponin of hecogenin, present in the medium age plant. At a period three months before flowering, the large quantity of manogenin saponin originally present in young plants has been replaced in large part by the saponin of gitogenin. The absence of the gitogenin saponin in the flower stalks indicates that it has collected in the roots of the flowering plant. This change is in accordance with the proposed scheme. In the course of the plants' growth, the saponin of hecogenin is stored. It is then discarded with the flower stalk. It is significant that the necessary enzyme needed for degrading hecogenin saponin to tigogenin saponin is lacking. This prominence of hecogenin in old plants is noted in other *Agave*. Another interesting fact is the singular occurrence of compounds of the cholestane series. This indicates that the bio-reduction of the 5,6-unsaturated compound, namely, kammogenin, has favored the formation of the manogenin saponin. Since the unsaturated compounds have not as yet been isolated from the *Agave*, these apparently must be rapidly reduced to the saturated compounds.

Other *Agave* have yielded compounds of the coprostane series, indicating that both courses of bio-reduction are possible in its species. In fact, representatives of both series were found in *Agave Lechuguilla* Torr. These were gitogenin and smilagenin. The saponins of these arose by two independent routes. On the other hand, compounds of the coprostane series may be favored. This is indicated by the presence of kammogenin and smilagenin saponins in *Yucca Harrimaniae* Trel.; and also by kammogenin and sarsasapogenin saponins in *Yucca brevifolia* Engelm. Obviously, the nature of the enzyme system plays an important role.

In *Agave gracilipes* Trel., the joint occurrence of manogenin, hecogenin, tigogenin and gitogenin in old and medium age plants favorably supports the proposed biogenetic relationship. The saponin of manogenin may have led to the formation of hecogenin and gitogenin saponins. The saponin of tigogenin may not only arise from hecogenin saponin in the manner discussed previously but also from the gitogenin saponin. The presence of hecogenin saponin in relatively large quantity in old plants clearly shows how the saponin of manogenin has been degraded. Once again, a singular occurrence of compounds of the cholestane series is shown.

The joint occurrence of yuccagenin–smilagenin and yuccagenin–sarsasapogenin in *Yucca flaccida* Haw. and *Yucca elata* Engelm., respectively, indicates the role this 5,6-unsaturated compound may play. In these plants, the intermediate saponin of samogenin was not encountered. It is particularly interesting to find that the saponin of yuccagenin, prominent in the immature plant, is replaced by the saponin of sarsasapogenin. The significance of this fact is discussed later. The possibility of the former being discarded with the flower stalk, however, has not been excluded since the flowers were not processed.

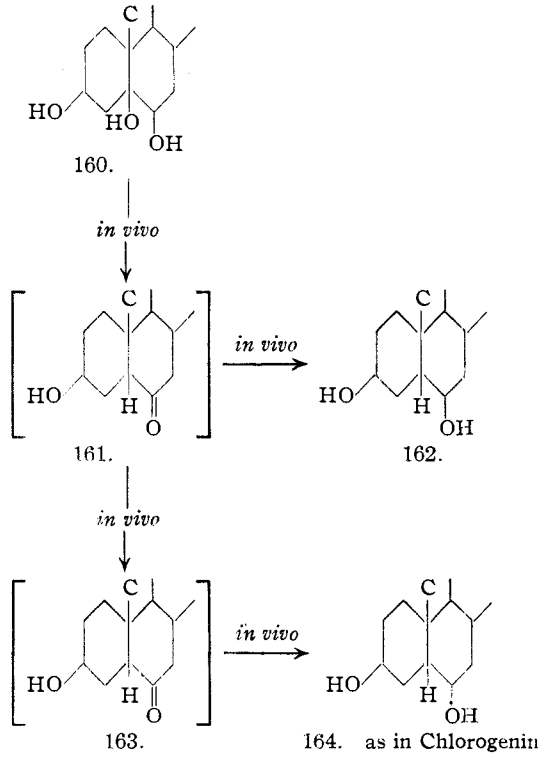
The formation of the saponin of hecogenin from the saponin of manogenin is more clearly shown in the study of *Manfreda maculosa* Hook. Thus, medium age plants contained predominantly manogenin saponin; however, in the flowering plant a large quantity of hecogenin saponin was present in the flower stalks and leaves. It is noted that the saponin of hecogenin is not further degraded but is discarded with the flower stalk.

In many plants, namely, *Agave Schottii* Engelm., *Yucca Whipplei* Torr. subsp. *intermedia*, and *Trigonella Foenum-graecum* L., the joint occurrence of gitogenin and tigogenin suggests a close biogenetic relationship. In accordance with the proposed scheme, the saponin of gitogenin is the immediate precursor of the saponin of tigogenin.

Many of the plants containing diosgenin have yielded other steroidal sapogenins with a Δ^5-3-hydroxy structure. These are yamogenin, pennogenin, nologenin and kryptogenin. Therefore, the nuclear structure in each case may be formed by the same biogenetic route. From the hydrolysis of the saponins of *Foenugreek* seeds, however, two saturated compounds, namely, tigogenin and gitogenin, were isolated along with diosgenin. Ac-

cording to the scheme the saponin of tigogenin may be formed either from the diosgenin saponin or from the gitogenin saponin. In the forty-six sources for diosgenin, this is the only case of the joint occurrence of tigogenin and diosgenin; consequently, it is more likely that the tigogenin saponin arose from the gitogenin saponin. As a matter of fact, the singular occurrence of Δ^5-3-hydroxy compounds indicates that these may well be formed by a separate biogenetic route. The proposed scheme takes this into account.

The saponin of chlorogenin (164) may also arise from a much hydroxylated nucleus such as 160. This could undergo the loss of a sugar group by enzymatic hydrolysis and dehydration to give the intermediate 161. The occurrence of a C-5 isomer[117] (162) of chlorogenin in *Digitalis purpurea*[73] suggests that this intermediate 161 has the *copro*-hydrogen at C-5. This compound would lead to this isomer (162) by reduction. However, it may first undergo a change to the *allo* compound (163) leading to the saponin of chlorogenin (164). The rearrangement of the *copro*-hydrogen to the *allo*-hydrogen is in accordance with the ready conversion of 3,6-diketocholanic acid and similar compounds of the coprostane series to the cholestane compounds in the laboratory.

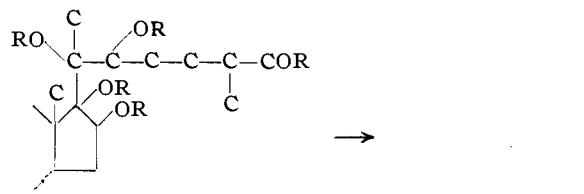


The role of the steroidal substances as sugar transports in the metabolism of the plant is strongly indicated by these studies. The biogenetic routes in both the nucleus and side-chain involve a successive loss of sugar groups from the saponins to produce simplified steroidal substances. This change in the side-chain is now discussed.

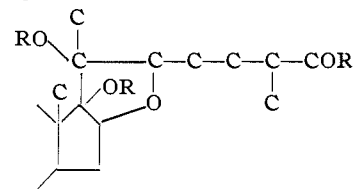
In an extensive study of *Beth* root, we have isolated four compounds which are chemically related. These are kryptogenin, nologenin, pennogenin and diosgenin. It is noteworthy that all of these have the same nuclear structure making them excellent compounds for a study of the side-chain biogenesis.

It may be recalled that the side-chains of these compounds have been interrelated by simple dehydration and reduction experiments. Thus, pennogenin (11) is derived from nologenin (10) by the loss of one molecule of water. The removal of a second molecule of water from pennogenin or the loss of two molecules of water from nologenin directly leads to the formation of kryptogenin (6). These reactions occur under the same conditions used in the hydrolysis of the plant saponins. It is significant, moreover, that these transformations of nologenin occur within the time alloted for the usual hydrolysis reaction. *Consequently, it is entirely possible that kryptogenin and pennogenin arise from nologenin during the acid hydrolysis of the plant saponins.* The possible course of these reactions has been discussed.

The saponin of nologenin (10) may be pictured as 165 or 166, the latter arising from the former by enzymatic hydrolysis of the sugar groups at C-16 and C-22 and dehydration. The formation of the tetrahydrofuran ring from a 1,4-dihydroxyl system has its counterpart in the laboratory. Thus, the dehydration of the phenyl Grignard product (115) from desoxytigogenin lactone (116) gives a tetrahydrofuran compound[22]; in addition, catalytic hydrogenation of kryptogenin (6) gives dihydrotigogenin (100).



165. Pro-nologenin saponin

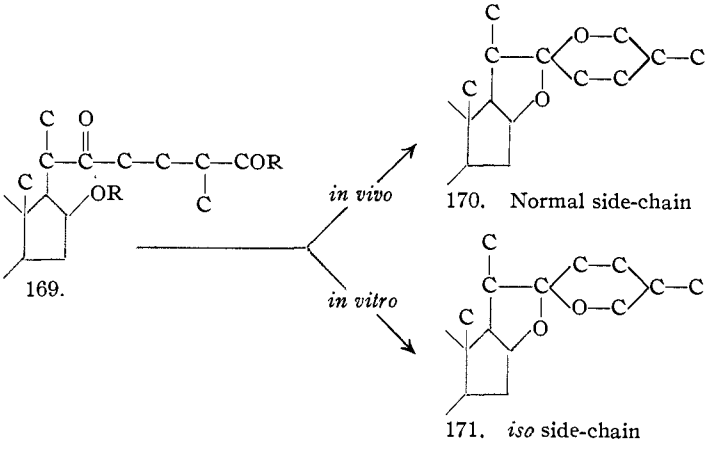


166. Nologenin saponin

The isolation of these many open side-chain sapogenins suggests that the spiro-ketal side-chain present in other steroidal sapogenins actually may be formed during the acid hydrolysis of the saponins, perhaps *via* an intermediate pseudo compound. This possibility, however, has several limitations. First, it does not explain the forma-

(117) Marker, Jones and Turner, THIS JOURNAL, **62**, 2537 (1940).

tion of the normal side-chain sapogenins such as yamogenin, neotigogenin, texogenin, etc.; second, it does not explain the formation of smilagenin since the latter is not formed by reversion of its pseudo-sapogenin. However, some intermediate sapogenin such as 169 could be formed. It is noted that this saponin possesses the open ketone form of the steroidal sapogenin side-chain. Removal of its sugar groups would allow the formation of the spiro-ketal side-chain. When this hydrolysis occurs in the laboratory under acid conditions, the steroidal sapogenins having the *iso* side-chain are formed. This follows from the fact that the *iso* configuration is the more stable form in acid medium. Thus, it is supposed that the *iso* side-chain is formed during the acid hydrolysis of the plant saponins. On the other hand, it is supposed that hydrolysis of this saponin in the plant followed by ring closure leads to the formation of the normal side-chain compounds. Since the latter are unaltered by short acid treatment, these are isolated with the side-chain intact. In this manner, the otherwise peculiar occurrence of *iso* and normal side-chain compounds in the same plant might be explained. For example, in the study of *Yucca Schottii* Engelm. an investigation of the flower stalks showed the presence of smilagenin (*iso* side-chain) while the fruit collected a few weeks later contained only sarsasapogenin (normal side-chain). Apparently, further hydrolysis of the saponin of smilagenin in the plant leads to the normal side-chain present in sarsasapogenin. Furthermore, it now seems probable that the simplest degradation product of the steroidal substances in *Yucca Schottii* Engelm. is the saponin of sarsasapogenin.



