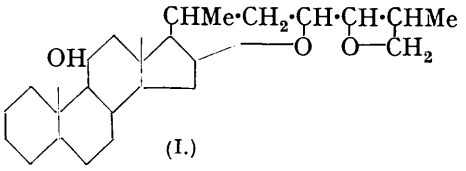
303. *Sapogenins. Part I. The Sapogenins of Sarsaparilla Root.*

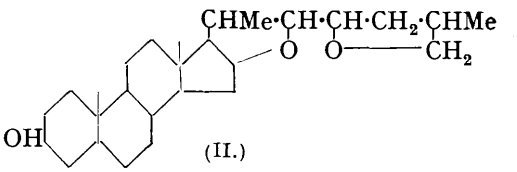
By F. A. Askew, S. N. Farmer, and G. A. R. Kon.

The so-called neutral sapogenins of *Digitalis* seeds (digitogenin, gitogenin, and tigogenin) are of considerable interest in view of their close connexion with the cardiac aglucones. Jacobs and Simpson (*J. Biol. Chem.*, 1934, **105**, 501) have shown that sarsasapogenin belongs to this group of compounds because both it and gitogenin yield on dehydrogenation with selenium 3′-methyl*cyclo*pentenophenanthrene together with a ketone $C_8H_{16}O$, which evidently represents the side chain common to these compounds. From the results of

degradation experiments (*ibid.*, 1935, **109**, 573) these authors tentatively put forward the formula (I) for sarsasapogenin. A similar structure (II) is adopted by Tschesche and



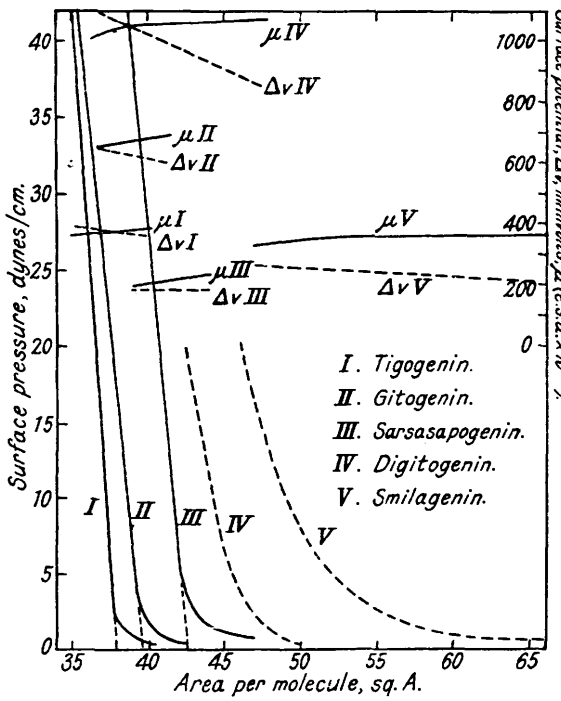
(I.)



(II.)

Hagedorn (*Ber.*, 1935, **68**, 1412, 2247; 1936, **69**, 797) for tigogenin, but the hydroxyl group common to it and the other two *Digitalis* sapogenins is placed on C_3, the position it occupies in all the sterols and cardiac aglucones. Such a formulation is supported by the formation of a ketone on heating the dibasic acid (gitogenic acid) produced by the oxidation of both tigogenin and gitogenin; by analogy with the behaviour of other sterol derivatives, this indicates that ring I has been opened. The opening of the ring takes place between the two hydroxyl-bearing carbon atoms of gitogenin, and the previous work of Windaus and Willerding (*Z. physiol. Chem.*, 1925, **143**, 33) and of Windaus and Linsert (*ibid.*, 1925, **147**, 277) can only be interpreted on the assumption that this takes place between C_3 and either C_2 or C_4. It has been proved that tigogenin is a cholestane derivative (*i.e.*, rings I and II are fused in the *trans*-position), and the opening of ring I in such a compound would be expected to take place preferentially between C_2 and C_3. It is also suggested that the precipitation of tigogenin by digitonin excludes a hydroxyl on C_4, as neither of the 4-cholestanols is precipitated; and tigogenone is not isomerised by acids or alkalis. The latter argument does not appear to be valid, because isomerisation is only to be expected, by analogy with the α-decalones, in an α-ketone of the *cis*-series. The evidence is thus fairly conclusive, even though it does depend on analogy to a great extent.

FIG. 1.



It occurred to us that valuable additional evidence regarding the position of the hydroxyl group in these compounds would be furnished by surface film measurements. Through the kindness of Dr. R. Tschesche we were able to examine the three *Digitalis* sapogenins and to compare them with sarsasapogenin and a closely related compound, *smilagenin*, which we have isolated from Jamaica sarsaparilla root.

