Stereochemistry of the Side-chain of the Steroidal Sapogenins : New Isomers of the Normal- and iso-*Sapogenins.*

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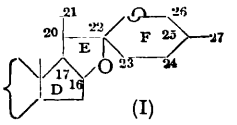
ψ-Sapogenins treated in solution with a trace of hydrochloric acid are converted by ring closure into isomers that are not identical with the original natural sapogenins. These *cyclo*-ψ-sapogenins are, under slightly acid conditions, in equilibrium with a small proportion of the open-chain ψ-sapogenins. Acetylation of the *cyclo*-ψ-compounds in pyridine gives monoacetates : boiling acetic anhydride yields diacetates of the ψ-compounds. Eight such *cyclo*-ψ-compounds have been prepared and their optical rotations and infrared absorption have been studied. The *cyclo*-ψ-compounds are believed to differ from the parent *iso*- or normal sapogenins only by virtue of stereoisomerism at $C_{(20)}$ or $C_{(22)}$ or both, but the configuration at these centres cannot yet be defined.

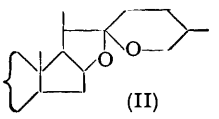
Steroidal sapogenins possessing the intact *spiro*ketal side chain were classified by Marker and his colleagues as " normal " or " *iso* " on the basis of their stability to acids ; a normal sapogenin gave rise to the corresponding *iso*-sapogenin on long treatment with mineral acid. This classification is supported by the infrared spectra of the sapogenins ; members of the normal series show in the 800—1100-cm.$^{-1}$ region a common absorption pattern that differs in detail from that displayed by the *iso*-compounds (Wall, Eddy, McClennan, and Klumpp, *Analyt. Chem.*, 1952, **24**, 1337 ; Jones, Katzenellenbogen, and Dobriner, *J. Amer. Chem. Soc.*, 1953, **75**, 158).

Corresponding members of the two series were thought to differ only in their configurations at $C_{(22)}$, as expressed in the partial formulæ (I) and (II) for the side-chains of the

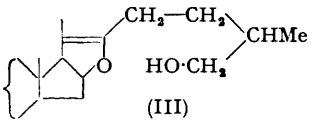
normal and the *iso*-compounds respectively. The evidence for this rested on the interconversion of such isomers with mineral acid and on the reported identity of the ψ-sapogenins (III) and dihydrosapogenins (IV) obtained from isomeric pairs by treatment with acetic anhydride and by catalytic hydrogenation respectively (cf. Fieser and Fieser, " Natural Products related to Phenanthrene," Reinhold, New York, 1949, p. 587; Shoppee and Shoppee, " Chemistry of Carbon Compounds," ed. E. H. Rodd, Vol. 2B, Elsevier, Amsterdam, 1953).



(I)



(II)



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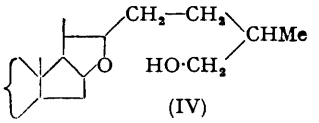


(IV)

In 1953 Scheer, Kostic, and Mosettig (*J. Amer. Chem. Soc.*, 1953, **75**, 4871) showed that, contrary to Marker's findings, sarsasapogenin (normal) and smilagenin (*iso*) furnished different ψ-sapogenins and dihydro-derivatives.* They isolated the $C_{(22)}$–$C_{(27)}$ chain of sarsasapogenin by degradation of the ψ-derivative and obtained (+)-α-methylglutaric acid; smilagenin, treated similarly, gave (−)-α-methylglutaric acid. It was thus demonstrated convincingly that the configuration at $C_{(25)}$ differs in the two sapogenins. It has been shown more recently, by similar means, that other normal sapogenins, *viz.*, *neo*tigogenin and sisalagenin (Callow and James, *J.*, 1955, 1671) belong to the same series as sarsasapogenin and that the *iso*-sapogenins, tigogenin, hecogenin, and diosgenin are similar to smilagenin in respect of configuration at $C_{(25)}$ (James, *Chem. and Ind.*, 1953, 1388; Callow and James, *loc. cit.*). The observation that samogenin and its normal isomer, markogenin, give different ψ-derivatives (Wall, Eddy, Serota, and Mininger, *J. Amer. Chem. Soc.*, 1953, **75**, 4437) is also in accordance with the generalisation that the normal and *iso*-sapogenins have opposite configurations at $C_{(25)}$ (cf. Farmer and Kon, *J.*, 1937, 414).

This conclusion calls for an explanation of the interconversion of normal and *iso*-compounds, which, it now appears, involves inversion at $C_{(25)}$, a centre that might be expected to be highly stable. A re-investigation of the conversion of normal into *iso*-sapogenins by mineral acid forms the subject of a separate paper (Callow and James, *loc. cit.*); we deal here with the alleged conversion of ψ-*iso*-sapogenins into a mixture of the corresponding normal and *iso*-compounds (Marker and Lopez, *J. Amer. Chem. Soc.*, 1947, **69**, 2373).

Observations made independently in our two laboratories led to a re-examination of the action of acid in trace amounts on ψ-sapogenins in solution and, as a result, new types of isomers have been discovered. Preliminary notes on this work have been published (Callow and James, *Chem. and Ind.*, 1954, 691; Dickson, Elks, Evans, Long, Oughton, and Page, *ibid.*, p. 692), and similar observations have been made in other laboratories on isomers of sarsasapogenin and smilagenin (Wall, Eddy, and Serota, *J. Amer. Chem. Soc.*, 1954, **76**, 2849; Wall and Serota, *ibid.*, p. 2850) and of diosgenin (Ziegler, Rosen, and Shabica, *ibid.*, p. 3865).

In one of our preliminary communications (Dickson *et al.*, *loc. cit.*) the name " *ana* "-sapogenin was suggested for the new isomers, which had then been obtained only in the *iso*-series. It is inappropriate to use the same name to cover the compounds since obtained from normal sapogenins, since there is certainly a difference in configuration at $C_{(25)}$ and there may be at $C_{(20)}$ or $C_{(22)}$. We would prefer, therefore, to adopt the suggestion made by Taylor (*Chem. and Ind.*, 1954, 1066) and to refer to the new isomers as *cyclo*-ψ-normal

* Trivial names are used for sapogenins throughout this paper; their constitutions are shown in Table 1.

or *cyclo*-ψ-*iso*-sapogenins; this nomenclature preserves the distinction between the $C_{(25)}$ isomers and, at the same time, suggests how the compounds arise.

