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

Sterols. CXIV. Sapogenins. XLIII. Oxidation Products from Tigogenin

BY RUSSELL E. MARKER, D. L. TURNER AND PAUL R. ULSHAFER

Reactions of sarsasapogenin studied in this Laboratory are not consistent with the assumptions of Fieser and his co-workers[1,2,3] based on the Tschesche–Hagedorn[10] formulation of the side-chain of the steroidal sapogenins. We have now carried out corresponding reactions with tigogenin. These confirm our earlier conclusions and give additional proof that the side chain is an inner ketal of a γ,δ'-dihydroxy ketone as in I.[4]

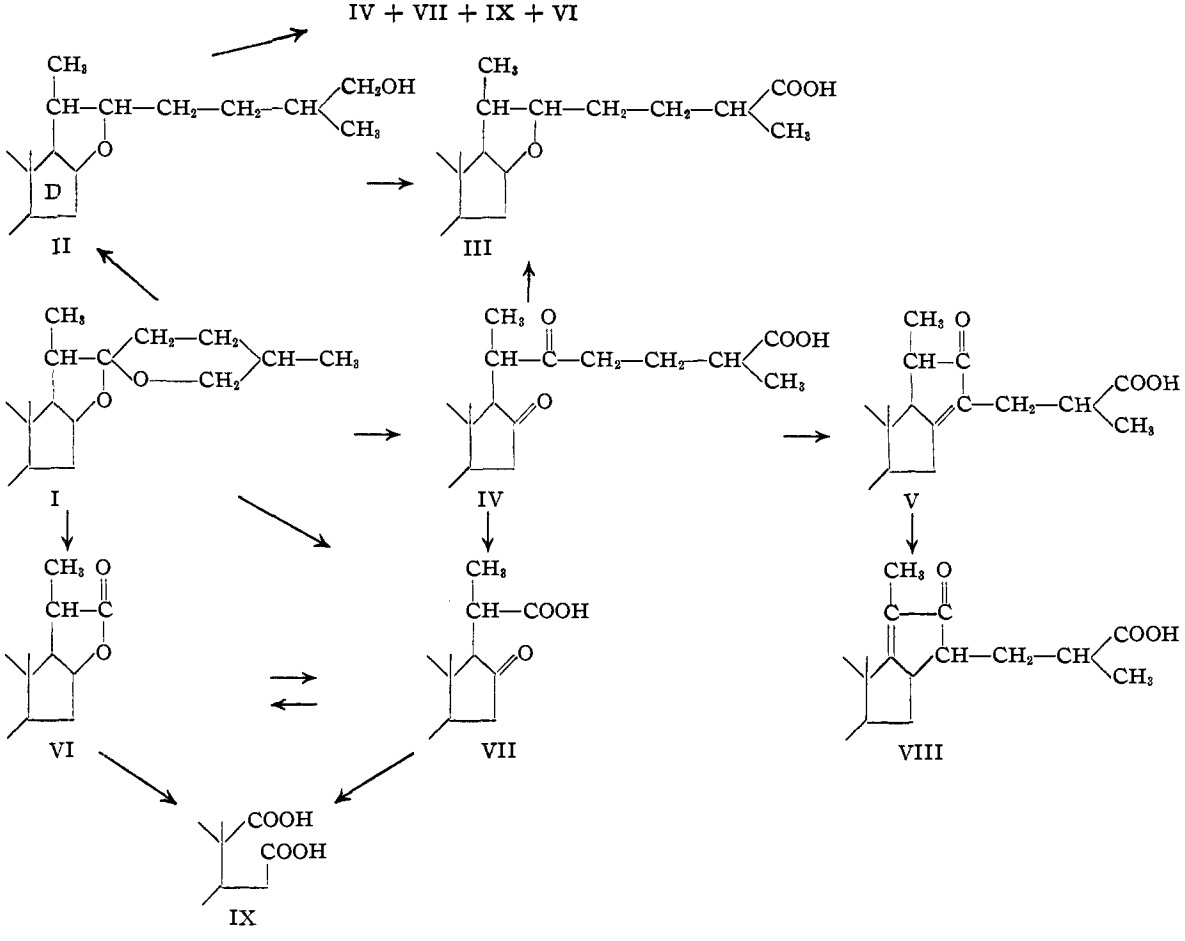
Oxidation of the sapogenins with chromic acid between 55 and 100° gives mixtures of the sapogenoic acid (III), the 16-keto acid (VII), the genin lactone (VI) and *etio*-bilianic acid (IX)[2,3,5,6] in addition to materials as yet not identified.