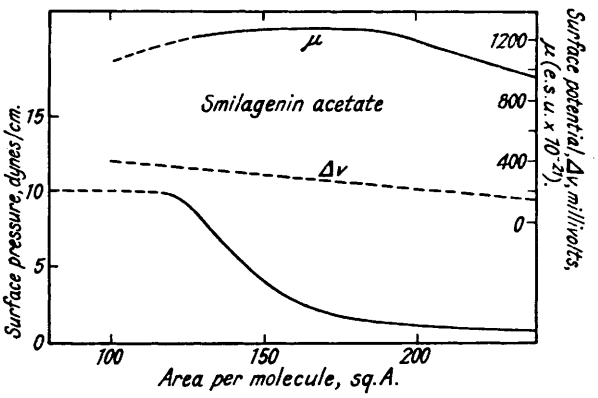
The measurements of surface pressure and potential were carried out as described by Adam, Askew, and Danielli (*Biochem. J.*, 1935, **29**, 1786), the results being summarised in Fig. 1. Three of the compounds, tigogenin, gitogenin, and sarsasapogenin, form stable, condensed, liquid films of small compressibility, having limiting areas at zero pressure of 38, 39·5, and 42 sq. A. respectively. These areas are close to those obtained with 3-hydroxy-compounds of the sterol series (Adam *et al.*, *loc. cit.*). Experience with films of sterols has shown that the area of 38 sq. A. cannot easily be reconciled with any other structure than that of a derivative of cholestane with a water-attracting group on C_3. The accepted

formulæ for tigogenin and gitogenin are thus fully confirmed. Examination of models shows that, for such compounds, either alteration of the ring structure from the cholestane to the coprostane type, or a shifting of the hydroxyl group from C_3 to C_2 or C_4 would increase the minimum cross-sectional area, measured on models, to about 40 sq. A. Thus the limiting area, 42 sq. A., found for sarsasapogenin would also be consistent with these alternative formulations; it is, however, not consistent with formulæ in which the hydroxyl group is far removed from the end of the molecule, as it would be on C_{11}. Films of such compounds would be expected to occupy much larger areas than those observed for sarsasapogenin, and to be much more compressible (compare the behaviour of cholestan-6-ol and $\Delta^{4:5}$-cholesten-7-ol, or ψ-cholesterol; Adam *et al.*, *loc. cit.*).

The other two compounds, digitogenin and smilagenin, form films which tend to occupy rather larger areas, but rapidly contract under slight increase of pressure, so that accurate measurements of area cannot be made. The condensed films formed in both cases are solid, but partially collapsed when examined ultramicroscopically. This behaviour is common among the structurally rather similar resinols, but is not yet fully understood.

The oxygen-containing rings in the side chain do not appear to exert much water-attraction, none of the hydroxyl compounds showing any tendency to form gaseous films (compare derivatives of oestrone; Adam, Danielli, Marrian, and Haslewood, *Biochem. J.*, 1932, **26**, 1233; Danielli, Marrian, and Haslewood, *ibid.*, 1933, **27**, 311). The *acetate* of smilagenin, however, in which the water-attracting properties of the hydroxyl group are diminished by acetylation, forms films of the gaseous type, the pressure rising continuously from low values at very large areas to 10 dynes/cm. at 120 sq. A. per molecule, at which area the film abruptly begins to collapse (Fig. 2). These data indicate that in this compound the molecules are lying flat on the surface, 120 sq. A. being close to the estimated minimum area occupied by a molecule of the sterol type oriented in this way (Adam *et al.*, *loc. cit.*). There is little difference between the areas occupied by tigogenin and gitogenin, although the latter has a second hydroxyl group on C_2; the films of gitogenin are, however, somewhat more compressible, and show much higher values of surface potential and μ.

FIG. 2.



These results thus fully confirm Tschesche and Hagedorn's formulæ for the *Digitalis* sapogenins, but make it necessary to revise formula (I) for sarsasapogenin, the hydroxyl group of which can only be accommodated on C_2, C_3, and C_4. The last position, although apparently in accord with the formation of a ketonic acid on oxidation (Jacobs and Simpson, *J. Biol. Chem.*, 1935, **109**, 573), is not probable, because it is now found that sarsasapogenin is precipitated by digitonin. We also find that sarsasapogenone is not isomerised by fairly vigorous treatment with alkali; this would indicate that it is not an α-ketone, but only if sarsasapogenin is a coprostane derivative. In any case, a difference in the position of the hydroxyl group is not sufficient to account for the difference between sarsasapogenin and tigogenin, because the hydroxyl-free lactones, $C_{22}H_{34}O_2$, which have been obtained from them (Jacobs and Simpson, *J. Biol. Chem.*, 1935, **110**, 565; Tschesche and Hagedorn, *Ber.*, 1935, **68**, 1412) are not identical; further experimental evidence on this point is required.