In *Agave huachucensis* Baker, however, this final loss of sugar groups and formation of the spiro-ketal side-chain in the plant apparently does not occur since only *iso* compounds were isolated. Only hecogenin (*iso* side-chain) was found in the fruit from this plant.

Another example of the change from *iso* compounds to the normal compounds is found in *Yucca elata* Engelm. Thus, yuccagenin (*iso* side-chain) is finally replaced by sarsasapogenin (normal side-chain). Here again, as the plant matures, the saponin in the form of 169 may go over to the normal closed side-chain.

Evidence for the absence of a closed side-chain in the saponins has been obtained by the catalytic hydrogenation of the saponins of gitogenin. Under the conditions employed, an aglucone having the dihydro side-chain should be obtained. Actually, gitogenin was obtained.

Other examples of the joint occurrence of normal and *iso* compounds are neotigogenin and tigogenin in *Chlorogalum pomeridianum* Kunth; texogenin and samogenin in *Yucca Schottii* Engelm.; and yamogenin and diosgenin in the *Dioscoreas*. In these cases, each pair are C-22 isomerides, indicating that they have a common origin.

It may be supposed that the saponins of the monohydroxysapogenins with the *iso* side-chain have either three or more sugar groups depending on whether the saponin is present as 169 or some further hydroxylated open chain compound. Similarly, the saponins of the dihydroxysapogenins with the *iso* side-chain might have four or more sugars attached. On the other hand, the saponins of the monohydroxysapogenins with the normal side-chain should have only one sugar group as in 170. Certain studies[40] on the saponins of steroidal sapogenins support these postulations. Thus, the saponin of digitogenin from *Digitalis purpurea* has five sugar groups; the saponin of gitogenin from the same plant has four sugar groups; and the saponin of tigogenin from *Chlorogalum pomeridianum* Kunth has six groups. The number of sugar groups present in the sarsasapogenin saponin from *Smilax ornata* Hook. has not been definitely determined. The proposed number of three sugar groups does not agree with the present scheme.

A discussion of the mode of biogenesis of the original saponins is beyond the scope of the present work. It does seem likely, however, that the original steroidal nucleus is formed from the condensation of sugar units.[118] This may account for the hydroxylated nucleus. The presence of large quantities of sugar in the plants as well as the occurrence of the sapogenins as glycosides favors this suggestion. The isolation of rhamnose[40] among the hydrolytic products from the saponins of *Chlorogalum pomeridianum* Kunth and the glucosides of the plant heart poisons is interesting. Its presence in the plant may supply the sugar unit from which the angular methyl groups in the steroidal nucleus are formed.

(118) Reichstein, "Chemie des Cortins und seiner Begleitstoffe," in Ruzicka and Stepp, "Ergebnisse der Vitamin und Hormonforschung," Akademische Verlagsgesellschaft, Leipzig, 1938, pp. 347 ff.

ISOLATION OF THE NEW SAPOGENINS

Apparatus and Method of Processing

The plants were processed for the steroidal sapogenin fractions by the following general procedure unless otherwise indicated. In most cases they could be ground satisfactorily for extraction by machines of three different types. The first machine was a small hay-cutter powered by a 2 h.p. a.c. motor. The second was an electrically powered meat grinder and the third was a specially constructed bench-saw. The saw unit of the latter consisted of a five-eighth-inch ball-bearing shaft equipped with thirty-six 5-inch dado blades powered by a three-fourths h.p. a.c. motor. It was mounted on a solidly constructed bench (38 × 24 × 24 inches) in such a manner that the blades protruded one inch above the center of the top-surface. When in operation the shredded material from the blades was directed downward through a chute to a suitable receiver.

The *Agave* were most conveniently chopped by the hay-cutter. The leaf-bases were first separated from the core and sliced into lengthwise strips. The slices and the core were passed through the hay-cutter four times. In this manner they were well chopped for alcoholic extraction. On the other hand, the *Yuccas* having stems 15–50 cm. in diameter were best ground by the bench-saw. The undried stems were cut into convenient lengths and the rough hard bark was removed before sawing. In cases where the samples were soft roots, leaves, or fruit, the meat-grinder was used.

The ground, undried samples were processed in batches of about 25 kg. The batch was extracted with 32 liters of 95% ethanol at moderate steam-bath temperature for twelve hours. A 30-gal. ash can with a suitable reflux condenser was a convenient container for this bulky operation.[119] The hot extract was strained through cheesecloth. The filter cake was washed with two 7-liter portions of hot ethanol and then squeezed dry in a modified wine press. The extract and washings contained in several 22-liter flasks or a 30-gal. ash can were evaporated under a slight vacuum to a sirup, which was further concentrated by passing a current of air over its surface. In several cases, when alcohol was difficult to stock, the plants were extracted with hot water. This was found to be a satisfactory method, although much slower since the aqueous extract had to be evaporated for the next step. For this purpose, the aqueous extract contained in 5-gal. cans was concentrated by heating on the steam-bath with a current of air passing over its surface. The concentrate was hydrolyzed by refluxing for two hours with 8 liters of 2 *N* ethanolic hydrochloric acid. The reaction mixture was cooled and filtered. When any considerable tar occurred, the tar was ground and digested several times with an equal volume of hot ethanol. The combined filtrates were diluted with 20 liters of ether and the solution was washed successively with water, 5% sodium hydroxide and water and evaporated. The fatty esters were hydrolyzed by refluxing the residue with three volumes of 10% alcoholic potash for thirty minutes. The cooled hydrolysis mixture was extracted with ether and the ethereal solution was washed with water and evaporated to a small volume. The crude sapogenin fraction which separated was dissolved in acetone and then treated with Norite.

It is difficult to generalize the crystallization procedures used in the isolation of the steroidal sapogenins since each plant species presented a unique case. However, since in most cases only one sapogenin predominated in the sapogenin fraction, a general procedure was followed for the choice of the correct solvent. The melting point of the crude sapogenin after one crystallization from acetone gave some idea as to its principal component. A melting point below 200° indicated the presence of the monohydroxy-sapogenins and one in the range of 210–240° indicated the dihydroxy-sapogenins or hecogenin. In the first case, methanol was the most promising solvent for recrystallization and in the latter case, ether was used. Frequently, the crude material obtained from acetone was converted to the acetate with boiling acetic anhydride for further purification. In all cases the acetates were prepared by refluxing an acetic anhydride solution of the sapogenin for thirty minutes and then removing the solvent *in vacuo*. The acetates with the exception of kryptogenin diacetate were hydrolyzed by refluxing with excess 10% ethanolic potassium hydroxide for thirty minutes.

Because of the extent of the work only the most important and representative isolation procedures are given below. The numerous changes of solvent, recrystallizations, mixed melting point determinations and interconversions between the genins and acetates are eliminated for the sake of brevity. The rest of the plants were worked through in similar ways. A complete list of the plants investigated, the locality of collection and the yield of particular sapogenins are given elsewhere.[3]

Identifications were made in all cases by analyses and mixed melting point determinations of the genins and their acetates. *In cases in which a mixture of two different genins with melting points in the same range did not give a melting point depression, a mixture of the corresponding acetates did (or vice versa) so that identification was really assured without the analyses.* For example, although the binary mixtures tigogenin–diosgenin, kammogenin–manogenin or samogenin–diosgenin did not give a good depression, the mixtures of the corresponding acetates did. Similarly, mixtures of samogenin–tigogenin acetates or gitogenin–manogenin acetates did not give good depressions but the mixtures of the free genins did. *In all*

(119) Authors, *Chem. Eng. News*, **20**, 373 (1942).

cases, however, analyses were used to confirm the identification by mixed melting points. It should be recalled that dimorphism is frequent among the genins and their acetates.

General Plant Characteristics

Although the saponin content of many plants varies with their phase in their life cycle some general characteristics have been observed between the plants investigated and the steroidal contents. Thus, the majority of the *Agaves* occurring in the United States contain as their principal steroidal sapogenin either hecogenin or manogenin, the former occurring more frequently than the latter. This is even the case with the Mexican *Agaves* with the exception that manogenin is generally the more common of the two. For the most part the *Agaves* occurring in Mexico give relatively smaller sapogenin fractions.

A large number of the *Agaves* collected in Mexico have incomplete botanical classification. These are called *Magueys* with the native names attached. Of particular interest are those *Magueys* which during the course of time the natives have singled out as the best sources for the manufacture of an intoxicating liquor known as tequila. These include *M. mescal azul, M. mescal bermejo, M. mescal chato* and *M. mescal mana larga.* We find that the steroidal fraction from each of these plants contains sitosterol. No sapogenins could be isolated.

