230. *Studies in the Synthesis of Cortisone. Part XIX.* Paper Chromatography of Some Steroidal* 11 : 12-*Diols and -Ketols.*

By S. G. Brooks, J. S. Hunt, A. G. Long, and B. Mooney.

Derivatives of tigogenin, tigogenin acetate, and tigogenone with oxygen-containing substituents at the 11-position have been made. Paper chromatography of these and of sapogenins with substituents at the 12-position and analysis of the results yield increments [ΔR_M, where $R_M = \log_{10}(1/R_F - 1)$] characteristic of each discrete polar group. The order of ΔR_M values matches the relative chemical properties of the groups. Interaction between adjacent polar groups reduces their activity, and paper chromatography shows that such influences, being subject to the familiar steric hindrances, intervene most markedly in reducing the polarity of equatorial hydroxyl groups. Consequently the simple premises of conformational analysis must be qualified for application to such compounds.

Attention to the methods of chromatography, including the use of solvents containing boric acid and exploitation of a reversible system, extend the scope of these studies.

In earlier Parts [1] of this series we successfully used paper chromatography for analysing mixtures and testing the homogeneity of sapogenins. We attempt now to appraise the results more critically, defining the intramolecular forces between the vicinal polar groups (in ring C in particular) and confirming thereby the structures of sapogenins containing such groups.

Besides simple sapogenins isolated from plants we needed 11- and 12-hydroxy- and -oxo-sapogenins for comparison with the triols and dihydroxy-ketones. Reduction of hecogenin (XI) provided rockogenin (X) and *epi*rockogenin (IX); [2] likewise 11-oxo-tigogenin (VII) (or its 3-acetate, VIIa) yielded 11α- (III) and 11β-hydroxytigogenin (V),[3] the 11β-epimer being efficiently made by reduction with lithium aluminium hydride or sodium borohydride and, by the latter means, without production of sufficient 11α-alcohol to be detectable on a paper chromatogram,[4] *i.e.*, <10%. Selective oxidation of the

* Part XVIII, *J.*, 1956, 4356.

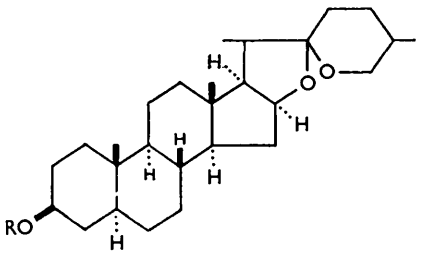
[1] (*a*) Part XIII, Elks and Phillipps, *J.*, 1956, 4320; (*b*) Part XV, Elks, Phillipps, Walker, and Wyman, *ibid.*, p. 4330; (*c*) Part XVI, Chapman, Elks, Phillipps, and Wyman, *ibid.*, p. 4344.

[2] Hirschmann, Snoddy, Hiskey, and Wendler, *J. Amer. Chem. Soc.*, 1954, **76**, 4013.

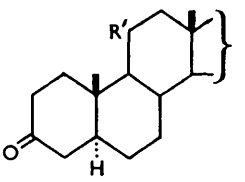
[3] Cf. Djerassi, Rosenkranz, *et al.*, *ibid.*, 1952, **74**, 1712; 1953, **75**, 1282.

[4] Klyne, *Ann. Reports*, 1954, **51**, 222; Allen, Bernstein, and Littell, *J. Amer. Chem. Soc.*, 1954, **76**, 6117.

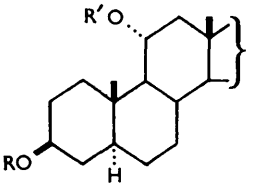
3β : 11β-diol (V) by the Oppenauer method gave 11β-hydroxytigogenone (VI), which yielded the 3 : 11-dione (VIII) when oxidised with potassium dichromate in acetone and dilute sulphuric acid.[5] This oxidant converted also the 3β : 11α- (III) and 3β : 11β-diol (V) into 11-oxotigogenone (VIII). Selective acetylation of these diols furnished the



(I) R = H †
(Ia) R = Ac



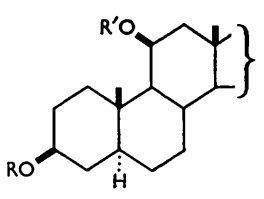
(II) R′ = H
(IV) R′ = α-OH
(IVa) R′ = α-OAc
(VI) R′ = β-OH
(VIa) R′ = β-OAc



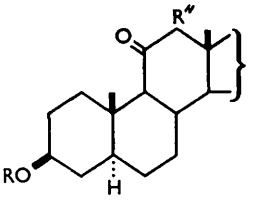
(III) R = R′ = H
(IIIa) R = Ac, R′ = H
(IIIb) R = R′ = Ac
(IIIc) R = H, R′ = Ac

† Tigogenin. The conformation of all the other compounds depicted above is that of tigogenin.

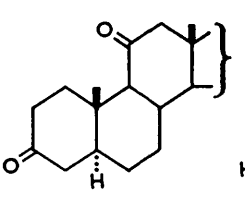
3β-acetoxy-11α- (IIIa) and -11β-alcohol (Va), the former in low yield.[6] Further acetylation of 11α-hydroxytigogenin acetate (IIIa) with acetic anhydride and pyridine gave the 3β : 11α-diacetate (IIIb), but these agents failed to esterify the 11β-hydroxy-ester (Va). We made 11β-acetoxytigogenin acetate (Vb) by using acetyl chloride and *NN*-dimethylaniline.[6b] Selective hydrolysis of the two diacetates (IIIb) and (Vb) gave the 11-acetates (IIIc) and (Vc). Final hydrolysis of the latter acetate (Vc) to the diol was tardy. Oxidation of 11β-acetoxytigogenin (Vc) with *N*-bromoacetamide in acetone gave the 11β-acetoxy-3-ketone (VIa), and acetylation of the ketol (IV) the 11α-acetoxy-ketone (IVa). We made the ketol (IV) by oxidation of the diol (III) with *N*-bromoacetamide.



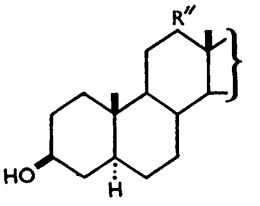
(V) R = R′ = H
(Va) R = Ac, R′ = H
(Vb) R = R′ = Ac
(Vc) R = H, R′ = Ac



(VII) R = R″ = H
(VIIa) R = Ac, R″ = H
(XII) R = H, R″ = α-OH
(XIII) R = H, R″ = β-OH



(VIII)

