Tetrahedron. Vol. 28, pp. 1289 to 1297. Pergamon Press 1972. Printed in Great Britain

NEW SOURCES OF STEROID SAPOGENINS—XIV[1]

25*S*-RUSCOGENIN AND SANSEVIERIGENIN, TWO NEW SPIROSTAN SAPOGENINS FROM *SANSEVIERIA TRIFASCIATA*

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(*Received in the UK* 10 *October* 1971; *Accepted for publication* 8 *November* 1971)

Abstract—From the leaves of *Sansevieria trifasciata* Prain β-sitosterol, ruscogenin (IIIa), neoruscogenin (Ia), and the two new spirostan sapogenins 25*S*-ruscogenin (IIa) and sansevierigenin (Va) have been isolated and the structures determined by chemical and spectroscopic methods.

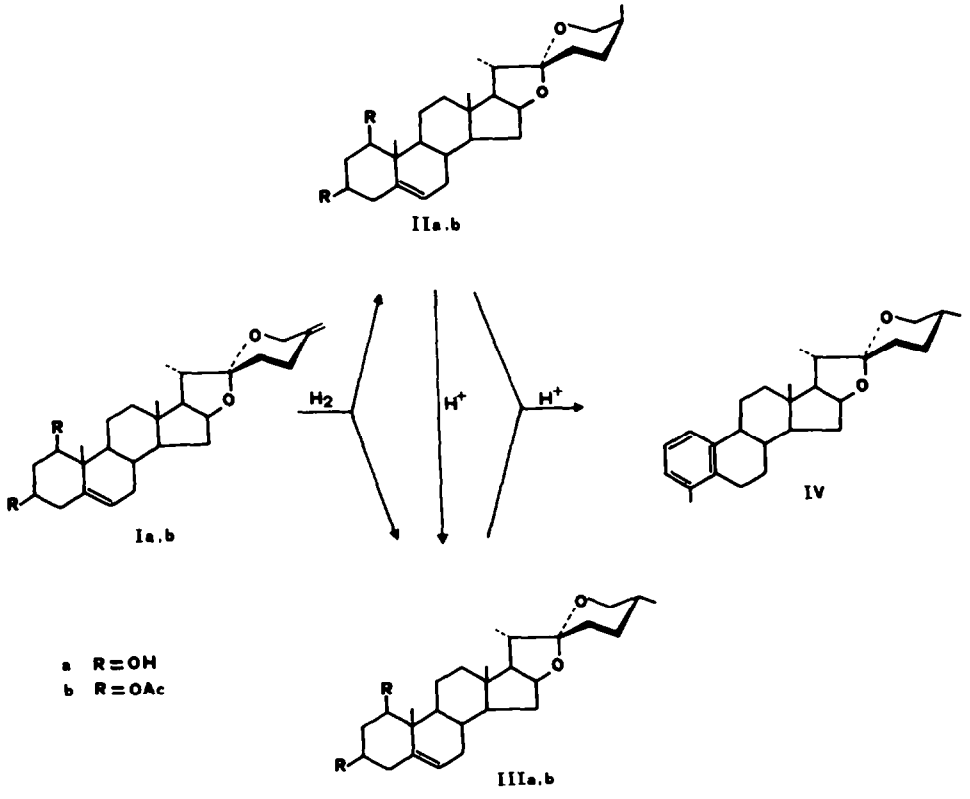
INTRODUCTION

SANSEVIERIA TRIFASCIATA Prain, an Agavacea of African origin is intensively cultivated on the Canary Isles. From the unhydrolyzable of its ethanolic extract we isolated β-sitosterol and five spirostan sapogenins. One was identified as ruscogenin (IIIa) by direct comparison with an authentic sample, and a second as neoruscogenin (Ia) by its physical and spectroscopic data.[2-4] The structures of 25*S*-ruscogenin (IIa) and sansevierigenin (Va), were established as (20*S*,22*R*,25*S*)-spirost-5-en-1β,3β-diol and (20*S*,22*S*,23*S*)-spirost-5,25(27)-dien-1β,3β,23-triol, respectively, this being the first time they are found in nature. The fifth spirostan sapogenin is currently being investigated.