In the present work, twenty-five species of *Dioscorea* in the amount of almost six thousand kilograms have been obtained. In the earlier studies, a systematic crystallization procedure had not been worked out; consequently, kryptogenin and yamogenin were not sought and only diosgenin, predominantly present, was isolated. In later work, however, twelve species were reinvestigated and the sapogenin fraction from each was crystallized essentially by one of two procedures (Figs. 8 and 9) to give yamogenin and kryptogenin as well as diosgenin. It is very likely that all of the Mexican *Dioscoreas* contain yamogenin and kryptogenin. Both of the latter occur in small amounts compared to the quantity of diosgenin.

In addition to *Beth* root many *Trillia* were investigated individually on a smaller scale. In several cases only diosgenin or kryptogenin could be isolated while in others both sapogenins occurred together. The different solubility of their acetates in acetic anhydride provided an excellent means for their separation. The more soluble sapogenins, namely, nologenin and pennogenin were not encountered. These results are significant in that they throw light upon some of our earlier work. Previously, we reported the isolation of diosgenin from *Beth* root. A thorough investigation of the mother liquors from its separation yielded no other sapogenin. These results appear to be in contrast to our present findings and those of Noller and co-workers[106] who isolated equal quantities of bethogenin (a derivative of kryptogenin) and diosgenin. A simple explanation of this is found in the fact that *Beth* root obtained from commercial houses consists of any of the *Trillium* species, depending upon which grows in the vicinity of collection. It is quite possible, furthermore, that the steroidal content of any one species changes with age and month of collection as it does in the *Agaves* and *Yuccas.*

The majority of the *Yuccas* investigated contained steroidal sapogenins. Smilagenin and sarsasapogenin were the more common occurring sapogenins. In general the *Yuccas* growing in the desert region of the southwestern United States contained sarsasapogenin and those growing in the southern and southeastern United States contained smilagenin. In addition six species growing in the coastal regions, namely, *Y. filamentosa* L. and the five sub-species of *Y. Whipplei* Torr. contained either gitogenin or tigogenin. The only exception to this "chemical boundary" is *Y. brevifolia* Engelm. which grows in California. It gave smilagenin. Smilagenin has been isolated from *Smilax ornata* Hook.[17] but only with difficulty. On the other hand, it can readily be obtained from the *Yuccas* since it is the principal steroidal constituent. As in the case with the *Agaves*, the Mexican *Yuccas* contained smaller steroidal fractions. *Yucca aloifolia* L., *Y. arkansana* Trel. and *Y. flaccida* Haw. are the best sources for smilagenin and *Yucca elata* Engelm., *Y. glauca* Nutt. and *Y. Treculeana* Carr. *var. caniculata* Hook. are the best sources for sarsasapogenin.

In general, the best sources found for the new sapogenins are: hecogenin, *Agaves Deserti* Engelm., *gracilipes* Trel. and *Toumeyana* Trel.; manogenin, *Agaves huachucensis* Baker, *utahensis* Engelm. and *bracteosa* S. Wats.; rockogenin, *Agave gracilipes* Trel.; agavogenin, *Agave huachucensis* Baker; lilagenin, *Lilium Humboldtii* Roezl and Leichtl.; kammogenin, *Yucca Schottii* Engelm. and *Samuela carnerosana* Trel.; samogenin and mexogenin, *Samuela carnerosana* Trel.; kryptogenin, pennogenin and nologenin, *Beth* root; texogenin and yuccagenin, *Yucca Schottii* Engelm.; yamogenin, *Dioscoreae* from Mexico.

Isolation, Experimental

Isolation of Hecogenin from *Hechtia texensis* **S. Wats.** Medium age plants, 14 kg., without roots were collected at Black Gap, Texas, in November, 1941. After extraction and hydrolysis the sapogenin fraction was crystallized first from acetone (m. p. 207–234°) and then from ether, m. p. 263–265°; wt. 0.5 g.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.5; H, 9.9.

The acetate crystallized from methanol, m. p. 241–243°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.9; H, 9.3.

Isolation of Manogenin from *Manfreda maculosa* **Hook.** —The entire medium age plants, 8 kg., were collected southeast of San Antonio, Texas, in November, 1941. The sapogenin fraction which separated from acetone, m. p. 225–230°, wt. 10.6 g., was recrystallized from ether to give manogenin, m. p. 241–243°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.5; H, 9.4.

When a solution of this substance in ethanol was treated with a 2% solution of digitonin in ethanol, there was formed immediately a heavy white precipitate of the digitonide.

Manogenin diacetate was crystallized from methanol, m. p. 253–255°.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 70.2; H, 8.6.

Isolation of **Rockogenin** from *Agave gracilipes* **Trel.** (**Fig. 3**).—Medium age and old plants, 700 kg. without roots, were collected 10 miles north of the El Capitan Mountain below the Texas–New Mexico border in March, 1942. The acid hydrolysis product was dissolved in 5 liters of ethanol and boiled with excess potassium hydroxide. The reaction mixture was diluted with 3 liters of water and extracted with 50 liters of ether. The ethereal solution (A) was evaporated to 5 liters, cooled and the solid was filtered and washed well with cold ether, wt. 1.2 kg. This fraction (B) was recrystallized from acetone and washed with ether. It was then acetylated and crystallized from acetone, m. p. and mixed m. p. with hecogenin acetate, 240–243°; wt. 40 g.

Anal. Calcd. for $C_{27}H_{44}O_6$: C, 73.7; H, 9.4. Found: C, 73.6; H, 9.3.

Hydrolysis of the above acetate gave hecogenin, m. p. and mixed m. p., 242°.

The filtrate (C) from the above acetate was evaporated and the residue was crystallized from ether followed by a recrystallization from acetone, m. p. and mixed m. p. with gitogenin diacetate, 240–244°; wt. 35 g. A mixture with the above acetate melted 200–215°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.4; H, 9.1.

Hydrolysis of this diacetate gave gitogenin, m. p. and mixed m. p., 264–266°.

The ether mother liquor (D) from the first crystallization of the crude unacetylated sapogenin fraction was evaporated and the residue was crystallized from acetone to give manogenin, m. p. and mixed m. p., 239–242°; wt. 87 g.

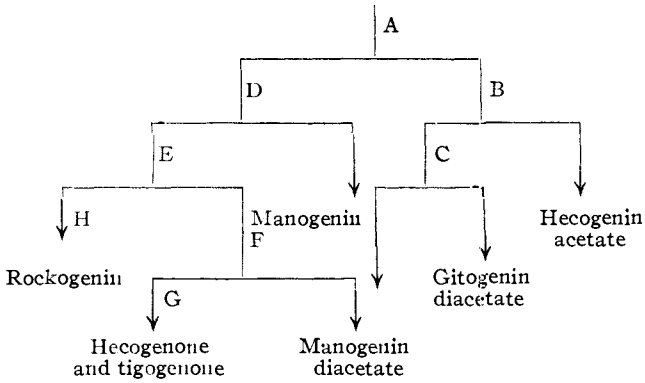


Fig. 3.—Crystallization of the sapogenin fraction from the March collection (700 kg.) of *Agave gracilipes* Engelm.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.4; H, 9.4.

When acetylated, this material gave manogenin diacetate which crystallized from acetone, m. p. and mixed m. p. 240–243°.

The filtrates (E) from the crystallization of manogenin were evaporated and the residue was acetylated and crystallized first from 2 liters and then from 200 cc. of methanol to give a first and second crop of crystals, respectively. The methanol mother liquor (H) was then boiled with excess potash and the product was extracted and crystallized from ether and then from methanol to give rockogenin, m. p. 220°; yield 5 g.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.8; H, 10.4.

Rockogenin diacetate crystallized from ether, m. p. 204–206°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 7.20; H, 9.5.

The first and second crops from above (F) were combined. Fractional crystallization from ether and acetone gave an additional 82 g. of manogenin diacetate. The mother liquors (G) after its removal were combined and evaporated. The residue was oxidized with an equal weight of chromic anhydride in acetic acid for thirty minutes. The reaction mixture was ether extracted and the ethereal solution was washed free of acids with sodium hydroxide solution. The neutral product was fractionally crystallized from ether to give hecogenone, m. p. and mixed m. p., 237–240°, wt. 10 g. and tigogenone, m. p. and mixed m. p. 207°, wt. 11 g.

Isolation of Agavogenin from *Agave huachucensis* **Baker** (**Fig. 4**).—Old plants without roots, 1360 kg., were collected in March, 1942, near Fort Huachuca, 50 miles east of Tucson, Arizona, along the Elgin–Canelo road. The concentrate from 3600 liters of alcoholic extract was dissolved in 336 liters of hot 70% alcohol and treated with a solution of 3 kg. of cholesterol (70% pure, from tuna fish oil) in 112 liters of 95% ethanol. The mixture was stirred thoroughly and allowed to stand overnight at 20°. The supernatant liquid was siphoned from the precipitate which had settled on the bottom of the kettle. The remaining alcoholic suspension of the precipitate was centrifuged and the precipitate was collected and washed successively with water, alcohol, ether and then dried at 70°; wt. 9.0 kg. The water wash removed the sugars and the ether removed the fats. The dried saponide was dissolved in 36 liters of pyridine and heated at steam-bath temperature for three hours. The solution was filtered through Kieselguhr and the filtrate was diluted with 360 liters of ether to precipitate the saponins. These were washed thoroughly with ether and dried at 70°; wt. 7.5 kg. The total saponins were hydrolyzed with 280 liters of 2 *N* alcoholic hydrochloric acid for three hours. The reaction mixture was concentrated to 200 liters *in vacuo*, cooled and diluted with 600 liters of water. The precipitated sapogenin fraction was allowed to settle to the bottom of the container and the supernatant liquid was siphoned off. The remaining water suspension of the sapogenins was filtered and the filter-cake was dried at 70°; wt., 1.8 kg.