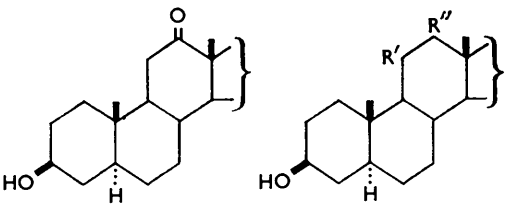


(IX) R″ = α-OH
(X) R″ = β-OH

Comparison of the properties of 11α- (III) and 11β-hydroxytigogenin (V), and, similarly of those of their esters, shows the following orders of activity : oxidation with *N*-bromoacetamide in acetone, 11β- > 3β- > 11α-; and, in contrast, oxidation with acidified aqueous potassium dichromate, 11β- > 11α- > 3β-; acetylation and hydrolysis of the ester groups, 3β- > 11α- ≫ 11β-. Oppenauer oxidation does not affect the 11β-hydroxy-group, and oxidation catalysed by metals[4] is probably similarly selective. *N*-Bromoacetamide has already been shown to oxidise 11β-alcohols in preference to their epimers and 3α-hydroxy-5β-steroids,[7] and *tert.*-butyl hypochlorite to oxidise a 3- in preference to an

[5] Bladon, Henbest, Jones, Lovell, and Woods, *J.*, 1954, 125.
[6] (*a*) Rosenkranz, Mancera, and Sondheimer, *J. Amer. Chem. Soc.*, 1954, **76**, 2227; (*b*) Crawshaw, Henbest, and Jones, *J.*, 1954, 731.
[7] Mancera, Romo, Sondheimer, Rosenkranz, and Djerassi, *J. Org. Chem.*, 1952, **17**, 1066; Herzog, Jevnik, and Hershberg, *J. Amer. Chem. Soc.*, 1953, **75**, 269; Hanze, Fonken, McIntosh, Searcy, and Levin, *ibid.*, 1954, **76**, 3179; Jones and Kocher, *ibid.*, p. 3682; cf. refs. 3, 4, and 12.

11α- or 11β-hydroxyl group.[8] However, chromatography of the products of some of our attempts at selective conversions betrayed their shortcomings (cf. Experimental section).



(XI)

(XIV) R′ = R″ = α-OH
(XV) R′ = β-OH, R″ = α-OH
(XVI) R′ = R″ = β-OH
(XVII) R′ = α-OH, R″ = β-OH

In assessing the intramolecular bonding in our sapogenins we have used their behaviour on paper chromatograms to test the relative strengths of their polar groups. In general, simple stanols with equatorial hydroxyl groups are more polar, and consequently have lower R_F values, than the isomers with axial groups,[9] although a few exceptions reveal unexpected steric effects.[10–12] Interaction between polar groups in steroids has not been studied by its effect on the R_F values, but the paper chromatography of sugars and cyclitols [13–15] suggests that vicinal equatorial hydroxy-groups engage in the strongest intramolecular bonding.

Martin [16] has propounded an explanation of partition chromatography by means of which a function $R_M = \log_{10}(1/R_F - 1)$ can be related to the free-energy change accompanying transport of the molecule from one phase to the other. To a satisfactory approximation (the R_M values being a measure of the chemical potential of the compound), the components of the molecule contribute additively; the function may therefore be likened to the parachor [17] or to Hammett's [18] substituent constant σ. Bate-Smith and Westall [19] showed that a further hydroxyl group introduced into the *ortho*-position of a phenol contributed less to the R_M value than one entering the *meta*-position. For our purpose, therefore, we deduced the contributions due to isolated hydroxy- and keto-groups in various positions in the steroidal sapogenins, with a view to calculating subsequently the discrepancies arising when such groups are juxtaposed. An earlier attempt to formulate the behaviour of steroids on paper chromatograms was based on different premises.[20]

Using the procedures devised by Sannié and Lapin [12] and by Heftmann and Hayden [21] for the paper chromatography of steroidal sapogenins, we produced chromatograms with discrepancies in the R_F values of $\geqslant 0{\cdot}03$, and with satisfactory reproducibility in the ΔR_M values calculated for the 11α- and 11β-hydroxy- and 11- and 12-oxo-groups of derivatives of tigogenin, tigogenin acetate, and tigogenone. The consistency, and the roundness of the spots on the chromatograms, as well as the propensity of the compounds to run in the solvent front in slightly more polar solvent systems, confirm the predominance of partition

[8] Fonken, Thompson, and Levin, *J. Amer. Chem. Soc.*, 1955, **77**, 172; ref. 7.
[9] Savard, *J. Biol. Chem.*, 1953, **202**, 557; *Recent Progr. in Hormone Res.*, 1954, **9**, 185.
[10] Kochakian and Stidworthy, *J. Biol. Chem.*, 1952, **199**, 607.
[11] Axelrod, *ibid.*, 1953, **205**, 173; *Recent Progr. in Hormone Res.*, 1954, **9**, 208; cf. Dirscherl and Gerhards, *Acta Endocrinol.*, 1955, **19**, 233.
[12] Sannié and Lapin, *Bull. Soc. chim. France*, 1952, 1080.
[13] Cifonella and Smith, *Analyt. Chem.*, 1954, **26**, 1132.
[14] Kowkabany, *Adv. Carbohydrate Chem.*, 1954, **9**, 303.
[15] (*a*) Isherwood and Jermyn, *Biochem. J.*, 1951, **48**, 515; (*b*) Mulvany, Agar, Peniston, and McCarthy, *J. Amer. Chem. Soc.*, 1951, **73**, 1255; (*c*) Posternak, Raymond, and Haerdi, *Helv. Chim. Acta*, 1955, **38**, 191.
[16] Martin, *Biochem. Soc. Symposia*, 1949, No. 3, p. 4; cf. ref. 19.
[17] Brimley and Barrett, "Practical Chromatography." Chapman and Hall, Ltd., London, 1953, p. 42.
[18] Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, 1940, p. 184.
[19] Bate-Smith and Westall, *Biochim. Biophys. Acta*, 1950, **4**, 427; Bradfield and Bate-Smith, *ibid.*, p. 441; Martin, *Discuss. Faraday Soc.*, 1949, **7**, 332.
[20] Grundy, Simpson, and Tait, *Nature*, 1952, **169**, 795.
[21] Heftmann and Hayden, *J. Biol. Chem.*, 1952, **197**, 47.

effects; appreciable adsorption on to the paper (which intervenes in the paper chromatography of some steroids [22]) is undesirable, especially as elution of adsorbed steroids defies a simple explanation, presumably owing to deformations in the molecules.[23] Our evidence indicates that the effects of substituents at the 3- and the 11-position are discrete. Calculations (recorded in the Experimental section) of molecular rotations confirm this impression, although they disclose anomalies for 11α- and 11β-acetoxy-groups.

Further calculations (see Table 1) show how published data yield increments attributable to the 11β- and 17α-hydroxy- and 11-oxo-substituents in corticoids, from

TABLE 1. *Derivatives of* 21-*hydroxypregn*-4-*ene*-3 : 20-*dione* (11-*deoxycorticosterone*).

