When refluxed with acetic anhydride it gave sarsasapogenin acetate; m. p. and mixed m. p. 126–128°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.8; H, 10.2.

The filtrate from the digitonide was evaporated to about 50 cc. Ether was added and the digitonin was filtered off. The filtrate was washed well with water and the solvent removed. The residue was crystallized from methanol; m. p. and mixed m. p. with *epi*-sarsasapogenin, 205–209°. When refluxed with acetic anhydride it gave a product which was crystallized from methanol; m. p. and mixed m. p. with *epi*-sarsasapogenin acetate, 190–195°.

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

Tigogenone.—The dog was fed a meat diet containing 7 g. of tigogenone. The feces were extracted and worked up as described above. The digitonin precipitable fraction gave a product which was crystallized from methanol; m. p. and mixed m. p. with tigogenin, 200–202°. This gave tigogenin acetate; m. p. and mixed m. p. with tigogenin acetate, 197–199°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.2; H, 10.3.

The fraction not precipitated by digitonin was crystallized from acetone; m. p. and mixed m. p. with an authentic sample of *epi*-tigogenin, 242–244°. This product gave *epi*-tigogenin acetate; m. p. and mixed m. p., 199–201°.

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

Summary

1. Diosgenin has been biologically converted to smilagenin and *epi*-smilagenin.

2. Similarly tigogenone and sarsasapogenone have been converted to the carbinols of both the alpha and beta configurations.

3. The significance of these facts has been discussed.

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

Sterols. CXLVIII. Sapogenins. LXII. The Structure of the Side Chain in the Dihydro-pseudosapogenins[1]

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

When dihydro-pseudosarsasapogenin (I) was oxidized with chromic anhydride at 15–18°, two products were obtained,[2] a C-27 keto acid (II) and 16-pregnenedione-3,20 (V). The latter arises from an acid oxidation intermediate which is hydrolyzed on extraction from ethereal solution with alkali.[3] In the same manner the oxidation of dihydropseudotigogenin gives an isomeric C-27 keto acid (VIII) together with 16-*allo*-pregnenedione-3,20 (VII).

On oxidation with chromic acid the two keto-acids, like the corresponding dihydropseudosapogenins, are converted to 16-pregnenedione-3,20 (V) and 16-*allo*-pregnenedione-3,30 (VII), respectively. This suggested that the formation of the keto acids involves only the oxidation of the two hydroxyl groups at C-3 and C-27. The presence of a single carbonyl group in each acid was indicated by the analyses of the oximes and semicarbazones.[4]

Clemmensen reduction of the acid (VIII) from dihydro-pseudotigogenin removed only one oxygen to give the 3-desoxy acid (IX). Catalytic reduction of both keto acids in neutral solution gave the corresponding 3-hydroxy acids (IV), (VI), (X). In the case of the acid from dihydropseudosarsasapogenin, a mixture of the epimeric carbinols resulted, the 3(α)-carbinol being present in greater quantity. The acid of the *allo* series gave the 3(β) carbinol. Bouveault reduction of the methyl ester of the acid (II) from dihydropseudosarsasapogenin gave *epi*-dihydro-pseudosarsasapogenin (III), identified as its *bis*-*p*-nitrobenzoate.[5]

These results establish definitely that the two keto acids are mono-ketones. The reactions can best be represented as in the accompanying chart.

The various reactions of the pseudosapogenins which have been reported[2,3,6,7] from this Laboratory are all consistent with the dihydrofuran formulation of the side-chain in these substances (XI).

(1) Original manuscript received June 25, 1941.

(2) Marker and Rohrmann, THIS JOURNAL, **62**, 521 (1940).