The formula of the lactone (VI) has been well established. Tschesche and Hagedorn[7] converted desoxytigogenin lactone to *etio-allo*-bilianic acid (IX). Similar transformations have been accomplished with desoxysarsasapogenin lactone,[8] and sarsasapogenin lactone.[9] Oxidation of sarsasapogenin lactone acetate at 90° gave 3-hydroxy-16-keto-*bis-nor*-cholanic acid[9] (VII). Oxidation at lower temperature of the unacetylated lactone gave the lactone-2,3(or 3,4) dibasic acid.[9] The latter was also made by the oxidation of sarsasapogenone with nitric acid.[9] In the case of tigogenin lactone which is more resistant to oxidation, chromic acid at 100° has been found to give the lactone 2,3-dicarboxylic acid,[10] a product obtained by Windaus[11] from "gitogenic acid" by nitric acid oxidation. We have now made the same acid by oxidizing gitogenin lactone with chromic acid at room temperature. Oxidation of tigogenone with nitric acid also gave a lactone dicarboxylic acid identical with that from tigogenin lactone or from gitogenin lactone. The lactone dibasic acid of Windaus melted at 238° and contained 0.5 mole of water. Our product melted at 244–245° and was free of water of crystallization.

The structure for the sapogenoic acids proposed by Tschesche and Hagedorn[12] was rendered untenable by the observations of Fieser and Jacobsen[2] and of Marker and Rohrmann.[13,14] Fieser and Jacobsen found that sarsasapogenoic acid (IV) gave a dioxime and that on treatment with alkali it formed anhydrosarsapogenoic acid (VIII) an alpha unsaturated ketone. They also reduced sarsasapogenoic acid to anhydrotetrahydro sarsasapogenoic acid (III). The assumption of the 1,4-diketone structure for the sapogenoic acids (IV) proposed by Marker and Rohrmann[13] explains these reactions readily. The condensations of sarsasapogenoic acid to form the anhydro acid and of tigogenoic acid (IV) to anhydrotigogenoic acid (VIII) as completed in this paper are analogous to the mesityl oxide type of reaction observed under similar conditions with beta-acetylpropiophenone[15,16] and with octanedione-3,6.[17] These reactions involve loss of the elements of water between one carbonyl group and the methyl or methylene group adjacent to the other with the formation of substituted cyclopentenones in agreement with the formulation of anhydrosapogenoic acid as V. It seems probable that V rearranges to VIII. Such a rearrangement accounts for the ability of the permanganate oxidation product of anhydrosarsasapogenoic acid to undergo the haloform reaction.[3] An intermediate substance (V) formed by the action of alkali on sarsasapogenoic acid has also been described by Marker and Rohrmann.[13] This type of rearrangement of alpha unsaturated ketones is similar to one observed by Ruzicka[18] and by Marker[19] in which 2-bromo-3-ketones gave 4-dehydro-ketones. It has now been found that tigogenoic acid (IV) also undergoes this condensation and forms anhydrotigogenoic acid (VIII).

