[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Sarsasapogenin. II. Sarsasapogenoic Acid

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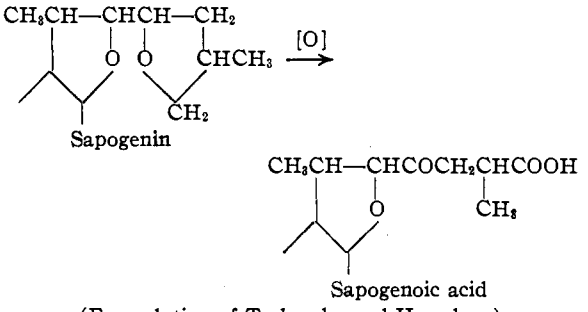
The observations reported in this paper are concerned with a study of sarsasapogenoic acid, a C_{27}-acid obtained as described in Part I[2] by the oxidation of sarsasapogenin acetate with chromic acid at 60–65° and subsequent gentle saponification of the acidic fraction. Under the conditions found most favorable for the isolation of this acid, the neutral fraction contains a small amount of the acetate of the hydroxy lactone $C_{22}H_{34}O_3$, together with a substance previously regarded[2] as unchanged sarsasapogenin acetate but now recognized as consisting in large part of an isomorphous substance of higher oxygen content, possibly the acetate of a hydroxysarsasapogenin. Sarsasapogenoic acid, which probably is a stereoisomer of Tschesche and Hagedorn's[3] tigogenoic acid ($C_{27}H_{42}O_5$), displays a number of interesting if perplexing properties, and a further study of the substance was undertaken with the idea that it might hold the key to the structure of the sapogenin side chain. The extent of experimentation has been somewhat limited by the low yield of this oxidation product of the rather difficultly accessible genin, the average yield being about 5 g. of acid from 20 g. of sarsasapogenin acetate, representing the material from some 20 lb. (9 kg.) of root.[4] Farmer and Kon[5] did not encounter the acid in the mixture obtained on conducting the oxidation at the temperature of the steam-bath with a larger amount of chromic acid, and oxidation at 32-33° by the procedure found by McMillan and Noller[6] to give a good yield of chlorogenoic acid diacetate proved to be unsatisfactory for the sapogenin under consideration. A slight difference in one part of a steroid molecule often alters considerably the susceptibility to attack by chromic acid at some other site.

Tschesche and Hagedorn[3] tentatively suggested that tigogenoic acid may be a γ-keto acid arising from the opening of one of the postulated five-membered oxide rings. They were not able, however, to demonstrate the presence of a carbonyl



(Formulation of Tschesche and Hagedorn)

group, the ethyl ester forming no oxime or semicarbazone under the usual conditions, and absorbing no hydrogen on attempted hydrogenation. Sarsasapogenoic acid is similarly inert to carbonyl reagents, the methyl ester, for example, being recovered unchanged after attempted oximation in boiling alcohol, and the acid yielding no semicarbazone under the usual conditions. McMillan and Noller[6] made a similar observation concerning chlorogenoic acid diacetate. On treating sarsasapogenoic acid or ester with carbonyl reagents under forcing conditions we were able to bring about reactions, but these all proved to be more profound than a simple condensation or addition involving only a carbonyl group. Thus the acid and its ester both react with two molecules of hydroxylamine at 130° and give products which appear to be unrelated but which contain two atoms of nitrogen (see chart). Paralleling the experience of Tschesche and Hagedorn, we found sarsasapogenoic acid to be very resistant to hydrogenation, but under certain conditions we were able in at least some experiments to effect a slow hydrogenation. On the above formulation one would expect to obtain a hydroxy acid or its lactone, but the substance isolated proved to be a free acid corresponding to the introduction of two or four hydrogen atoms and the loss of a molecule of water; the compound evidently is an anhydrohydro acid and not a lactone. This loss of water in some way other than by lactonization is surprising; it cannot be attributed to the presence of water of crystallization in the starting material (m. p. 193°) for this gives a normal ester, acetate and methyl ester benzoate.

The elimination of a molecule of water was observed also in a curious reaction of sarsasa-

(1) Du Pont Research Fellow.