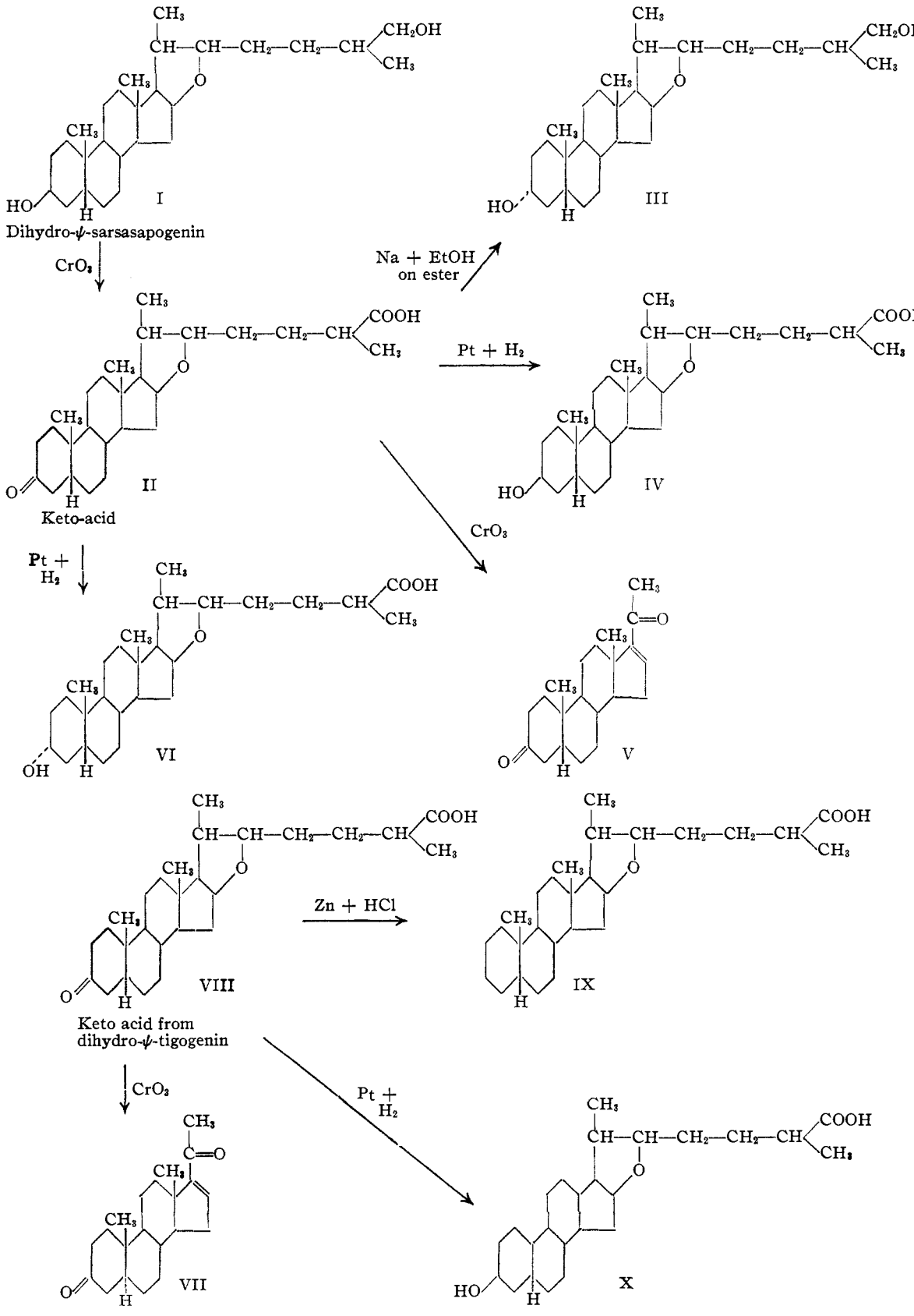
(3) (a) Marker, *et al.*, *ibid.*, **63**, 774 (1941); (b) Marker *et al.*, *ibid.*, **63**, 779 (1941).

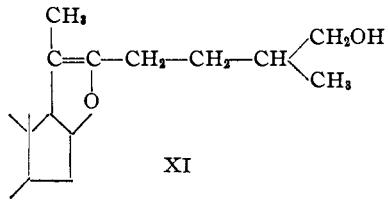
(4) We are unable to duplicate the preparation of the *bis*-semicarbazone previously reported.[2] The analytical data as previously given for the acid and ester are correct for the mono-keto compound.

(5) Marker, Rohrmann and Jones, THIS JOURNAL, **62**, 648 (1940).

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

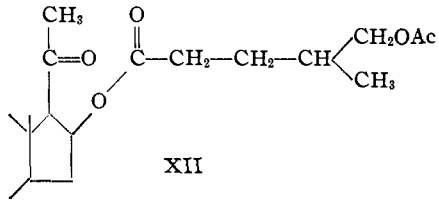
(7) Marker, Jones and Kreuger, *ibid.*, **62**, 2532 (1940).


I
Dihydro-ψ-sarsasapogenin
III
CrO3
Na + EtOH
on ester
II
Keto-acid
Pt + H2
IV
CrO3
Pt +
H2
VI
V
VIII
Keto acid from
dihydro-ψ-tigogenin
Zn + HCl
IX
CrO3
Pt +
H2
VII
X



XI

The structure of the side-chain in the oxidation products has been established as (XII).[2,3] Con-



XII

sequently the oxide bridge must be assumed to be present in dihydro-pseudosapogenins as well as in the pseudosapogenins. That the third oxygen atom is of oxide function is also indicated by the formation of diacetates from the dihydro-pseudosapogenins and by the properties of the C_{27} keto acids described in the present paper. This leads to a structure for the side-chain of dihydro-pseudosapogenins (I) which is identical with one previously assigned to the dihydrosapogenins.[8,9] The dihydrosapogenins can be oxidized to monoketo acids, anhydrotetrahydro-sarsasapogenoic acid[9] and anhydrotetrahydrotigogenoic acid,[10,11] which were assigned constitutions identical with these of the acids described in the present paper. Oxidation of the diacetates of the dihydrosapogenins at higher temperatures gave the same products which were obtained from the sapogenins: the C_{22} lactone, the sapogenoic acid, the 16-keto acid, etc.,[9,10] in extreme contrast to the behavior of the dihydro-pseudosapogenins on oxidation. This may be due to a difference of configuration at C-20 and C-22. Sterols differing only stereochemically are known to give different products of oxidation in other cases.[12]

We thank Parke, Davis and Company for their assistance.