Marker and Lopez (*loc. cit.*) treated a number of ψ-*iso*-sapogenins in ethanolic solution with hydrochloric acid, added to a concentration of about 0·5N, and kept the solutions overnight in a refrigerator. The products, precipitated by water and recrystallised, yielded *iso*-sapogenins, and from the mother-liquors were isolated "*neo*"-sapogenins, which had lower melting points and, refluxed with acetic anhydride, yielded acetates of "*neo*"(*i.e.*, normal)-sapogenins. Our own findings were in complete contrast. We observed, for example, that adding hydrochloric acid, to a concentration of, say, 0·01N, to a solution of ψ-hecogenin in ethanol led to a change in specific rotation from +100° to a steady value of +35·5° within 3 hours. A similar change occurred in ethyl acetate solution. The conditions of the reaction were modified for preparative purposes and the product was found to be a new isomer, *cyclo*-ψ-hecogenin; the only other material found was a small amount of hecogenin in the mother-liquors. A precise repetition of Marker and Lopez's experiment with ψ-hecogenin again yielded the new isomer as the sole product; no normal sapogenin could be found in this or in any other cyclisation of a ψ-*iso*-sapogenin.

cyclo-ψ-Hecogenin was distinguished from hecogenin by a lower melting point and a higher optical rotation and by many differences in the infrared absorption spectrum (see below).

cyclo-ψ-Hecogenin forms a monoacetate (presumably at the 3-hydroxy-group) on treatment with acetic anhydride and pyridine. The infrared spectra of the free alcohol and the acetate are similar in the 800—1000-cm.$^{-1}$ region. Elementary analysis of both alcohol and acetate indicates that *cyclo*-ψ-hecogenin is isomeric with ψ-hecogenin and hence with hecogenin itself.

cyclo-ψ-Hecogenin resembles hecogenin and differs from the open-chain ψ-compound in having no free hydroxyl group at $C_{(26)}$ (as shown by formation of a monoacetate), by its transparency at *ca.* 217 mμ (cf. Cameron, Evans, Hamlet, Hunt, Jones, and Long, *J.*, in the press) and by its failure to give a colour with tetranitromethane. The ease of formation of *cyclo*-ψ- from ψ-hecogenin is best explained by assuming that it arises by reclosure of ring F to give a *spiro*ketal differing from the parent *iso*-sapogenin only in the configuration at $C_{(20)}$ or $C_{(22)}$ or both.

cyclo-ψ-Hecogenin is efficiently converted into hecogenin by brief treatment with boiling alcoholic hydrogen chloride. On treatment with boiling acetic anhydride, it gives a high yield of ψ-hecogenin diacetate; in this behaviour it differs from the *iso*-sapogenin, which requires prolonged treatment with acetic anhydride at about 200° to produce the ψ-derivative.

When ψ-tigogenin, 11-oxo-ψ-tigogenin, ψ-smilagenin, and ψ-diosgenin (all ψ-*iso*-sapogenins) were submitted to mild acid treatment, they too yielded *cyclo*-ψ-compounds with properties, both physical and chemical, similar to those of *cyclo*-ψ-hecogenin. In particular, all the *cyclo*-ψ-*iso*-sapogenins showed characteristic infrared absorption between 800 and 1100 cm.$^{-1}$. This is discussed in more detail below.

When ethanolic solutions of ψ-normal sapogenins were treated with a trace of acid only small, or even undetectable, changes in optical rotation occurred within a short period. Nevertheless, addition of water caused precipitation of *cyclo*-ψ-compounds with optical rotations similar to those of the ψ-compounds from which they were derived. The *cyclo*-ψ-compounds of the normal series have a characteristic type of infrared absorption spectrum different from that of the *cyclo*-ψ-*iso*-derivatives. Like the latter, they yield diacetates of the parent ψ-sapogenins when refluxed with acetic anhydride, and monoacetates of *cyclo*-ψ-sapogenins with acetic anhydride in pyridine. When they are boiled with dilute hydrochloric acid the normal sapogenins are obtained. *cyclo*-ψ-Sarsasapogenin, *cyclo*-ψ-*neo*tigogenin, and *cyclo*-ψ-sisalagenin were prepared from the corresponding ψ-compounds and are described in the Experimental section.

A study of the properties of solutions of ψ- and *cyclo*-ψ-sapogenins has disclosed the existence in acid medium of an equilibrium between the two types. Acidification of an alcoholic solution of either ψ-hecogenin or *cyclo*-ψ-hecogenin caused the rotation to change to an intermediate value, corresponding to about 1 part of the former and 9 of the latter.

A similar observation was made with 11-oxotigogenin derivatives. In the normal series, the rotations of the ψ- and *cyclo*-ψ-compounds were too close together to allow the method to be used.

Again, acidification of an alcoholic solution of 11-oxo*cyclo*-ψ-tigogenin caused the appearance of an absorption maximum at 216 mμ with about one-tenth of the intensity of that of 11-oxo-ψ-tigogenin; a solution of the latter, on acidification, gave the same reduced absorption. The pair of isomers derived from sarsasapogenin behaved similarly, indicating that the same equilibrium exists in the normal series. With other compounds results were less clear cut; the same general effect was noted, but the weak maxima were either displaced or obscured by an increased general absorption.

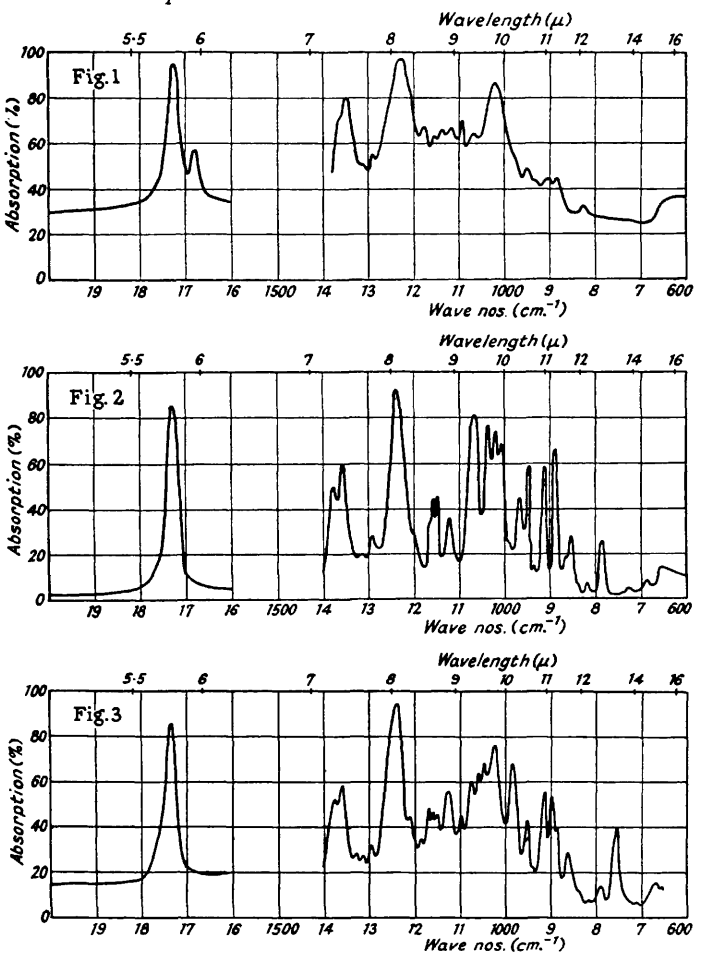
Further evidence came from paper-chromatographic studies. In neutral or pyridine-containing solvents the sapogenins used in this work behaved in the expected manner as pure compounds: isomeric normal, *iso*-, and *cyclo*-ψ-compounds differing only in the side chains could not easily be distinguished from one another, but the ψ-*iso*- and ψ-normal compounds were much more polar and, although differences between them individually could hardly be observed, as a group they could easily be distinguished from the other four isomers. In acidic solvents the ψ-sapogenins gave two spots, one behaving like the ψ-compound, the other like the *cyclo*-ψ-compound. Similarly, *cyclo*-ψ-compounds could be resolved under these conditions into two components, the more polar behaving as a ψ-compound. This is further evidence that the ψ-sapogenins form in an acidic medium an equilibrium mixture with the *cyclo*-ψ-compounds and confirmed our previous conclusion that the latter predominate. In the solvents used for chromatography the transformation of one compound into the other is not so quick that separation of the components is prevented, but we noticed that the ψ-normal sapogenins were more difficult to trace in such mixtures than the ψ-*iso*-compounds. We attribute this difference to the further changes suffered by the equilibrium mixtures, which are themselves of limited stability and pass finally into the natural normal and *iso*-sapogenins. We believe that this state is reached more rapidly in the normal series, so that the ψ-normal sapogenins are more difficult to detect in acid solutions than are the ψ-*iso*-compounds.