One-half of the sapogenin fraction was hydrolyzed with an excess of alcoholic potash for thirty minutes. The reaction mixture after cooling to 35° was extracted with 80 liters of ether and the extract (A) was washed with water and evaporated to four liters. The precipitate was filtered from the cold mixture, washed thoroughly with ether and dried; wt., 595 g. The total product (B) was acetylated and crystallized from ether followed by a crystallization from acetone, m. p. and mixed m. p. with manogenin diacetate, 245–247°; yield 26 g.

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

When hydrolyzed the diacetate gave manogenin, m. p. and mixed m. p., 240-243°.

The acetate mother liquors (C) were combined and evaporated and the residue was crystallized from methanol to give an intermediate fraction, wt. 20 g., which was further treated with Girard reagent T[120] as described below. The portion soluble in methanol (D) was hydrolyzed, crystallized from ether and then acetone and reacetylated. The product was crystallized from ether and acetone, m. p. and mixed m. p. with gitogenin diacetate, 242–244°; wt. 65 g.

Hydrolysis of the above diacetate gave **gitogenin**, m. p. and mixed m. p., 264–267°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 75.1; H, 10.0.

(120) Girard and Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936).

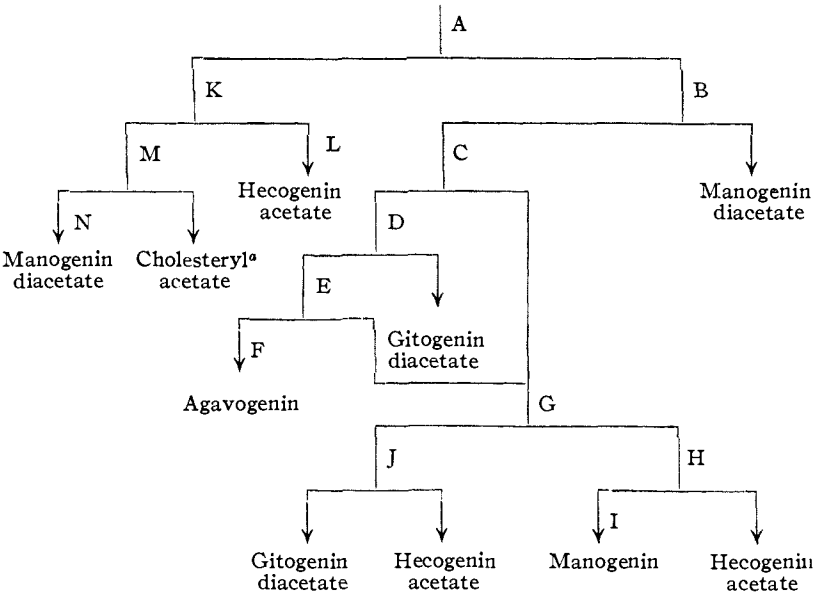
The acetate mother liquors (E) after the removal of gitogenin diacetate were combined and evaporated. The residue was crystallized from methanol, m. p. 200–225°, wt. 102 g. This fraction combined with that mentioned above was treated with Girard reagent T[120] as described below. The mother liquors (F) after removal of this material were hydrolyzed and crystallized from ether–methanol to give a small intermediate fraction. The filtrate was further evaporated and the residue was crystallized from ether to give agavogenin, m. p. 242°; wt. 5 g.; a mixture with 12-dihydromanogenin melted 240–242°.

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

This product was saturated to bromine–acetic acid and forms an alcohol-insoluble digitonide.

The acetate crystallized from methanol, m. p. 228–231°; a mixture with 12-dihydromanogenin triacetate melted 228–231°.

Anal. Calcd. for $C_{33}H_{50}O_8$: C, 69.0; H, 8.8. Found: C, 68.6; H, 8.7.



[a] Cholesterol carried through from the precipitation of the saponins.

Fig. 4.—Crystallization of the sapogenin fraction from the March collection (1360 kg.) of *Agave huachucensis* Baker.

The total intermediate fractions (G) from above, wt. 122 g., were refluxed with 50 g. of Girard reagent T in 1.8 liters of ethanol for thirty minutes. The mixture was diluted with an equal volume of water and extracted with 4 liters of ether. The aqueous layer was reëxtracted with 1.5 liters of ether and then heated on the steam-bath with 250 cc. of concentrated hydrochloric acid for thirty minutes. The Girard ketone fraction (H) precipitated. This was extracted with ether and the ethereal solution was washed with water and evaporated to yield 12 g. of hecogenin acetate, m. p. and mixed m. p., 241–243°.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.6; H, 9.3.

From the mother liquors (I) by fractional crystallization from ether was obtained manogenin, m. p. and mixed m. p., 249–251°, wt. 1 g. Mixtures of this with hecogenin, manogenin, kammogenin and gitogenin acetates melted 20-30° lower than either component. This diol was probably formed during the acid hydrolysis of the Girard ketone derivative.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.2; H, 9.7.

The ethereal solution of the Girard non-ketone fraction (J) was washed with water and evaporated until crystals started to appear, cooled and filtered, m. p. 217–235°, wt. 42 g. This material was fractionally crystallized from ether to give a first fraction of hecogenin acetate, m. p. and mixed m. p., 251–252°, wt. 19 g., and a second fraction of gitogenin diacetate, m. p. and mixed m. p., 241–243°; wt. 15 g.

The ether filtrate and washing (K) from the crystallization of the original sapogenin fraction from above were combined and evaporated. The residue, wt. 270 g., was acetylated and treated with 75 g. of Girard reagent T in 1 liter of ethanol in a like manner as described above. The ketone fraction (L) was crystallized from ether to give 5 g. of hecogenin acetate, m. p. and mixed m. p., 246–247°. The non-ketone fraction (M) was crystallized from methanol, m. p. and mixed m. p. with cholesteryl acetate, 113–114°; wt. 64 g. The mother liquor (N) from this product was concentrated and cooled to give 27 g. of semi-solid material. This was hydrolyzed and crystallized from ether to give 6 g. of material melting 225–230° which after acetylation crystallized from methanol, m. p. 256–257°, wt. 5.5 g.; a mixture with manogenin diacetate (250°) melted 250–255°.

Isolation of Lilagenin from *Lilium Humboldtii* **Roezl and Leichtl.**—The bulbs, 4.23 kg., containing 50% water were ground and processed. The sapogenin fraction after treatment with Norite was crystallized from ether, m. p. 230–241°; wt. 0.9 g. Recrystallization from ether, m. p. 245-252°, followed by acetylation and crystallization from acetone and then methanol gave lilagenin diacetate, m. p. 150–155°. A mixture with chlorogenin diacetate (151°) melted 130–144°.

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

Hydrolysis of the diacetate and crystallization from methanol gave lilagenin, m. p. 242–246°. A mixture with yuccagenin (252°) melted 215–235°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 73.5; H, 9.8. Found: C, 75.4; H, 9.9.

Isolation of Kammogenin from *Yucca Harrimaniae* **Trel.**—Roots, 7 kg., from old plants were collected at Zion Park, Utah, in December, 1941. The sapogenin fraction which precipitated from acetone, m. p. 220–221°, wt. 6 g., was acetylated and crystallized from ethyl acetate as plates, m. p. and mixed m. p. with sarsasapogenin acetate, 140–142°; yield 2 g.

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

Hydrolysis of the acetate gave sarsasapogenin which crystallized from ether as flat needles, m. p. and mixed m. p., 202–203°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 78.0; H, 10.6.

The mother liquors after removal of the sarsasapogenin acetate were combined and evaporated. The residue was hydrolyzed with 10% alcoholic potassium hydroxide for thirty minutes. The reaction mixture was cooled and extracted with ether. After washing with water the ethereal solution was evaporated to 40 cc. and cooled to 0°. The crystals which appeared were filtered (194–210°) and recrystallized from ether, m. p. 215–230°, wt. 1.4 g. This material was acetylated and the product after treatment with Norite was crystallized once from acetone and then twice from methanol to give kammogenin di-

acetate, m. p. 240–243°. When it was crystallized from acetone, the diacetate of kammogenin melted at 255–257°. These are probably polymorphic forms. A mixture with manogenin diacetate (255°) melted 220–230°.

Anal. Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.5; H, 8.5.

This material readily decolorized an acetic acid solution of bromine.

When treated with an alcoholic solution of potassium hydroxide, it gave kammogenin which crystallized from methanol as white needles, m. p. 242°. A mixture with manogenin (243°) gave no depression in melting point.

Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.9; H, 9.1. Found: C, 72.6; H, 9.1.

When an alcoholic solution of this genin was added to a 2% solution of digitonin, a flocculent precipitate of the digitonide formed.

Isolation of Samogenin and Mexogenin from *Samuela carnerosana* Trel. (Fig. 5).—The caudex, 272 kg., from young plants was collected along the Rio Grande River at Black Gap in the Big Bend region of Texas in March. The alcoholic concentrate from the extraction of the plants was dissolved in 192 liters of ethanol and hydrolyzed with 48 liters of concentrated hydrochloric acid for three hours. The reaction mixture was then extracted with 160 liters of butyl alcohol. The latter solution was washed several times with water and then with 10% caustic solution. The alkali wash caused the separation of a considerable amount of tarry substance which was not investigated. The alcohol layer was then heated under reflux for two hours with a 10% caustic solution. The alcohol layer was washed free of alkali and evaporated *in vacuo*. The solid residue was powdered and thoroughly extracted with 160 liters of acetone. The acetone solution was evaporated.

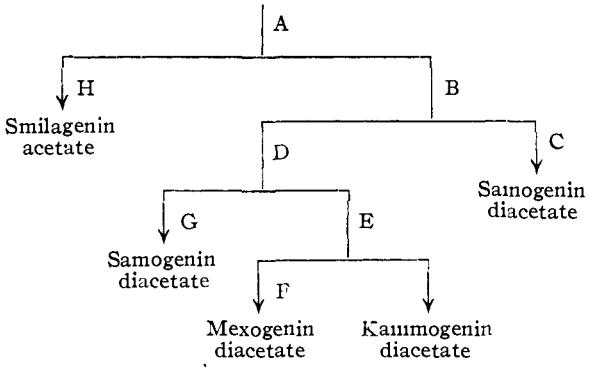


Fig. 5.—Crystallization of the sapogenins from the March collection (272 kg.) of *Samuela carnerosana* Trel.