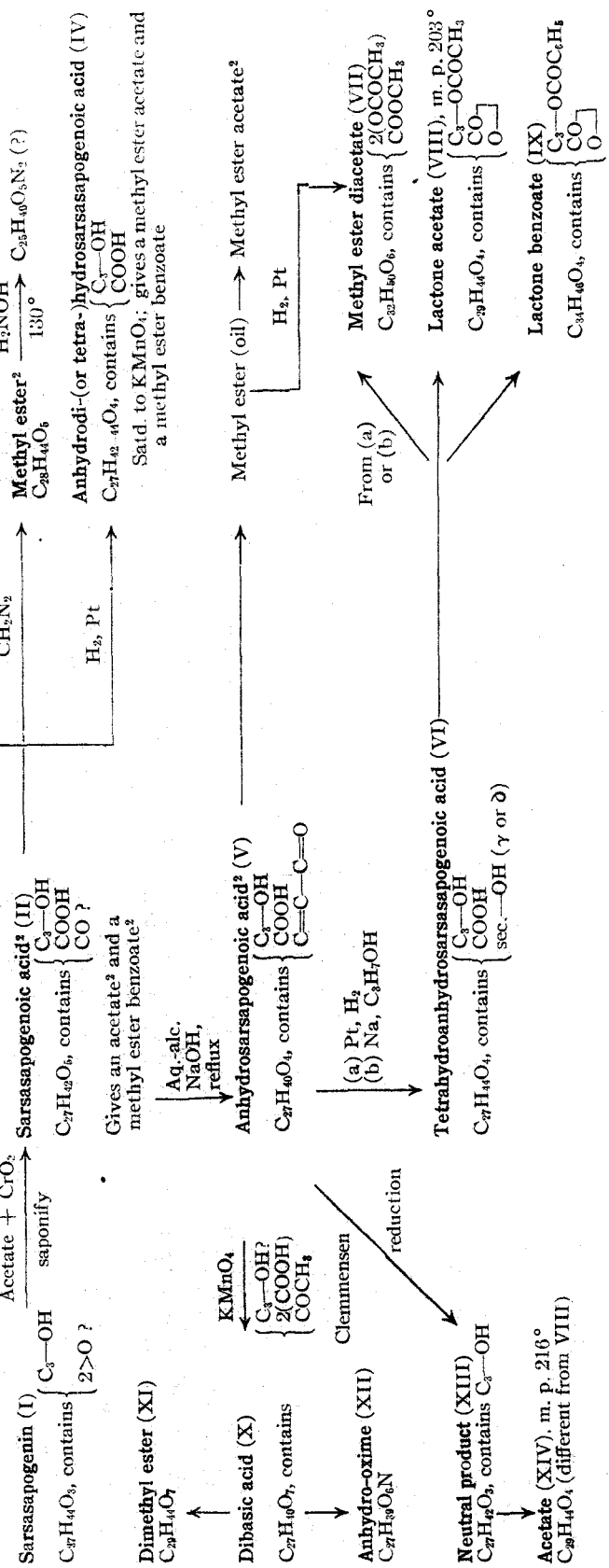
(2) Fieser and Jacobsen, THIS JOURNAL, **60**, 28 (1938).

(3) Tschesche and Hagedorn, *Ber.*, **68**, 1412, 2247 (1935).

(4) We are greatly indebted to E. R. Squibb and Sons for generous supplies of sirupy glycoside extracted from Mexican (sarsaparilla) root under the supervision of Dr. J. M. Ort.

(5) Farmer and Kon, *J. Chem. Soc.*, 414 (1937).

(6) McMillan and Noller, THIS JOURNAL, **60**, 1630 (1938).



pogenoic acid with alkali.[2] When heated with dilute aqueous alcoholic alkali for two hours, the sapogenoic acid ($C_{27}H_{42}O_5$) is converted into a higher melting acid $C_{27}H_{40}O_4$, designated anhydrosarsasapogenoic acid. This substance is quite reactive and, as indicated in the chart, has been converted into a number of transformation products. The presence of a carboxyl group and of the original C_3-hydroxyl group was established by the preparation of a crystalline methyl ester acetate. The anhydro acid rapidly absorbs two moles of hydrogen in the presence of platinum oxide catalyst giving a tetrahydro acid, and the same compound (VI) was obtained by reduction with sodium and *n*-propyl alcohol. The latter reaction suggested the presence of a conjugated system, and this inference was fully confirmed by a determination of the absorption spectrum kindly carried out for us by Dr. T. J. Webb at the Merck Research Laboratories. The absorption maximum at 243 mμ (Fig. 1) clearly indicates that the substance is an α,β-unsaturated ketone. The four oxygen atoms are therefore present as a hydroxyl, carboxyl, and conjugated carbonyl group, and it is clear that both of the original oxide rings of the sapogenin have been severed. A further significant inference can be drawn from the behavior of tetrahydroanhydrosarsasapogenoic acid (VI). On esterification with diazomethane followed by acetylation, this gave a crystalline methyl ester diacetate (VII), as shown by the analysis and saponification equivalent. The same substance (VII) was also obtained from the anhydro acid (V) in a different sequence: esterification, hydrogenation, and acetylation. The free acid group

is thus characterized, one of the acetylated hydroxyls must be that originally present in the nucleus at C_3, and the other is a new secondary alcoholic group arising from the addition of hydrogen to the carbonyl group of the anhydro acid. When the tetrahydroanhydro acid was acetylated without prior esterification it yielded, in place of a diacetate, a lactone acetate (VIII); a lactone benzoate (IX) was similarly obtained on benzoylation. The new hydroxyl group formed in the reduction of the anhydro acid must, therefore, be in a position with respect to the carboxyl group favorable for lactone formation, and it is concluded that the carbonyl group of anhydrosarsasapogenoic acid is γ or δ to the carboxyl group.

From its composition, anhydrosarsasapogenoic acid must contain either one isolated double bond, in addition to that conjugated with the carbonyl group, or a newly formed carbon ring. The fact that the tetrahydro acid resists further hydrogenation and is saturated to permanganate points to the presence of a new ring.

The anhydro acid was altered on attempted oximation at 130° but gave a non-crystalline product, and the methyl ester acetate was attacked only slowly by chromic acid at 95° and afforded no crystalline material. The free acid, however, is oxidized smoothly and rapidly by alkaline permanganate and gives in good yield a nicely crystalline dibasic acid (X), m. p. 207°, of the composition $C_{27}H_{40}O_7$. The reaction

$$C_{27}H_{40}O_4 + 3[O] \longrightarrow C_{27}H_{40}O_7$$

involving the production of a new carboxyl group, can only be interpreted as follows

$$\left\{\begin{matrix}-CH \\ \| \\ -C-\end{matrix}\right. \longrightarrow \left\{\begin{matrix}-COOH \\ >CO\end{matrix}\right.$$

One of the unsaturated carbon atoms carries a hydrogen atom and the other is joined to two carbon residues, and since no loss of carbon atoms occurs on severing the ethylenic linkage it is evident that the double bond is inserted in a carbon chain which is joined to the main body of the molecule on both sides of the point of unsaturation. This is perhaps a further indication of the presence of a fifth ring in the anhydro acid. For further characterization the dibasic acid X was heated with hypoiodite in dioxane–alkali, according to the haloform test of Fuson and Tullock,[7] and it yielded iodoform. The carbonyl group generated in the oxidation therefore is

(7) Fuson and Tullock, THIS JOURNAL, **56**, 1638 (1934).