Comparison of the infrared spectra of the *cyclo*-ψ-sapogenins with those of the other classes of isomers leads to certain conclusions. In the spectra of normal sapogenins the 920—915-cm.$^{-1}$ absorption band has 3—4 times the intensity of the 899—894-cm.$^{-1}$ band, whereas in the *iso*-compounds the relations are reversed, the 899—897-cm.$^{-1}$ band having 1·3—2·5 times the intensity of the 920—916-cm.$^{-1}$ band (see Wall, Eddy, McClennan, and Klumpp, *loc. cit.*; Jones, Katzenellenbogen, and Dobriner, *loc. cit.*). Bands at 851 cm.$^{-1}$ in the normal series and at 863 cm.$^{-1}$ in the *iso*-series are also valuable means of distinguishing between them. In other respects there are no major differences. In the ψ-series, the type of absorption changes; there is a dominating band with a maximum at 1045—1030 cm.$^{-1}$, and bands in the rest of the fingerprint region are relatively subdued particularly at the lower frequencies. A band of medium intensity at 1695—1690 cm.$^{-1}$ (olefinic ether) is characteristic, but may be obscured by the C=O stretching band in the spectra of ketonic compounds. The absorption spectra of the corresponding ψ-normal and ψ-*iso*-sapogenins were said to be " essentially identical " by Wall, Eddy, and Serota (*loc. cit.*). We should agree with this, with the reservation that in Nujol mulls, where the crystalline structure is retained, differences may be considerable. This was observed, for instance, with ψ-hecogenin and ψ-sisalagenin diacetates. These compounds are sufficiently soluble for measurements to be made in solution at concentrations at which " essential identity " of absorption could be confirmed.

In contrast to the other classes of isomers, the *cyclo*-ψ-sapogenins show distinct differences between the normal and the *iso*-series. The fingerprint region shows a series of sharp, well-defined peaks, indicating the closure, from the open-chain ψ-compound, of a ring system with structure analogous to, but not identical with, that in the original sapogenins. In both normal and *iso*-series the bands at 920—916 and at 902—892 cm.$^{-1}$, present in the original sapogenins and lost in the ψ-compounds, reappear in the *cyclo*-ψ-compounds, but are then of nearly equal intensity in both series. The *cyclo*-ψ-*iso*-compounds show characteristic absorption peaks at about 1360, 1070, 1012, 920, 895, 856, and

785 cm.$^{-1}$, and the *cyclo*-ψ-normal compounds at about 1360, 1080, 1048, 982, 916, 901, and 868 cm.$^{-1}$. The two types of spectra are sufficiently distinct to indicate that some factor of structure or interaction of groups differs in the normal and the *iso*-series. The features discussed are illustrated by the representative spectra of ψ-tigogenin diacetate (Fig. 1, C.S. No. 202 *), *cyclo*-ψ-tigogenin acetate (Fig. 2, C.S. No. 203) and *cyclo*-ψ-*neo*tigogenin acetate (Fig. 3).

Infrared spectra of: FIG. 1, *ψ-Tigogenin diacetate;* FIG. 2, cyclo-*ψ-tigogenin acetate;* FIG. 3, cyclo-ψ-neo-*tigogenin acetate. All* 1% *solutions in* CS_2; 0·8 *mm. cell; Perkin–Elmer double-beam spectrophotometer with a sodium chloride prism.*



The optical properties of our compounds are presented in Table 1. The values of $\Delta[M]$ for the change sapogenin ⟶ *cyclo*-ψ-sapogenin vary little for the normal compounds, but cover a rather wider range in the *iso*-series. Nevertheless, there is an obvious difference between the values in the two series, a fact made the more remarkable by the close similarity between $\Delta[M]$ values for the change sapogenin ⟶ ψ-sapogenin in both of the series. The significance of these results is discussed below.

To return to the work of Marker and Lopez (*loc. cit.*), it is apparent that they did not

* Spectra thus denoted have been deposited with the Chemical Society. Photocopies, price 3*s.* 0*d.* per copy per spectrum, may be obtained from the General Secretary, The Chemical Society, Burlington House, Piccadilly, London, W.1, on application stating the C.S. No.

TABLE 1. *Specific rotations and molecular rotation differences of steroid sapogenins, ψ-sapogenins, and* cyclo-*ψ-sapogenins.*

Parent compounds (*A*) Name	Substituents	[α]D	ψ-Isomers (*B*) [α]D	*cyclo*-ψ-Isomers (*C*) [α]D	Δ[*M*D] *B* − *A*	Δ[*M*D] *C* − *A*
iso-*Sapogenins* (25 *D*) *						
Smilagenin	3β-OH; 5β-H	− 67°	+ 24°	− 61°	+378°	+ 25°
Tigogenin	3β-OH; 5α-H	− 67	+ 23	− 67·5	+375	− 2
11-Oxotigogenin ...	3β-OH; 5α-H; 11-O:	− 33	+ 76	− 29	+469	+ 17
Hecogenin	3β-OH; 5α-H; 12-O:	+ 7	+100	+ 21	+400	+ 62
Diosgenin	3β-OH; Δ5	−123	− 28	−113	+393	+ 41
Normal sapogenins (25 *L*)						
Sarsasapogenin ...	3β-OH; 5β-H	− 77	+ 12	+ 27	+370	+432
*neo*Tigogenin	3β-OH; 5α-H	− 75	+ 11	+ 25	+358	+416
Sisalagenin	3β-OH; 5α-H; 12-O:	− 3	+ 91	+ 95	+411	+428

Mean values: Δ[*M*D] (*B* − *A*) { *iso*-sapogenins ..... +404°; normal sapogenins ..... +380

Δ[*M*]D (*C* − *A*) { *iso*-sapogenins ..... + 29; normal sapogenins ..... +425

For the three epimeric pairs: Δ[*M*D] {25 *D* minus 25 *L*} { sapogenins ..... + 41; ψ-sapogenins ..... + 46; *cyclo*-ψ-sapogenins ..... −410

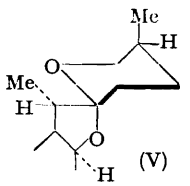
* In common with other workers in this field we have related the configuration at $C_{(25)}$ to that of the α-methylglutaric acid obtained by oxidation, and adopted the convention, currently accepted for α-substituted acids, of using the prefixes *D*- and *L*- (cf. James, *loc. cit.*, Ziegler *et al.*, *loc. cit.*, Klyne and Stokes, *J.*, 1954, 1979; Dickson, Page, and Rogers, *J.*, 1955, 443; Ziegler, Rosen, and Shabica, *J. Amer. Chem. Soc.*, 1955, **77**, 1223; Bijvoet, *Endeavour*, 1955, **14**, 71).

obtain the *cyclo*-ψ-isomers upon cyclisation of their ψ-*iso*-sapogenins. This is suggested by the difference in melting point between their product and ours in, for example, the cyclisation of ψ-hecogenin, but more cogent evidence comes from the fact that their method of acetylation (boiling with acetic anhydride) would have converted any *cyclo*-ψ-*iso*-compounds into the ψ-*iso*-sapogenin diacetates, which would have been unmistakable both from their low melting points and from their elementary analyses. We can offer no explanation for the differences between their results and ours, and because of these differences we can throw no light upon the remarkably ready inversion at $C_{(25)}$ implied by their work.