(1) Fieser, "Chemistry of Natural Products Related to Phenanthrene," 2nd edition, 1937.
(2) Fieser and Jacobsen, *ibid.*, **60**, 28 (1938).
(3) Fieser and Jacobsen, *ibid.*, **60**, 2753 (1938).
(4) Marker and Rohrmann, *ibid.*, **61**, 846 (1939).
(5) Marker and Rohrmann, *ibid.*, **61**, 1285 (1939).
(6) Marker and Rohrmann, *ibid.*, **61**, 2722 (1939).
(7) Tschesche and Hagedorn, *Ber.*, **68**, 1412 (1935).
(8) Farmer and Kon, *J. Chem. Soc.*, 414 (1937).
(9) Marker and Rohrmann, THIS JOURNAL, **62**, 76 (1940).
(10) Tschesche and Hagedorn, *Ber.*, **69**, 797 (1936).
(11) Windaus and Linsert, *Z. physiol. Chem.*, **147**, 275 (1925).
(12) Tschesche and Hagedorn, *Ber.*, **68**, 2247 (1935).
(13) Marker and Rohrmann, THIS JOURNAL, **61**, 2072 (1939).
(14) Marker and Rohrmann, *ibid.*, **61**, 3477 (1939).
(15) Borsche and Fels, *Ber.*, **39**, 1922 (1906).
(16) Borsche and Menz, *ibid.*, **41**, 190 (1908).
(17) Blaise, *Compt. rend.*, **158**, 708 (1914).
(18) Ruzicka, Plattner and Aeschbacher, *Helv. Chim. Acta*, **21**, 866 (1938).
(19) Marker, Wittle and Flambeck, THIS JOURNAL, **61**, 1333 (1939).

CHART A.—Side chains of sapogenin oxidation products according to Marker and co-workers.[4,5,6,9,13,14]



We have prepared a dioxime of tigogenoic acid under conditions similar to those used by Fieser for the preparation of sarsasapogenoic acid dioxime. This supports the diketone structure of the sapogenoic acids.

The evidence of reduction is even more significant for the structure of the sapogenoic acids. Acetonylacetone on reduction gives 2,5-dimethyltetrahydrofuran.[20] If the 1,4-diketone structure is correct for tigogenoic and sarsasapogenoic acids then these should also give tetrahydrofurans. This was found to be true of sarsasapogenoic acid[13] and the proof has now been extended to tigogenoic acid (IV). Tschesche and Hagedorn[12] were unable to effect the catalytic hydrogenation of tigogenoic acid, but this has now been accomplished with Adams catalyst using acetic acid as the solvent. The product was tetrahydroanhydrotigogenoic acid (III) identical with an acid obtained by the oxidation of the mono acetate of dihydrotigogenin (II). This oxidation was conducted at room temperature. Any alteration in the tetrahydrofuran ring of dihydrotigogenin is highly improbable under these conditions.

If the formulation of Fieser were correct, it should be possible to oxidize sapogenoic acids to the C-22 lactones. The chromic acid oxidation of dihydrosapogenins (II) like that of the sapogenins themselves gave a mixture of lactone (VI), sapogenoic acid (IV), 16-keto acid (VIII) and *etio*-bilianic acid (IX).[14] The permanganate oxidation of sapogenins gave only lactone, 16-keto acid and sapogenoic acid.[21] The 16-keto acid was recovered unchanged when oxidation with permanganate was attempted. Similarly the lactone could not be oxidized with permanganate. However, no lactone was obtained by the oxidation of sapogenoic acids; chromic acid gave the 16-keto acid and *etio*-bilianic acid. Calcium permanganate was without effect. Oxidation with nitric

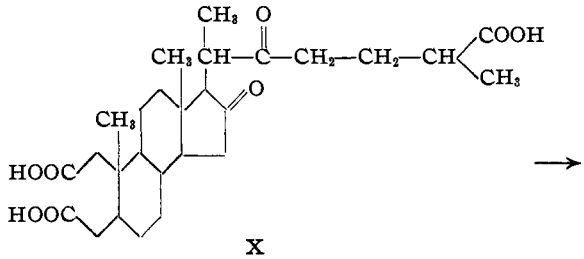
(20) Sabatier and Mailhe, *Ann. chim.*, [8] **16**, 70 (1909).