RESULTS AND DISCUSSION

25*S*-Ruscogenin (IIa), $C_{27}H_{42}O_4$, double m.p. 194–196° and 212–214°, $[\alpha]_D$ −112°, was eluted together with ruscogenin (IIIa) and neoruscogenin (Ia), being separated from them by preparative column chromatography of the corresponding acetates. IIa forms a diacetate (IIb), $C_{31}H_{46}O_6$, the IR spectrum of which lacks OH absorptions. Hence, two of the four O atoms in IIa are present as OH groups, the other two forming part of a (20*S*,22*R*,25*S*)-spirostan ring as is seen from the positions and relative intensities of the bands at 985, 918, 900 and 850 cm^{-1}.[5] This is confirmed by the NMR spectrum of IIb (Table 1): the pattern and the chemical shifts of the 2H—C_{26} are characteristic of an axial (25*S*) 25-Me; furthermore, the deshielding of 0·01 ppm which is observed when changing from $CDCl_3$ to C_6D_6, is compatible only with an axial 25-Me.[6] The absorptions at 3030, 2830 and 830 cm^{-1} in the IR spectrum of IIb indicate the presence of a Δ^5.[7] This, as well as the probable location of the two OH groups with β configuration at C_1 and C_3 is also deduced from the fact that the chemical shifts of the Me groups at C_{13}, C_{10} and C_{20} coincide for the acetates of 25*S*-ruscogenin (IIb) and ruscogenin (IIIb). The spectroscopic study thus provides structure (20*S*,22*R*,25*S*)-spirost-5-en-1β,3β-diol for IIa.

In order to prove this by chemical methods, IIb was treated under normal conditions of isomerization at C_{25} by refluxing it with HCl/EtOH (1:2), affording a



compound (IV), $C_{27}H_{38}O_2$, which in the IR does not possess any OH absorptions. The inversion of the configuration at C_{25} is deduced from the position and intensities of the bands at 980, 920, 900 and 860 cm^{-1}, characteristic of a (20*S*,22*R*,25*R*)-spirostan ring;[5] the peaks at 3060, 3020, 775 and 735 cm^{-1} as well as several weak absorptions between 2000 and 1700 cm^{-1} indicate the presence of a 1,2,3-trisubstituted aromatic ring.[8a] In the NMR spectrum of IV (Table 1) the multiplet at 6·57* ($W_{\frac{1}{2}}$ = 12 Hz; 2H—C_{26}) and a broad singlet at 8·40 (2H—C_{23}) provide further evidence for a (20*S*,22*R*,25*R*)-spirostan ring,[6,9] while a multiplet (3H) centered at approximately 2·93 is assigned to aromatic protons and a singlet (3H) at 7·80 to the Me group situated now on the aromatic nucleus. As compound IV must be formed by a rearrangement similar to the one observed in systems of the type 1,4-dien-3-one,[10] which excludes position C_1 for the aromatic Me group, we deduce that its structure must be (25*R*)-19-nor-4-methyl-spirost-1,3,5-triene. Indeed, Takeda *et al.*[11] isolated from *Metanarthecium luteo-viride* Maxim. a spirostan sapogenin, called luvigenin, with this structure. Its physical and spectroscopic data coincide with those of our compound IV. To prove that a 5-en-1β,3β-diol system under the above conditions suffers this type of aromatization, ruscogenin (IIIa) was treated in the same way, yielding luvigenin (IV). By milder acid treatment (HCl/EtOH = 1:4) 25*S*-ruscogenin (IIa) was isomerized at C_{25} without aromatization of ring A; the acetylated reaction product proved to be identical in all respects with ruscogenin acetate (IIIb). On the

* All chemical shifts are given in τ-values.