One-half of the residue was hydrolyzed with an excess of alcoholic potash for thirty minutes. The reaction mixture, cooled to 35°, was extracted with 40 liters of ether and the extract was washed with water and evaporated. The residue was separated into an acetone-soluble fraction (H) and an acetone-insoluble fraction (B). The latter weighing 148 g. was further crystallized from ether to give an ether-soluble fraction (D) and an ether-insoluble fraction (C), wt. 46 g.

The ether-insoluble fraction (C) was acetylated and crystallized from methanol and ether to give samogenin diacetate, m. p. 195–198°; yield 30 g. Hydrolysis of the diacetate gave samogenin, m. p. 208–214°.

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

The sapogenins in the ether-soluble fraction (D) were acetylated and treated with 40 g. of Girard reagent T in 1 liter of ethanol as described for *A. huachucensis* Baker. The ketone fraction (E) was crystallized from ether–pentane, m. p. 195–200°, wt. 34 g. This material was recrystallized from 125 cc. of ether to give a small ether-insoluble fraction, m. p. and mixed m. p. with kammogenin diacetate, 258–261°, wt, 1.5 g.

Anal. Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.5; H, 8.7.

The ether filtrate (F) after the removal of kammogenin diacetate was diluted with pentane, concentrated and cooled to give mexogenin diacetate, m. p. 204–206°; yield 25 g.

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

The Girard non-ketone fraction (G) was fractionally crystallized from methanol to give an additional 7 g. of samogenin diacetate, m. p. and mixed m. p., 195–198°. Other fractions were destroyed in a fire.

The acetone-soluble fraction (H) from above was concentrated, cooled and filtered to give a dark semi-solid mass. This material was hydrolyzed with excess 20% alcoholic potash for thirty minutes. The product from methanol (172–180°) was acetylated and treated with Girard reagent T as described previously. In this case, however, no ketone fraction was obtained. The non-ketone fraction was crystallized from acetone, m. p. and mixed m. p. with smilagenin acetate, 148–150°; yield 30 g.

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

Hydrolysis of the acetate gave smilagenin, m. p. and mixed m. p., 184–186°.

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

Kryptogenin, Pennogenin and Nologenin, New Sapogenins from the *Trillium* Species (Fig. 6).—Twenty lots of undried *Beth* root (*Trillium erectum* L. and allied species) weighing 11,400 kg. were collected at seven different localities in the Blue Ridge Mountain region of the southeastern states. All of these consisted largely of young plants. Collections were made by the following drug companies: Penick, Ashville, N. C.; Wilcox, Boone, N. C.; Phillips, Wilkesboro, N. C.; Greer, Marion, Va.; Greer, Lenoir, N. C.; and Blue Ridge, West Jefferson, N. C. The sapogenin content varied between the lots but in general diosgenin accounted for 35–60% of the total crystalline sapogenin content, kryptogenin for 20–54% and pennogenin for 18–20%. In addition small amounts of nologenin and fesogenin were obtained. The last named compound has been found to be identical with anhydrokryptogenin and probably arises from kryptogenin during the alkaline hydrolysis.

Twenty lots of concentrate from the alcoholic extraction of the total 11,400 kg. of undried roots (2,800 kg. of dried root) were processed in this Laboratory. The water content of the roots averaged 65–75%. In each case, the thick extract, averaging 34 kg. from 560 kg. undried root, was diluted with two liters of ethanol and then defatted with three 15-liter portions of ether. Most of the ether containing the fats was removed by decantation. Upon heating the thick gum gently on the steam-bath more ether separated. This was removed and the gum was then heated to 70° with a stream of air passing over its surface to remove the last traces of ether. The gum was dissolved in 25 liters of 95% ethanol contained in a 35-gal. steam jacketed copper kettle and hydrolyzed with 5 liters of concentrated hydrochloric acid at the boiling point for two and one-half hours. The hydrolysis mixture after cooling was diluted with 100 liters of ether. The ethereal solution was washed with 40 liters of water, 22 liters of 5% sodium hydroxide, and 60 liters of water, and then evaporated. An acetone solution (*ca.* 4 liters) of the residue was cooled at salt–ice temperature to give the crude crystalline sapogenin fraction, wt. 700 g.

The crystallization procedures for the isolation of the steroidal sapogenins were different in the first three 34-kg. runs; however, in later runs a general procedure was used which could be duplicated. A typical run on one of the samples is given below.

The sapogenin fraction (B), 700 g., was acetylated with 2.1 liters of boiling acetic anhydride for thirty minutes.

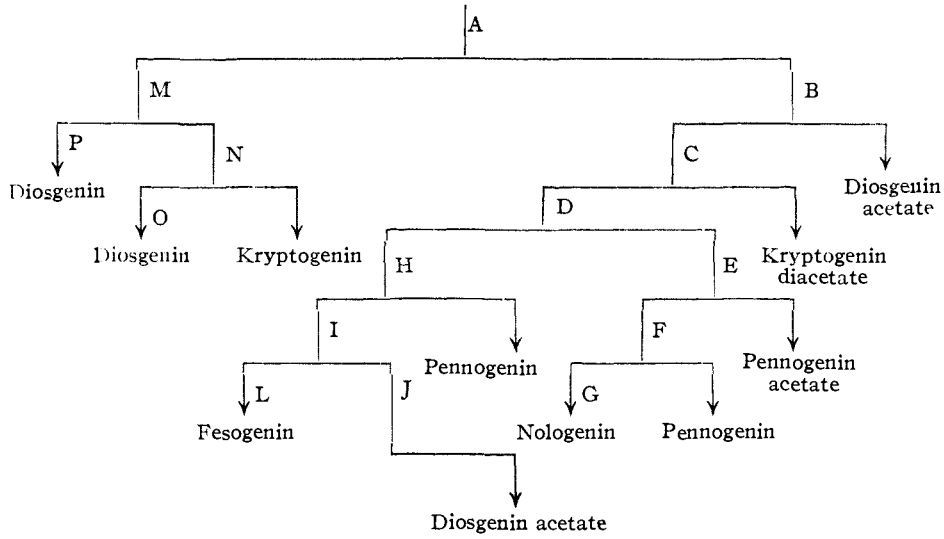


Fig. 6.—Crystallization of the sapogenin fraction from *Beth* root (560 kg.).

After cooling the acetylation mixture to room temperature, the crystalline diosgenin acetate was filtered and washed with cold methanol, m. p. and mixed m. p., 196–198°, yield 310 g.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.2; H, 9.6.

Hydrolysis gave diosgenin crystallized from acetone, m. p. and mixed m. p., 212–214°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.2; H, 10.4.

The acetic anhydride mother liquors (C) and the residue from the evaporation of the methanol wash were combined and the solution was evaporated *in vacuo* on the steam-bath to one-third volume. The solution was cooled in an ice–salt bath for two hours. The crystalline solid was filtered and slurried with one liter of cold methanol and refiltered to give kryptogenin diacetate, m. p. 152–153°; yield, 140 g.

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 diacetate as described later gave kryptogenin which was crystallized from acetone, m. p. 187–189°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.1; H, 9.7.

The mother liquor (D) from kryptogenin diacetate and the methanol wash were evaporated and the residues were combined and hydrolyzed with 150 g. of sodium hydroxide in one liter of ethanol at steam-bath temperature for one-half hour. The hydrolysis mixture was extracted with ether (20 liters). The ethereal solution after washing with water was concentrated until crystallization started, cooled and filtered to give the crude pennogenin fraction (E), m. p. 236–240°; wt. 55 g. Recrystallization from acetone gave material melting 242–244°; wt. 28 g. This material was further purified by acetylation with 90 cc. of boiling acetic anhydride under reflux for thirty minutes. The acetylation mixture was cooled to room temperature slowly and filtered. The gelatinous precipitate was washed with a small volume of acetic anhydride and air dried overnight. After six crystallizations from acetone (material melting 1, 172–176°; 2, 176–180°; 3, 177–182°; 4, 189–191°; 5, 194–195°) pure pennogenin acetate was obtained, m. p. 198–200°; wt. 2 g. An additional 8 g. was obtained from the mother liquor of crystallizations 4, 5 and 6.

Anal. Calcd. for $C_{29}H_{44}O_5$: C, 73.7; H, 9.4. Found: C, 73.6; H, 9.6.

Hydrolysis of the acetate gave pennogenin which was crystallized from ether, m. p. 245–247°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.7; H, 10.0.

The mother liquors from crystallizations 1, 2 and 3 were combined and evaporated and the residue (F) was repeatedly crystallized from ethanol to give material melting 182–183°, wt. 7 g. The latter was purified further by hydrolysis and crystallization from ether to give pennogenin, m. p. 243–245°; wt. 6 g.

The mother liquors from the above 182–183° fraction were combined and evaporated and the residue (G) was crystallized from ether–pentane mixture to give material, m. p. 152–153°, wt. 6 g. Hydrolysis of this material followed by crystallization from ether gave nologenin as small plates, m. p. 265–267°. This substance is relatively insoluble in ether. A mixture with chlorogenin (274°) melted 248–250°.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.3; H, 9.9. Found: C, 72.3; H, 9.5.

It forms an insoluble digitonide in alcohol.

Nologenin diacetate was prepared and was crystallized from an ether–pentane mixture and then from ethanol at room temperature as needles, m. p., 179–180°.

Anal. Calcd. for $C_{31}H_{48}O_7$: C, 69.9; H, 9.1. Found: C, 70.0; H, 9.2.

The mother liquor (H) from the crystallization of the crude pennogenin fraction (236–240°) was further concentrated to give a second crop, m. p. 224–227°, 20 g., which after repeated crystallizations from acetone gave additional crude pennogenin, m. p. 234–235°, wt. 7 g., further purified as the acetate. After the removal of this second crop, the oily residue (I) was taken up in a small volume of ether and diluted with a larger volume of ligroin. The solution was allowed to evaporate slowly, whereby a third crop precipitated, wt. 20 g. A solution of the latter (J) in 50 cc. of acetic anhydride was refluxed for thirty minutes and then cooled to room temperature to give diosgenin acetate, m. p. and mixed m. p., 200–202°, wt. 3 g.