				R_M Values [a]			
Derivative	B_1	B_2 [b]	B_3	Sakal and Merrill [c,g]	Schmidt *et al.* [h]	Hofmann *et al.* [i]	Pechet [j]
11β-Hydroxy-	0·53	−0·27	0·75	−0·27	0·35	0·43	−0·75
17α-Hydroxy-	0·43	−0·37	0·60	−0·37	0·69	0·75	−0·83
11-Oxo-	0·25	−0·48	0·48	—	—	—	−0·91
Parent compound	−0·79	−1·28	−0·48	−1·06	0·95	1·00	−1·10
Group					ΔR_M Values [d]		
11β-Hydroxy-	1·31	1·01	1·23	0·79	0·61 [e]	0·57 [e]	0·34
17α-Hydroxy-	1·22	0·91	1·08	0·69	0·27 [e]	0·25 [e]	0·27
11-Oxo-	1·04	0·80	0·95	—	—	—	0·19 [a]
Comparison of calculated [f] *and observed* R_M *values.* [a]							
11β : 17α-Dihydroxy- (Cortisol)							
Calc.	1·74	0·64	1·83	0·42	0·07	0·18	−0·38
Obs.	1·51	0·95	2·00	0·25	0·04	0·25	−0·16
17α-Hydroxy-11-oxo- (Cortisone)							
Calc.	1·47	0·43	1·55	—	—	—	−0·64
Obs.	1·28	0·60	1·69	0·00	0·05	0·27	−0·41

[a] Calc. from Bush's results (for solvent systems B_1, B_2 and B_3) (*Biochem. J.*, 1952, **50**, 370) and the other sources cited. [b] The R_F values in this system were too high for accurate calculation. [c] Dried paper. The separations secured were not very sharp; undried papers gave poorer results. [d] Calc. from the four rows above in the Table. [e] Signs reversed. [f] Calc. from figures in the two sections above in the Table. [g] Sakal and Merrill, *Science*, 1953, **117**, 451. [h] Schmidt, Staudinger, and Bauer, *Biochem. Z.*, 1953, **324**, 128. [i] Hofmann and Staudinger, *ibid.*, 1951, **322**, 230. [j] Pechet, *J. Clin. Endocrinol. Metab.*, 1953, **13**, 1542.

which R_M and R_F values for cortisone and cortisol can be derived in fair agreement with those found by experiment. These results again refute the existence in the dissolved steroid of strong intramolecular hydrogen bonds implicating hydroxy- and keto-substituents at the 11-position. Although 17α-hydroxypregnan-20-ones contain bonds of this type, which are detectable by infra-red spectroscopy [24] and by anomalous optical rotations,[25] they have not been noticed in the 17α : 21-dihydroxy-20-ketone system. We shall discuss this below.

Table 1 contains results for aqueous solvent systems. Burton, Zaffaroni, and Keutmann [26] devised means of resolving mixtures of corticoids in non-aqueous solvent systems containing, for example, propylene glycol or formamide as the polar component. This practice has been widely adopted, but the R_F values of corticoids in such systems may be too low for direct measurement; therefore the results are generally given as R_T values, the R_T of a compound being proportional to the ratio of its R_F to that of a standard.[9] Provided that the R_F of the standard is small compared to unity and to $1/R_T$, $\Delta R_M = -\log_{10} R_T$ approximately, where ΔR_M is the contribution of the group distinguishing the steroids under comparison. Applying this equation to the data of

[22] Cf. Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 3232.
[23] Brooks, Klyne, and Miller, *Biochem. J.*, 1953, **54**, 212.
[24] Jones, Humphries, Herling, and Dobriner, *J. Amer. Chem. Soc.*, 1952, **74**, 2820; cf. Fukushima, Dobriner, Heffler, Kritchevsky, Herling, and Roberts, *ibid.*, 1955, **77**, 6585.
[25] Norymberski, *J.*, 1954, 762; cf. Bloom, Agnello, and Laubach, *Experientia*, 1956, **12**, 27.
[26] Burton, Zaffaroni, and Keutmann, *J. Biol. Chem.*, 1951, **188**, 763.

Burton *et al.*, we find ΔR_M values as follows for the groups mentioned in Table 3: 17α-hydroxy-, 0·85; 11β-hydroxy-, 0·69; 11-oxo-, 0·30. The aggregate ΔR_M value calculated for the substituents at the 11- and the 17-position is 1·16 in cortisone and 1·55 in cortisol, in fair agreement with the increments of 1·39 and 1·77 deduced from the data given for these compounds and 11-deoxycorticosterone. Savard's results [9] with systems composed of propylene glycol and light petroleum or toluene testify to the excellence of the resolution and afford the ΔR_M values set out in Table 2, the approximate equation being used when the implicit assumptions seemed justifiable. The weaker influence of the 17α-hydroxy-group in the 21-deoxy series reveals the hydrogen-bonding in these compounds, mentioned above, and the low polarity of the 21-hydroxyl and enhanced activity of the 17-hydroxyl in the 20-oxo-17 : 21-diols suggest that in these compounds the primary displaces the tertiary hydroxyl in the association with the keto-group.

TABLE 2. ΔR_M *Values for hydroxyl groups.*[a]

Mobile phase [b]	6α	6β	11α	11β	14α	16α	17α [c]	17α [d]	21 [c]
Light petroleum ...	1·92	1·33	1·70	1·19	1·36	1·70	0·92	1·17	0·71
	(0·05)	(0·11)	(0·15)	(0·09)	(0·18)				
Toluene	1·88	1·40	1·46	1·15	—	1·96	0·98	1·32	*ca.* 0·4
		(0·18)		(0·20)			(0·17)		

[a] Calc. from Savard's data [9] from the equation $\Delta R_M = -\log_{10} R_T$ (see text). Values in parentheses indicate limits of error, where two or more calculations could be made. [b] Stationary phase: propylene glycol. [c] In 17α : 21-dihydroxy-20-oxopregnanes. [d] In 17α-hydroxy-20-oxopregnanes. [e] 11-Oxo, $\Delta R_M = 0·79$ (0·12).

The high resolving power of the solvent systems used in our initial experiments hindered extension of these methods to dihydroxy-keto- and trihydroxy-sapogenins, because in conditions suitable for these the monohydroxy-compounds had R_F values too high to permit accurate calculation. We overcame this difficulty by reversing the roles of the phases: with the predominantly aqueous phase flowing, we achieved adequate resolving power and satisfactory R_F and R_M values (cf. refs. *h*—*j* of Table 1) and, the phases having been transposed, the more polar sapogenins outdistanced the monohydroxy-compounds. With the most suitable reversible solvent system (described in the Experimental section as D) corticoids and their 21-acetates, most of which were more polar than the sapogenins, were separated satisfactorily when the predominantly aqueous phase was used as the stationary component; ΔR_M values calculated in this way for the 11β-hydroxy- and 11-oxo-groups in these compounds were nearly equal (but of opposite sign) to the corresponding increments obtained from the R_F values of the sapogenins run with the phases reversed (see Table 3). Such agreement suggests a likely extension of the present methods satisfactorily to steroids of widely differing polarities.