Smilagenin is clearly closely related to sarsasapogenin and was doubtless encountered by Power and Salway (J., 1914, **105**, 201) in the course of their examination of Jamaica sarsaparilla, although they referred to it as sarsasapogenin; the two compounds are, however, distinct, and the new name is suggested to obviate confusion. Smilagenin is more soluble and lower-melting than its isomeride, and differs from it in its rotation; both its

benzoate and its acetate, however, melt higher than those of sarsasapogenin. Smilagenin is also precipitated by digitonin, although very slowly, and differs in this respect from epi-*sarsasapogenin*, which we have prepared for comparison. The *deoxy*-compound, obtained by the reduction of *smilagenyl chloride*, appears to be different from deoxysarsasapogenin, and this would indicate that the two aglucones differ in some manner more fundamental than the steric arrangement of the hydroxyl group.

Experimental.

Sarsasapogenin.—The isolation of this compound from Vera Cruz sarsaparilla was carried out as described by Jacobs and Simpson (*loc. cit.*); the purification of the crude aglucone is, however, most conveniently carried out by running a 4—5% benzene solution through a column of Merck's activated alumina, which absorbs the coloured impurities, and recovering the purified compound by evaporation under reduced pressure. The m. p. is found to be 197—198° after crystallisation from acetone, or somewhat lower than observed by Jacobs and Simpson, but the rotation * agrees well with their value: $[\alpha]_D^{25°}$ − 75°, $[\alpha]_{5461}^{25°}$ − 89°, $\alpha_{5461}/\alpha_D = 1{\cdot}18$ ($c = 0{\cdot}498$ in chloroform).

Digitonide. A mixture of 100 mg. of digitonin in 10 c.c. of 90% alcohol and 28 mg. of sarsasapogenin in 3·8 c.c. of 95% alcohol was kept at room temperature for 2 days, 48 mg. of an amorphous precipitate having then separated. This was dissolved in a little pyridine, and the digitonin precipitated with ether; from the filtered solution 6 mg. of sarsasapogenin were recovered and identified.

Acetate. The aglucone was boiled with an excess of acetic anhydride for an hour, the reagent distilled off under reduced pressure, and the residue recrystallised from methanol, in which it is sparingly soluble, forming flattened needles up to 1 cm. in length, m. p. 144—145°; the lower-melting preparations described by previous authors were evidently impure. The rotation is $[\alpha]_D^{25°}$ − 70·2°, $[\alpha]_{5461}^{25°}$ − 83·1° $\alpha_{5461}/\alpha_D = 1{\cdot}18$ ($c = 1{\cdot}183$ in chloroform) (Found: C, 76·1; H, 10·4. $C_{29}H_{46}O_4$ requires C, 75·9; H, 10·1%).

Benzoate. No benzoate was formed on treating sarsasapogenin as described by Kaufmann and Fuchs (*Ber.*, 1923, **56**, 2527), and the compound of m. p. 124° which they obtained cannot have been the pure *ester*. This was obtained by keeping a solution of 0·3 g. of aglucone and 0·7 g. of freshly distilled benzoyl chloride in 9 c.c. of pyridine for 3 hours, then adding an excess of dilute sulphuric acid. The crude solid was digested with methanol and recrystallised from ethyl alcohol, forming stout transparent rods, m. p. 170—171° (Found: C, 78·6; H, 9·1. $C_{34}H_{48}O_4$ requires C, 78·4; H, 9·3%).

epi*Sarsasapogenin.*—A solution of 0·25 g. of sarsasapogenone in 200 c.c. of ether was kept vigorously stirred and treated with 7·5 g. of sodium in several portions, water being run in dropwise at the same time. The dried ethereal solution gave on evaporation a solid residue, which was recrystallised once from *cyclo*hexane and twice from acetone; it formed needles, m. p. 204—206° (mixed m. p. with sarsasapogenin about 187°) and gave no precipitate with digitonin (Found: C, 77·5; H, 10·6. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·7%); $[\alpha]_D$ − 71°, $[\alpha]_{5461}$ − 84° ($c = 0{\cdot}721$ in chloroform); $[\alpha]_{5461}/[\alpha]_D = 1{\cdot}19$. Sarsasapogenin was recovered unchanged after a similar treatment with sodium in moist ether.

Acetate. This was prepared as described above, and crystallised from acetone in plates, m. p. 192—193° (Found: C, 76·1, 75·9; H, 10·1, 10·1. $C_{29}H_{46}O_4$ requires C, 75·9; H, 10·1%).