(21) Marker and Rohrmann, THIS JOURNAL, **62**, 222 (1940).

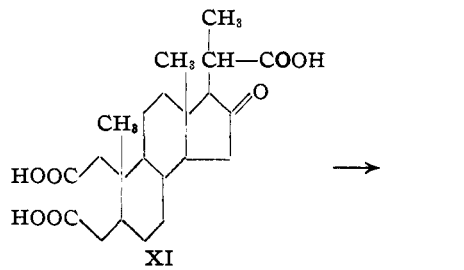
acid gave the 16-keto acid. *It has not been possible under any conditions to get the lactone from the oxidation of sapogenoic acids.*

These observations have been confirmed in the study of the oxidation of tigogenoic acid acetate. As with sarsasapogenoic acid there was no trace of lactone in the reaction products, but 3-hydroxy-*etio-allo*-bilianic acid was readily isolated.

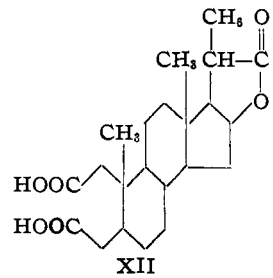
The gitogenoic diacid (X) obtained by Windaus and Schneckenburger[22] by the oxidation of gitogenin with chromic acid has been prepared from tigogenin, 3-dehydrotigogenin lactone being formed as a by-product. The acid has the same structure of the side-chain as tigogenoic acid (IV). Windaus and Linsert[11] have oxidized this acid with nitric acid and obtained an acid to which they gave the formula $C_{21}H_{30}O_7$. Fieser has suggested a structure for this tribasic acid on page 332 of his book.[1] We have now disproved this structure and established the acid as 16-keto-*bis-nor-allo-iso*-lithobilianic acid (XI),[22] analogous to the 16-keto acid from sarsasapogenoic acid except for the opening of ring A. The proof of this structure was obtained by catalytic reduction. Reduction of 16-keto-*bis-nor*-cholanic acids gives characteristic sapogenin lactones. Thus Marker and Rohrmann[5] obtained sarsasapogenin lactone from the 16-keto-acid of sarsasapogenin. We now find the product from the reduction of the acid of Windaus and Linsert (XI) to be identical with the acid lactone (XII) obtained from both gitogenin lactone and tigogenin lactone.



X



XI



XII

Finally the observations of Marker and Rohrmann[14] on the oxidation of the diacetate of dihydrosarsasapogenin have been extended by a study of the oxidation of the diacetate of dihydrotigogenin (II) with chromic acid. The products obtained were tigogenin lactone, 3-dehydrotigogenoic acid and 3-hydroxy-*etio-allo*-bilianic acid. The 3-hydroxy-*etio-allo*-bilianic acid had not been obtained previously. It was oxidized to 3-keto-*etio-allo*-bilianic acid which gave no depression in melting point when mixed with the 3-keto-*etio-allo*-bilianic acid from *allo*-pregnanetriol-3,16,20.[23] The anhydrides of the bilianic acids from dihydrotigogenin and *allo*-pregnanetriol were also identical.

We wish to thank Parke, Davis and Company for their generous help.

Experimental Part

The tigogenin lactone and tigogenoic acid were prepared according to Tschesche and Hagedorn.[7]

Oxidation of Gitogenin Lactone.—To a solution of 100 mg. of gitogenin lactone in 20 cc. of acetic acid was added a solution of 200 mg. of chromic anhydride in 10 cc. of 90% acetic acid. After standing one hour at 25° the solution was diluted with water, the precipitated product was filtered and crystallized from dilute acetic acid, m. p. 244–245°.

Anal. Calcd. for $C_{22}H_{32}O_6$: C, 67.3; H, 8.2. Found: C, 67.3; H, 8.2.