Experimental Part

Oxidation of Dihydropseudotigogenin.—To a solution of 8 g. of dihydropseudotigogenin in 400 cc. of acetic acid at 15° was added a solution of 6.4 g. of chromic anhydride in 50 cc. of 60% acetic acid. The temperature was maintained at 10–15° for thirty minutes. The product was poured into water and then extracted with ether. The ethereal solution was washed free of acetic acid with water. The acid was then removed with 10% sodium hydroxide solution. The alkaline layer was allowed to stand for thirty minutes and then reëxtracted with ether. The C_{27} keto acid separated in the alkaline layer as an insoluble sodium salt. This was filtered and decomposed with dilute hydrochloric acid. It was taken up in ether and crystallized after evaporation to a small volume; yield about 0.8 g. It was recrystallized from ethyl acetate, m. p. 207–209°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.3; H, 9.6. Found: C, 75.2; H, 9.7.

The neutral fraction from the above oxidation gave material which was crystallized from acetone, m. p. 211–213°. When mixed with 16-*allo*-pregnene-3,20-dione (m. p. 210–212°) there was no depression in melting point.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 80.1; H, 9.7.

Semicarbazone of the Above *allo*-Keto-acid (VIII).—This was prepared in the usual manner by boiling for two hours in aqueous ethanol with semicarbazide hydrochloride and potassium acetate; recrystallized from ethanol, m. p. 210–213° dec.

Anal. Calcd. for $C_{28}H_{45}O_4N_3$ (mono-semicarbazone): C, 68.9; H, 9.3. Calcd. for $C_{29}H_{46}O_4N_6$ (bis-semicarbazone): C, 64.2; H, 8.5. Found: C, 68.9; H, 9.2.

Oxime of *allo*-Keto-acid (VIII).—This was prepared by a method similar to that used for the semicarbazone and recrystallized from methanol as colorless needles which decomposed at 232–234°.

Anal. Calcd. for $C_{27}H_{43}O_4N$: C, 72.8; H, 9.7. Found: C, 72.8; H, 9.7.

Methyl Ester of *allo*-Keto-acid (VIII).—This was prepared in ether with diazomethane and recrystallized from methanol, m. p. 138°.

Anal. Calcd. for $C_{28}H_{44}O_4$: C, 75.6; H, 10.0. Found: C, 75.3; H, 9.8.

Oxidation of *allo*-Keto-acid (VIII).—To a solution of 1.4 g. of the above keto-acid in 115 cc. of acetic acid was added a solution of 1.4 g. of chromic anhydride in 28 cc. of 80 per cent acetic acid at 28°. The mixture was allowed to stand for seventy-five minutes at 28–30°. It was poured into water and extracted with ether. The ethereal solution was washed to remove acetic acid. The acid fraction was extracted with 20% potassium hydroxide solution; it was allowed to stand for one hour and then re-extracted with ether. The united ether extracts gave crystalline material on evaporation. This was recrystallized from acetone, m. p. 212–213°. When mixed with 16-*allo*-pregnene-3,20-dione, there was no depression in melting point.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 80.0; H, 9.5.

Oxidation of Dihydropseudosarsasapogenin (I).—This followed the procedure given for the oxidation of dihydropseudotigogenin except that the sodium salt was not sepa-

(8) Marker and Rohrmann, THIS JOURNAL, **61**, 846 (1939).
(9) Marker and Rohrmann, *ibid.*, **61**, 2072 (1939).
(10) Marker, Turner and Ulshafer, *ibid.*, **63**, 763 (1941).
(11) Anhydrotetrahydro-tigogenoic acid was incorrectly designated "tetrahydroanhydro-tigogenoic acid" in the theoretical section of the paper of ref. 10.
(12) *Cf.* Marker *et al.*, *ibid.*, **61**, 3317 (1939).

rated by filtration. The acid product was crystallized from acetone, m. p. 233–236°. When mixed with the acid previously prepared[2] there was no depression in melting point.

