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

Sterols. LXXXIII. Oxidation Products of Sarsasapogenin. Studies on the C_{22} Lactone

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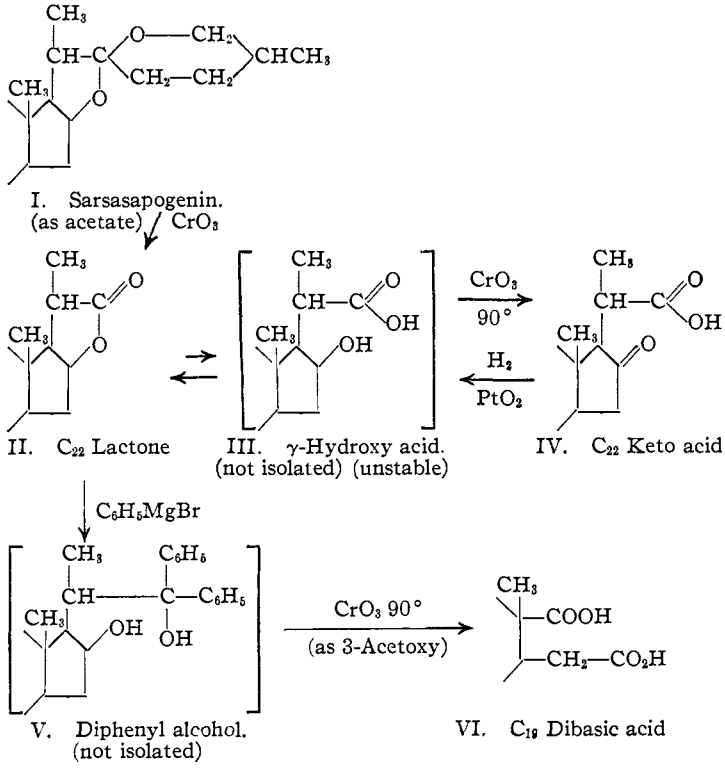
Perhaps one of the most striking characteristics common to all of the more extensively studied steroid sapogenins is the formation of lactones upon rather vigorous oxidation with chromic anhydride (after adequate protection of the nuclear hydroxyl groups).[1] Sarsasapogenin,[2] smilagenin,[3] tigogenin,[4] chlorogenin,[5] gitogenin[6] and digitogenin,[6] have thus been oxidized to C_{22} lactones.



While all these lactones undoubtedly contain the same number of carbon atoms, it should be pointed out that many of the analyses reported for the substances do not distinguish between a C_{22} and a C_{23} compound. Our analytical results on the sarsasapogenin lactone and its various derivatives are in accord with the C_{22} composition. However, it is evident, as we pointed out previously,[7] that the final proof of the structure and composition of the lactone will depend upon its *stepwise* degradation to a known sterol derivative, an achievement not yet reported.

That the lactone (II) has a very slight tendency to exist as a γ-hydroxy acid (III) is indicated by the fact that the lactone acetate upon rather vigorous oxidation with chromic anhydride gives rise to some of the C_{22} keto acid (IV) which was previously obtained from a similar oxidation of sarsasapogenin acetate.[8]

The most fruitful process of degrading the sarsasapogenin lactone to a known product has been by the method of Tschesche and Hagedorn[4] involving the oxidation of the diphenyl Grignard addition product (V) of the lactone. By this method Farmer and Kon[3] degraded the desoxy lactone to *etio*-bilianic acid. We have carried out a similar degradation of the hydroxy lactone to obtain the C_{19} dibasic acid identical with that previously obtained by the chromic anhydride oxidation of the acetates of sarsasapogenin,[9] sarsasapogenoic acid[9] and dihydrosarsasapogenin.[10] This acid is most likely 3-hydroxy-*etio*-bilianic acid. The fact that we were unable to oxidize the C_{22} keto acid with chromic anhydride after acetylation with boiling acetic anhydride is of interest. This suggests that the γ-keto acid has a tendency to exist in a lactone form which would be stabilized to oxidation by acetylation (*cf.* levulinic acid).