Oxidation of Tigogenone with Nitric Acid.—To a suspension of 1 g. of tigogenone in 10 cc. of glacial acetic acid was added 15 cc. of nitric acid (d. 1.50). Brown fumes were liberated and the solid soon dissolved. The mixture was heated at 90° for one hour, diluted with water and the precipitated solid was filtered. It was dissolved in ether and shaken with 5% sodium hydroxide solution. The alkaline layer was acidified with hydrochloric acid and extracted with ether. The product was crystallized from dilute acetic acid, m. p. 242–244°. It gave no depression in melting point when mixed with tigogenin-2,3-diacid lactone (XII) prepared by the oxidation of gitogenin lactone and of tigogenin lactone with chromic acid.

Anal. Calcd. for $C_{22}H_{32}O_6$: C, 67.3; H, 8.2. Found: C, 67.1; H, 8.2.

Gitogenoic-2,3 Diacid.—To a solution of 20 g. of tigogenin in 1 liter of acetic acid at 90–95° was added a solution of 44 g. of chromic anhydride in 50 cc. of water and 100 cc.

(22) Windaus and Schneckenburger, *Ber.*, **46**, 2628 (1913).

(23) Marker and Wittle, THIS JOURNAL, **61**, 855 (1939).

of acetic acid over a period of thirty minutes with stirring. It was heated for an additional ninety minutes, cooled and methanol added. The solution was concentrated to about 200 cc., water and ether were added and the ethereal layer was shaken with water. The acid was removed with sodium hydroxide solution. The aqueous layer was acidified and extracted with ether. Upon evaporation of the ether, crystalline material separated, m. p. 216–219°. It was crystallized from ether and from dilute acetic acid. It gave no depression in melting point when mixed with a sample prepared by the chromic acid oxidation of gitogenic acid.

Anal. Calcd. for $C_{27}H_{40}O_5 \cdot {}^1/_2H_2O$: C, 64.5; H, 8.2. Found: C, 64.4, 64.2; H, 8.2, 8.4.

The neutral fraction gave a product melting at 254° which gave no depression when mixed with 3-dehydrotigogenin lactone.

Oxidation of Gitogenoic-2,3-diacid with Nitric Acid.—To one gram of the acid was added 10 cc. of fuming nitric acid. It was allowed to stand at room temperature for three hours, water was added and the product was extracted with ether. The ethereal solution was washed well with water. Upon evaporation of the ether, the product crystallized. It was filtered and recrystallized from ether and from acetic acid. This was 16-keto-*bis-nor-allo-iso*-lithobilianic acid (XI) obtained by Windaus and Linsert[11] but assigned an incorrect structure by them, m. p. 295–298° dec.

Anal. Calcd. for $C_{22}H_{32}O_7$: C, 64.6; H, 7.9. Found: C, 64.2; H, 7.7.

A mixture of 200 mg. of the above acid, 400 mg. of Adams catalyst, 50 cc. of absolute ethanol and 50 cc. of ether was shaken with hydrogen at 45 pounds pressure for fifteen hours. The mixture was filtered, the filtrate evaporated and the residue was crystallized from dilute acetic acid, m. p. 244°. It gave no depression in melting point when mixed with the 2,3-diacid lactone of gitogenin (XII).

Anal. Calcd. for $C_{22}H_{32}O_6$: C, 67.3; H, 8.2. Found: C, 66.9; H, 8.3.

Oxidation of the Diacetate of Dihydrotigogenin.—A mixture of 12 g. of dihydrotigogenin and 50 cc. of acetic anhydride was refluxed for thirty minutes. The excess acetic anhydride was evaporated *in vacuo* and the residue was dissolved in 200 cc. of acetic acid. The well stirred solution was heated at 90–95° on a steam-bath while 24 g. of chromic anhydride in 125 cc. of 80 per cent. acetic acid was added over a period of two hours. Heating was continued for two more hours. The solution was cooled, 20 cc. of methanol was added and the solvent was removed *in vacuo*. The residue was extracted with water and ether. The ethereal solution after washing with water was shaken with 3 per cent. sodium hydroxide solution to remove the acidic material.