TABLE 1. CHEMICAL SHIFTS (τ-SCALE)[a]

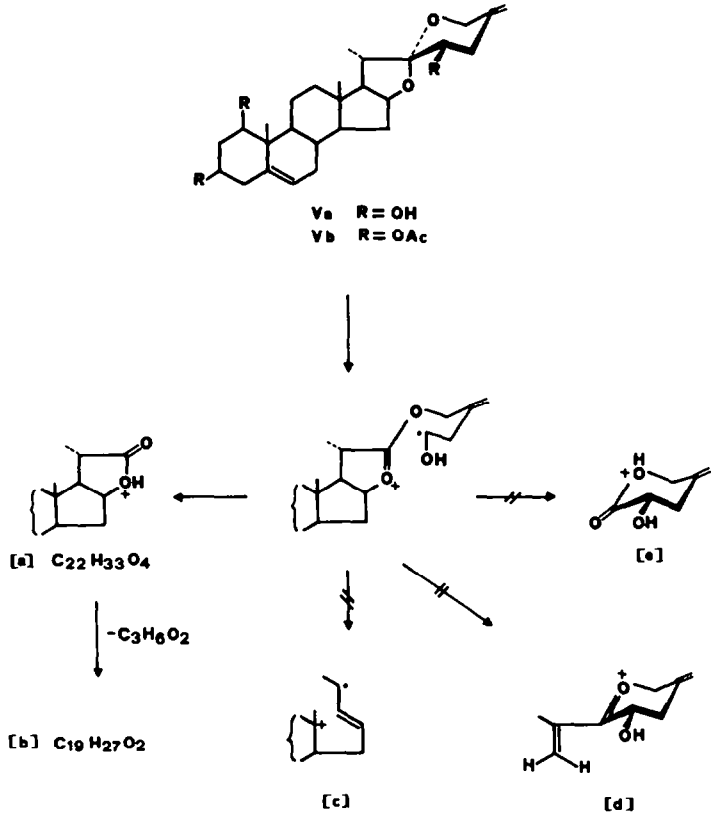
Compound	Solvent	H—C_6	2H—C_{26}	13-Me	10-Me	20-Me	25-Me	3H—$C_{1,2,3}$
25*S*-Ruscogenin acetate (IIb)	$CDCl_3$	4·37	5·95, 6·13; 6·61, 6·80	9·22	8·85	9·04	8·94	—
		m [10]	m [5]	s	s	d (6)	d (6)	
	C_6D_6	4·60	5·80, 5·98; 6·57, 6·76	9·20	8·94	8·82	8·93	—
		m [10]	m [6]	s	s	d (6)	d (6)	
Ruscogenin acetate (IIIb)	$CDCl_3$	4·40	6·57	9·22	8·86	9·06	—	—
		m [10]	m [11]	s	s	d (6)		
	C_6D_6	4·60	6·45	9·20	8·94	8·81	9·33	—
		m [10]	m [11]	s	s	d (6)	d (7)	
Luvigenin (IV)	$CDCl_3$	—	6·57	9·19	7·80[b]	9·00	9·21	2·93
			m [12]	s	s	d (6)	d (6)	m [10]
Sansevierigenin acetate (Vb)	$CDCl_3$	4·38	5·64, 5·84; 6·11, 6·31	9·17	8·85	9·07	5·18[c]	—
		m [10]	AB [4]	s	s	d (6)	s [6]	
	C_6D_6	4·58	5·53, 5·73; 6·05, 6·26	9·19	8·92	8·77	5·29[c]	—
		m [10]	AB [4]	s	s	d (6)	s [5]	
Neoruscogenin acetate (Ib)	$CDCl_3$	4·38	5·60, 5·80; 6·05, 6·26	9·21	8·84	9·05	5·27[c]	—
		m [12]	AB [4]	s	s	d (6)	s [6]	
	C_6D_6	4·60	5·40, 5·61; 5·94, 6·14	9·21	8·93	8·85	5·28[c]	—
		m [10]	AB [4]	s	s	d (6)	s [6]	

[a] Coupling constants J in parentheses, $W_{\frac{1}{2}}$ in brackets (both in Hz).
[b] Me group at C_4.
[c] $CH_2{=}C_{25}$.

other hand, catalytic hydrogenation of the known neoruscogenin acetate (Ib) gave IIb together with its C_{25}-epimer (IIIb). By the same method Robert *et al.*[3] obtained IIb, but this is the first time that 25*S*-ruscogenin (IIa) is isolated from a natural source. These results confirm that its structure must be (20*S*,22*R*,25*S*)-spirost-5-en-1β,3β-diol.