Two views have been expressed as to the structures of these new isomers. Wall, Eddy, and Serota (*J. Amer. Chem. Soc.*, 1954, **76**, 2849; cf. also Wall and Serota, *ibid.*, p. 2850), from a consideration of the reactions of the various isomers, have put forward the structures (V), (VI), (VII), and (VIII) for the side chains of the normal, *iso*-, *cyclo*-ψ-normal, and *cyclo*-ψ-*iso*-compounds respectively. Taylor (*loc. cit.*), on the other hand, on the basis of conformational analysis, and considering the probable mechanism of cyclisation of ψ-sapogenins, has assigned structures (V), (IX), (X), and (VIII) to the respective isomers; a similar view is taken by Ziegler, Rosen, and Shabica (*loc. cit.*) about their cyclisation of ψ-diosgenin. (These authors, following Marker, call their product " neodiosgenin " but it appears to be identical with our *cyclo*-ψ-diosgenin and is distinguished from yamogenin, the normal isomer of diosgenin, by the quoted infrared bands; cf. Jones *et al.*, *loc. cit.*)

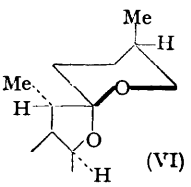
The existing evidence does not appear to be convincingly in favour of either of these theories but some of Wall's reasoning, in particular, is made untenable by our demonstration of the equilibrium between ψ- and *cyclo*-ψ-compounds in acid (including acetic acid) solution. Thus, Wall uses in his argument the facts that *cyclo*-ψ-compounds are much more readily oxidised by chromic acid than are the sapogenins themselves and again that the dihydro-compounds obtained by catalytic reduction of *cyclo*-ψ-compounds are less stable to chromic acid than are the dihydrosapogenins. However, in view of our findings it cannot be said that *cyclo*-ψ-compounds, as such, have ever been oxidised or reduced; their apparent reactions are a most certainly those of the corresponding ψ-compounds in which the asymmetry at $C_{(20)}$ and $C_{(22)}$ is lost. For this reason, arguments based on the reactions of the dihydro-*cyclo*-ψ-compounds or of the *cyclo*-ψ-compounds in acid medium have no bearing on the configuration of the latter compounds at $C_{(20)}$ or $C_{(22)}$. We can add our own

observations on the hydrogenation of *cyclo-ψ*-compounds. Hydrogenation of *cyclo-ψ*-tigogenin acetate in acetic acid containing sodium acetate was similar to that of *ψ*-tigogenin in being rapid and in giving (after acetylation) the same product. Hydrogenation of tigogenin acetate was very slow and gave a different dihydro-compound (cf. Wall, Eddy, and Serota, *loc. cit.*). We have attempted to carry out the reductions in neutral medium, but without success.



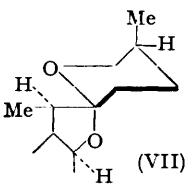
(V)

Wall : normal
Taylor : normal



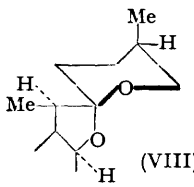
(VI)

Wall : *iso*



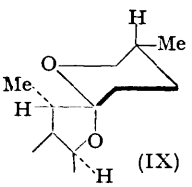
(VII)

Wall : *cyclo-ψ*-normal



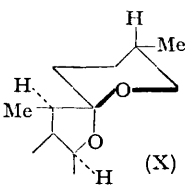
(VIII)

Wall : *cyclo-ψ-iso*
Taylor : *cyclo-ψ-iso*



(IX)

Taylor : *iso*



(X)

Taylor : *cyclo-ψ*-normal

We have examined the optical properties of our compounds to see whether they lend support to one or other of the above views. The similarity in the values of $\Delta[M]$ for the changes *iso-* ⟶ *ψ-iso-* (+404°) and normal ⟶ *ψ*-normal sapogenins (+380°) is in accord with Taylor's formulation, if the asymmetric centre at $C_{(25)}$ is assumed to exert no marked vicinal effect; however, after making this assumption it is difficult to reconcile Taylor's view with the very different values of $\Delta[M]$ for the changes sapogenin ⟶ *cyclo-ψ*-sapogenin in the two series (+29° and +425°, respectively) since in each precisely similar changes are involved at $C_{(20)}$ and $C_{(22)}$.

It would, perhaps, be possible to reconcile the molecular-rotation data with the scheme of Wall *et al.*, if it were to be supposed that the vicinal action of the 22-O- and the 20-Me group was of the same order of magnitude as that of the 6-O- and $C_{(4)}$ in 6-acetoxyl derivatives of coprostane and cholestane, in which the relative disposition of the groups is analogous. By use of Barton and Klyne's mean figures (*Chem. and Ind.*, 1948, 755) it is seen that the change of $C_{(4)}$ from α to β in the 6α-acetoxy-compounds entails a change of +291° in $[M]$, whereas change of $C_{(4)}$ from α to β in the 6β-acetoxy-compounds entails a change of −44°. However, the analogy that has been drawn is rather an example of the surprising effects of " vicinal action "—a phenomenon not susceptible to quantitative assessment—than an argument for the scheme of Wall *et al.*

It appears to us that the existing evidence permits the assignment of configurations to $C_{(16)}$, $C_{(17)}$, and $C_{(25)}$ only, of the asymmetric centres in rings E and F. The configurations of the normal and the *iso*-sapogenins at $C_{(25)}$, which have been discussed above, seem beyond dispute. Marker and Turner (*J. Amer. Chem. Soc.*, 1941, **63**, 767) have converted diosgenin into cholesterol by reactions that probably do not involve $C_{(17)}$ and therefore suggest that the former, in common with all the naturally occurring sterols, has its side chain attached in the β-configuration to that carbon atom. We do not, however, agree with Wall, Eddy, and Serota (*loc. cit.*) that identity of configuration at $C_{(20)}$ is also implied : the reactions involved the Clemmensen reduction of the latent $C_{(22)}$ carbonyl group of diosgenin and if that group existed, even transitorily, isomerisation at the neighbouring asymmetric centre would clearly be possible.

Scheer, Kostic, and Mosettig (*loc. cit.*), by proving that dihydrosarsasapogenin and

dihydrosmilagenin differ only at $C_{(25)}$, have shown that the parent sapogenins (and, by inference, normal and *iso*-sapogenins, in general) have identical configurations at $C_{(16)}$, $C_{(17)}$, and $C_{(20)}$; the situation is less clear at $C_{(22)}$, where inversion might have occurred during cleavage of the $C_{(22)}$–O bond (cf. Wall and Serota, *loc. cit.*).