The ethereal solution containing the neutral fraction was evaporated to a sirup, which was refluxed with a 1% alcoholic potassium hydroxide solution. The solution was diluted with water and the mixture was shaken with ether until the solid dissolved. The alkaline layer was washed several times with ether and then was separated and acidified with hydrochloric acid. The mixture was extracted with ether. The ether was evaporated leaving a crystalline residue which was crystallized from ether–pentane and from ethanol: yield 680 mg., m. p. 231–233°. It gave no depression in melting point when mixed with an authentic sample of tigogenin lactone.

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.3; H, 9.9. Found: C, 76.4; H, 9.8.

The ether layer from the above alkali removal of the lactone was evaporated and the residue dissolved in 20 cc. of acetic acid at room temperature. A solution of 1.5 g. of chromic anhydride in 20 cc. of acetic acid was added slowly. After thirty minutes water was added and the product was taken up in ether. The ethereal solution was washed well with water and with dilute sodium hydroxide. The alkaline layer was acidified and extracted with ether. The solvent was removed and the residue was crystallized from dilute methanol, m. p. 184–185°. When mixed with 3-dehydrotigogenoic acid prepared by the mild oxidation of tigogenoic acid there was no depression in melting point.

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

The alkaline washings containing the acid material from the oxidation were heated on the steam-bath for one hour to complete hydrolysis. The solution was cooled and acidified with hydrochloric acid. The acids were dissolved in ether–pentane and the solvent evaporated to 25 cc. Upon standing in a refrigerator the product crystallized. It was recrystallized from ether, yield 1.1 g., m. p. 244–247° dec. This was 3-hydroxy-*etio-allo*-bilianic acid.

Anal. Calcd. for $C_{19}H_{30}O_5$: C, 67.4; H, 8.9. Found: C, 67.6; H, 9.2.

This acid was oxidized with chromic anhydride in acetic acid at room temperature for thirty minutes. The resulting product was crystallized from ether–pentane and from acetone, m. p. 256–258° dec. It gave no depression in melting point when mixed with 3-keto-*etio-allo*-bilianic acid prepared by the oxidation of *allo*-pregnanetriol-3,16-20.[23]

Upon refluxing the keto compound with acetic anhydride for one hour, it formed an anhydride which was crystallized from ethanol, m. p. 220–223°. It gave no depression in melting point when mixed with 3-keto-*etio-allo*-bilianic anhydride prepared from an authentic sample.

Tigogenoic Acid Dioxime.—A mixture of tigogenoic acid (1 g.), potassium acetate (1 g.), hydroxylamine hydrochloride (0.66 g.) and methanol (60 cc.) was heated to 130° for three hours in a bomb tube. Two-thirds of the solvent was removed and the mixture was poured into water and filtered, yield 820 mg. It was recrystallized from methanol. It turned brown on heating at 230° and decomposed to a black tar with evolution of gas at 250°.

Anal. Calcd. for $C_{27}H_{44}O_5N_2$: N, 5.75. Found: N, 5.76.

Anhydrotigogenoic Acid.—To a solution of 1 g. of tigogenoic acid in 50 cc. of alcohol was added 2 g. of potassium hydroxide in 10 cc. of water. It was refluxed for two hours, cooled, water was added and the acid was precipitated by the slow addition of dilute hydrochloric acid. This was filtered, dried and crystallized from acetone, in which it is quite insoluble, m. p. 256–258°.

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

Anhydrotetrahydrotigogenoic Acid.—(a) To a solution of 300 mg. of tigogenoic acid dissolved in 150 cc. of acetic acid was added 300 mg. of Adams catalyst and the mixture was shaken under hydrogen at 45 pounds pressure for ten hours. The solution was filtered and the solvent was removed *in vacuo*. The residue was crystallized from acetone, m. p. 203–205°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.2. Found: C, 75.0; H, 10.1.