Sansevierigenin (Va), $C_{27}H_{40}O_5$ (high resolution MS), m.p. 234–237°, $[\alpha]_D$ −125°, was obtained by preparative column chromatography after eluting the mixture of Ia, IIa and IIIa. It has three OH groups as may be deduced on the one hand from the presence of the fragments at *m/e* 426 ($C_{27}H_{38}O_4$), 408 ($C_{27}H_{36}O_3$) and 390 which correspond to the loss of one, two and three water molecules from the molecular ion, and on the other hand from the fact that its acetate (Vb), which in the IR has no OH absorptions, exhibits in the NMR spectrum (C_6D_6) three singlets at 8·20, 8·25 and 8·27 (3H each). The IR spectrum of Vb lacks the characteristic bands of the spirostan ring: the peaks at 3030, 2830 and 830 cm^{-1} correspond to a $\Delta^{5,7}$ and those at 3073 and 870 cm^{-1} to a methylene group.[8b] Both assignments are confirmed by the NMR spectrum ($CDCl_3$, see Table 1) which shows a multiplet centred at 4·38 (1H, $W_{\frac{1}{2}} = 12$

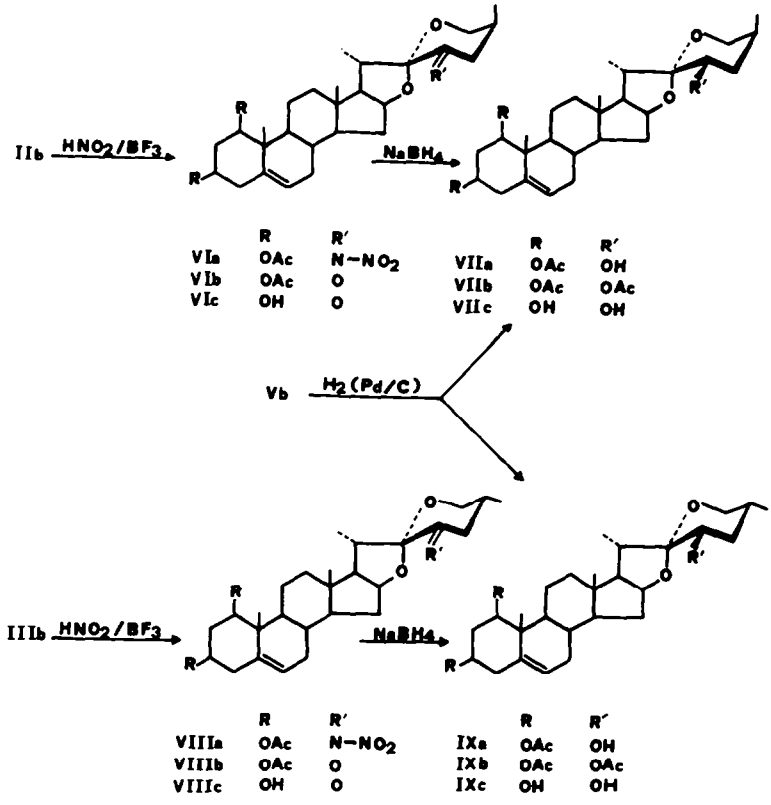
Hz; H—C_6) and a broad singlet at 5·18 (2H, $W_{\frac{1}{2}}$ = 6 Hz: $\begin{matrix} R \\ \diagdown \\ \quad C{=}CH_2 \\ \diagup \\ R' \end{matrix}$). By comparing the position and aspect of the NMR signals for the 2H—C_{26} (AB-system) with those of neoruscogenin acetate (Ib), the methylene group in Vb may be located at C_{25}. On the other hand, the fact that the chemical shifts of the 10-Me (in $CDCl_3$ as well as C_6D_6) of Vb coincide with those of the acetates of ruscogenin (IIIb) and neoruscogenin (Ib), confirms the presence of a Δ^5 in Vb, at the same time situating two of the acetyl groups at C_1 and C_3, both in β configuration. As regards the third OH present in Va, the mass spectrum easily permits locating it at C_{23} because the fragmentation pattern