Simpson and Jacobs[11] observed that sarsasapogenin upon oxidation with sodium hypobromite yielded a dibasic acid which upon further oxidation with nitric acid yielded a lactone dibasic acid. We have obtained a similar acid by the oxidation

(1) Strain, "The Sterols and Related Compounds" in "Gilman's Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1938, Chap. 15, p. 1346.
(2) Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936).
(3) Farmer and Kon, *ibid.*, 414 (1937).
(4) Tschesche and Hagedorn, *Ber.*, **68**, 1412 (1935).
(5) McMillan and Noller, THIS JOURNAL, **61**, 1630 (1939).
(6) Marker and Rohrmann, *ibid.*, **61**, 2724 (1939).
(7) Marker and Rohrmann, *ibid.*, **61**, 846 (1939).
(8) Marker and Rohrmann, *ibid.*, **61**, 1285 (1939).
(9) Marker and Rohrmann, *ibid.*, **61**, 2722 (1939).
(10) Marker and Rohrmann, *ibid.*, **61**, 3477 (1939).
(11) Simpson and Jacobs, *J. Biol. Chem.*, **108**, 573 (1935).

TABLE I

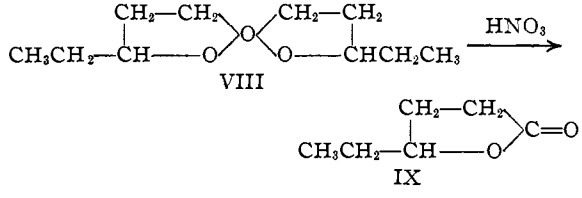
Substance	Composition	M. p., °C.	Analysis Calcd. C	Calcd. H	Found C	Found H
Lactone acetate[a]	$C_{24}H_{36}O_4$	184.5–185.5	74.15	9.37	74.26	9.30
Lactone benzoate[b]	$C_{29}H_{38}O_4$	207.5–209	77.28	8.49	77.16	8.49
Hydroxylactone	$C_{22}H_{34}O_3$	199–200	76.25	9.89	76.35	10.04
3-Keto lactone	$C_{22}H_{32}O_3$	184–185	76.69	9.37	76.55	9.37
Desoxy lactone	$C_{22}H_{34}O_2$	128–129	79.93	10.37	79.88	10.37

[a] The acetate was purified by sublimation in high vacuum at 130–140°. [b] The benzoate was prepared in pyridine solution with benzoyl chloride at room temperature and crystallized from acetone–pentane.

of sarsasapogenin lactone with chromic anhydride. The same acid was obtained by the direct oxidation of sarsasapogenone with nitric acid. While no comparison was made between our acid and that of Simpson and Jacobs the substances are very probably identical, as the melting points of the free acids and of the methyl esters are in good agreement. While this lactone dibasic acid is undoubtedly either a 2 || 3 or a 3 || 4 diacid there is insufficient evidence at present to distinguish between the two possibilities, especially in view of recent work[12] on the acids of the coprostane series resulting from the oxidation of ring A.

The question regarding the origin of the C_{22} lactones is of great interest but there is little information available on the course of the oxidation. However, it is now fairly certain that the sapogenoic acids are not intermediates in the formation of the lactone.[13,14,15]

The fact that nitric acid exerts a specific action on the side chain to yield lactones[11,15,16] is of considerable interest. In this connection it is probably significant that diethyl oxetone (VIII), a substance of the ketone spiro acetal type and consequently analogous to our proposed structure of the sapogenin side chain[7] (I), is oxidized by nitric acid to yield caproic lactone (IX) and propionic acid.[17]



In previous work concerning the hydrogenation of the C_{22} keto acid[8] a C_{22} lactone was obtained in two forms, m. p. 188° and m. p. 198°, which appeared to be polymorphic or possibly steroisomers. That the two forms are polymorphic is indicated by the fact that they yielded the same acetates and upon oxidation gave the same 3-keto-lactones.

We wish to thank Parke, Davis and Company for their generous help and assistance in the various phases of this work.

Experimental Part

Sarsasapogenin Lactone (II).—The lactone and its derivatives were prepared as described by previous workers. Our analytical data are summarized in Table I.