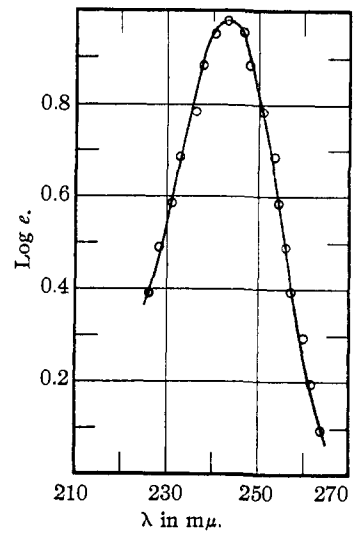


Fig. 1.—Anhydrosarsasapogenoic acid (V) in absolute alcohol.

present in the form of an acetyl group, and the knowledge of the oxidation can be elaborated thus

$$\left\{\begin{matrix}-CH \\ \| \\ -CCH_3\end{matrix}\right. \longrightarrow \left\{\begin{matrix}-COOH \\ -COCH_3\end{matrix}\right.$$

The conjugated carbonyl group of anhydrosarsasapogenoic acid may be attached to either of the two unsaturated carbon atoms. A further requirement is that this group must be placed γ (or δ) to the carboxyl group, and from the evidence of the absorption spectrum it appears that the carboxyl group is not conjugated with the 1,4-system. The anhydro acid, therefore, must contain one of the groupings

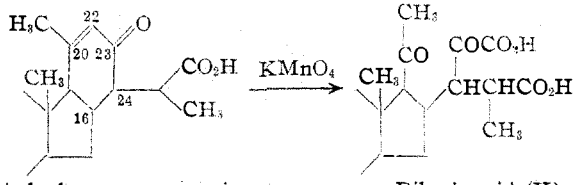
$$-C(CH_3)=CHCOC\dot{C}\dot{C}COOH \text{ or}$$

$$-CH=C(CH_3)CO\dot{C}\dot{C}\dot{C}COOH$$

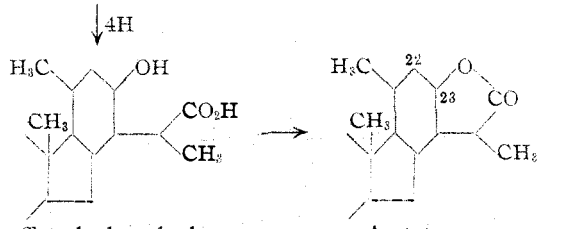
Only the first of these groupings can be accommodated to the cholesterol side chain, and it remains only to place the carbon bridge connecting the residues on the two sides of the double bond. Inspection of the possible structures shows that the choice lies between the six-ring structure indicated in the partial formula and a seven- or eight-ring structure; the six-ring formula is the more likely both from the point of view of ring size and because ring closure in this case would involve an activated position α- to the carbonyl group.

It is inferred that the anhydro acid probably has the structure shown and that it contains a new

ring formed by condensation between the C_{24}-position of the side chain and position 16 of Ring D. According to this formulation the di-

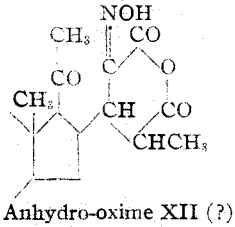


Anhydrosarsasapogenoic acid (V) — Dibasic acid (X)



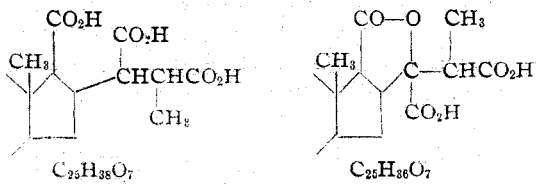
Tetrahydroanhydrosarsasapogenoic acid (VI) — Acetate lactone (VIII)

basic acid produced on permanganate oxidation has two carbonyl groups, but since they are both subject to considerable hindrance the formula is perhaps not inconsistent with the observation that the dimethyl ester does not react with hydroxylamine at 130°. The free acid does react under these conditions and yields an anhydro-oxime. It is known only that the substance is not a β,γ-unsaturated enol lactone (negative Legal test), but with so many functional groups present any formulation, such as that shown, is purely conjectural. Uncertain also is the struc-



Anhydro-oxime XII (?)

ture of a nicely crystalline product obtained in one small-scale hypohalite oxidation of the dibasic acid. Preliminary analyses indicate that probably two carbon atoms are lost in the oxidation and that the composition is $C_{25}H_{38}O_7$ or possibly $C_{25}H_{36}O_7$. Since a titration with a small sample



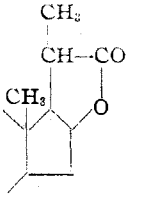
$C_{25}H_{38}O_7$ — $C_{25}H_{36}O_7$

Hypohalite oxidation product (?)

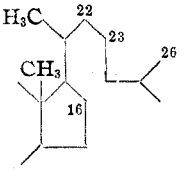
(in the cold) pointed to a dibasic acid, the second structure shown is the more probable.

While the nature of these little studied transformations is still highly uncertain, the formulation given above for tetrahydroanhydrosarsasapogenoic acid is reasonably secure; all of the functional groups have been characterized adequately, particularly by conversion to the lactone acetate and benzoate. As shown on the reaction chart, anhydrosarsasapogenoic acid was also converted in poor yield by Clemmensen reduction into a neutral product giving an acetate of the same composition as the acetate lactone VIII. The substances melt at not greatly different temperatures but show a distinct depression when mixed. The acetate resulting from the Clemmensen reduction product may be a δ-lactone with the oxygen attached at the 22-position, or, considering the opportunity for the migration of the double bond in the acid mixture, it may be a stereoisomeric γ-lactone different from VIII in the configuration at C_{23}.