We have been able to confirm the anomalous behaviour of rockogenin (X) and *epi*rockogenin (IX) in some solvent systems; [12] when they run in the expected order, *i.e.*, with the former (12β-hydroxy; equatorial) behaving as the more polar epimer, the separation is small. Most of our calculations pertain to systems in which these epimers run normally. The anomaly may be attributed to the formation of solvates that detract mostly from the polarity of the more hydrophilic groups; a similar explanation has been adduced in the course of comments on peculiarities in the paper chromatography of sugars.[15a] Or we could presume that the 12β-hydroxyl tends to form a bond with one of the oxygen atoms in the *spiro*ketal system or is hampered by groups or atoms in rings E and F (*e.g.*, at the 20-position),[27] just as the 19α(equatorial)-substituent in the triterpenoid nucleus [28] suffers interference from the hydrogen atoms at $C_{(12)}$. It is noteworthy in the present context that the rotational contribution (ΔM_D −9°) of the 12β-hydroxyl group to

[27] (*a*) Callow, Dickson, Elks, Evans, James, Long, Oughton, and Page, *J.*, 1955, 1966; (*b*) cf. Mueller, Stobaugh, and Winniford, *J. Amer. Chem. Soc.*, 1953, **75**, 4888.

[28] Ames, Beton, Bowers, Halsall, and Jones, *J.*, 1954, 1905; cf. Klyne, *Experientia*, 1956, **12**, 119; Rothman and Wall, *J. Amer. Chem. Soc.*, 1956, **78**, 1744; Hassall and Reyle, *Chem. and Ind.*, 1956, 487.

the molecular rotation of rockogenin is abnormal, whereas that (ΔM_D + 107°) of the 12α-hydroxyl in *epi*rockogenin is normal; Barton and Klyne[29] give +50° and +93° as average values for such contributions. The effects of changes in the side chain on the stability of the 12-hydroxy-11-keto-steroids have been discussed in another paper.[1c]

TABLE 3. ΔR_M [a] *Values for various groups* [b] *in steroidal sapogenins.*

	3-Oxo	3β-OH	3α-OH	11-Oxo	11α-OH	11β-OH	12-Oxo	12α-OH	12β-OH	15β-OH
Solvent DR [c]	0·83	1·09 [c]	—	0·62 [f]	1·29	0·95 [f]	0·69	1·13	1·18	0·40
	0·69 [d]	0·94 [d]	1·09 [d]	—	—	—	—	—	—	—
Solvent E ...	—	—	—	0·53	1·34	0·93	0·91	1·16	1·30	0·56

The values for the substituents at the 3-position were calculated with deoxysmilagenin as the reference compound, and all the others from tigogenin (I) or tigogenone (II). [b] In the tigogenin series, unless otherwise stated; cf. previous footnote and footnotes *d* and *e*. [c] Signs of ΔR_M values reversed. [d] For 5β-series, *i.e.*, smilagenin and related compounds. For the 3β-OH in diosgenin, ΔR_M = 1·19. [f] Mean ΔR_M values calc. from R_F values of cortisone, cortisol, and Reichstein's compound S and their 21-acetates, run in solvent D: 11-oxo-, 0·60; 11β-OH, 0·94.

Using the figures in Table 3, we could assess the differences (δ) between the calculated and observed R_M values for the 12-hydroxy-11-oxo- and 11 : 12-dihydroxy-tigogenins. Table 4 shows the satisfactory agreement between the conclusions reached when a solvent system was used ordinarily or (probably with slightly greater accuracy) with the usual functions of the phases reversed. The conclusions testify to bonding in the 12β-hydroxy-11-ketone (XIII) stronger than in its epimer (XII); indeed, so marked is the difference that the latter behaves as the more polar form, in spite of the slightly greater polarity of the hydroxyl group in rockogenin (X) compared with that in *epi*rockogenin (IX). The ketolic glycosides leptoside and inertoside (in another solvent system) are not so anomalous, for the latter (with a 12α-hydroxy-group) outruns its epimer;[30] however, the isomeric 11α-hydroxy-12-ketone intermedioside runs further than both, intramolecular bonding

TABLE 4. *Detection of intramolecular bonding (by δ values).*

	Solvent system DR [a] R_M Values			Solvent system E R_M Values		
Tigogenin deriv.	Calc.[b]	Found	δ [c]	Calc.[c]	Found	δ [c]
12α-Hydroxy-11-oxo-	1·15	0·75	0·40	0·57	0·37	0·20
12β-Hydroxy-11-oxo-	1·20	0·39	0·81	0·71	−0·14	0·85
11α : 12α-Dihydroxy-	1·82	0·83	0·99	1·38	0·69	0·69
11β : 12α-Dihydroxy-	1·48	0·95	0·53	0·97	1·12	−0·15
11β : 12β-Dihydroxy-	1·53	0·72	0·81	1·11	0·53	0·58
11α : 12β-Dihydroxy-	1·87	0·87	1·00	1·52	0·79	0·73

[a] Signs of R_M and δ values reversed. [b] From the increments given in Table 3. [c] For meaning, see text.

obviously outweighing the superiority in polarity that the unaffected 12-oxo- and equatorial 11α-hydroxy-groups would otherwise bestow on it. (These last compounds are 14β-hydroxy-steroids with rings C and D *cis*; the steric effects would therefore differ in detail from those considered for the sapogenins.) Anomalies in the rates of oxidation of 11-hydroxy-12-oxo-steroids have also been observed.[31]

The figures in Table 4 also reveal appreciable vicinal effects in the diastereoisomeric 11 : 12-dihydroxytigogenins. Although the order of running defies any simple explanation, the δ values are rational, the most marked interplay occurring between the two equatorial hydroxy-groups and between the 11α(equatorial)- and 12α(axial)-groups. The doubly axial 11β : 12α-diol system is least affected by intramolecular forces. The axial 12α-hydroxyl group allows strong bonding in the 11α : 12α-dihydroxy-system, but the 11β-hydroxy-group in 11β : 12α- and 11β : 12β-dihydroxy-tigogenin is evidently reluctant to be so engaged.

[29] Barton and Klyne, *Chem. and Ind.*, 1948, 755.
[30] Hegedüs, Tamm, and Reichstein, *Helv. Chim. Acta*, 1955, **38**, 98; Schindler, *ibid.*, 1956, **39**, 64.
[31] Archer, Lewis, Martini, and Jackman, *J. Amer. Chem. Soc.*, 1954, **76**, 4915.

Addition of boric acid [32,33] to the solvents used for our paper chromatograms markedly affected the progression of derivatives containing the 11α : 12α- and 11β : 12β-diol systems. This influence is most aptly expressed as the change (ΔR_M^B) in R_M value brought about by the addition of the acid. The figures compared in Table 5 show that the results are nearly the same when the roles of the solvents are reversed. The use of boric acid and reversal of the phases permit separation and identification of each of the four diastereoisomeric forms of 11 : 12-dihydroxy-tigogenin, the unmodified systems being of inadequate resolving power. The hydroxyl groups spanned by the acid might in this way be protected, for example, from oxidation.[34]

TABLE 5. *Effects of boric acid on the paper chromatography of the 11 : 12-dihydroxytigogenins.*

vic.-Diols (tigogenin derivatives)	R_M Values			R_M [a] Values		
	Solvent D	Solvent DB	ΔR_M^B	Solvent DR	Solvent DBR	ΔR_M^B
11α : 12α- (e, a)	−0·11	−0·91	0·80	0·83	0·18	0·65
11β : 12α- (a, a)	0·21	0·05	0·16	0·95	0·69	0·27
11β : 12β- (a, e)	−0·37	−0·69	0·32	0·72	0·29	0·43
11α : 12β- (e, e)	0·02	−0·04	0·06	0·87	0·66	0·21

[a] Signs of the R_M and ΔR_M^B values reversed; for the meaning of the latter function, see text.