Finally, it has been reasoned by Mueller, Stobaugh, and Winniford (*J. Amer. Chem. Soc.*, 1953, **75**, 4888) that the β-configuration at $C_{(17)}$ fixes the configuration at $C_{(16)}$ as β also. Since the transformations, sapogenin $\rightleftharpoons$ ψ-sapogenin $\rightleftharpoons$ *cyclo*-ψ-sapogenin would be expected to involve only the asymmetric centres at $C_{(20)}$ and $C_{(22)}$, we believe that normal and *cyclo*-ψ-normal sapogenins have the configuration 16β : 17β : 25*L* and that the *iso*- and *cyclo*-ψ-*iso*-compounds have the configuration 16β : 17β : 25*D*. The evidence as to configuration at $C_{(20)}$ and $C_{(22)}$ rests on much less firm foundations and has, indeed, led to contradictory opinions; we consider, therefore, that in the absence of direct evidence it is premature to assign configurations at these centres to any of the isomers.

Added in Proof.—Scheer, Kostik, and Mosettig ((*J. Amer. Chem. Soc.*, 1955, **77**, 641), Ziegler, Rosen, and Shabica (*ibid.*, p. 1223), and Wall, Serota, and Eddy (*ibid.*, p. 1230) have reported results of experiments on the sapogenin side-chain which do not differ in principle from their earlier results referred to in this paper.

Experimental

M. p.s were taken on a Kofler block and are corrected. Ultraviolet absorptions were determined on ethanol solutions and optical rotations on chloroform solutions unless otherwise specified.

Infrared absorptions were determined on two Perkin–Elmer Model 21 double-beam spectrophotometers with rock-salt prisms. In one laboratory the potassium bromide disc method was used as a routine, and most of the spectrograms deposited with the Society are taken by this method. In the other laboratory, solutions in CS_2 and CCl_4 were measured wherever possible; less soluble materials were examined as mulls in Nujol or in solution in bromoform, chloroform, or pyridine. Where measurement in CS_2 was possible, only figures for this solution are given, unless other media gave notably different results. Solvent absorption masked absorption peaks in some instances, but sufficient characteristic bands could be observed to identify the various classes of isomers.

The preparation of the ψ-sapogenins used in this work is described in the papers of Cameron *et al.* and of Callow and James (*locc. cit.*).

Treatment of ψ-Hecogenin with Hydrochloric Acid by the Method of Marker and Lopez (loc. cit.). —A solution of ψ-hecogenin (4 g.) in ethanol (100 ml.) and concentrated hydrochloric acid (4 ml.) was left overnight at 0°; some crystalline solid separated during this time. The mixture was poured into water. The precipitated solid crystallised from methanol as plates (3·08 g.), m. p. 190—196°, $[\alpha]_D^{22}$ +12·5°, the infrared spectrum of which indicated that they were essentially *cyclo*-ψ-hecogenin (see below): further crystallisation from slightly alkaline methanol gave the pure methanol solvate, m. p. 218—221°, $[\alpha]_D^{20}$ +18·5°.

The filtrate from the precipitated material was evaporated; the residue (0·86 g.) had the characteristic infrared absorption of a *cyclo*-ψ-sapogenin and yielded ψ-hecogenin diacetate on being boiled with acetic anhydride.

Preparation of cyclo-ψ-*Sapogenins.*—Cyclisation of ψ-sapogenins was carried out in ethanol or ethyl acetate. Both methods are exemplified for ψ-hecogenin; the other ψ-compounds were cyclised by essentially the same techniques, except in the few cases where differences are noted.

cyclo-ψ-*Hecogenin.*—(*a*) ψ-Hecogenin (7·3 g.) was dissolved in ethanol (100 ml.), and N-hydrochloric acid (1 ml.) was added. Crystallisation of the product began almost at once. After 30 min. the solid was collected (5·1 g.; m. p. 206—217°), and a further crop (1·6 g.) obtained by dilution of the filtrate with water.

(*b*) A solution of ψ-hecogenin (5 g.) in ethyl acetate (250 ml.), which had $[\alpha]_D^{20}$ +86°, was treated with one drop of concentrated hydrochloric acid, and the mixture was shaken. Within a minute $[\alpha]_D^{20}$ had fallen to +17° and was stable at this value. After being washed with water (3 × 250 ml.), the solution was evaporated on the water-bath under reduced pressure to the point of crystallisation, and the crystalline product was collected.

cyclo-ψ-*Hecogenin* separated from methanol with solvent of crystallisation (removed at 130°/0·5 mm. in 2 hr.), and the crystals had m. p.s depending on the rate of heating, *e.g.*, 221—

231° or 210—222°. From ethanol it separated in needles containing 1 mol. of ethanol, m. p. 196—212° after a change of crystal form at 145° (Found, in air-dried substance: C, 73·4; H, 10·0; after drying at 136°/0·2 mm.: C, 74·8; H, 9·85. $C_{27}H_{42}O_4,C_2H_6O$ requires C, 73·1; H, 10·15. $C_{27}H_{42}O_4$ requires C, 75·3; H, 9·8%). $[\alpha]_D^{20}$ was +21°, $[\alpha]_{5461}^{21}$ +29° (*c*, 1·153), and $[\alpha]_D^{22}$ +22°, $[\alpha]_{5461}^{21}$ +29° (*c*, 0·565 in EtOH). The substance showed no appreciable absorption in the ultraviolet region above 205 mμ. Infrared max. (in CS_2) were at 3620, 1036 (equatorial hydroxyl), 1708 (ketone), 1068, 1012, 922, 895, 856, and 785 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin). Spectrum in KBr: (C.S. No. 176). The substance gave no colour with tetranitromethane.

The *acetate* was prepared by keeping a solution in acetic anhydride and pyridine (1 : 1) overnight at room temperature. From methanol or ethyl acetate it formed crystals with m. p. dependent on the rate of heating, 205—208° or 215—223°, $[\alpha]_D$ +9° (Found: C, 74·0; H, 9·4. $C_{29}H_{44}O_5$ requires C, 73·7; H, 9·4%). $\nu_{max.}$ (in CS_2): 1735 and 1235 cm.$^{-1}$ (acetate), and the characteristic bands found in the alcohol.

Formation of ψ-Hecogenin Diacetate from cyclo-*ψ-Hecogenin.*—*cyclo-ψ*-Hecogenin (0·5 g.) in acetic anhydride (20 ml.) was boiled under reflux for 45 min. The solvent was then evaporated and the residue crystallised from methanol to give *ψ*-hecogenin diacetate (0·41 g.) as plates, m. p. 94—96°, $[\alpha]_D^{20}$ +71° (*c*, 0·56), identified by mixed m. p. and infrared spectrum (C.S. No. 188) (Found: C, 72·5; H, 9·1. Calc. for $C_{31}H_{46}O_6$: C, 72·3; H, 9·0%).

Formation of Hecogenin from cyclo-*ψ-Hecogenin.*—*cyclo-ψ*-Hecogenin (0·25 g.) in methanol (25 ml.) with concentrated hydrochloric acid (2·5 ml.) was boiled under reflux for 30 min. On cooling, hecogenin (0·23 g.) crystallised in plates, m. p. 260—263°, $[\alpha]_D^{22}$ +7° (*c*, 0·81), identified by mixed m. p. and infrared spectrum.

cyclo-*ψ-Smilagenin.*—This was prepared from *ψ*-smilagenin by method (*a*), and by method (*b*) but in methyl acetate. *cyclo-ψ*-Smilagenin separated from methanol as plates, m. p. 111—115°, resolidifying and melting at 183—186·5°, $[\alpha]_D^{21}$ −61°, $[\alpha]_{5461}^{21}$ −72° (*c*, 0·394). $\nu_{max.}$ (in KBr) 1365, 1075, 1015, 919, 897, 858, and 785 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin) (C.S. No. 177). Wall, Eddy, and Serota (*loc. cit.*) give m. p. 185° and $[\alpha]_D^{25}$ −60° for their "20-*iso*smilagenin." Another specimen of *cyclo-ψ*-smilagenin separated from acetone in small rhombs, m. p. 157—165°, $[\alpha]_D^{22}$ −65° (*c*, 1·0). It was characterised by its infrared spectrum and analysis (Found: C, 77·9; H, 10·7. Calc. for $C_{27}H_{44}O_3$: C, 77·8; H, 10·65%).