After removal of the third fraction (J), the solution was evaporated and the residue was hydrolyzed with alcoholic potash. The product was ether extracted, after treatment with Norite, was crystallized from acetone over a period of a month to give fesogenin, m. p. 180°.

Anal. Calcd. for $C_{27}H_{40}O_3$: C, 78.6; H, 9.8. Found: C, 78.3; H, 9.8.

The acetone soluble fraction (M) from the crystallization of the crude sapogenin fraction was evaporated and

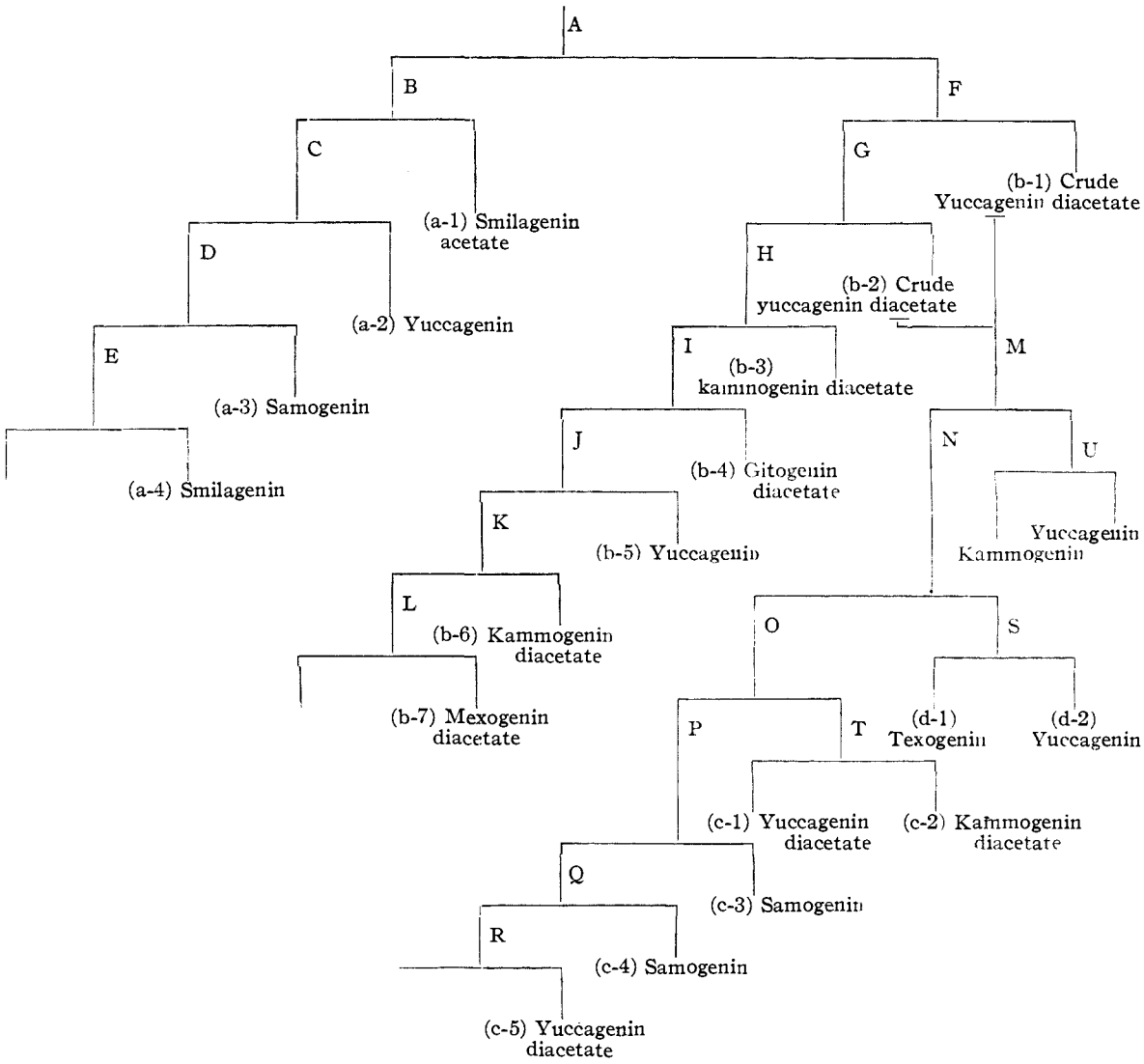


Fig. 7.—Crystallization of sapogenin fraction from the March collection (682 g.) of *Yucca Schottii* Engelm.

the oily residue was extracted with 15 liters of cold ligroin. The soluble fraction (P) after the removal of the ligroin was hydrolyzed and crystallized from methanol to give diosgenin, wt. 94 g. The cold ligroin-insoluble fraction (N) was then extracted with three 5-liter portions of hot ligroin. The hot ligroin-insoluble fraction was recrystallized from acetone to give kryptogenin, wt. 80 g. The first hot ligroin extract gave non-crystalline material, while the other two extracts (O) gave a total of 15 g. of diosgenin crystallized from acetone.

Isolation of Texogenin and Yuccagenin, New Sapogenins, from *Yucca Schottii* Engelm. (Fig. 7).—The caudex, 682 kg., from old plants was collected south of Sonoita, Arizona, in March, 1942. The sapogenin fraction was crystallized from 7 liters of ether to give an ether-insoluble product (F) which was thoroughly washed with cold ether; wt. 1360 g. This material was acetylated and crystallized from methanol (12 liters) to give crude yuccagenin diacetate (b-1), m. p. 168–175°, wt. 720 g. The latter was further purified as the pseudosapogenin as described below. From the mother liquor was obtained a second crop of yuccagenin diacetate (b-2) weighing 52 g. which was combined with the first crop (b-1). The total methanol mother liquors after removal of the crude yuccagenin diacetate were concentrated and cooled to give material which was recrystallized from ether and then acetone to give kammogenin diacetate (b-3), m. p. and mixed m. p., 257°; yield 74 g.

Anal. Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.5; H, 8.5.

Hydrolysis of this diacetate gave kammogenin which crystallized from ether, m. p. and mixed m. p., 242–244°.

The total mother liquors after removal of kammogenin diacetate (b-3) were evaporated and the residue was crystallized from acetic anhydride and then from acetone to give gitogenin diacetate (b-4), m. p. and mixed m. p., 242–243°; yield 16 g.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.0; H, 9.5.

Hydrolysis of this diacetate gave gitogenin which was crystallized from ether, m. p. and mixed m. p., 266–268°.

The total mother liquors after the removal of gitogenin diacetate (b-4) were evaporated and the residue was hydrolyzed with hot alcoholic potash. The hydrolysis mixture was diluted with water and the precipitated solids were extracted with ether (6 liters). Material which did

not dissolve was filtered, wt. 40 g., and crystallized from acetone to give yuccagenin (b-5), m. p., 241–243°; yield 30 g. This was converted to the diacetate, m. p. and mixed m. p., 178°. From the ether-soluble fraction from the hydrolysis mixture were obtained three crops of crystals, each melting 190–203°, which were combined, wt. 203 g. This material was acetylated and crystallized from methanol to give an insoluble fraction, m. p. 150–169°, 150 g., which was further crystallized from ether–pentane, methanol and ether to give kammogenin diacetate (b-6), m. p. and mixed m. p., 254–255°; yield 21 g.

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

The methanol filtrate from the 150–169° fraction was evaporated. The residue was hydrolyzed and crystallized from ether to give material melting 225–229°, wt. 12 g. This was acetylated and crystallized from pentane to give mexogenin diacetate (b-7), m. p. and mixed m. p. 206–208°, yield 10 g.

Anal. Calcd. for $C_{31}H_{46}O_7$: C, 70.2; H, 8.7. Found: C, 69.9; H, 8.7.

Hydrolysis of the diacetate gave mexogenin which was crystallized from ether, m. p. and mixed m. p., 245–246°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.6; H, 9.5. Found: C, 72.9; H, 9.6.

Mexogenin forms an insoluble digitonide in 70% alcohol and is saturated to bromine in acetic acid.

The solvent was removed from the ether soluble fraction (B) from the hydrolysis of the glycosides and the residue after one crystallization from methanol was acetylated. The product (a-1) was crystallized from methanol and then from acetone, m. p. and mixed m. p. with smilagenin acetate, 150–152°; yield 24 g.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.1; H, 9.9.

Hydrolysis of the acetate gave smilagenin which was crystallized from methanol, m. p. and mixed m. p., 183–184°.

The mother liquors from the crystallization of the smilagenin acetate were combined with the original mother liquor and the total was evaporated. The residue was hydrolyzed with excess alcoholic potash and the product was allowed to crystallize over a period of one month. The semi-solid material was washed with 8 liters of ether. The residue, m. p. 215–222°, 50 g., was crystallized from methanol to give yuccagenin (a-2), m. p. and mixed m. p., 240–243°; yield 32 g. Acetylation gave yuccagenin diacetate, m. p. and mixed m. p., 177–179°. The ether wash from this fraction (a-2) was concentrated to 2 liters and cooled to give a second fraction, m. p. 150–183°, wt. 80 g. Recrystallization of this material from ether gave samogenin (a-3), m. p. and mixed m. p., 205–206°; yield 31 g.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 75.1; H, 10.2.

Samogenin diacetate crystallized from methanol as long needles, m. p. and mixed m. p., 199–200°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.1; H, 9.5.

The ethereal mother liquor after the removal of the samogenin fraction was further concentrated to give a third fraction which was purified to give smilagenin (a-4), m. p. and mixed m. p., 184–186°; yield 72 g.