With the knowledge gained of the nature of anhydrosarsasapogenoic acid, the next step is to attempt to trace the manner of its formation and the structure of this part of the sapogenin molecule. The carbonyl group at C_{23} and the terminal carboxyl group must have arisen by the rupturing of one of the original oxide linkages at C_{23} and C_{26} in the chromic acid oxidation, and the degradative experiments of Farmer and Kon[5] have established the attachment of an oxide bridge at C_{16}. The presence of a double bond in the anhydro acid between positions C_{20} and C_{22} is understandable only on the assumption that an oxide ring originally extended to one of these positions. The former position is eliminated by the observation that derivatives of the sapogenin can



C_{22}-Saturated lactone

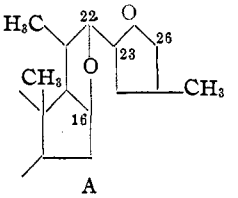


Position of oxide linkages

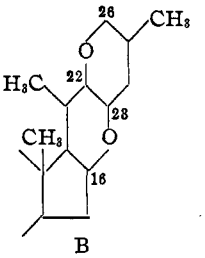
be oxidized to saturated C_{22}-lactones in which the carbon atom in question is not hydroxylated.[8,5] The evidence therefore indicates that the oxidic linkages of the sapogenin are joined at positions 16, 22, 23, and 26. The only structures for the

(8) Simpson and Jacobs, *J. Biol. Chem.*, **110**, 565 (1935).

sapogenin admissible on this basis are those shown in Formulas A and B, and only the first of these accounts for the appearance of a carbonyl group



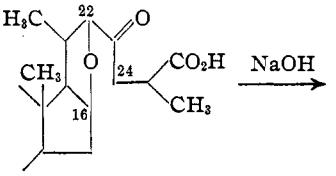
A



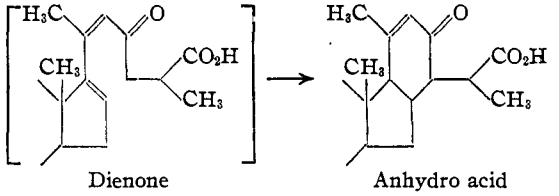
B

at C_{23} and a terminal carboxyl group in the oxidation, as demonstrated in the characterization of the anhydro acid. The structure A, which is the formulation tentatively suggested by Tschesche and Hagedorn,[8] thus accords best with the observations reported.

Sarsasapogenoic acid is then a γ-keto acid, corresponding also to the formulation of Tschesche and Hagedorn. The remarkable transforma-



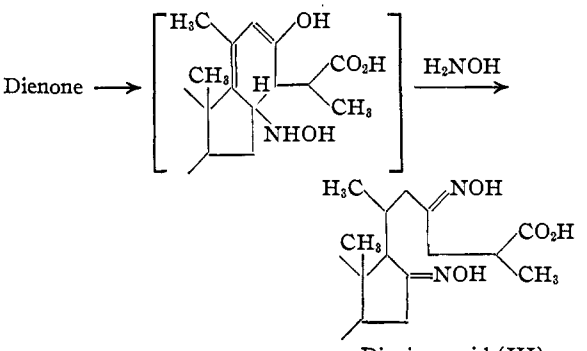
Sarsasapogenoic acid



Dienone Anhydro acid

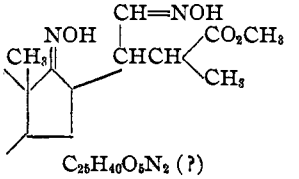
tion brought about by the action of alkali involves elimination of the oxide bridge and a condensation between positions 16 and 24. Position 16 is a point of attachment of the oxygen atom, and the methylene group at position 24 is adjacent to an activating carbonyl group, which may account for the disposition to cyclize under the influence of alkali. The ready cleavage of the oxygen bridge may also be attributable to the activating influence of the carbonyl group adjacent to C_{22}, and the reaction possibly involves the intermediate formation of a dienone.

This hypothetical dienone may be an intermediate in other reactions of the sapogenoic acid, cleavage of the oxide ring occurring under the forcing conditions required to overcome the resistance of the carbonyl to additions. The inert character of this group may be associated with a hindrance arising from a special steric arrangement, although this does not seem very plausible, or to a tendency for the substance to exist in the lactol form. If the reaction with hydroxylamine at 130° involves cleavage of the oxide ring, one molecule of the reagent may add to the ends of the conjugated system of the dienone or dienone oxime giving, by a succession of ketonizations, the saturated dioxime-acid of the formula shown.



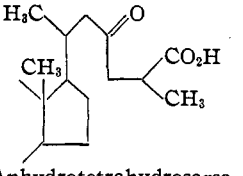
Dioxime-acid (III)

The reaction of the sapogenoic methyl ester with hydroxylamine at 130° seems to result in the loss of three carbon atoms and may follow some other course; a purely speculative formulation satisfying the present analytical data is as follows.



$C_{25}H_{40}O_5N_2$ (?)

The product obtained from sarsasapogenoic acid in some experiments in a slow hydrogenation may be either a di- or a tetrahydride; the analytical data do not distinguish between the two possibilities but agree slightly better with the tetrahydride formula. Possibly the activated oxide ring is eliminated by hydrogenolysis, or the dienone is slowly formed and saturated with hydrogen, giving in either case a saturated γ-keto acid. The substance may also be a satu-



Anhydrotetrahydrosarsasapogenoic acid, $C_{27}H_{44}O_4$ (?)

rated dihydride with a linkage between C_{16} and C_{24}, as in the anhydro acid.