The acetate separated from *n*-hexane–ethyl acetate as small rhombs, m. p. 135—142°, $[\alpha]_D^{23}$ −53° (*c*, 1·01) (Found: C, 75·8; H, 10·2. Calc. for $C_{29}H_{46}O_4$: C, 75·9; H, 10·1%). $\nu_{max.}$ (in CS_2) 1730 and 1230 (acetate), 1070, 1018, 920, 895, 856, and 785 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin). Another specimen separated from methanol as plates, m. p. 151—154°, $[\alpha]_D^{21}$ −55°, $[\alpha]_{5461}^{21}$ −63° (*c*, 0·192). Wall, Eddy, and Serota (*loc. cit.*) give m. p. 160°, $[\alpha]_D^{25}$ −49°.

cyclo-*ψ-Tigogenin.*—This was prepared from *ψ*-tigogenin by method (*b*), the specific rotation dropping from +22·5° to −54°. cyclo-*ψ-Tigogenin* crystallised from methanol in needles (61% yield), m. p. 198—202°, $[\alpha]_D^{20}$ −67·5° (*c*, 0·64) (Found: C, 77·8; H, 10·75%). $\nu_{max.}$ (in Nujol) 3500 (OH), 1071, 1015, 920, 894, 857, and 787 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin). Spectrum in KBr: (C.S. No. 178).

The *acetate* formed plates (from methanol), m. p. 177—179°, $[\alpha]_D^{20}$ −71° (*c*, 0·69) (Found: C, 76·1; H, 10·3. $C_{29}H_{46}O_4$ requires C, 75·9; H, 10·1%). $\nu_{max.}$ (in CS_2) 1730, 1240 (acetate), 1068, 1012, 920, 893, 854, and 788 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin) (Fig. 2, C.S. No. 203).

Boiled under reflux with acetic anhydride, *cyclo-ψ*-tigogenin yielded *ψ*-tigogenin diacetate as a gum which had the infrared absorption characteristics of the authentic substance, and gave, on hydrolysis, *ψ*-tigogenin, m. p. and mixed m. p. 182—187°.

Boiling methanolic hydrochloric acid converted *cyclo-ψ*-tigogenin into tigogenin.

11-*Oxo*cyclo-*ψ-tigogenin.*—Prepared from 11-oxo-*ψ*-tigogenin in 73% yield by method (*b*) in methyl acetate, and precipitated by addition of aqueous sodium acetate, 11-*oxo*cyclo-*ψ-tigogenin* crystallised from ethanol in prisms, m. p. 223—226°, $[\alpha]_D^{21}$ −28° (*c*, 0·994) (Found: C, 75·5; H, 9·7. $C_{27}H_{42}O_4$ requires C, 75·3; H, 9·8%). $\nu_{max.}$ (in CS_2) 3620 and 1035 (equatorial OH), 1707 (ketone), 1070, 1012, 918, 895, 855, and 784 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin). Spectrum in KBr, C.S. No. 179.

The *acetate* separated from ethyl acetate in leaflets with m. p. 207—223°, depressed on admixture with 11-oxotigogenin acetate, and $[\alpha]_D^{20}$ −40° (*c*, 1·08) (Found: C, 73·9. H, 9·2. $C_{29}H_{44}O_5$ requires C, 73·7; H, 9·4%). $\nu_{max.}$ (in CS_2) 1731, 1235 (acetate), 1709 (ketone), 1070, 1012, 917, 892, 854, and 784 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin).

11-Oxo*cyclo-ψ*-tigogenin was converted by boiling acetic anhydride into 11-oxo-*ψ*-tigogenin diacetate and by boiling methanolic hydrochloric acid into 11-oxotigogenin, both products being identified by comparison with authentic specimens.

In another preparation of 11-oxo*cyclo*-ψ-tigogenin, the conditions were those of Marker and Lopez (*loc. cit.*) (see above). The crystals which separated from solution were the *cyclo*-ψ-compound (60% yield after recrystallisation): the residue after evaporation of the mother-liquors was 11-oxotigogenin since, on being refluxed with acetic anhydride for 30 min., it yielded 11-oxotigogenin acetate (11%), m. p. 220—224°, $[\alpha]_D^{22}$ −40° (*c*, 1·0), identified by mixed m. p. and infrared spectrum.

cyclo-ψ-*Diosgenin*.—This was prepared from ψ-diosgenin by method (*a*) in 92% yield. Recrystallised from acetone, it formed needles, m. p. 193—198°, $[\alpha]_D^{22}$ −122° (*c*, 0·53). $\nu_{max.}$ (in CS_2) 3620 and 1042 (equatorial OH), 1665 and 835 (trisubstituted ethylene), 1072, 1012, 920, 895, 855, and 788 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin). Spectrum in KBr, C.S. No. 180. Ziegler *et al.* (*loc. cit.*) give m. p. 197—201°, $[\alpha]_D$ −122°, for their "neodiosgenin," prepared from ψ-diosgenin by mild acid treatment. A specimen labelled "ψ-nitogenin" from the collection of the late Professor G. A. R. Kon (cf. Kon and Weller, *J.*, 1939, 800; Marker, Wagner, Goldsmith, Ulshafer, and Ruof, *J. Amer. Chem. Soc.*, 1943, **65**, 1248) was found to consist of *cyclo*-ψ-diosgenin. Recrystallised twice from acetone, it gave needles, m. p. 186—191°, $[\alpha]_D^{21}$ −120° (*c*, 0·985) (Found: C, 78·3; H, 10·0. Calc. for $C_{27}H_{42}O_3$: C, 78·2; H, 10·2%). Its infrared spectrum in CS_2 was identical with that of *cyclo*-ψ-diosgenin, prepared as above.

The *cyclo*-ψ-diosgenin gave a colour with tetranitromethane and a positive Liebermann–Burchardt test.