The crude yuccagenin diacetate (b-1; b-2) weighing 772 g. was heated in a sealed tube with 750 cc. of acetic anhydride at 200° for ten hours. The excess solvent was removed *in vacuo* and the product was crystallized from methanol to give a mixture of pseudoyuccagenin and pseudokammogenin triacetates, m. p., 142–144°; wt. 303 g. This material was further purified by reverting it to the sapogenins by alkaline and acid hydrolysis. Thus, a portion, 25 g., was hydrolyzed with a boiling solution of 15 g. potassium hydroxide in 400 cc. of ethanol for thirty minutes. The alkaline hydrolysis mixture was diluted with one liter of ethanol and then acidified with 30 cc. of concentrated hydrochloric acid. After filtering the precipitated potassium salt, the filtrate was refluxed one hour with an additional 50 cc. of acid, then concentrated *in vacuo* to one-third volume and diluted with water. The precipitated solid was filtered and dried, wt. 18 g. This material was acetylated and then allowed to stand with excess semicarbazide acetate in pyridine–alcohol mixture for one week at room temperature. The mixture was diluted with water and the precipitated solid was filtered and washed with water and dried. The non-ketone material consisting largely of yuccagenin diacetate was removed by digestion of the dried precipitate with ether. It was crystallized from methanol as needles, m. p. and mixed m. p. with yuccagenin diacetate, 176–178°; yield 9.8 g. The semicarbazone fraction, wt. 8 g., in 2 liters of ethanol was hydrolyzed with 200 cc. of dilute sulfuric acid (1:1) for forty-five minutes at reflux temperature. The mixture was ether extracted and the product was hydrolyzed with alcoholic potash and then crystallized from ether as needles, m. p. and mixed m. p. with kammogenin, 242°; yield 5.7 g. This material was further identified as kammogenin diacetate, m. p. and mixed m. p., 260–262°.

Another 25-g. portion (M) after alkaline and acid hydrolysis was acetylated and fractionally crystallized from acetone to give 1.9 g. of kammogenin diacetate and 2.8 g. of yuccagenin diacetate.

The mother liquor from this material (M) was given an alkaline hydrolysis followed by an acid hydrolysis, thus reconverting the pseudosapogenins to the sapogenins. These are separated into methanol-soluble (O) and methanol-insoluble (S) fractions.

The insoluble fraction (S), m. p. 208–210°, wt. 125 g., was recrystallized from acetone-ethyl acetate to give yuccagenin, (d-2), m. p. and mixed m. p., 241–243°, wt. 50 g.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.8. Found: C, 75.2; H, 9.7.

The mother liquor after the removal of yuccagenin was evaporated and the residue was crystallized from acetone to give texogenin (d-1), m. p. 172–175°; yield 31 g.

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

The acetate crystallized from methanol as needles, m. p., 172–173°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.8; H, 9.3.

The methanol-soluble fraction (O) was evaporated and the residue was washed with 5 liters of boiling ether. The ether-insoluble material was acetylated and fractionally crystallized from methanol to give kammogenin acetate (c-2), m. p. and mixed m. p., 255–256°, wt. 2 g. Hydrolysis of the latter gave kammogenin, m. p. and mixed m. p., 240–242°. A second fraction consisted of yuccagenin diacetate (c-1), m. p. and mixed m. p., 177–178°, 4 g., further identified as the free genin, m. p. and mixed m. p., 242–243°.

The ether wash (5 liters) from above was evaporated and the residue was crystallized from methanol and then acetone to give samogenin (c-3), m. p. and mixed m. p., 205–207°, 14 g., further identified as the acetate, m. p. and mixed m. p., 196–198°. The mother liquors after the removal of samogenin were evaporated and the residue was fractionally crystallized from acetone to give an additional 14 g. of samogenin (c-4) and 3 g. of yuccagenin purified as the acetate (c-5).

Yamogenin, A New Steroidal Sapogenin from the *Dioscoreas*.—Diosgenin has been found to be the principal sapogenin from the hydrolysis of the saponins from the *Dioscoreas*. This is especially true of the species growing in the United States. It has been found, however, that the sapogenin fraction from *Dioscorea testudinaria*, a species from Mexico, consists of a mixture of sapogenins difficult to separate directly by crystallization from the usual solvents. Subsequent studies on other *Dioscoreas* from Mexico eventually led to methods which have proved

very effective in perfecting the separation. These procedures are summarized in Figs. 8 and 9.

By the first procedure (Fig. 8), the crude sapogenin fraction (A) from acetone is acetylated with three times its weight of boiling acetic anhydride. Gradual cooling of the acetylation mixture (B) to room temperature causes the separation of almost pure diosgenin acetate. Concentration of the acetic anhydride mother liquor (C) followed by cooling at salt–ice temperature gives a second crop of crystals consisting of a mixture of kryptogenin diacetate and yamogenin acetate. The latter can be separated by crystallization from the usual solvents. It is very difficult to separate mixtures of diosgenin and yamogenin as such; however, crystallization of the acetates as indicated above permits separation.

The second procedure (Fig. 9) differs from the first in several ways. First, the crude sapogenin fraction (A) is crystallized from a relatively larger volume of acetone, thus placing most of the yamogenin in the acetone-soluble fraction (D). Yamogenin is isolated from the latter (D) as the acetate only after the total steroidal material from (D) has been treated with alkali. Such treatment converts kryptogenin to fesogenin, a more soluble compound, and simplifies the isolation of yamogenin acetate. Second, kryptogenin diacetate occurs as the major constituent of the soluble acetate fraction (C) and is readily separated. Diosgenin acetate is isolated in the same manner by both procedures.

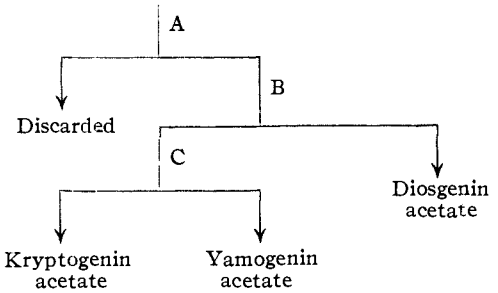


Fig. 8.—Crystallization of the sapogenin fraction from a *Dioscorea* (200 kg.), procedure No. 1.

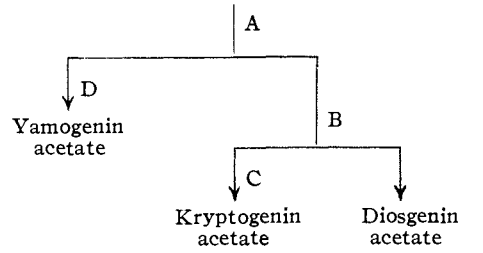


Fig. 9.—Crystallization of the sapogenin fraction from a *Dioscorea* (200 kg.), procedure No. 2.

A typical example is given of the processing of *Dioscorea bulbifera* L. The concentrate, 20 kg., from the alcoholic extraction of 225 kg. of undried roots was defatted with 20 liters of ether and then partially hydrolyzed by heating at 75° for forty-five minutes with 24 liters of 20% aqueous ethanol containing 4 liters of concentrated hydrochloric acid. The hydrolysis mixture was cooled to room temperature by the addition of ice and the precipitate was allowed to settle. The supernatant liquid was decanted or siphoned. The precipitate was either filtered and dried for treatment described below or further treated directly.

1. In the latter case, the suspension was dissolved in an equal volume of ethanol (8–12 liters) and treated with 200 cc. of concentrated hydrochloric acid per liter of alcoholic solution for two hours at the boiling point of the mixture. The hydrolysis mixture was cooled, decanted from the tars and extracted with 60 liters of ether. The ethereal solution was washed with 20 liters of water, 10 liters of 5% sodium hydroxide, and 20 liters of water, and then concentrated until a considerable amount of solid had crystallized. After standing at room temperature for several days in an unstoppered 22-liter flask, the crystalline material was filtered, washed with 300 cc. of cold ether, and then slurried with 2 liters of methanol at room temperature to give crude diosgenin; wt. 315 g. This was purified by acetylation and crystallization from acetic anhydride and then acetone–ethyl acetate mixture, m. p. and mixed m. p. with diosgenin acetate, 200–202°; yield, 210 g.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.2; H, 9.6.

2. In a second run, the precipitate from the partial hydrolysis mixture was filtered, washed with water and dried; yield, 1.3 kg. from 100 kg. of undried roots. This material was further hydrolyzed by treatment with 1.2 liters of concentrated hydrochloric acid in 5 liters of ethanol at steam-bath temperature for three hours. The sapogenin fraction was then extracted with ether in the usual manner.

For the separation of kryptogenin and yamogenin as well as diosgenin from the sapogenin fraction from *D. bulbifera* L., the ether extract was concentrated to one liter and allowed to stand overnight at room temperature, giving an ether-soluble fraction and an ether-insoluble fraction, wt. 46 g. The latter was acetylated with 140 cc. of boiling acetic anhydride for thirty minutes. The acetylation mixture was allowed to cool to room temperature. The crystals were filtered and washed with cold methanol to give crude diosgenin acetate, m. p. 180–188°; wt. 32 g. The acetic anhydride mother liquor combined with the residue from the evaporation of the methanol wash was concentrated to 70 cc. and then cooled in a salt–ice bath for one hour. The crystallized material was filtered and washed free of acetic anhydride with cold methanol, m. p. 130–138°; wt. 9 g. Recrystallization from acetone gave crude kryptogenin diacetate, m. p. 141–147°; wt. 7 g. For purification this material was crystallized from acetone to give a high melting intermediate fraction, m. p. 148–167°; wt. 2 g. The acetone mother liquor from its separation was further concentrated to give kryptogenin diacetate, m. p. and mixed m. p., 151–153°; wt. 5 g.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.3; H, 9.0. Found: C, 72.3; H, 8.8.

The solvent was removed from the ether-soluble fraction from above and the residue was crystallized from 1 liter of acetone to give an acetone-insoluble fraction, wt. 50 g. This was purified by acetylation with 150 cc. of acetic anhydride followed by crystallization at room temperature from the acetylation mixture. Crude diosgenin acetate, m. p., 178–183°, 34 g., was obtained. The acetic anhydride mother liquor was concentrated to one-half volume and cooled at salt–ice temperature for one hour to give material melting 129–135°; wt. 5 g. After four crystallizations from acetone, pure yamogenin acetate was obtained, m. p., 180–182°.