While many of the new compounds described

need to be characterized much more fully in order to establish the structures, the evidence regarding the structure of the anhydrosapogenoic acid is now secure and the only formulation of the sapogenin side chain consistent with this structure is that suggested by Tschesche and Hagedorn.[3]

Experimental Part[9]

Extraction of the Genin.—In working further batches of the sirupy extract from Mexican (sarsaparilla) root we followed a suggestion kindly made by Dr. J. C. E. Simpson and simplified the process by omitting the initial extraction with ligroin. The gum was defatted during the precipitation of the crude glycoside by running a 3.5-kg. portion in a thin stream into 10 l. of vigorously stirred ether. The resulting viscous gum was then processed as before,[2] centrifuging the aqueous solution of the gum prior to hydrolysis, when clarification was necessary. One batch of 18.2 kg. of crude sirup yielded 195 g. of pure sarsasapogenin, which is about the amount previously reported;[2] another lot of 20.0 kg. processed in the same way gave only 67 g. of genin. We were not able to determine the variable factor involved.

Notes on the Preparation and Characterization of Sarsasapogenoic Acid.—In studying further the oxidation of sarsasapogenin acetate,[2] material (3.8 g.), m. p. 141–145°, recovered from the neutral fraction and previously designated as "unchanged sarsasapogenin acetate" was crystallized repeatedly from acetone–chloroform to yield 2 g. of flat needles melting constantly at 165–167°. This material resembles sarsasapogenin acetate in crystalline form but melts about 20° higher. On saponification with alcoholic potassium hydroxide this gave a product crystallizing from acetone–chloroform in elongated plates, m. p. 216–218° (av. of three analyses: C, 76.09; H, 10.06). The crystals are similar in appearance to those of sarsasapogenin but the m. p. is distinctly higher. This substance on reacetylation and crystallization as above gave material, m. p. 158–160°, apparently identical with the original acetate (no depression). This was analyzed with the following results.

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.76; sapon. equiv., 475. Found: C, 73.20, 73.21; H, 9.71, 9.93; sapon. equiv., 487.

These analyses suggest that the substance is the acetate of a hydroxysarsasapogenin, but the carbon content of the saponified material is about 1% higher than that required for the normal desacetyl compound. All that can be said is that the substance recovered from the neutral fraction is definitely not pure sarsasapogenin acetate and probably is an oxidation product, or contains such a substance.

Among other attempts to improve the yield of sarsasapogenoic acid, sarsasapogenin acetate (4 g.) was oxidized by the exact procedure worked out by McMillan and Noller[6] for the conversion of chlorogenin diacetate into the sapogenoic acid diacetate in good yield (32–33°), and the precipitated material was extracted with aqueous sodium carbonate from ether. Acidification of the extract gave 2 g. of solid, but no crystalline product could be obtained from this material. After gentle saponification, as in our original procedure,[2] and crystallization from dilute acetone 0.4 g. of crude sarsasapogenoic acid was obtained, m. p. 188–190°. The neutral fraction yielded (from aqueous acetone) 0.32 g. of needles, m. p. 162–166°. On conducting the oxidation (4 g. of acetate, 6 g. chromic anhydride) initially at 35–37° and then at 45°, no crystalline sapogenoic acid could be isolated.

Trials were made of the action of chromic anhydride on sarsasapogenoic acid acetate (0.85 g.) at 60–65° and at 90–95°, but in each instance the only crystalline product isolated from the acidic fraction after saponification was unchanged sarsasapogenoic acid (0.2 g.) and there was no appreciable neutral fraction, indicating the absence of the acetate lactone $C_{24}H_{36}O_4$.

An attempted Clemmensen reduction of sarsasapogenoic acid (0.5 g.), using 20 cc. of benzene, 70 cc. of alcohol, and 20 cc. of 6 *N* hydrochloric acid with fresh additions of concentrated acid (ten hours) gave a resin which formed dense clusters of fine needles (0.3 g.) from ether–hexane, but satisfactory crystals could not be obtained free from gum. Reduction with sodium and *n*-propyl alcohol gave an even less promising product.

Heated with semicarbazide acetate in alcohol for two hours, sarsasapogenoic acid gave only non-crystalline material. When treated with hydroxylamine under similar conditions, the methyl ester was recovered unchanged. Oximation of the methyl ester at 130° for three hours gave in a poor yield a substance crystallizing from dilute methanol in fine needles, m. p. 169–171° (Found: C, 66.96, 67.21; H, 8.68, 9.30; N, 6.41, 6.49. $C_{25}H_{40}O_5N_2$ requires C, 66.92; H, 8.99; N, 6.25). Because of the very low yield, the substance was not studied further.

Dioxime-Acid (III) from Sarsasapogenoic Acid.—A mixture of 500 mg. of sarsasapogenoic acid, 0.33 g. of hydroxylamine hydrochloride, and 0.5 g. of potassium acetate in 35 cc. of absolute methanol was heated in a sealed tube at 130° for three hours. The solution was concentrated and diluted with water and the finely divided solid which separated was collected, dried, and crystallized by dissolving it in about 150 cc. of alcohol and concentrating the solution to a volume of 35 cc. The product then separated in small leaves, and after a second crystallization there was obtained 350 mg. of pure material which, when dried in vacuum at 80° over phosphorus pentoxide, melted with decomposition at 247° in a bath preheated to 235°.

Anal. Calcd. for $C_{27}H_{44}O_5N_2$: C, 68.03; H, 9.31; N, 5.76; neut. equiv., 477. Found: C, 67.86, 67.88; H, 9.18, 9.16; N, 5.89, 5.98; neut. equiv., 461.

