3032 cu. Å. The number of glucose residues per unit cell is then

$$\frac{3032 \times 1.240 \times 6.06 \times 10^{-1}}{162.1} = 14.05 \cong 14$$

The monoclinic space groups allowed for an optically active molecule are C_2^1-$P2$, C_2^2-$P2_1$, and C_2^3-$C2$. Many reflections of the form (hkl) with $(h + k)$ odd are present so that C_2^3-$C2$ is eliminated. Intense reciprocal lattice goniometer patterns were made rotating the crystal about the zone [100]. All reflections of the form $(0kl)$ were observed to $(\sin\theta)/\lambda = 0.56$, except the odd orders of $(0k0)$, through (090), which were absent. The space group can then be taken as C_2^2-$P2_1$. This space group requires an even number of molecules per unit cell so we must have two molecules of seven glucose residues each.

Summary

1. The molecular weights of the Schardinger α- and β-dextrins have been accurately determined by X-ray diffraction and crystal density measurements.

2. The α-dextrin contains six glucose residues per molecule and has been renamed cyclohexaamylose; the β-dextrin contains seven glucose residues and has been renamed cycloheptaamylose.

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

Sterols. CXLVII. Sapogenins. LXI. The Bio-reduction of Steroids

BY RUSSELL E. MARKER, R. B. WAGNER AND PAUL R. ULSHAFER

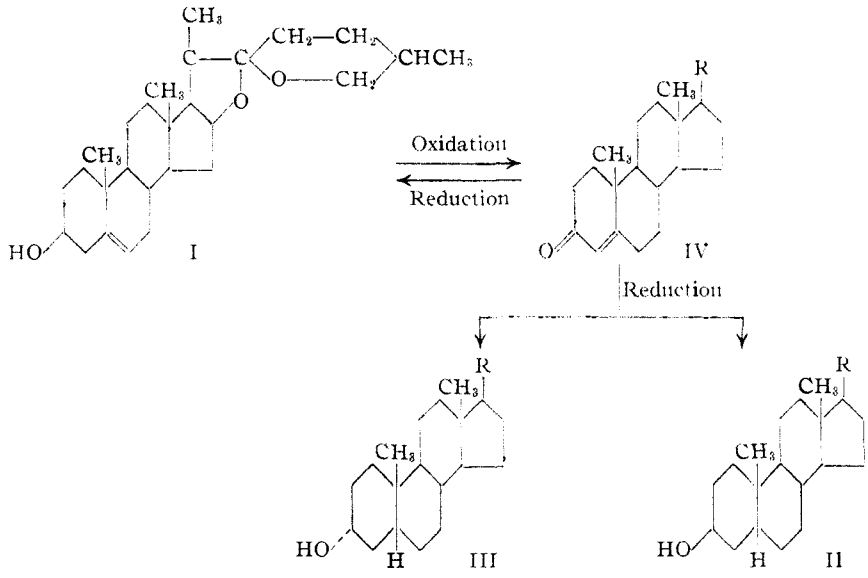
It has been postulated that Δ^5-3-hydroxysteroids arise as reduction products of Δ^4-3-ketosteroids under biological conditions.[1] Using Δ^4-dehydrotigogenone as a model substance for the bio-reduction process, we[3] have recently shown that this when administered to a dog on a biscuit diet gave diosgenin (I), smilagenin (iso-sarsasapogenin) (II) and *epi*-smilagenin (*epi*-iso-sarsasapogenin) (III). These results not only support our hypothesis, but also support the conception that cholestenone is an intermediate in the formation of coprosterol in the organism[1,5] since the products, (I), (II) and (III) correspond in nuclear structure to cholesterol, coprosterol and *epi*-coprosterol. The sapogenin derivatives having the characteristic side-chain act as effective indicators and are not subject to the suggestions of Fieser and Wolfe.[2]

We have now extended this work along the line of that of Rosenheim and Webster[4] who showed that β-sitosterol administered together with brain-powder to rats was converted into an isomeride of sitostanol which they named *copro*-sitostanol. The latter agrees in composition and properties with 24-ethylcoprostanol-3(β). Since a coprostane derivative has not been obtained directly from a Δ^5-3-hydroxysteroid by chemical action Rosenheim and Webster's experiment indicates a Δ^4-3-ketosteroid as a very probable intermediate for the formation of the *copro*-sitostanol.

Accordingly, diosgenin (I) was administered to a dog fed on a meat diet containing small portions of pig brain. The non-saponifiable fraction of the feces gave smilagenin (II) and *epi*-smilagenin (III) products which correspond to coprosterol and its epimer. This and the previous conversion support the hypothesis of Schoenheimer[5] that there is a reversible biological reaction of the type cholestenone $\rightleftarrows$ cholesterol. Δ^4-Dehydrotigogenone (IV) may be reduced by one enzyme system to smilagenin (II) and *epi*-smilagenin (III) or by another enzyme system converted to diosgenin (I). The present work indicates the reversible reaction involving the following oxidation–reduction mechanism; diosgenin (I) $\underset{[R]}{\overset{[O]}{\rightleftarrows}}$ Δ^4-dehydrotigogenone (IV) $\xrightarrow{[R]}$ smilagenin (II) and *epi*-smilagenin (III). These reactions are summarized in the accompanying chart.