An attempt to reduce the hydroxylactone with phosphorus and hydriodic acid (sp. gr. 1.7) at 160° gave only non-crystalline neutral products. The products were very insoluble in methanol and distilled in high vacuum at 90°, suggesting substances of a hydrocarbon nature. The lactone showed no tendency to esterification when allowed to stand for several days with absolute ethanol and concentrated sulfuric acid.

epi-**Lactone.**—A mixture of 500 mg. of the keto lactone, 500 mg. of Adams catalyst, and 80 cc. of 98% ethanol was shaken with hydrogen at 3 atmospheres pressure at room temperature for three hours. The mixture was filtered and the filtrate evaporated *in vacuo*. The residue was taken up in 10 cc. of hot 95% ethanol and to this solution was added a hot solution of 500 mg. of digitonin in 15 cc. of 80% ethanol. The mixture after standing at 0° was filtered. The filtrate was diluted with water and the precipitated solid taken up with ether. Evaporation of the ether gave a residue which crystallized from ether–acetone–pentane as white needles, m. p. 198–200°. This gave a 10° depression with the 3-β-hydroxylactone, m. p. 199–200°.

Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.25; H, 9.89. Found: C, 76.33; H, 9.95.

The *epi*-lactone when refluxed for twenty minutes with acetic anhydride yielded the *epi-lactone acetate* which crystallized from ether–pentane as white crystals, m. p. 159–160°.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.2; H, 9.35. Found: C, 73.9; H, 9.2.

Oxidation of Hydroxylactone to Lactone Dibasic Acid.—To a well-stirred solution of 2 g. of hydroxy lactone in 50 cc. of glacial acetic acid heated at 50–55° was added a solution of 3 g. of chromic anhydride in 20 cc. of 80% acetic acid over a period of three hours. The solution was stirred for an additional two hours at 55°. The mixture was then evaporated *in vacuo* to a volume of 30 cc. The residual material was diluted with water and the precipi-

(12) Marker, Plambeck, Wittle, Rohrmann, Krueger and Ulshafer, THIS JOURNAL, **61**, 3317 (1939).
(13) Fieser and Jacobsen, *ibid.*, **60**, 2753 (1938).
(14) Marker and Rohrmann, *ibid.*, **61**, 2072 (1939).
(15) Windaus and Linsert, *Z. physiol. Chem.*, **147**, 275 (1925).
(16) Jacobs and Simpson, *J. Biol. Chem.*, **105**, 501 (1934).
(17) Fittig and Dubois, *Ann.*, **256**, 141 (1889).

tated solid extracted with ether. The ethereal extract was washed well with water and the solution evaporated to a volume of 30 cc., compact white crystals separated. These were recrystallized from methanol–ether to give a product with m. p. 285–288° dec.

Anal. Calcd. for $C_{22}H_{32}O_6$: C, 67.3; H, 8.2. Found: C, 67.0; H, 8.4.

When treated with an ethereal solution of diazomethane the acid yielded a *dimethyl ester* which crystallized from ether–pentane as fine white needles, m. p. 170–171°.

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 68.5; H, 8.6. Found: C, 68.2; H, 8.7.

Oxidation of Sarsasapogenone with Nitric Acid.—To a suspension of 1 g. of sarsasapogenone in 9 cc. of glacial acetic acid was added 15 cc. of nitric acid (d. 1.50). Brown fumes were liberated and the solid dissolved almost at once. The mixture was heated at 90° for one hour. The solution was diluted with water and the precipitated solid collected and washed with water. The solid was dissolved in ether and shaken with 3% sodium hydroxide solution.

The neutral fraction which would not crystallize was not investigated further. The sodium hydroxide extract was acidified with hydrochloric acid and extracted with ether. Evaporation of the ether gave compact white crystals which were recrystallized from ether–acetone, m. p. 285–289° dec. This gave no depression with the sample of dibasic lactone acid obtained previously, m. p. 285–288° dec.

Anal. Calcd. for $C_{22}H_{32}O_6$: C, 67.3; H, 8.2. Found: C, 67.0; H, 8.3.

With diazomethane the acid yielded a product which crystallized from ether–pentane as fine white needles, m. p. 170–171.5°. This gave no depression with the sample obtained above, m. p. 170–171°.