It seems necessary to adhere strictly to the conditions specified in order to obtain the pure dioxime in satisfactory yield, for non-crystalline products were obtained when the oximation was conducted at a slightly higher temperature or for a longer time, or on varying the proportion of the reagents. Attempted hydrolysis of the dioxime acid with methyl alcoholic hydrochloric acid gave no crystalline product.

Anhydrodi-(or tetra)-hydrosarsasapogenoic Acid (IV).—One-half gram of carefully purified sarsasapogenoic acid (from aqueous acetone, m. p. 190–191°) in 40 cc. of pure

(9) All melting points are corrected. Analyses by Mrs. Verna R. Keevil, Dr. C. Fitz, and the Arlington Laboratories.

acetic acid was shaken with hydrogen at atmospheric pressure in the presence of 0.1 g. of Adams catalyst. Absorption of hydrogen continued slowly for fifteen to twenty hours; the apparent consumption was about 1 mole of hydrogen, but this could not be determined accurately. The solvent was partially removed in vacuum, and after dilution with water a solid product was obtained. The dried material when crystallized from 90% acetone yielded 380 mg. of short needles, m. p. 171–178°. This material was apparently nearly pure; after four more crystallizations from the same solvent the melting point range was 174–184°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Calcd. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25. Found: C, 75.01, 74.97; H, 10.18, 10.06. Earlier analyses of a less highly purified sample gave the results: C, 75.59, 75.15; H, 9.92, 10.25.

The reduced acid is markedly less soluble in acetone than sarsasapogenoic acid. It is soluble in alkali but does not decolorize permanganate.

Although the absorption of hydrogen proceeded steadily if slowly in the above experiment, hydrogenation subsequently was accomplished only in a few of several trials with various preparations of catalyst. Even so it usually was necessary to continue shaking for a prolonged period, work up the mixture, separate a small amount of the less soluble reduced acid, and submit the recovered starting material to further hydrogenation. No reduction was observed with Raney nickel in absolute alcohol at 80° and 2000 lb. (133 atm.) pressure of hydrogen. Using Adams catalyst and glacial acetic acid, 1 g. of acid shaken with hydrogen at 90–100° and 500 lb. (33 atm.) pressure for six hours yielded 30 mg. of the acid IV, m. p. 173–183°.

Methyl Ester Acetate of IV.—Treated with diazomethane, the reduced acid IV gave an oily methyl ester mobile at −15°. On acetylation with acetic anhydride and potassium acetate this yielded a product which became crystalline on being moistened with methanol. Crystallization from aqueous methanol gave leaves melting at 64–66° with evolution of gas. After repeated crystallization from the same solvent the substance formed glistening hexagonal plates, m. p. 64–66°, dec. For analysis the sample was dried at room temperature over phosphorus pentoxide.

Anal. Calcd. for $C_{30}H_{46}O_5 \cdot {}^1/_2CH_3OH \cdot {}^1/_2H_2O$: C, 71.59; H, 9.65; solvent of crystallization, 4.89. Calcd. for $C_{30}H_{46}O_5 \cdot {}^1/_2CH_3OH \cdot {}^1/_2H_2O$: C, 71.31; H, 10.01; solvent of crystallization, 4.87. Found: C, 71.57, 71.50; H, 10.06, 9.93; loss in weight at 110° and 5 mm., 4.99, 4.97.

Methyl Ester Benzoate of IV.—The liquid methyl ester from 0.3 g. of the acid IV was heated with 1 cc. of benzoyl chloride in 4 cc. of dry pyridine for thirty minutes, and the mixture was diluted with water and extracted with ether. After washing with acid and alkali and evaporating the ether, the residue was crystallized from methanol. This gave silvery leaves, m. p. 136.5–139°, and after repeated crystallization the substance melted, when dried at 100° and 15 mm., at 138.5–140.5°.

Anal. Calcd. for $C_{35}H_{48}O_5$: C, 76.64; H, 8.87. Calcd. for $C_{35}H_{50}O_5$: C, 76.32; H, 9.15. Found: C, 76.37, 76.35; H, 9.06, 8.83.

Tetrahydroanhydrosarsasapogenoic Acid (VI). (a) **By Catalytic Hydrogenation of V.**—One-half gram of the anhydro acid (m. p. 244–246°, dec.) in 50 cc. of absolute alcohol with 0.1 g. of Adams catalyst was shaken with hydrogen at atmospheric pressure. The reaction proceeded at a regular rate and stopped with the absorption of 2 moles of gas in four to five hours. When concentrated and diluted with water the solution deposited 480 mg. of flat needles which effervesce on melting at 182°. Further crystallization from aqueous alcohol, ether–hexane, or benzene–acetone did not greatly change the decomposition point. Other preparations melted with effervescence at temperatures ranging from 179 to 188°. The product is soluble in alkali but does not decolorize permanganate.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25. Found: C, 74.99; H, 10.57.

(b) **With Sodium and Alcohol.**—A solution of 0.5 g. of the anhydro acid V in 20 cc. of *n*-propyl alcohol was refluxed for one hour while adding 2 g. of sodium in small portions. The solution was diluted with a large volume of water, acidified with dilute sulfuric acid, and the chalky precipitate was collected and crystallized from aqueous methanol. Needles were obtained (300 mg.) melting with effervescence at 170°. Further crystallization from aqueous methanol and from ether–hexane gave material melting at 181° with decomposition and showing no depression when mixed with the sample prepared by catalytic hydrogenation.