EXPERIMENTAL

Unless other conditions are mentioned, the description below is of our regular practice. Measurements of optical rotation and ultraviolet absorption were on solutions of the steroids in $CHCl_3$, and of infrared absorption on CS_2 solutions. The method of measuring infrared absorption has been described elsewhere.[35] Grade O alumina (Peter Spence and Sons Ltd., Widnes, Lancs.) was used for chromatography. The products of reactions were either precipitated by water from water-miscible solvents, washed with water, and dried, or extracted into a water-immiscible solvent, washed with aqueous sodium hydrogen carbonate and water, dried ($MgSO_4$), and filtered, the solvent being then distilled to dryness under reduced pressure. The structures of compounds identified with authentic specimens were confirmed by mixed m. p. and infrared spectroscopy. M. p.s were measured with a Kofler block.

All the sapogenins described herein gave in the infrared absorption characteristic of *iso*spirostans,[36] but only the positions of maxima due to carbonyl groups are mentioned.

Molecular-rotation Differences.—Acyloxy-groups at the 11-position affect the contributions at the 3-position. Thus for acetylation and oxidation (to the 3-ketone) of the 3β-hydroxyl group, the following ΔM_D values were observed : [37] (I), −54°, +60°; (VII), −41°, +77°; (III), −15°, +57°; (IIIc), +37°, +104°; (V), −54°, +46°; (Vc), −2°, +46°.

Contributions (ΔM_D) from the substituents at the 11-position for derivatives of tigogenin (I), tigogenin acetate (Ia), and tigogenone (II) are as follows. 11α-Acetoxy : (IIIc), −183°; (IIIb), −114°; (IVa), −139°. 11-Oxo : (VII), +123°; (VIIa), +136°; (VIII), +140°. 11α-Hydroxy : (III), −70°; (IIIa), −31°; (IV), −73°. 11β-Hydroxy : (V), +25°; (Va), +25°; (VI), +9°. 11β-Acetoxy : (Vc), +72°; (Vb), +60°; (VIa), +124°.

Paper Chromatography.—The solvents were run downwards in the usual type of apparatus,[27a] kept at 30° ± 0·5°; the solvents ("AnalaR" or distilled) were kept at this temperature for several hours before use. The papers (Whatman No. 2) were exposed to the vapour over the mixture for at least 40 hr. before solvent was allowed to run over them. If the mixture separated into two phases, the paper was exposed in the equilibration stage to both. In two-phased mixtures, the less aqueous layer provided the moving phase, unless the functions were reversed (such conditions being designated by the letter R after the description of the solvent;

[32] Boeseken, *Adv. Carbohydrate Chem.*, 1949, **4**, 189; Zittle, *Adv. Enzymol.*, 1951, **12**, 493; cf. Kowkabany, ref. 14.
[33] Angyal and McDonald, *J.*, 1952, 686.
[34] Cf. Zittle, ref. 32, p. 500; Staple, *Nature*, 1955, **176**, 1125; Brutcher, Roberts, Barr, and Pearson, *J. Amer. Chem. Soc.*, 1956, **78**, 1507.
[35] Dickson, Page, and Rogers, *J.*, 1955, 443.
[36] See ref. 27*a* for literature.
[37] Cf. Fieser and Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp. New York, 1949, p. 208.

the running times were then increased by about 50%). Mixtures designated by the letter B contained 8·5% (w/v) of boric acid, and the papers were dipped in 5% (w/v) aqueous boric acid and dried at room temperature before they were used.

The sapogenins were located on the chromatograms by Komarowsky's reagent,[27a] by means of which about 4 μg. were detectable; phosphomolybdic [38] and trichloroacetic acid [21] were less sensitive as reagents.

The R_F values of several selected sapogenins varied by a standard mean deviation of $\pm 0{\cdot}03$ on more than a dozen chromatograms and were reproducible when the specimens were included in mixtures.

The R_F values included in Table 6 were obtained in the above fashion. Values given elsewhere in this Experimental section were obtained with solvent system F4 (see below). R_M values accurate enough for most purposes were calculated from a plot of $R_M = \log_{10}(1/R_F - 1)$; the resolving power ($\Delta R_F/\Delta R_M$) was obviously greatest when $R_F = 0{\cdot}5$. When $R_F < 0{\cdot}2$, $R_M = -\log_{10} R_F$ approx.

Solvent Systems.—In this section light petroleum means the fraction, b. p. 60—80°, and the chloroform was alcohol-free. Times for the running solvent to move about 35 cm. are given. Systems A to E were based on data in reports by Heftmann and Hayden [21] and by Bush,[39] and F to I on systems used by Sannié and Lapin [12] and by Callow *et al.*[27a]

(A) Light petroleum (400), ethanol (10), toluene (100), water (90), 95 min. (B) As (A), except toluene (50), 95 min. (C) As (A), but no toluene, 140 min. (D) Light petroleum (250), methanol (600), toluene (500), water (300), 95 min. (E) As (C), except ethanol (200), 100 min. (F) Light petroleum (500), chloroform (50), acetic acid (5), 110 min. (G) As (F), except light petroleum (250), and benzene (250), 95 min. (H) Chloroform (50), acetic acid (5), benzene (500), 120 min. (I) Light petroleum (500), acetic acid (10), 95 min. (J) Light petroleum (500), methanol (10), 105 min. (Tetrahydrofurfuryl alcohol, *NN*-dimethylformamide, 2-methoxyethanol, nitromethane, and ethyl acetate could be used without advantage instead of methanol.) (K) Light petroleum (500), pyridine (10), 105 min. System F4 was the same as F, except that it was used at room temperature, equilibrated overnight, and run on Whatman No. 4 papers; the separations were then not quite as sharp as with F.

The following compounds used in this work as starting materials had m. p.s and rotations in agreement with specimens described in the literature: [40] tigogenin (I), R_F 0·85 (sublimes readily at 170—180°/10^{-1} mm.); tigogenin acetate (Ia), R_F 0·95; hecogenin (XI), R_F 0·74; hecogenin acetate (XIa), R_F 0·89; rockogenin (X); *epi*rockogenin (IX). Spectra of Nujol mulls of tigogenin gave a peak at 1665 cm.$^{-1}$, absent from the spectra of solutions and from all the spectra of the acetate (Ia), from which it was derived or into which it was converted.

Professor D. H. R. Barton provided us with specimens of deoxysmilagenin, *epi*sarsasapogenin, smilagenin, smilagenone, digitogenin, and sarsasapogenin from the late Professor Kon's collection.[41] They appeared pure by paper chromatography. (The two last-named were also obtained from Light & Co., Poyle, Colnbrook, Bucks.) Professor Kon's two samples [42] of 3-methylsarsasapogenin, m. p. 179—180° and 188—189°, are mixtures whose properties are discussed below. His specimen of gitogenin (m. p. 273—277°) was contaminated with a more polar sapogenin (see Table 6).