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

Hydrolysis and crystallization from ether gave yamogenin, m. p. 198–200°. A mixture with diosgenin melted ten degrees lower.

Isolation of the Saponin of Gitogenin from *Manfreda virginica* L.[121]—The undried roots and rhizomes weighing 25 kg. were ground in a food chopper and the mass was refluxed for four hours with 16 liters of 95% ethanol. The extract was filtered and the filter cake was washed well with alcohol. The alcohol was distilled *in vacuo* until considerable foaming occurred. The solid material which separated was filtered and dried; wt. 93 g. This material is the gitonide of gitogenin. A mixture of the crude saponide and 400 cc. of dry pyridine[122] was heated on the steam-bath for ten hours. To the resulting solution was

(121) Several new saponins were isolated in the course of the plant studies. Only two of these are described here.

(122) Schoenheimer and Dam, *J. Biol. Chem.*, 110, 461 (1935).

added 4 liters of ether. The saponin of gitogenin which precipitated was filtered, washed well with ether and dried; wt. 60 g. It was dissolved in ethanol and treated with Norite. Crystallization from ethanol gave material m. p. 275° dec.

The ether filtrate from the saponin precipitation was washed thoroughly with water, 10% hydrochloric acid and water. The ethereal solution was concentrated and the product was crystallized several times from methanol as white needles, m. p. and mixed m. p. with gitogenin, 267–268°; yield 2.5 g.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.9; H, 10.0.

A solution of 2.5 g. of the saponin in one liter of 95% ethanol, 200 cc. of water and 200 cc. of hydrochloric acid was refluxed for two hours. The product was extracted with ether and crystallized twice from methanol as fine white needles, m. p. and mixed m. p. with gitogenin, 265–266°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.9; H, 10.1.

The acetate was prepared with boiling acetic anhydride and crystallized from methanol, m. p. with gitogenin diacetate, 242–244°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 72.2; H, 9.5.

Since digitonin readily forms saponides with the steroidal sapogenins, it is not at all surprising to find that the glucoside of gitogenin forms a saponide with gitogenin. It is uncertain whether the gitogenin occurs free in the plant or whether its presence here is due to enzymatic hydrolysis of its glucoside. Gitogenin has been reported to occur free in *Digitalis* leaves.[16]

Isolation of the Saponin of Gitogenin from *Yucca filamentosa* L.—The rhizomes, 5 kg., from medium age plants were collected 10 miles west of Huntsville, South Carolina, on the road to Columbia, S. C., in August, 1941. The concentrate from the alcoholic extract was defatted by stirring with ether and decanting the ethereal layer from the gum. The process was repeated twice. The gum was then heated to remove the remaining ether. The residue was taken up in 6 liters of 90% alcohol and the hot solution was treated with 27 g. of cholesterol dissolved in 1 liter of 95% ethanol. The mixture was diluted with 200 cc. of water and allowed to cool. The precipitated solid was filtered, washed with 85% alcohol until colorless and dried, wt. 87 g. The saponide was decomposed with pyridine and the solution was filtered. The filtrate was diluted with ether and the precipitated solid was filtered, washed with ether and dried, wt. 67 g. The solid was crystallized several times from 85% alcohol and then from 95% alcohol to give a white solid, m. p. 263–265° dec.

Anal. Found: C, 56.34; H, 7.60.

A solution of 8 g. of this saponin in one liter of 95 ethanol was refluxed with 200 cc. of water and 200 cc. of concentrated hydrochloric acid for two hours. Water was added and the product was ether extracted. The ethereal solution was washed with water and the solvent removed. The residue was crystallized from methanol as white needles, m. p. and mixed m. p. with gitogenin, 265–268°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 74.9; H, 10.1.

The acetate was prepared and was crystallized from methanol as needles, m. p. and mixed m. p. with gitogenin diacetate, 240–242°.

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

This saponin is reasonably soluble in ethanol and may be used to precipitate sterols for isolation and purification in place of digitonin, which has long been used for this purpose. In the same way that digitonin is used to separate enantiomorphic sterols, its alcoholic solution gives a flocculent precipitate with solutions of diosgenin, smilagenin or cholesterol, but does not form an insoluble saponide with *epi*-smilagenin which has the alpha configuration at C-3. It also resembles digitonin in that its alcoholic solution forms a precipitate with phenol.

To 80% alcoholic solutions containing 10 mg. of cholesterol, diosgenin, smilagenin, phenol and *epi*-smilagenin were added 2-cc. portions of a 2% alcoholic solution of the saponin. The mixtures were allowed to stand overnight. In the first four cases an insoluble saponide formed, but *epi*-smilagenin gave no precipitate even after standing several weeks.

In a second run, the sapogenin fraction from 10 kg. of rhizomes was separated into acetone-soluble and acetone-insoluble fractions by leaching with one liter of acetone. The insoluble fractions were crystallized from methanol, m. p., 245–250°; wt. 25 g. Two more crystallizations from methanol gave pure gitogenin, m. p. and mixed m. p., 270–272°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.3. Found: C, 75.0; H, 10.4.

Gitogenin diacetate crystallized from methanol as needles, m. p. and mixed m. p., 241–243°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.1; H, 9.4. Found: C, 71.9; H, 9.6.

The acetone-soluble fraction was evaporated and the residue after an alkaline hydrolysis was treated with 15 g. of digitonin in ethanol to give 10.3 g. of digitonide. After decomposition with pyridine (30 cc.), the sapogenin fraction, 1.5 g., was isolated in the usual manner and crystallized twice from acetone to give impure gitogenin, m. p. 260–261°; a mixture with gitogenin (272°) melted 266–268°.

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Dr. D. L. Turner and Dr. J. M. Vandenbelt. We thank Parke, Davis and Company for their assistance.

Summary

1. Twelve new sapogenins and two new pro-sapogenins have been isolated. The original isolation procedure for each is described. Additional sources for these have been reported previously.[3]

2. Chemical interrelationships between these and the previously characterized sapogenins have been carried out. Structures for the new sapogenins and the pro-sapogenins have been proposed.

3. Neochlorogenin, β-neochlorogenin, 7-ketogitogenin, 7-ketoyuccagenin and 6-ketotigogenin have been prepared.

4. The positions of the hydroxyl groups in digitogenin have been further established at C-2, C-3 and C-15.

5. Diosgenin and tigogenin have been interrelated with kryptogenin, further illustrating the spiro-ketal nature of the sapogenin side-chain.

6. Bethogenin has been shown to be a derivative of kryptogenin.

7. A biogenetic relationship of the sapogenins has been proposed and correlated with the seasonal changes of the steroidal content of various plants.

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(123) Original manuscript received June 26, 1944.

NOTES

The *cis-trans* Isomers of 1,3-Dichloropropene

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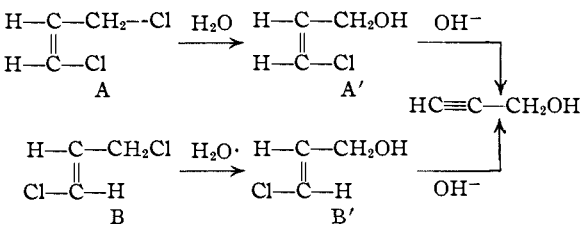
The terms *alpha* and *beta* were formerly used to designate, respectively, the low and high boiling isomers of 1,3-dichloropropene.[1] On the basis of comparative studies of the rates of hydrolysis of the isomeric dichlorides as catalyzed by the cuprous chloride–chloride ion complex and of the comparative rates of dehydrochlorination of the corresponding 3-chloro-2-propen-1-ols the *alpha* isomer has been assigned the *trans* and the *beta* isomer the *cis* configuration.[2] In surveying the evidence on which these assignments of structure are based we have noted that there is still doubt as to the correct geometric configurations of the two compounds.

For purposes of discussion the two dichlorides will be designated A and B and the corresponding alcohols as A′ and B′, as represented below.



The catalytic action of cuprous chloride in the hydrolysis of allyl chloride in dilute hydrochloric acid solution has been explained on the assumption that the reaction is facilitated by formation of an intermediate complex between the olefin and $CuCl_2^-$.[3] Since the rate of hydrolysis of *beta* 1,3-dichloropropene under these conditions is much more rapid than that of the *alpha* isomer, it has been assumed that formation of an olefin–$CuCl_2^-$ complex occurs more readily with the *beta* than with the *alpha* isomer.[2] Hatch and Roberts have made the further assumption that the factors influencing the formation of such a complex are similar to those involved in the mercuration of an olefin. Since *cis*-methyl cinnamate and *cis*-stilbene are mercurated faster than the corresponding *trans* isomers,[4] they have concluded that beta 1,3-dichloropropene is the *cis* isomer. Though Hatch and Roberts do not show structural formulas, it seems likely that the *cis* isomer to which they refer should be represented by formula A, in which the two hydrogen atoms attached to the ethylenic linkage are *cis* with respect to each other.

In a mercuration reaction of the type under consideration it has been shown that the mercurating agent, methoxymercuric acetate, adds to the double bond of the olefin.[5] On the other hand the hydrolysis of a dichloropropene is a substitution reaction. However the formation of a complex between dichloropropene and $CuCl_2^-$, resulting in an enhanced rate of hydrolysis of the unsaturated halide, might be explained on the assumption that the complex were formed by addition of the $CuCl_2^-$ to the double bond of the olefin. There is still no experimental evidence to indicate that the mechanism of formation of this complex would be similar to or different from that of the

(1) Hatch and Moore, THIS JOURNAL, **66**, 285 (1944).

(2) Hatch and Roberts, *ibid.*, **68**, 1196 (1946).

(3) Hatch and Estes, *ibid.*, **67**, 1730 (1945).

(4) Thomas and Wetmore, *ibid.*, **63**, 136 (1941), have assigned the *cis* configuration to the 2-butene isomer which mercurates faster.

(5) (a) Wright, *ibid.*, **57**, 1993 (1935); (b) Romeyn and Wright, *ibid.*, **69**, 697 (1947).