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

Sterols. CXVI. Sapogenins. XLV. The Isosarsasapogenin Configuration

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In previous papers from this Laboratory[1,2,3,4] it has been suggested that the sapogenins with the isosarsasapogenin configuration, *i. e.*, smilagenin, tigogenin, diosgenin, etc., differ from those of the sarsasapogenin configuration at C-22 and not at C-20 or C-16. This is supported by the observation that sarsasapogenin and smilagenin give the same pseudogenin[3] and the same dihydrogenin.[1] It has now been found that persulfuric acid oxidizes smilagenin to the same pregnanetriol-3(β),-16,20 which was obtained from sarsasapogenin.[5]

Sarsasapogenin reacts with methylmagnesium iodide and with ethylmagnesium bromide.[6] This reaction has now been applied to diosgenin, tigogenin and smilagenin. Smilagenin with ethylmagnesium bromide gave an isomer of the product from sarsasapogenin. The smilagenin product gave a depression of melting point of 7° when mixed with that from sarsapogenin. However, the diacetates did not give any depression in mixed melting point. There was a melting point elevation when the acetates were mixed in the proportion 1 : 1. Examination of the crystals of the free materials showed that the two products are similar in crystal habit and form but differ in sign of elongation and in visible refractive index. They are probably optical isomers.

On the basis of the spiro-ketal structure of the sapogenins of Marker and Rohrmann[1] having a reactive position at C-22 it would seem probable that the Grignard reaction with sarsasapogenin gave 22-ethyl-dihydrosarsasapogenin. The fact that smilagenin gave an isomer of this supports the assignment of the different configurations at C-22 to the sarsasapogenin type and the isosarsasapogenin type of genins.

The reaction with ethylmagnesium bromide and diosgenin gave 22-ethyl-dihydrodiosgenin which also has two esterifiable hydroxyl groups. Catalytic reduction of this gave 22-ethyl-dihydrotigogenin identical with material obtained by the direct treatment of tigogenin with ethylmagnesium bromide. Support for the structure assigned to these Grignard products was obtained by the oxidation of the tigogenin product with chromic acid to the expected keto acid.

We have also applied the persulfuric acid oxidation[5,7] method to tigogenin and to *epi*-tigogenin and obtained the corresponding pregnanetriols to which in analogy with previous work[7] can be assigned the structure of *allo*-pregnanetriol-3(β),-16,20 and *allo*-pregnanetriol-3(α),16,20, respectively. The action of hydrogen peroxide on tigogenin gave *allo*-pregnanetriol-3(β),16,20.

It is interesting that the *epi-allo*-pregnanetriol differs from Marrian's triol.[5] The difference probably lies in the configuration of the side chain.

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Experimental Part

***allo*-Pregnanetriol-3(β),16,20.**—(a) A solution of 1.0 g. of tigogenin in 100 cc. of glacial acetic acid was allowed to stand at 25° for four days with the reagent of von Baeyer and Villiger prepared from 2.0 g. of potassium persulfate.[8] The mixture was then poured into water and the product was taken up in ether. The residue remaining after evaporation of the ether was dissolved in ethanolic potash and the solution was refluxed for fifteen minutes. This was poured into water and the precipitated solid was taken up in ether. Removal of the ether gave a product (400 mg.) which was dissolved in acetone. After the removal of a small quantity of insoluble material, the mother liquor was evaporated and the residue was recrystallized from ether, m. p. 235–237°.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.95; H, 10.8. Found: C, 74.8; H, 11.0.

(b). A mixture of 6 g. of tigogenin acetate, 50 cc. of 30% hydrogen peroxide and 300 cc. of acetic acid was heated overnight at 70°. The solution was concentrated *in vacuo*, water was added and the product was taken up in ether. The ether was washed with water and the solvent was evaporated. The residue was hydrolyzed with hot ethanolic potassium hydroxide, extracted with ether, and the solvent was removed to a small volume. Crystals which separated from this solution have a m. p. of 206–208°. When mixed with tigogenin, m. p. 204–207°, there was no depression in melting point. The material in the mother liquors was crystallized from acetone, m. p. 234–236°. When mixed with the product from (a) above there was no depression in melting point. When refluxed

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 846 (1939).
(2) Marker and Rohrmann, *ibid.*, **62**, 647 (1940).
(3) Marker, Rohrmann and Jones, *ibid.*, **62**, 648 (1940).
(4) Marker, Rohrmann and Jones, *ibid.*, **62**, 1162 (1940).
(5) Marker and Turner, *ibid.*, **62**, 2540 (1940).
(6) Marker and Rohrmann, *ibid.*, **62**, 900 (1940).
(7) Marker, *et al.*, *ibid.*, **62**, 525 (1940).
(8) Marker and Turner, *ibid.*, **62**, 2540 (1940).

in acetic anhydride it gave a **triacetate.** This was recrystallized from acetone, m. p. 166°.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.1; H, 9.2. Found: C, 70.4; H, 8.9.

A **tribenzoate** was made by treatment with benzoyl chloride and pyridine. Recrystallized from acetone it melted at 204°.