Tigogenone (II).—Tigogenin (I) (10 g.) was oxidised [5] in refluxing "AnalaR" acetone (500 ml.) with a solution of 0·33M-potassium dichromate in 4N-sulphuric acid (30 ml.; 1·25 equiv.). After 5 min. the green solution was poured into water (2 l.), and the precipitate, after being washed with water and dried, crystallised from acetone as needles (7·9 g., 80%) of tigogenone (II), m. p. 204—208° (subliming at 140—170°/10^{-5} mm.), $[\alpha]_D^{29}$ −50° (*c* 0·51), −35° (*c* 0·58 in pyridine), $\nu_{max.}$ 1710 cm.$^{-1}$, R_F 0·92. Its 2 : 4-*dinitrophenylhydrazone* formed yellow needles, m. p. 260—266°, $[\alpha]_D^{25}$ −44° (*c* 0·45), $\lambda_{max.}$ 368·5 mμ (ε 29,100), on chromatography on alumina and crystallisation from ethyl acetate (Found: N, 9·55. $C_{33}H_{46}O_6N_4$ requires N, 9·4%).

[38] Kritchevsky and Kirk, *Arch. Biochem. Biophys.*, 1952, **35**, 346; cf. ref. 27*a*.
[39] Bush, *Biochem. J.*, 1952, **50**, 370.
[40] Wall, Krider, Rothman, and Eddy, *J. Biol. Chem.*, 1952, **198**, 533; Klass, Fieser, and Fieser, *J. Amer. Chem. Soc.*, 1955, **77**, 3829; cf. ref. 17.
[41] Kon, Soper, and Woolman, *J.*, 1939, 1201; Marker and Rohrmann, *J. Amer. Chem. Soc.*, 1939, **61**, 943.
[42] Bolt and Backer, *Rec. Trav. chim.*, 1937, **56**, 1139; Farmer and Kon, *J.*, 1937, 414; Kuwada and Miyasaka, *J. Pharm. Soc. Japan*, 1938, **58**, 540; Barton, *Experientia*, 1955, Suppl. II, p. 121; B.P. 738,576; U.S.P. 2,713,061.

TABLE 6. R_F *Values in various solvent systems.*

No.	Compound	A	B	C	D	E
1	Deoxysmilagenin	1·00	1·00	1·00	1·00	0·95
2	Sarsasapogenin	0·91	0·90	0·90	0·95	0·93
3	*epi*Sarsasapogenin	0·84	0·78	0·74	0·90	0·86
4	Smilagenin	0·93	0·90	0·90	0·95	0·93
5	Tigogenin (I)	0·88	0·80	0·77	1·00	0·93
6	Diosgenin	—	0·80	0·77	1·00	0·93
7	Smilagenone	0·99	1·00	0·98	1·00	0·94
8	Tigogenone (II)	0·99	0·97	0·98	1·00	0·94
9	11-Oxotigogenone (VIII)	0·98 *	0·87	0·80 *	1·00	0·85
10	3-Methylsarsasapogenin	0·95	0·92	0·73 0·87	1·00	0·91
11	Hecogenin (XI)	0·50	0·31	0·15	0·95	0·62
12	11-Oxotigogenin (VII)	0·71	0·50	0·33	0·90	0·73
13	Rockogenin (X)	0·43 *	0·26	0·22 *	0·83	0·40
14	*epi*Rockogenin (IX)	0·30 *	0·14	0·15	0·80	0·48
Tigogenin derivatives						
15	11α-Hydroxy- (III)	0·25	0·13	0·10 *	0·78	0·38
16	11β-Hydroxy- (V)	0·50	0·36	0·22	0·90	0·61
17	12α-Hydroxy-11-oxo- (XII)	0·16 *	0·09 *	0·03 *	0·72	0·30
18	12β-Hydroxy-11-oxo- (XIII)	0·53	0·35 *	0·23	0·88	0·58
19	11α : 12α-Dihydroxy- (XIV)	0·15 *	0·10 *	0·07 *	0·56	0·17
20	11β : 12α-Dihydroxy- (XV)	0·07 *	0·04 *	0·02 *	0·38	0·07
21	11β : 12β-Dihydroxy- (XVI)	0·14 *	0·08 *	0·03 *	0·70	0·23
22	11α : 12β-Dihydroxy- (XVII)	0·13 *	0·08 *	0·05 *	0·49	0·14
23	Gitogenin	—	0·05 0·12	0·03 * 0·07	0·70 0·80	0·37 0·68
24	Digitogenin	0·07	0·04	0·03		0·37

No.	F	G	H	I	J	K	DB	DR	DBR
1	0·98	1·00	1·00	0·97	1·00	0·97	1·00	0·02	0·00
2	0·90	0·94	0·98	0·87	0·75	0·83	1·00	0·15	0·14
3	0·84	0·92	0·98	0·80	0·51	0·71	1·00	0·20	0·18
4	0·87	0·95	0·98	0·89	0·75	0·83	1·00	0·15	0·15
5	0·89	0·93	0·98	0·79	0·53	0·73	0·98	0·20	0·20
6	0·85	0·95	0·98	0·77	0·52	0·71	0·98	0·24	0·20
7	0·95	1·00	1·00	0·93	0·90	0·85	1·00	0·09	0·00
8	0·95	1·00	1·00	0·95	0·90	0·84	1·00	0·12	0·00
9	0·90	1·00	1·00	0·81	0·65	0·66	1·00	0·30	0·05
10	0·93	0·99	1·00	0·87 0·94	0·77 0·87	0·80 0·85	1·00	0·25 0·19	0·00
11	0·48	0·95	0·95	0·30	0·11	0·29 *	0·98	0·55	0·35
12	0·64	0·90	0·95	0·50	0·24	0·43	0·98	0·58	0·35
13	0·41 *	0·77	0·89	0·27 *	0·19 *	0·15	0·90	0·79	0·70
14	0·30 *	0·61	0·84	0·15 *	0·08 *	0·15	0·80	0·77	0·68
Tigogenin derivatives									
15	0·20	0·65	0·85	0·11 *	0·04 *	0·12	0·85	0·83	0·70
16	0·47	0·83	0·95	0·30	0·13	0·29	0·93	0·69	0·50
17	0·09	0·45	0·76	0·08 *	0·04 *	0·11 *	0·75	0·85	0·75
18	0·44	0·79	0·91	0·28	0·13	0·26 *	0·83	0·71	0·50
19	0·11 *	0·31*	0·57	0·06 *	0·04 *	0·12	0·89	0·87	0·60
20	0·10 *	0·34 *	0·59	0·04 *	0·04 *	0·03	0·47	0·90	0·83
21	0·15 *	0·55 *	0·73	0·10 *	0·04 *	0·13 *	0·83	0·84	0·66
22	0·09 *	0·30 *	0·51	0·08 *	0·04 *	0·12 *	0·52	0·88	0·82
23	0·09 0·18	—	0·73	0·06 0·11	0·06 *	0·11 *	0·70 0·80	0·77 0·66	0·70 0·46
24	0·11	0·45	0·71	0·08 *	0·04 *	0·08 *	0·73	0·83	0·74

* R_F value measured for the head of a streak; in other cases spots were well-defined circles.