(b) To a solution of 12 g. of the monoacetate of dihydrotigogenin in 200 cc. of acetic acid at 30° was added a solution of 4 g. of chromic anhydride in 50 cc. of 90% acetic acid. After standing one hour, the solution was diluted with water and extracted with ether. The ether was removed and the residue was hydrolyzed with alcoholic potassium hydroxide and crystallized from acetone, m. p. 203–305°. When mixed with the above sample it gave no depression in melting point.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 75.0; H, 10.2. Found: C, 74.8; H, 10.0.

Oxidation of Tigogenoic Acid.—A solution of 3 g. of chromic anhydride in 15 cc. of 80% acetic acid was added dropwise with stirring over a period of one hour to a solution of 1.5 g. of tigogenoic acid acetate in 30 cc. of glacial acetic acid at 85°. The stirring and heating was continued for an additional two hours. Methanol was added and the solvent was removed *in vacuo*. The residue was extracted with water and ether. The ethereal extract was washed well with water and a 3% potassium hydroxide solution. Upon evaporation of the ether there was no residue, thus showing the absence of lactonic material.

The alkaline extract was heated on a steam-bath for thirty minutes, cooled, acidified with hydrochloric acid and extracted with ether. The ether was evaporated to 10 cc. and allowed to stand in a refrigerator for several days. Only a small amount of crystalline material was obtained. This melted at 244–247° and gave no depression in melting point when mixed with 3-hydroxy-*etio-allo*-bilianic acid.

Anal. Calcd. for $C_{19}H_{30}O_5$: C, 67.4; H, 8.9. Found: C, 67.1; H, 9.1.

Summary

Various oxidation products have been obtained from tigogenin and their structures have been discussed. Further support is given to the ketal structure of the steroidal sapogenin side chain.

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Sterols. CXV. Sapogenins. XLIV. The Relation between Diosgenin and Cholesterol

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The assumption that the carbon skeleton of the side-chain in the steroidal sapogenins is identical with that of cholesterol has been based on the isolation of α-methylglutaric acid from the oxidation products of digitogenic acid[1] and the occurrence of a substance thought to be methyl isohexyl ketone among the products obtained by treating sarsasapogenin with selenium.[2] However, the α-methylglutaric acid might come from the nucleus rather than from the side-chain[3] and the substance of Ruzicka and Van Veen is probably not methyl isohexyl ketone.[4] By converting diosgenin to cholesterol we have now obtained conclusive proof that the steroidal sapogenins have the same carbon skeleton as cholesterol, and that they have 27 carbon atoms. This conversion also provides additional evidence for the 5,6 position of the double bond in diosgenin.

(1) Windaus and Willerding, *Z. physiol. Chem.*, **143**, 33 (1925).
(2) Ruzicka and Van Veen, *ibid.*, **184**, 69 (1939).
(3) *Cf.* Fieser, "Chemistry of Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1937, p. 326.
(4) Jacobs and Simpson, *J. Biol. Chem.*, **105**, 501 (1934).

Diosgenin was reduced by the method of Marker and Rohrmann[5] to tetrahydrodiosgenin (I) a substance similar to tetrahydrosarsasapogenin to which the structure of 3,16,27-trihydroxycoprostane was assigned.[5] Catalytic reduction of the double bond in tetrahydrodiosgenin gave tetrahydrotigogenin which is 3,16,27-trihydroxycholestane.

The hydroxyl groups in tetrahydrodiosgenin were replaced by bromine atoms using phosphorus tribromide. The product was treated with less than an equimolar quantity of potassium acetate and then reduced with sodium in propyl alcohol. Two substances were separated from the reduction mixture by distillation at reduced pressure. These were identified as Δ^5-cholestene and cholesterol. The Δ^5-cholestene was reduced catalytically with hydrogen to cholestane.

Tetrahydrodiosgenin forms a triacetate. The oxidation of this with selenious acid followed by hydrolysis gives a substance similar to the Δ^5-3,4-

(5) Marker and Rohrmann, THIS JOURNAL, **61**, 846 (1939).