The *acetate* formed leaflets (from ethyl acetate), m. p. 183—212° (transition at 165°), $[\alpha]_D^{21}$ −118° (*c*, 0·51) (Found: C, 76·4; H, 9·5. $C_{29}H_{44}O_4$ requires C, 76·3; H, 9·7%). $\nu_{max.}$ (in CS_2) 1732 and 1238 (acetate), 1665 and 835 (trisubstituted ethylene), 1072, 1014, 918, 894, 855, and 786 cm.$^{-1}$ (*cyclo-ψ-iso*-sapogenin).

cyclo-ψ-Diosgenin was converted by boiling acetic anhydride into ψ-diosgenin diacetate and by boiling ethanolic hydrochloric acid into diosgenin, the products being identified by comparison with authentic specimens.

cyclo-ψ-*Sarsasapogenin*.—This was prepared from ψ-sarsasapogenin by method (*a*), the product being precipitated after 10 min. by addition of water. The precipitate, at first oily, slowly solidified and was recrystallised from methanol, from which it separated in needles, m. p. 99—102°, then resolidifying and melting at 172—179°, $[\alpha]_D^{18}$ +27°, $[\alpha]_{5461}^{18}$ +31° (*c*, 0·679) (Found, in air-dried substance: C, 75·6; H, 10·7; and, after drying at 100°/0·1 mm.: C, 78·3, 78·3; H, 11·3, 10·8. Calc. for $C_{27}H_{44}O_3,CH_4O$: C, 74·95; H, 10·8. Calc. for $C_{27}H_{44}O_3$: C, 77·8; H, 10·65%). $\nu_{max.}$ (in CS_2) 3620, 1030 (axial OH), 1360, 1080, 1046, 982, 917, 902, and 869 cm.$^{-1}$ (*cyclo*-ψ-normal sapogenin). Spectrum in KBr, C.S. No. 181. The equal intensity of the bands at 917 and 902 cm.$^{-1}$ is to be noted. Wall, Eddy, and Serota (*loc. cit.*) report m. p. 176—177°, $[\alpha]_D^{25}$ +31·9°.

cyclo-ψ-Sarsasapogenin could not be prepared by method (*b*): the product was sarsasapogenin, and this result seems to be an indication of the greater instability of *cyclo*-ψ-normal as compared with the *cyclo-ψ-iso*-compounds.

cyclo-ψ-Sarsasapogenin acetate separated in plates from methanol, m. p. 167—170°, $[\alpha]_D^{19}$ +27°, $[\alpha]_{5461}^{19}$ +32° (*c*, 0·515) (Found: C, 76·05; H, 10·0. Calc. for $C_{29}H_{46}O_4$: C, 75·9; H, 10·1%). $\nu_{max.}$ (in CS_2) 1732, 1249, 1235 (axial acetate), 1080, 1047, 982, 919, 902, and 868 cm.$^{-1}$ (*cyclo*-ψ-normal sapogenin). Spectrum in KBr, C.S. No. 182. Wall, Eddy, and Serota (*loc. cit.*) give m. p. 167°, $[\alpha]_D^{25}$ +30°.

cyclo-ψ-Sarsasapogenin on being refluxed with acetic anhydride gave an uncrystallisable gum having an infrared spectrum with the characteristics of that of a ψ-sapogenin diacetate; hydrolysis gave ψ-sarsasapogenin, identified by comparison with an authentic specimen.

cyclo-ψ-neo*Tigogenin*.—Prepared by method (*a*) from ψ-*neo*tigogenin, cyclo-ψ-neo*tigogenin* separated from ethanol in rhomboid plates containing solvent of crystallisation which, after a transition at 140°, melted at 168° and resolidified to needles melting at 186—189°, $[\alpha]_D^{21}$ +25°, $[\alpha]_{5461}^{21}$ +30° (*c*, 0·616) (Found: in substance dried at 130° *in vacuo*: C, 77·4; H, 10·4. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·65%). $\nu_{max.}$ (in CS_2) 3620 and 1038 (equatorial OH), 1362, 1080, 1048, 984, 918, 902, and 868 cm.$^{-1}$ (*cyclo*-ψ-normal sapogenin). The spectrum showed similar bands in potassium bromide (C.S. No. 183), a double peak at 1052 and 1045 cm.$^{-1}$ and the equality of the bands at 921 and 902 cm.$^{-1}$ being notable features.

The *acetate* separated from methanol as plates, m. p. 169·5—173°, $[\alpha]_D^{20}$ +11°, $[\alpha]_{5461}^{20}$ +13° (*c*, 0·33) (Found: C, 76·05; H, 10·0. $C_{29}H_{46}O_4$ requires C, 75·9; H, 10·1%). $\nu_{max.}$ (in CS_2) 1731 and 1240 (acetate), 1080, 1048, 985, 916, 900, and 865 cm.$^{-1}$ (*cyclo*-ψ-normal sapogenin) (Fig. 3). Spectrum in KBr, C.S. No. 184.

When *cyclo-ψ-neo*tigogenin was boiled with acetic anhydride a non-crystalline product was

obtained, having an infrared spectrum with the characteristics of that of a ψ-sapogenin diacetate; hydrolysis gave ψ-*neo*tigogenin, identified by comparison with an authentic specimen.

cyclo-ψ-*Sisalagenin*.—This was prepared by general method (*a*) from ψ-sisalagenin. The value of $[\alpha]_D$ in the ethanol solution changed from +86° to +95° during the reaction. cyclo-ψ-*Sisalagenin* separated from methanol as plates, m. p. 180—184°; another specimen had m. p. 177—182·5° and resolidified to needles, m. p. 192°, $[\alpha]_D^{21}$ +95°, $[\alpha]_{5461}^{21}$ +116° (*c*, 0·758) (Found: C, 74·8, 75·7; H, 9·7, 9·75. $C_{27}H_{42}O_4$ requires C, 75·3; H, 9·8%). $\nu_{max.}$ (in CS_2) 1705 (ketone), 3620 and 1038 (equatorial OH), 1362, 1082, 1048, 979, 918, 902, and 868 cm.$^{-1}$ (*cyclo*-ψ-normal sapogenin). Spectrum in KBr, C.S. No. 185.

The *acetate* had m. p. 204—208°, $[\alpha]_D^{20}$ +93°, $[\alpha]_{5461}^{20}$ +112° (*c*, 0·405) (Found: C, 73·7; H, 9·2. $C_{29}H_{44}O_5$ requires C, 73·7; H, 9·4%). $\nu_{max.}$ (in CS_2) 1735 and 1240 (acetate), 1708 (ketone), 1080, 1049, 980, 916, 901, and 868 cm.$^{-1}$ (*cyclo*-ψ-normal sapogenin). Spectrum in KBr, C.S. No. 186.

Refluxed with acetic anhydride, on one occasion *cyclo*-ψ-sisalagenin yielded the acetate, but on a second ψ-sisalagenin diacetate was obtained. This crystallised from light petroleum (b. p. 40—60°) in plates, m. p. 112—113°, $[\alpha]_D^{22}$ +77°, $[\alpha]_{5461}^{22}$ +94°. $\nu_{max.}$ (in CS_2) 1732 and 1238 (acetate), 1706 (ketone), 1690, and 1028 cm.$^{-1}$ (ψ-sapogenin) (Spectrum in KBr, C.S. No. 187). The substance was identical with material, m. p. and mixed m. p. 112—113°, prepared from ψ-sisalagenin by acetylation with acetic anhydride in pyridine (Callow and James, *loc. cit.*).

Studies of the Equilibrium between ψ- and cyclo-ψ-*Sapogenins*.—(i) *Optical rotation*. A 0·565% solution of *cyclo*-ψ-hecogenin in ethanol had $[\alpha]_D^{22}$ +22°, unchanged after 24 hr., but on the addition of 10% by volume of 0·1N-hydrochloric acid in 90% ethanol the specific rotation rose within 15 min. to +32°. Under the same conditions hecogenin showed no mutarotation. The same rotation could be approached from ψ-hecogenin. A 0·328% solution in ethanol had $[\alpha]_D$ +100°; addition of 0·1N-hydrochloric acid (1 ml.) to the solution (18 ml.) caused the rotation to drop to +35·5° in 180 min.; there was no further change in 24 hr.