Anal. Calcd. for $C_{42}H_{48}O_6$: C, 77.7; H, 7.5. Found: C, 77.9; H, 7.3.

***allo*-Pregnanetriol-3(α),16,20.**—*epi*-Tigogenin (370 mg.) dissolved in 20 cc. of glacial acetic acid was treated with the von Baeyer reagent from 1 g. of potassium persulfate.[7] The procedure followed that given above. The product was recrystallized from ether–pentane and then from acetone, m. p. 210–212°.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.95; H, 10.8. Found: C, 75.0; H, 10.7.

The **triacetate** was recrystallized from acetone, m. p. 148–150°.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.1; H, 9.2. Found: C, 70.4; H, 9.1.

Pregnanetriol-3(β),16,20 from Smilagenin.—A solution of 2.0 g. of pure smilagenin in 100 cc. of acetic acid was treated with the von Baeyer reagent from 4 g. of potassium persulfate as described above. This gave 500 mg. of crystals melting at 223–226°. When mixed with pregnanetriol-3(β),16,20 from sarsasapogenin there was no depression in melting point.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.9; H, 10.8. Found: C, 74.9; H, 11.0.

Reaction of Diosgenin with Ethylmagnesium Bromide.—A solution of 5 g. of diosgenin in 200 cc. of ether was added to an ethereal solution of ethylmagnesium bromide prepared from 6.1 g. of magnesium and 27.5 g. of ethyl bromide and 100 cc. of ether. The ether was distilled off and the thick residual liquid was dissolved in 50 cc. of benzene and refluxed for sixty hours. The reaction mixture was decomposed with cold dilute hydrochloric acid and the solid was taken up in ether. The ether insoluble material was recrystallized from alcohol, m. p. 211–214°.

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.3; H, 10.9. Found: C, 78.4; H, 11.0.

When treated with *p*-nitrobenzoyl chloride in pyridine at 30° for twenty-four hours the substance formed a di-*p*-nitrobenzoate which crystallized from ether and ethyl acetate, m. p. 183–184°.

Anal. Calcd. for $C_{43}H_{54}O_9N_2$: C, 69.5; H, 7.3. Found: C, 69.8; H, 7.4.

22-Ethyldihydrotigogenin.—A solution of 2.5 g. of the product obtained from the reaction of diosgenin with ethylmagnesium bromide in 200 cc. of acetic acid was shaken with 500 mg. of Adams catalyst under a pressure of 35 pounds of hydrogen for three hours. The product crystallized from ethyl alcohol as white plates, m. p. 192–194°.

Anal. Calcd. for $C_{29}H_{50}O_3$: C, 77.9; H, 11.3. Found: C, 77.9; H, 11.3.

When treated with *p*-nitrobenzoyl chloride it gave a di-*p*-nitrobenzoate which crystallized from ether and ethyl acetate, m. p. 183–184°.

Anal. Calcd. for $C_{43}H_{56}O_9N_2$: C, 69.3; H, 7.6. Found: C, 69.3; H, 7.8.

(b) The procedure described for diosgenin was followed using tigogenin. The solid formed upon decomposition of the Grignard complex was extracted with ether. The ether insoluble material was recrystallized from alcohol to give material of m. p. 203–206° which did not depress the melting point of tigogenin. From the alcoholic mother liquors of the ether soluble material, colorless crystals were obtained, m. p. 192°, which did not depress the melting point of the hydrogenated product from the reaction of diosgenin with ethylmagnesium bromide.

Oxidation of 22-Ethyldihydrotigogenin.—To a solution of 2 g. of 22-ethyldihydrotigogenin in 50 cc. of acetic acid was added a solution of 2 g. of chromic anhydride in 50 cc. of 80% acetic acid. The mixture was allowed to stand at 15° for one hour; it was poured into water and extracted with ether. The ethereal solution was washed with water and then with 3% sodium hydroxide solution. The alkaline water layer was acidified and the precipitated acid was taken up in ether and crystallized from methanol–ether, m. p. 221–223°.

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.9; H, 10.1. Found: C, 76.1; H, 10.1.

Reaction of Smilagenin with Ethylmagnesium Bromide.—The procedure previously described was repeated using smilagenin. The ether soluble material from the decomposed reaction mixture was recrystallized from ether–pentane, ether–alcohol and ethyl acetate giving colorless needles, m. p. 161–162°. This gave a 7° depression with the product from the reaction of sarsasapogenin with ethylmagnesium bromide, m. p. 161–162°.

Anal. Calcd. for $C_{29}H_{50}O_3$: C, 77.9; H, 11.3. Found: C, 77.5; H, 11.2.

Both 22-ethyldihydrosarsasapogenin and 22-ethyldihydrosmilagenin crystallize in prismatic form. The crystals appear to be tetragonal or orthorhombic. The interference figure is probably biaxial in both. However, 22-ethyldihydrosarsasapogenin has a continuous negative sign of elongation and a visible refractive index between 1.535–1.540, while 22-ethyldihydrosmilagenin has a continuous positive sign of elongation and refractive index with limits 1.505–1.510.