Another observation in the present work is that the bio-reduction of keto-sapogenins gives hydroxy compounds of both alpha and beta configuration. This is contrary to the earlier statements[1] that reduction *in vivo* of 3-ketosteroids appears to give only alpha compounds. Tigogenone and sarsasapogenone having the cholestane and the coprostane configuration, respectively, were administered to a dog fed on a meat diet. In the case of the tigogenone the feces contained both

(1) Marker, THIS JOURNAL, **60**, 1725 (1938).
(2) Fieser and Wolfe, *ibid.*, **63**, 1485 (1941).
(3) Marker, Wittbecker, Wagner and Turner, *ibid.*, **64**, 818 (1942).
(4) Rosenheim and Webster, *J. Soc. Chem. Ind.*, **XL**, 486 (1941)
(5) Schoenheimer, Rittenberg and Graff, *J. Biol. Chem.*, **111**, 185 (1935).



tigogenin and *epi*-tigogenin. Similarly, the sarsasapogenone gave both sarsasapogenin and its epimer.

We thank Parke, Davis and Company for their assistance.

Experimental Part

The Biological Conversions of Diosgenin.—A 10-kg. dog was fed daily a mixture of 150 g. of meat, 50 g. of pig-brain and 3 g. of diosgenin for three consecutive days. After these feedings the dog was maintained for three additional days on a meat diet. Its feces were collected during the entire period and immediately ground up in acetone. The residue was further extracted with ether. The solvent was removed and the residue hydrolyzed with alcoholic potassium hydroxide. The product was extracted with ether and the ethereal solution washed with water. The solvent was removed and the residue was refluxed with 30 cc. of acetic anhydride for thirty minutes. After cooling the crystalline product was filtered and washed with cold ether; m. p. and mixed m. p. with an authentic sample of diosgenin acetate, 197–200°; yield 5.2 g.

The filtrate was vacuum distilled and the residue was refluxed for thirty minutes with alcoholic potassium hydroxide. Water was added and the product extracted with ether. The solvent was removed and the residue was heated on a steam-bath for two hours with 50 cc. of pyridine and 10 g. of succinic anhydride. After cooling, ether was added and the pyridine was removed by washing the ethereal solution with dilute hydrochloric acid. The ether layer was then washed well with potassium carbonate solution. The aqueous layer was acidified and extracted with ether. The solvent was removed and the residue was refluxed with alcoholic potassium hydroxide for thirty minutes. Water was added and the product was extracted with ether. The solvent was removed and the residue was dissolved in a small amount of 95% alcohol. To this was added a solution of 15 g. of digitonin in 700 cc. of 95% ethanol. After standing for two hours in the ice-box the digitonide was filtered and washed well with alcohol. The filtrate was concentrated to about 50 cc., ether was added and the excess digitonin filtered off. The filtrate was washed well with water, the solvent was removed and the residue was crystallized from methanol, m. p. and mixed m. p. with an authentic sample of *epi*-smilagenin 217–220°; yield 0.2 g. of good material.

When refluxed with acetic anhydride it gave an acetate which was crystallized from methanol and from acetone; m. p. and mixed m. p. with an authentic sample of *epi*-smilagenin acetate, 158–160°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 76.0; H, 10.2.

The digitonide was decomposed with pyridine and the product thus obtained was converted to the acetate and crystallized from ether to give an additional crop of diosgenin acetate. The filtrate from this was fractionally crystallized from methanol and from acetone to give a product; m. p. and mixed m. p. with an authentic sample of smilagenin acetate, 127–130°; yield 0.1 g. of good material.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.8; H, 10.0.

Sarsasapogenone.—The dog was fed meat containing 7 g. of sarsasapogenone divided into three daily feedings. Its feces were collected for six consecutive days and immediately ground up with acetone. After a thorough extraction with acetone the feces were extracted with ether. The combined extracts were evaporated and the residue was hydrolyzed with alcoholic potassium hydroxide for thirty minutes. Water was added and the product was extracted well with ether. The solvent was removed and the residue was heated on a steam-bath for two hours with 25 cc. of pyridine and 10 g. of succinic anhydride. Ether was added and the pyridine was removed by washing the ethereal solution with dilute hydrochloric acid. The succinates were removed by shaking with potassium carbonate solution. The aqueous layer was acidified, extracted with ether and the solvent removed. The residue was hydrolyzed by refluxing with alcoholic potassium hydroxide. The neutral fraction was extracted with ether and the solvent was removed; yield 2.7 g.

The residue was dissolved in alcohol and 10 g. of digitonin in 500 cc. of ethanol was added. After standing at room temperature for several hours the digitonide was filtered, washed with alcohol and dried. It was then decomposed by warming on a steam-bath for one hour with pyridine. Ether was added and the digitonin was filtered. The filtrate was washed well with water and dilute hydrochloric acid. The solvent was evaporated and the residue was crystallized from ethanol and from methanol; m. p. and mixed m. p. with an authentic sample of sarsasapogenin, 199–200°.

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).