11-Oxotigogenone (VIII), made in 66% and 78% yield respectively by similar oxidations of 11-oxotigogenin (VII) and 11β-hydroxytigogenone (VI) (see below), needles (from ether–hexane), m. p. 245—248°, $[\alpha]_D^{25}$ −16° (*c* 1·2), $\nu_{max.}$ 1710 cm.$^{-1}$, R_F 0·90 (lit.,[3] m. p. 236—238°, $[\alpha]_D^{20}$ −19°).

11α-*Hydroxytigogenin* (III).—11-Oxotigogenin acetate (VIIa) (2 g.)[43] was reduced[44] with

[43] (*a*) Schmidlin and Wettstein, *Helv. Chim. Acta*, 1953, **36**, 1241; (*b*) Cornforth, Osbond, and Phillipps, *J.*, 1954, 907.

[44] Heusser, Anliker, and Jeger, *Helv. Chim. Acta*, 1952, **35**, 1537; Oliveto, Hershberg, *et al.*, *J. Amer. Chem. Soc.*, 1953, **75**, 1505, and earlier papers.

sodium (20 g.) in refluxing propan-1-ol (400 ml.). The metal was added during 1 hr., and the solution refluxed for another 1·5 hr. The crystallised product (III) (1·59 g., 87%) occurred as rhombs, m. p. 220—222°, $[\alpha]_D^{26}$ −78° (*c* 0·91), R_F 0·35.

11α-*Hydroxytigogenin acetate* (IIIa) was made from the 3-alcohol (III) (0·74 g.), pure pyridine (5 ml.), and acetic anhydride (0·23 g., 1·3 equiv.), kept for 22 hr. at 0°. Chromatography of the product on alumina (Grade H) and crystallisation from acetone of the solid eluted with ether afforded the 3-*acetate* (IIIa) as needles (60 mg., 7%), m. p. 202—204°, $[\alpha]_D^{25}$ −74° (*c* 0·5), $\nu_{max.}$ 1730 and 1240 cm.$^{-1}$, R_F 0·73 (Found : C, 73·1; H, 9·9. $C_{29}H_{46}O_5$ requires C, 73·4; H, 9·8%). Methanol eluted a fraction (0·33 g.) identified as the original diol (III), acetylation of which with pyridine (2 ml.) and acetic anhydride (5 ml.) at 90—100° for 1 hr. gave the diacetate (IIIb), needles (from acetone–hexane), m. p. 173—175°, $[\alpha]_D^{27}$ −80° (*c* 0·64), $\nu_{max.}$ 1730 and 1238 cm.$^{-1}$, R_F 0·93.

11α-*Acetoxytigogenin* (IIIc).—A solution of the foregoing diacetate (IIIb) (0·8 g.) in methanol (34 ml.) containing potassium hydrogen carbonate (0·22 g., 1·4 equiv.) and water (4 ml.) was refluxed for 1·6 hr. The isolated product (0·77 g.) crystallised from acetone as needles of the 11-*acetate* (IIIc) (0·39 g., 53%), m. p. 175—178°, $[\alpha]_D^{18}$ −95° (*c* 0·85), $\nu_{max.}$ 1730 and 1240 cm.$^{-1}$, R_F 0·80 (Found : C, 73·6; H, 9·7. $C_{29}H_{46}O_5$ requires C, 73·4; H, 9·8%).

11-Oxotigogenin (VII) (made by hydrolysis of its 3-acetate [44]), rhombs (from acetone–hexane), m. p. 224—227°, $[\alpha]_D^{22}$ −34° (*c* 0·98), $\nu_{max.}$ 1705 cm.$^{-1}$, R_F 0·81 (lit.,[3] m. p. 223—225°, $[\alpha]_D^{20}$ −29°).

11β-*Hydroxytigogenin* (V).—Reduction of 11-oxotigogenin acetate (VIIa) (0·5 g.) in absolute ether (200 ml.) with lithium aluminium hydride (0·5 g.) in ether (100 ml.) (to which the steroid was added) was complete in 20 min. at the b. p.[3] Crystallisation from acetone of the isolated steroid [which gave a faint spot on a paper chromatogram in the position expected for the 3β : 11α-diol (III)] afforded prisms (3·44 g., 75%), m. p. 200—205°, $[\alpha]_D^{19}$ −53°. Recrystallisation gave the pure diol (V), m. p. 208—212°, $[\alpha]_D^{18}$ −56° (*c* 0·77), R_F 0·61 (lit.,[3] m. p. 202—204°, $[\alpha]_D^{20}$ −49°).

Reduction of the ketol acetate (VIIa) (5 g.) in refluxing alcohol (120 ml.) and water (20 ml.) containing potassium hydrogen carbonate (2 g.) and sodium borohydride (2·6 g.) was complete within 1·5 hr. The crude product appeared homogeneous (by paper chromatography), and crystallisation from acetone afforded the pure diol (V) (4·21 g., 92%). The derived acetate (Va) occurred as needles, m. p. 230—235°, $[\alpha]_D^{22}$ −63° (*c* 0·83), $\nu_{max.}$ 1735 and 1242 cm.$^{-1}$, R_F 0·86 (Found : C, 73·45; H, 9·6. Calc. for $C_{29}H_{46}O_5$: C, 73·4; H, 9·8%) (lit.,[3] m. p. 225—227°, $[\alpha]_D^{20}$ −47°, and [44] m. p. 222—224°, $[\alpha]_D^{20}$ −55°).

11β-*Acetoxytigogenin Acetate* (Vb).—The 3β : 11β-diol (V) (3·4 g.) in " AnalaR " benzene (50 ml.), toluene (25 ml.), *NN*-dimethylaniline (20 ml.), and acetyl chloride (6 ml.) was refluxed gently under nitrogen for 20 hr. Crystallisation from acetone of the isolated steroid afforded rhombs (3·76 g., 93%) of the 3β : 11β-*diacetate* (Vb), m. p. 102—106°. Recrystallisation gave the analytical specimen, m. p. 103—107°, $[\alpha]_D^{20}$ −38° (*c* 0·94), $\nu_{max.}$ 1732 and 1240 cm.$^{-1}$, R_F 0·91 (Found : C, 71·8; H, 9·6. $C_{31}H_{48}O_6$ requires C, 72·1; H, 9·4%).*

11β-*Acetoxytigogenin* (Vc), made by hydrolysis (1·5 hr.) of the foregoing diacetate (Vb) (3·2 g.) in refluxing methanol (90 ml.) and water (15 ml.) containing potassium hydrogen carbonate (2·5 g.), crystallised from acetone as needles (2·03 g., 69%), m. p. 208—210°. A recrystallised specimen had m. p. 214—215°, $[\alpha]_D^{24}$ −41° (*c* 1·02), $\nu_{max.}$ 1734 and 1240 cm.$^{-1}$, R_F 0·73 (Found : C, 73·5; H, 9·85. $C_{29}H_{46}O_5$ requires C, 73·4; H, 9·8%).