is typical of spirostan sapogenins with an electronegative substituent at this C atom,[12] being absent the fragments [d] and [e] which contain ring F, as well as [c] which is of great relative abundance in spirostan sapogenins without such a substituent at C_{23}. In addition, the high intensity and the mass of fragment [a] ($C_{22}H_{33}O_4$) and the fact that it looses one and two water molecules ($C_{22}H_{31}O_3$ and $C_{22}H_{29}O_2$) restrict the position of the third OH to C_{23} and that of the methylene group to C_{25}.

When calculating[13] the chemical shifts of the Me groups at C_{13}, C_{10} and C_{20} in Vb, we find that those for the configuration 23*R* (axial OAc at C_{23}: 9·21, 8·86, 8·98 in

$CDCl_3$ and 9·26, 8·93, 8·73 in C_6D_6) are in great discrepancy with the experimental ones (Table 1). On the other hand, the values calculated for the configuration 23*S* (equatorial OAc at C_{23}: 9·16, 8·85, 9·07 in $CDCl_3$ and 9·19, 8·93, 8·81 in C_6D_6) are in complete agreement with the observed ones.* Thus, the spectroscopic results indicate structure (20*S*,22*S*,23*S*)-spirost-5,25(27)-dien-1β,3β,23-triol for Va.



This was confirmed chemically by preparing VIIb and IXb as follows: treatment of 25*S*-ruscogenin acetate (IIb) with HNO_2 + BF_3[15] yielded its 23-nitrimine (VIa),† $C_{31}H_{44}O_8N_2$, IR 1645 (C=N), 1580 and 1321 cm^{-1} (NO_2),[17] which by adsorption on neutral Al_2O_3 (grade III) was hydrolyzed to give 23-keto-25*S*-ruscogenin acetate (VIb). Saponification afforded the alcohol VIc, $C_{27}H_{40}O_5$ (*m/e* 444), IR 1730 cm^{-1} (C_{23}=O)[18], the mass spectrum of which shows the characteristic fragmentation pattern of a 23-ketone:[12] the absence of fragments containing ring F, the loss of CO from the molecular ion (*m/e* 416), and the presence of the ions [a] and [b] (*m/e* 361 and 287) are only compatible with structure VIc. Reduction of VIb with $NaBH_4$ in EtOH[19–21] yielded VIIa and finally acetylation of this compound the triacetate

* These chemical shifts are calculated combining those of the acetates of isoplexigenin A, B and D with those of the acetates of tigogenin, diosgenin, gitogenin and neoruscogenin. For the values of the isoplexigenins in $CDCl_3$, see[14]. In C_6D_6 the chemical shifts of the 13-, 10- and 20-Me of the acetates of isoplexigenin A and D are 9·18, 9·33, 8·78, and 9·40, 9·25, 8·70, respectively.

† A more detailed study of the reaction of spirostan sapogenins with HNO_2 + BF_3 is reported in [16].

VIIb. By the same method we obtained from ruscogenin acetate (IIIb) the 23-nitrimine VIIIa, $C_{31}H_{44}O_8N_2$, IR 1646, 1577, 1320 cm^{-1}, which after hydrolysis on alumina gave the 23-ketone VIIIb. Saponification yielded the alcohol VIIIc, $C_{27}H_{40}O_5$ (*m/e* 444), IR 1730 cm^{-1} (C_{23}=O),[18] its mass spectrum exhibiting the same fragmentation pattern as the C_{25}-epimer VIc. Reduction of VIIIb with $NaBH_4$ afforded IXa which was acetylated to give IXb.

On the other hand, by selective hydrogenation of Vb with Pd/C we obtained the two epimers at C_{25}, VIIb and IXb, which proved to be identical in all respects with the triacetates VIIb and IXb prepared previously. Hence, the structure of sansevierigenin (Va) is established as (20*S*,22*S*,23*S*)-spirost-5,25(27)-dien-1β,3β,23-triol.