Methyl Ester Diacetate VII. (a) **From Tetrahydroanhydrosarsasapogenoic Acid.**—On treating 0.46 g. of the tetrahydro compound VI (prepared by catalytic hydrogenation) with diazomethane in ether there was obtained a non-crystalline residue. This was taken up in acetic anhydride (10 cc.) and heated with potassium acetate (0.1 g.). Treatment with water afforded a solid product and on crystallization from methanol this gave 380 mg. of lustrous leaves, m. p. 158–160°. Further crystallization from methanol and from ether–hexane raised the melting point (of the well-dried material) to 159.5–161°.

Anal. Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50; sapon. equiv., 177. Found: C, 72.58, 72.40; H, 9.33, 9.29; sapon. equiv., 181.

The same compound was obtained from a sample of VI prepared by reduction with sodium and alcohol; the purified substance melted at 160–162° and the mixed m. p. was 160–161.5°.

(b) **From the Methyl Ester of Anhydrosarsasapogenoic Acid.**—The oily ester prepared from V with diazomethane was hydrogenated as in (a) and the crude reaction product treated with acetic anhydride and potassium acetate. This gave material which crystallized from methanol as glistening leaves, m. p. 159–161°, and there was no depression when mixed with the first sample of diacetate described above.

Lactone Acetate VIII.—A solution of 200 mg. of tetrahydroanhydrosarsasapogenoic acid and 50 mg. of potassium acetate in 3 cc. of acetic anhydride was heated slowly to boiling during forty-five minutes; treatment with water gave 180 mg. of solid material. This crystallized from 80% methanol in flat needles, m. p. 200–203° (dried in vacuum at 80°).

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71; sapon. equiv., 228. Found: C, 76.58, 76.11; H, 9.77, 9.51; sapon. equiv., 233.

The compound is insoluble in cold alkali, saturated to permanganate and tetranitromethane, and does not react with diazomethane.

Lactone Benzoate IX.—The tetrahydro acid VI (100 mg.) was heated at 50–55° for one hour with benzoyl chloride (1 cc.) in pyridine (3 cc.). After decomposing the excess benzoyl chloride the mixture was extracted with ether and the ether layer was washed with acid and sodium bicarbonate solution, dried and evaporated. When the oily residue was moistened with petroleum ether and allowed to stand in the cold room for several days, 50 mg. of a fine powder separated, m. p. 218–223°, dec. Repeated crystallization from acetone–hexane gave flat, bluntly tapering needles melting gradually with slight yellowing at 225–235° (well dried).

Anal. Calcd. for $C_{34}H_{46}O_4$: C, 78.72; H, 8.94. Found: C, 78.54; H, 8.86.

The benzoate is insoluble in cold alkali and does not react with diazomethane.

Clemmensen Reduction of Anhydrosarsasapogenoic Acid: Neutral Product XIII.—A mixture of 500 mg. of the acid, 20 cc. of alcohol, 2 g. of amalgamated zinc, and 5 cc. of concentrated hydrochloric acid was refluxed for five and one-half hours, with the further addition during this period of two 5-cc. portions of acid. The solution was poured into water and the finely divided solid collected and taken into ether. After washing with water the solution was extracted with sodium hydroxide solution, the alkaline extract depositing 20 mg. of solid when acidified. The ethereal solution of the neutral fraction was evaporated, and the residue yielded a crystalline product (100 mg.), m. p. 212–219°, from ether–hexane. Twice recrystallized from ether–hexane, the substance was obtained as rectangular plates (75 mg.), m. p. 226–229° (dried in vacuum).

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.11; H, 10.39.

Acetylation of the Neutral Product: Acetate XIV.—After heating 50 mg. of the product XIII with acetic anhydride and potassium acetate, treatment with water gave a solid. This crystallized from aqueous methanol as long, flat needles, m. p. 212.5–214°. Repeated crystallization from the same solvent brought the m. p. of well dried material to 214–216°.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.32; H, 9.44.

This acetate is different from the lactone acetate VIII from tetrahydroanhydrosarsasapogenoic acid, for a mixture of the two samples began to melt somewhat below 185°.

Oxidation of Anhydrosarsasapogenoic Acid: Dibasic Acid X.—One-half gram of the anhydro acid V was suspended in 20 cc. of water and neutralized with sodium hydroxide. The solution was cooled to 0° and a 2% solution of potassium permanganate was dropped in with stirring until the color was no longer discharged, the end-point being very apparent. The solution was filtered through a thin layer of Norite to remove manganese dioxide and the nearly colorless filtrate was acidified with dilute sulfuric acid. The precipitate was dried at room temperature in vacuum over sulfuric acid and crystallized from ether–hexane, giving 350 mg. of prisms, m. p. 193–203°, dec. After two crystallizations from ether–acetone–hexane, it melted at 206–207°, dec.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46; neut. equiv., 238. Found: C, 68.20; H, 8.84; neut. equiv., 232.

Attempted Clemmensen reduction of the acid gave a non-crystalline product. Gas is evolved at the melting point of the dibasic acid, and this is not carbon dioxide. When heated at 235° under nitrogen the acid was converted into a brittle glass which was separated with bicarbonate into neutral and acidic fractions, but neither yielded crystals.

Dimethyl Ester XI.—The dibasic acid X (200 mg.) with excess diazomethane in ether gave a solid ester which crystallized from ether–hexane in fine, silky needles, m. p. 163.5–165°, and on recrystallization melted at 164.5–165° after thorough drying.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.79. Found: C, 69.26, 69.01; H, 8.84, 9.12.

The ester was recovered unchanged on attempted oximation in refluxing alcohol for three hours and in methanol at 130° for two hours.