11β-*Acetoxytigogenone* (VIa).—11β-Acetoxytigogenin (Vb) (1·5 g.) in " AnalaR " acetone (80 ml.) was treated with *N*-bromoacetamide (1·0 g.) and set aside for 18 hr. at room temperature. Crystallisation from acetone of the isolated steroid gave needles (1·07 g., 72%), m. p. 185—192°. Two recrystallisations gave the pure *acetate* (VIa), m. p. 190—194°, $[\alpha]_D^{19}$ −31° (*c* 1·1), $\nu_{max.}$ 1732 and 1238, and 1715 cm.$^{-1}$, R_F 0·88 (Found : C, 73·8; H, 9·3. $C_{29}H_{44}O_5$ requires C, 73·7; H, 9·4%).

Oxidation of 11α-*Hydroxytigogenin* (III).—The diol (III) (0·155 g.) in acetone (5 ml.) was oxidised at room temperature with 1·33N-potassium dichromate in 5N-sulphuric acid (0·59 ml., 1·1 equiv.). After the oxidant had been used up, the steroid (0·165 g.), $[\alpha]_D^{23}$ −45°, was isolated.

* Callow and James (*J.*, 1956, 4739) give m. p. 141—142·5°, $[\alpha]_D^{21}$ −35°, for this compound, but have no specimen available for comparison. In a redetermination our material, now 2½ years old, had m. p. 134—136°, but its infrared absorption was unchanged. Isomorphism is probably responsible for the discrepancies.

Paper chromatography showed that it consisted of a mixture of at least three compounds, probably the dione (VIII), 11-oxotigogenin (VII), and the original diol (III) (in order of increasing polarity). Chromatography on alumina (Grade H) afforded pure specimens (20, 30, and 30 mg.) of these compounds, eluted with benzene, ether, and chloroform respectively.

11α-*Hydroxytigogenone* (IV).—A solution of the diol (III) (1 g.) in " AnalaR " acetone (70 ml.) containing *N*-bromoacetamide (0·646 g., 2 equiv.) was set aside at room temperature for 18 hr. The isolated *ketol* (IV) (0·99 g.) crystallised from acetone as rods (0·8 g., 80%), m. p. 212—214°, $[\alpha]_D^{19}$ −64° (*c* 0·63), $\nu_{max.}$ 1714 cm.$^{-1}$, R_F 0·65 (Found : C, 75·2; H, 9·8. $C_{27}H_{42}O_4$ requires C, 75·3; H, 9·8%). 11α-*Acetoxytigogenone* (IVa) formed plates (from acetone–hexane), m. p. 185—189°, $[\alpha]_D^{19}$ −73° (*c* 1·0), $\nu_{max.}$ 1732 and 1240, and 1718 cm.$^{-1}$, R_F 0·93 (Found : C, 73·8; H, 9·35. $C_{29}H_{44}O_5$ requires C, 73·7; H, 9·4%).*

Oxidation of 11β-*Hydroxytigogenin* (V).—(i) *With* N-*bromoacetamide*. The diol (V) (0·5 g.) and *N*-bromoacetamide (0·323 g., 2 equiv.) were kept in " AnalaR " acetone (35 ml.) at room temperature for 16 hr. The crude product appeared by paper chromatography to contain the dione (VIII) and the 11-ketone (VII). Chromatography on alumina afforded a pure specimen (65 mg.) of the former (eluted with benzene); ether eluted material inert to Brady's reagent from which pure 11-oxotigogenin (VII) (0·10 g.) was obtained crystalline.

(ii) *With potassium dichromate*. The diol (V) (0·3 g.) in " AnalaR " acetone (10 ml.) at room temperature was oxidised with 1·33N-potassium dichromate in 5N-sulphuric acid (1·045 ml., 1·1 equiv.). The product (0·28 g.) seemed (by paper chromatography) to contain a trace of the original diol (V) with the ketol (VII) and dione (VIII). Chromatography on alumina (Grade H) afforded the last-named in the benzene eluate (0·115 g.); crystallisation afforded a pure specimen (55 mg.). Crystallisation of the material eluted by ether gave the 11-ketone (VII) (0·125 g.). The diol (V) was not isolable.

(iii) *Oppenauer method*. The 3β : 11β-diol (V) (2 g.) in toluene (80 ml.) and anhydrous *cyclo*hexanone (15 ml.) was refluxed with aluminium *iso*propoxide (1 g.) in toluene (10 ml.) for 1 hr., the solvents were distilled off with steam, and 11β-*hydroxytigogenone* was isolated with ethyl acetate and crystallised from methanol as prisms (1·28 g., 68%), m. p. 222—227°, $[\alpha]_D^{21}$ −45°. Recrystallised material had m. p. 225—228°, $[\alpha]_D^{20}$ −46° (*c* 0·91), $\nu_{max.}$ (in Nujol) 1694 cm.$^{-1}$, R_F 0·78 (Found : C, 75·1; H, 9·9. $C_{27}H_{42}O_4$ requires C, 75·3; H, 9·8%). The compound gave an immediate precipitate with Brady's reagent.

3-*Methylsarsasapogenin*.—Professor Barton having been kind enough to make available to us the late Professor Kon's collection of sapogenin specimens, we were able to examine samples of 3-methylsarsasapogenin. Although the action of methylmagnesium halides on 3-oxo-steroids generally gives mixtures of epimeric stanols, Farmer and Kon [42] reported no difficulty in isolating a product from sarsasapogenone that they pronounced " clearly homogeneous." Paper chromatography of two of Professor Kon's specimens of 3-methylsarsasapogenin revealed two components in each : the higher-melting specimen predominated in the component with the higher R_F value and the other contained the two in nearly equal amounts. We examined the infrared absorption with Dr. Page's help. Although the spirostan system introduces absorption in the 1000—1100 cm.$^{-1}$ region overlying the relatively weak bands due to the C–O frequencies in the carbinol groups, the lower-melting specimen showed a peak at 1028 cm.$^{-1}$, probably attributable to a 3β(axial)-hydroxyl group, and the other a peak at 1060 cm.$^{-1}$, befitting the absorption of the equatorial 3α-hydroxyl group.[45] Supplies of these materials were too exiguous for further work; therefore we can presume only that the components are the epimeric 3α-hydroxy-3β-methyl- and 3β-hydroxy-3α-methyl-spirostan. If so, then the latter epimer is the less hydrophilic, notwithstanding the equatorial configuration of its hydroxy-group.

GLAXO LABORATORIES, LTD., GREENFORD, MIDDLESEX. [*Received, August 29th*, 1956.]

[45] Rosenkrantz *et al.*, *J. Biol. Chem.*, 1952, **195**, 503, 509; *J. Amer. Chem. Soc.*, 1953, **75**, 903; 1955, **77**, 2237; Furst, Kuhn, Scotoni, and Günthard, *Helv. Chim. Acta*, 1952, **35**, 951; Cole, Jones, and Dobriner, *J. Amer. Chem. Soc.*, 1952, **74**, 5571; cf. Dauben, Hoerger, and Freeman, *ibid.*, p. 5206; Cole, *J.*, 1952, 4969.