To a solution of 300 mg. of the acid in 70 cc. of ethanol was added a solution of 1.0 g. of semicarbazide hydrochloride in 5 cc. of water and a solution of 1.0 g. of potassium acetate in 5 cc of water. This was refluxed for two hours. The mixture was poured into water, the product was filtered and washed with water and ether. It was recrystallized from ethanol and decomposed at 236°.

Anal. Calcd. for $C_{28}H_{45}O_4N_3$: C, 68.9; H, 9.3. Found: C, 68.7; H, 9.3.

The **oxime** was prepared similarly. It was recrystallized from methanol and melted at 232–234°.

Anal. Calcd. for $C_{27}H_{43}O_4N$: C, 72.8; H, 9.7. Found: C, 72.4; H, 9.6.

Methyl Ester of the Above Keto-acid (II).—This was prepared with diazomethane in ether and was recrystallized from pentane as prismatic needles, m. p. 116.5°. Upon standing for two years the ester previously described had changed in melting point from 85–87° to 112°, apparently changing crystalline form. This gave no depression with the above sample.

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

Semicarbazone of the Methyl Ester of the Above Keto Acid (II).—This was prepared in the usual manner and recrystallized from methanol. It decomposed at 225°.

Anal. Calcd. for $C_{29}H_{47}O_4N_3$: C, 69.4; H, 9.4. Found: C, 69.3; H, 9.4.

Bouveault Reduction of the Methyl Ester of (II).—Sodium (2.5 g.) was added to a solution of 2.0 g. of methyl ester in 20 cc. of absolute ethanol (dried over magnesium methylate). The mixture was boiled for one hour, then an additional 25 cc. of absolute ethanol and 2 g. of sodium was added. After boiling for two additional hours the mixture was poured into water and extracted with ether. The ethereal solution was washed with water and evaporated. The residue was a mixture difficult to separate. It was dissolved in dry pyridine and treated with an excess of *p*-nitrobenzoyl chloride. The *p*-nitrobenzoate was isolated in the usual manner and crystallized from acetone, m. p. 206–208°. Mixed with the bis-*p*-nitrobenzoate of epidihydropseudosarsasapogenin, m. p. 208–9°, the m. p. was 206–208°, yield 0.5 g.

Anal. Calcd. for $C_{41}H_{54}O_9N_2$: C, 68.5; H, 7.6. Found: C, 68.7; H, 7.4.

Clemmensen Reduction of the Keto-acid (II) from Dihydropseudosarsasapogenin.—To a refluxing solution of 1 g. of the keto acid in 250 cc. of ethanol containing 40 g. of amalgamated zinc (20-mesh) was added 70 cc. of concentrated hydrochloric acid during a period of three hours. The mixture was refluxed for an additional hour, poured into water and extracted with ether. The residue remaining after evaporation was crystallized from methanol, m. p. 81.5–82.5.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 78.1; H, 10.7.

Catalytic Reduction of the Keto-acid (II) from Dihydropseudosarsasapogenin.—A mixture of 1.0 g. of acid and 0.5 g. of platinum oxide catalyst in 200 cc. of absolute ethanol was shaken with hydrogen at 3 atm. for two hours. The catalyst was removed and 200 cc. of ethanol was added together with a hot solution of 8.0 g. of digitonin in 500 cc. of 85% ethanol. The digitonide which separated was decomposed with pyridine in the usual manner. The 3(β)-hydroxy acid was crystallized from acetone, m. p. 189–190°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.25. Found: C, 74.6; H, 10.3.

The fraction which did not precipitate with digitonin was crystallized from ether, m. p. 181–183°. This is the 3(α)-hydroxy acid.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.25. Found: C, 74.6; H, 10.3.

The **acetate** was prepared by treatment with acetic anhydride in pyridine in the usual manner. It was crystallized from methanol, m. p. 197–199°.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.4; H, 9.8. Found: C, 73.5; H, 9.6.

Catalytic Reduction of the Keto-acid (VIII) from Dihydropseudotigogenin.—The keto acid was reduced by the procedure given for the reduction of the acid from dihydropseudosarsasapogenin except that the digitonin separation was unnecessary. The product was crystallized from methanol, m. p. 240–241°.

It was quite insoluble in acetone, ethyl acetate and chloroform.

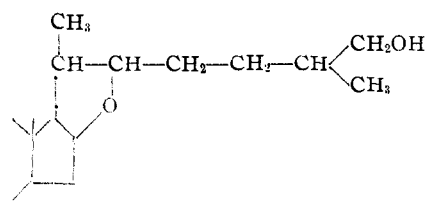
Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.25. Found: C, 75.1; H, 10.3.

The **acetate** was prepared by refluxing with acetic anhydride. It was recrystallized from ethyl acetate, m. p. 179–181°.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.4; H, 9.8. Found: C, 73.7; H, 9.7.

Summary

Evidence has been presented indicating that the side chain in the dihydropseudosapogenins has the structure



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