Smilagenin.—Powdered grey Jamaica sarsaparilla was extracted with alcohol, and the extract concentrated as decribed by Jacobs and Simpson (*loc. cit.*); no solid genin was precipitated after acid hydrolysis even on cooling with ice. The liquid was then repeatedly shaken with benzene and water, causing a copious precipitation of a brick-red solid, which was removed by filtration. The benzene solution was separated from the aqueous one, roughly dried, and passed through a long column of active alumina, the column being successively coloured brown, brownish-red, and pink; there was a greenish colouring matter at the bottom of the column, which was not retained. The benzene solution on evaporation deposited *smilagenin* in very poor yield (about 1 g. from 10 lb. of root). It was fractionally crystallised from acetone, long silky needles, m. p. 183—184° (m. p. 180° on mixing with sarsasapogenin), being obtained; no other compounds appeared to be present. It is more soluble than sarsasapogenin and appears to form a hydrate when crystallised from methyl alcohol (compare Power and Salway, *loc. cit.*)

* We are indebted to Dr. R. K. Callow, of the National Institute for Medical Research, Hampstead, for determining the rotations recorded in this paper.

(Found : C, 77·6, 77·7; H, 10·7, 10·9. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·7%). The rotation of smilagenin in chloroform is lower than that of sarsasapogenin, but higher in methyl alcohol : $[\alpha]_D^{25°}$ — 69°, $[\alpha]_{5461}^{25°}$ — 80°, α_{5461}/α_D = 1·17 (c = 0·311 in chloroform), $[\alpha]_D^{25°}$ — 61°, $[\alpha]_{5461}^{25°}$ — 70°, α_{5461}/α_D = 1·15 (c = 0·339 in methyl alcohol).

Digitonide. The precipitation with digitonin was carried out as described above, but the mixture had to be kept for 6 days; a comparable yield of precipitate was then obtained. It was decomposed as before, and the aglucone identified by mixed m. p.

The *acetate*, prepared as described on p. 1402, crystallised from methanol in fine silky needles resembling the aglucone in appearance, m. p. 150—151° (mixed m. p. with sarsasapogenin acetate *ca.* 118°), $[\alpha]_D^{25°}$ — 59·6°, $[\alpha]_{5461}^{25°}$ — 68·9° (c = 0·251 in chloroform), $[\alpha]_{5461}/[\alpha]_D$ 1·16 (Found : C, 76·1; H, 10·1. $C_{29}H_{46}O_4$ requires C, 75·9; H, 10·1%).

The *benzoate*, prepared in pyridine solution, crystallised from ethyl alcohol in fine needles, m. p. 181—181·5° (mixed m. p. with sarsasapogenin benzoate *ca.* 153°) (Found : C, 78·5; H, 9·3. $C_{34}H_{48}O_4$ requires C, 78·4; H, 9·3%).

Smilagenone.—0·5 G. of smilagenin in 6 c.c. of acetic acid and 0·3 c.c. of water were treated during an hour with 0·13 g. of chromic acid in 2 c.c. of acetic acid and 0·27 c.c. of water, added dropwise. The solution was then kept at 55° for 15 mins., diluted with water, and extracted with ether. The extract gave the *ketone* on washing, drying, and evaporation; fine needles, m. p. 156—157°, from acetone (Found : C, 77·8; H, 10·3. $C_{27}H_{42}O_3$ requires C, 78·2; H, 10·2%). The ketone forms an oxime, crystallising in long glistening needles, m. p. 189°.

Smilagenyl Chloride.—8 G. of phosphorus pentachloride in 7 c.c. of carbon disulphide were run during 25 mins. into an ice-cold solution of 1 g. of smilagenin (dried in a vacuum at 110° for an hour) in 8 c.c. of dry chloroform. The solution was treated with water, washed with sodium carbonate, dried, and evaporated. The residue of *chloride* (0·2 g.) formed fine needles, m. p. 194—195°, after crystallisation from acetone (Found : C, 74·6; H, 10·0; Cl, 8·1. $C_{27}H_{43}O_2Cl$ requires C, 74·5; H, 10·2; Cl, 8·2%).

Deoxysmilagenin.—The chloride (0·16 g.) in 16 c.c. of boiling amyl alcohol was gradually treated with 1 g. of sodium, the solution being then boiled for a further 1½ hours. The cooled solution was washed with water and distilled in steam, the residue in the flask being extracted with ether. On evaporation and crystallisation from methyl alcohol, the *deoxy*-compound was obtained in long needles, m. p. 132—133° (Found : C, 81·3; H, 11·1. $C_{27}H_{44}O_2$ requires C, 80·9; H, 11·1%).

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