Anhydro-oxime XII from the Dibasic Acid.—The acid X (200 mg.) was heated at 135° for three hours with an excess of hydroxylamine acetate in absolute methanol. The solvent was partially evaporated and the residue treated with water. The white solid was dried and taken into ether–acetone; on long standing there was obtained 0.2 g. of crystalline material, m. p. 263–266°, dec. (effervesces and becomes dark brown). Further crystallization from methanol–acetone gave glistening microneedles (95 mg.), m. p. 268°, dec. The substance gives a negative Legal test.

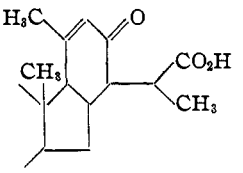
Anal. Calcd. for $C_{27}H_{39}O_6N$: C, 68.47; H, 8.30; N, 2.96. Found: C, 68.32; H, 8.88; N, 3.33.

Haloform Oxidation of the Dibasic Acid X.—A solution of 0.6 g. (m. p. 204–206°, dec.) of the dibasic acid in 15 cc. of dioxane and 6 cc. of 10% sodium hydroxide was treated at 60° with iodine–potassium iodide until the color persisted for two minutes.[7] No iodoform separated on dilution with water, but a halogen-containing acid was obtained on acidification. This was redissolved in 5% alkali and warmed with iodine–potassium iodide solution on the steam-bath, and after three minutes yellow crystals of iodoform (odor) separated, m. p. 117–122°. The alkaline solution was chilled and acidified, and the dried precipitate after two crystallizations from dilute acetone yielded 50 mg. of glistening, small leaves, m. p. 212–213°, dec. (gas evolution). Preliminary analyses gave the results: C, 66.82, 67.00; H, 8.75, 8.97; neut. equiv., 233; $C_{23}H_{34}O_7$ requires: C, 66.93; H, 8.09; $C_{25}H_{38}O_7$ requires: C, 66.63; H, 8.50. On attempting to recover the material used in determining the neutralization equivalent it was found that the substance had undergone alteration (isomerization?).

Summary

From a study of a number of transformation products of sarsasapogenoic acid and anhydro-

sarsasapogenoic acid it is concluded that the latter substance is an unsaturated γ-keto acid containing the grouping shown. The sapogenin and the sapogenoic acid very probably are correctly represented by the formulas of Tschesche and Hagedorn.



CONVERSE MEMORIAL LABORATORY
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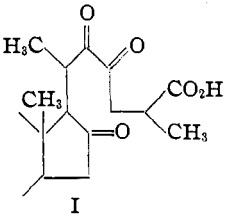
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Sarsasapogenin. III. Concerning Desoxysarsasapogenin and the Degradation of the C_{22}-Hydroxy Lactone

BY LOUIS F. FIESER AND ROBERT P. JACOBSEN[1]

Simpson and Jacobs[2] prepared desoxysarsasapogenin by reduction of the sapogenyl chloride with sodium and amyl alcohol, but the over-all yield from the sapogenin was only about 14% and the method was employed only after other possible methods of removing the hydroxyl group had been considered and abandoned as unsatisfactory. Attempted reduction of sarsasapogenone by the Wolff–Kishner method was unsuccessful, and Clemmensen reduction of the ketone was considered unsuitable because of the known sensitivity of the side chain to acids.[3] In our experiments on the acid cleavage of the side chain,[4] we had observed that this sensitivity is manifested particularly when acetic acid is employed as the solvent but that cleavage occurs much less readily in alcoholic solution. It therefore seemed possible that with this solvent reduction of the ketone might be accomplished at an acidity below that at which cleavage occurs. It was also thought that the addition of an immiscible solvent, as in Martin's[5] procedure, might provide added protection for the sensitive steroid. These hopes were realized, for on refluxing sarsasapogenone for five days with amalgamated zinc in a two-phase medium of alcohol, benzene, and 6 *N* hydrochloric acid, pure desoxysarsasapogenin was obtained in an over-all yield of 43.5% from sarsasapogenin.

The comparatively simple new method of preparing the desoxysapogenin may have other applications, and this consideration has prompted us to report the observation at the termination of the fellowship work of the junior author, even though the investigation of the desoxy compound has not yet reached a very advanced stage. In connection with our work on the oxidation of sarsasapogenin acetate,[4,6] we hoped to extend a brief observation of Simpson and Jacobs[2] and gain further information concerning the acidic substances resulting from the oxidation of desoxysarsasapogenin with chromic acid. Simpson and Jacobs were interested chiefly in the lactone $C_{22}H_{34}O_2$, which they obtained in 15–18% yield on conducting the reaction at 75°, but they investigated incidentally the acidic material encountered as a by-product. Neither the acidic product nor the material obtained from it on esterification yielded homogeneous crystals, but the oily ester on reaction with semicarbazide acetate gave in very small yield a pure substance having the composition of the disemicarbazone methyl ester of an acid characterized by the analyses as either $C_{27}H_{40}O_5$ or $C_{27}H_{42}O_5$. Simpson and Jacobs were inclined to accept the first formula and to regard the oxidation product as a triketo acid with one inert carbonyl group, as in I. A similar oxidation product of sarsasapogenin



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acetate was isolated by Farmer and Kon[7] as the methyl ester acetate, apparently of the acid $C_{27}H_{40}O_6$. We conducted the oxidation at the

(1) Du Pont Research Fellow.
(2) Simpson and Jacobs, *J. Biol. Chem.*, **110**, 565 (1935).
(3) Jacobs and Simpson, *ibid.*, **105**, 501 (1934).
(4) Fieser and Jacobsen, THIS JOURNAL, **60**, 28 (1938).
(5) Martin, *ibid.*, **58**, 1438 (1936).
(6) Fieser and Jacobsen, *ibid.*, **60**, 2753 (1938).
(7) Farmer and Kon, *J. Chem. Soc.*, 414 (1937).