Oxidation of Lactone Acetate to C_{22}-Keto Acid.—To a solution of 800 mg. of lactone acetate, m. p. 183–184°, in 30 cc. of glacial acetic acid was added a solution of 2 g. of chromic anhydride in 40 cc. of 80% acetic acid. The resulting solution was heated on the steam-bath at 90° for three hours. The mixture was diluted with water and extracted with ether. The ethereal extract was washed well with water and then extracted with 3% sodium hydroxide solution. The sodium hydroxide extract was heated for twenty minutes on the steam-bath, cooled, acidified with hydrochloric acid and the solid acid extracted with ether. The ether upon slow evaporation deposited compact white crystals. These, after crystallization from ether–methanol, melted at 285–287° dec. and gave no depression with a sample of the C_{22} keto acid, m. p. 285–287°.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.9; H, 9.5. Found: C, 73.0; H, 9.6.

The neutral fraction consisted of unchanged lactone acetate.

Degradation of Hydroxylactone to C_{19}-Dibasic Acid.—To a Grignard reagent prepared from 1.25 g. of magnesium, 9 g. of phenyl bromide and 45 cc. of ether was added a solution of 2 g. of hydroxy lactone, m. p. 199–201°, over a period of ten minutes. The mixture was refluxed on the steam-bath for two hours after which the solvent was removed. The residue was dissolved in 50 cc. of dry pyridine and treated with 5 cc. of acetic anhydride at room temperature for eighteen hours. The mixture was decomposed with water and extracted with ether. After washing well with dilute hydrochloric acid the ether was evaporated to give a yellow non-crystalline sirup.

This was dissolved in 50 cc. of acetic acid and to the resulting solution heated at 90–95° was added 8 g. of chromic anhydride in 70 cc. of 80% acetic acid over a period of one hour. The mixture was heated for an additional two hours, the excess oxidizing agent was destroyed with ethanol and the mixture concentrated *in vacuo* to a volume of about 50 cc. The residual mixture was diluted with water and the precipitated solid taken up in ether. The residue remaining upon evaporation of the ether was refluxed for thirty minutes with an excess of ethanolic potassium hydroxide. The solid which separated upon dilution with water was taken up in ether and discarded. The alkaline water layer was acidified with hydrochloric acid and the acids taken up in ether. Evaporation of the ether gave a residue which crystallized from chloroform as white crystals, m. p. 218–220°; yield, 150 mg. This gave no depression in melting point with a sample of the C_{19}-dibasic acid, m. p. 220–222°.

Anal. Calcd. for $C_{19}H_{30}O_5$: C, 67.4; H, 8.9. Found: C, 67.2; H, 8.8.

Further Characterization of C_{22} Keto Acid.[8]—Treatment of the methyl ester of the keto acid with boiling acetic anhydride for thirty minutes followed by decomposition of the excess acetic anhydride with water yielded a *methyl ester acetate* which crystallized from ether–pentane as compact white crystals, m. p. 198–199.5°.

Anal. Calcd. for $C_{25}H_{38}O_6$: C, 71.7; H, 9.1. Found: C, 71.5; H, 9.1.

Treatment of the acid with hydroxylamine hydrochloride under the usual conditions gave an *oxime* which crystallized from aqueous methanol as small compact white crystals, m. p. 206–208° dec.

Anal. Calcd. for $C_{22}H_{35}O_4N$: C, 70.0; H, 9.3; N, 3.7. Found: C, 69.8; H, 9.2; N, 3.8.

Acetylation of the acid with boiling acetic anhydride gave a non-crystalline product. Oxidation of this product with chromic anhydride in acetic acid at 90–95° followed by alkaline hydrolysis of the total products yielded largely the original acid and none of the C_{19} dibasic acid was isolated.

The lactone, m. p. 186–188°, obtained by the catalytic hydrogenation of the C_{22} keto acid in ethanol–hydrochloric acid solution upon refluxing with acetic anhydride gave an acetate, m. p. 183–184°. This gave no depression with an authentic sample of the normal lactone acetate, m. p. 184.5–185.5°. Oxidation of the lactone with chromic anhydride at 25° gave the 3-ketolactone, m. p. 184–185°. This gave no depression with an authentic sample of the 3-ketolactone, m. p. 184–185°.

Summary

Oxidation of the acetate of the C_{22} lactone from sarsasapogenin with chromic anhydride yielded the C_{22} keto acid.

The C_{22} lactone has been degraded to the C_{19} dibasic acid.

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