When refluxed with an excess of acetic anhydride the product gave a diacetate which crystallized from aqueous methanol as colorless needles, m. p. 89–91°. A mixture of equal quantities of this and the diacetate of the product from the reaction of sarsasapogenin with ethylmagnesium bromide, m. p. 89–91°, gave a melting point of 89–95°.

Anal. Calcd. for $C_{33}H_{54}O_5$: C, 74.7; H, 10.3. Found: C, 74.8; H, 10.2.

Summary

1. The reaction of diosgenin, tigogenin and smilagenin with ethylmagnesium bromide gave the corresponding 22-ethyldihydrosapogenins.

2. *allo*-Pregnanetriols-3,16,20 were prepared from tigogenin and *epi*-tigogenin.

3. Evidence has been discussed indicating that the configuration of the side-chain in sarsasapogenin and isosarsasapogenin differs at C-22.

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Sterols. CXVII. Sapogenins. XLVI. The Structure of Pseudosapogenins

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When the steroidal sapogenins are heated with acetic anhydride at 200° they are converted into pseudosapogenins[1,2,3] which are important intermediates in the preparation of the steroidal hormones.[4,5,6] In previous papers we have presented a discussion of various formulas[7,8,9] which may be assigned to the pseudosapogenins. The experimental evidence available at that time suggested formula I in preference to formula II for these compounds. Points have been raised by Drs. Crooks, Wittle and Whitmore which convince us that II may be a peculiar tautomeric form of I. We have continued our study of the chemical structure of these compounds and have obtained evidence to this effect.

It had been observed that in the oxidation of the pseudosapogenins or their diacetates, it was only after alkaline hydrolysis of the crude oxidation product that crystalline Δ^{16}-pregnenedione-3,20 compounds or Δ^{16}-pregnenol-3-one-20 compounds could be isolated. This suggested that the conversion to the pregnenolone was a secondary reaction brought about by the action of alkali.

Pseudotigogenin diacetate, pseudodiosgenin diacetate and dihydro-pseudotigogenin (tetrahydropseudodiosgenin) diacetate have now been oxidized with chromic acid and the intermediate oxidation products have been obtained in crystalline form. The products from pseudotigogenin diacetate and dihydro-pseudotigogenin diacetate are identical and have the composition $C_{31}H_{48}O_7$. The oxidation product from pseudodiosgenin diacetate has the Δ^5-double bond and was obtained in good yield without protecting the bond with bromine before oxidation. Catalytic reduction of this in neutral solution gave the same product obtained from pseudotigogenin diacetate and dihydro-pseudotigogenin diacetate.

Hydrolysis of the oxidation product of pseudotigogenin diacetate with ethanolic potash or by boiling with alcohol containing hydrochloric acid gave Δ^{16}-*allo*-pregnenol-3(β)-one-20, while similar hydrolysis of the oxidation product from the diacetate of pseudodiosgenin gave $\Delta^{5,16}$-pregnadienol-3(β)-one-20. In both cases the yield was practically quantitative. The reduction of the oxidation product from pseudotigogenin diacetate using the method of Meerwein–Ponndorff or by catalytic hydrogenation with Adams catalyst followed by hydrolysis gave in both cases the same product. This is apparently an *allo*-pregnanetriol, differing from that recently obtained from tigogenin by persulfuric acid oxidation.[10] The reduction of the oxidation product from pseudodiosgenin diacetate using the Meerwein reaction gave a similar unsaturated triol which was reduced catalytically to the triol obtained from the oxidation product of pseudotigogenin diacetate. These products cannot be obtained in alkaline solution which hydrolyzes the oxidation products. The reduction of the oxidation product of pseudotigogenin diacetate with sodium in dry alcohol gave the expected *allo*-pregnanediol-3(β),20(α).

The oxidation product of dihydro-pseudotigogenin diacetate was oxidized at room temperature with chromic acid to 3-hydroxy-*etio*-*allo*-bilianic acid identical with the acid previously described.[11] There were no neutral products in this oxidation.

The intermediate oxidation products of the pseudosapogenins can best be explained by formula III corresponding to formula II for the pseudosapogenins. It is impossible to explain the diketo acid[7,9] formed as an intermediate

(1) Marker and Rohrmann, THIS JOURNAL, **62**, 518 (1940).
(2) Marker, Rohrmann and Jones, *ibid.*, **62**, 648 (1940).
(3) Marker and Rohrmann, *ibid.*, **62**, 898 (1940).
(4) Marker, Tsukamoto and Turner, *ibid.*, **62**, 2525 (1940).
(5) Marker, *ibid.*, **62**, 2543 (1940).
(6) Marker, *ibid.*, **62**, 2621 (1940).
(7) Marker and Rohrmann, *ibid.*, **62**, 521 (1940).
(8) Marker and Rohrmann, *ibid.*, **62**, 896 (1940).
(9) Marker, Jones and Krueger, *ibid.*, **62**, 2532 (1940).
(10) Marker, Turner, Wagner and Ulshafer, *ibid.*, **63**, 772 (1941).
(11) Marker, Turner and Ulshafer, *ibid.*, **63**, 763 (1941).