Similar observations were made with 11-oxo*cyclo*-ψ-tigogenin. A solution of 0·2 g. in a mixture of chloroform (7 ml.) and ethanol (10 ml.) showed $[\alpha]_D$ −24°. Two minutes after the addition of a drop of concentrated hydrochloric acid the solution had $[\alpha]_D$ −17°. In the same concentration an acidified solution of 11-oxo-ψ-tigogenin had $[\alpha]_D$ −15°. Little further change was observed in either case with increase in time or quantity of acid at room temperature. If linear relations are assumed the rotation figures indicate the presence of 92% of *cyclo*-ψ-sapogenin in the equilibrium mixture.

(ii) *Ultraviolet absorption*. An ethanolic solution of 11-oxo*cyclo*-ψ-tigogenin showed negligible absorption at 216 mμ; addition of a trace of hydrochloric acid caused the rapid appearance of a peak at 216 mμ ($E_{1\,cm.}^{1\%}$ 12). 11-Oxo-ψ-tigogenin in ethanol showed $\lambda_{max.}$ 215 mμ ($E_{1\,cm.}^{1\%}$ 115), changed on acidification to $\lambda_{max.}$ 215 mμ ($E_{1\,cm.}^{1\%}$ 13).

Similarly, *cyclo*-ψ-sarsasapogenin in neutral ethanolic solution showed general absorption only at 215 mμ ($E_{1\,cm.}^{1\%}$ 10); on acidification, the solution had $\lambda_{max.}$ 215 mμ ($E_{1\,cm.}^{1\%}$ 25). The corresponding figures for ψ-sarsasapogenin were 215 mμ ($E_{1\,cm.}^{1\%}$ 145) and 207—217 mμ ($E_{1\,cm.}^{1\%}$ 25).

(iii) *Paper-partition chromatography*. Paper chromatography on Whatman No. 2 papers in an acidic solvent (cf. Sannié and Lapin, *Bull. Soc. chim. France*, 1952, 1080) and another containing pyridine shows how the ψ- and *cyclo*-ψ-compounds change in acid circumstances. The *cyclo*-ψ-normal compounds generate the normal sapogenins more quickly than the corresponding *cyclo*-ψ-*iso*-compounds give the *iso*-sapogenins (see footnote to Table 2). The ψ-sapogenins were applied to the paper in pyridine solutions, which were stored at 0°. Unless stated otherwise, the chromatograms were equilibrated for 2 days and then run at 30° ± 1°. The solvents ran downwards. Ethanolic phosphomolybdic acid (10% w/v) (cf. Kritchevsky and Kirk, *Arch. Biochem. Biophys.*, 1952, **35**, 346) or Komarowsky's reagent (cf. Nogare and Mitchell, *Analyt. Chem.*, 1953, **25**, 1376) revealed the sapogenins as lasting blue or yellow spots when the papers were heated gently. Komarowsky's reagent was made for this purpose as follows: A 2% (w/v) solution (10 ml.; if necessary percolated through an alumina pad) of *p*-hydroxybenzaldehyde in ethanol was mixed just before use with concentrated sulphuric acid (0·4 ml.). Papers thus sprayed did not last long. The R_F values are given in Table 2.

Hydrogenation of cyclo-ψ-*Tigogenin*.—Platinum oxide (0·5 g.) was reduced under hydrogen in acetic acid (15 ml.) containing anhydrous sodium acetate (0·1 g.). *cyclo*-ψ-Tigogenin acetate (0·6 g.) in glacial acetic acid (15 ml.) containing anhydrous sodium acetate (0·05 g.) was then added and the hydrogenation continued at 20°/766 mm. The uptake ceased after 30 min. at 34 ml. of hydrogen (1·1 mol.). After filtration the solvent was removed by evaporation and the product was crystallised from methanol, giving needles, m. p. 145—151°. Acetylation with

acetic anhydride in pyridine overnight gave dihydro-ψ-tigogenin diacetate as needles (from methanol), m. p. 123—124°, unchanged by two further recrystallisations. The m. p. of a mixture with dihydro-ψ-tigogenin diacetate (see below) was 123—124°, and of a mixture with dihydrotigogenin diacetate (m. p. 114—116°) was 110—114°. $[\alpha]_D^{20}$ was −17° (*c*, 0·65) (Found: C, 74·2; H, 9·9. Calc. for $C_{31}H_{50}O_5$: C, 74·1; H, 10·0%). $\nu_{max.}$ (in CS_2): 1734, 1236 (acetate), and 1020 cm.$^{-1}$. There was no vinyl ether band at 1690 cm.$^{-1}$. No appreciable absorption in

TABLE 2.

	Solvent: pyridine (8); light petroleum, b. p. 100—120° (15); water (3)			Solvent: light petroleum (100); chloroform (10); acetic acid (1)		
Parent sapogenin	Named form	*cyclo*-ψ-	ψ-	Named form	*cyclo*-ψ-	ψ-
Tigogenin	0·95	0·90	0·50	0·82	0·82, 0·30	0·80, 0·30
*neo*Tigogenin ...	0·95	0·87	0·48	0·80	0·80	0·80, 0·25 *
Sarsasapogenin	0·95	0·95	0·61	0·89	0·90	0·85, 0·41 *
Smilagenin	0·95	0·95	—	0·89	0·89	—
Hecogenin	0·71	0·69	0·07	0·40	0·40	0·38, 0·06 *
Sisalagenin	0·73	0·72	0·07	0·50	0·50, 0·33	0·50, 0·35, 0·06 *
11-Oxotigogenin	0·76	0·76	0·09	0·62	0·46, 0·12	0·46, 0·10

* These fractions were detectable only if the equilibration was omitted, so that exposure to the acid-containing solvent lasted only for the time of development (*ca.* 1·5 hr.).

the ultraviolet was found above 200 mμ. The compound gave no colour with tetranitromethane.

Dihydro-ψ-tigogenin diacetate slowly decolorised a solution of bromine in acetic acid; dihydrotigogenin diacetate failed to react under these conditions (cf. Marker, Jones, and Krueger, *J. Amer. Chem. Soc.*, 1940, **62**, 2532).

Hydrogenation of ψ-Tigogenin.—ψ-Tigogenin (1·0 g.) was hydrogenated as described for the *cyclo*-ψ-compound, but with twice the quantity of acetic acid; the theoretical volume of hydrogen was taken up in 11 min. The product was acetylated with pyridine and acetic anhydride at 100° for 30 min. The product, after crystallisation from methanol, had m. p. 123—124°, unchanged by further crystallisation, and $[\alpha]_D^{20}$ −15·5° (*c*, 0·775). Marker *et al.* (*J. Amer. Chem. Soc.*, 1940, **62**, 3003; 1947, **69**, 2167) give m. p. 122—124°.

Hydrogenation of Tigogenin Acetate.—Tigogenin acetate (2·0 g.) was hydrogenated as described for the *cyclo*-ψ-compound. Uptake of hydrogen was extremely slow and some 60 hr. were required for completion of the reduction. Acetylation with pyridine–acetic anhydride at 100° for 30 min. gave dihydrotigogenin diacetate (2·0 g.) with m. p. 114—116°, unchanged by further crystallisation from methanol, and $[\alpha]_D^{20}$ −12·5° (*c*, 0·91). Doukas and Fontaine (*J. Amer. Chem. Soc.*, 1953, **75**, 5355) give m. p. 116—117° and $[\alpha]_D^{20}$ −15°.

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