EXPERIMENTAL

The m.ps, determined on a Kofler block, are uncorrected. Solvent used for recrystallization was MeOH unless otherwise stated. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 141 polarimeter and the IR spectra on a Perkin-Elmer 237 spectrophotometer. NMR spectra were taken with a Perkin-Elmer R-10 instrument (60 MHz) using TMS as internal reference. The mass spectra were recorded on Hitachi Perkin-Elmer RMU-7 and AEI MS-902 mass spectrometers. All the chromatographic adsorbents were Merck products. The spray reagent for TLC was H_2SO_4—AcOH—H_2O (4:80:16). Column chromatography was performed on silica gel 0·2–0·5 mm, dry column chromatography on silica gel 0·05–0·2 mm and preparative TLC on silica gel $PF_{254+366}$ (thickness 0·5 mm). The acetylations were realized with Ac_2O in pyridine and the saponifications with 2% KOH in MeOH, in both cases leaving the reaction mixture at room temp for 12 hr.

Isolation of the sapogenins. The air-dried leaves of *Sansevieria trifasciata* (58 kg), collected in the south of Tenerife (Abama-Guía de Isora), were finely cut and extracted several times with EtOH in a soxhlet. After combining and filtering the cold ethanolic extracts, they were concentrated *in vacuo*, diluted with the same volume of water and degreased with benzene in a liquid–liquid extractor. After adding conc H_2SO_4 to the aq ethanolic extract till it was 2N, it was refluxed for 6 hr, then poured into water, neutralized with $NaHCO_3$ and filtered. The ppt was dissolved in $CHCl_3$ and washed several times with 10% KOH aq and water. Evaporation of the solvent afforded the crude mixture of sapogenins (504 g), which was chromatographed on a column. Elution with $CHCl_3$ and $CHCl_3$–MeOH gave first β-sitosterol, then a mixture of steroid sapogenins, and last sansevierigenin which was purified by chromatographing it several times on dry columns eluting with benzene–EtOAc (6:4). The mixture of steroid sapogenins, consisting of 25*S*-ruscogenin, neoruscogenin, ruscogenin and a fourth still unknown compound, was acetylated and then separated by chromatography on dry columns with benzene–EtOAc as eluents, first on silica gel alone obtaining 25*S*-ruscogenin acetate, and then impregnated with 20% $AgNO_3$ separating the remaining three sapogenins.

β-*Sitosterol* (1·5 g), m.p. 136–138°, $[\alpha]_D$ −36° (*c*, 0·32): IR spectrum superimposable with that of an authentic sample.

Ruscogenin acetate IIIb (0·39 g), m.p. 194–196°, $[\alpha]_D$ −82° (*c*, 0·200). (Found: C, 72·55; H, 9·20. Calc. for $C_{31}H_{46}O_6$: C, 72·34; H, 9·01%); IR spectrum superimposable with that of an authentic sample; NMR: see Table 1.

Neoruscogenin Ia (0·40 g), m.p. 196–198°, $[\alpha]_D$ −118° (*c*, 0·240). (Found: C, 75·66; H, 9·18. Calc. for $C_{27}H_{40}O_4$: C, 75·66; H, 9·41%). Its *acetate* (Ib) could not be obtained in crystalline form. (Found: C, 72·41; H, 8·45. Calc. for $C_{31}H_{44}O_6$: C, 72·63; H, 8·65%): $\nu_{max}^{CS_2}$ 3065, 875 (CH_2=), 3030, 2830, 838 (Δ^5), 1745, 1240 cm^{-1} (OAc): NMR: see Table 1.

25*S*-*Ruscogenin* IIa (4·0 g), double m.p. 194–196°, 212–214°, $[\alpha]_D$ −112° (*c*, 0·220). (Found: C, 75·45; H, 9·97. $C_{27}H_{42}O_4$ requires: C, 75·31; H, 9·83%). *Acetate* (IIb), m.p. 182–185°, $[\alpha]_D$ −88° (*c*, 0·210). (Found: C, 72·12; H, 9·04. $C_{31}H_{46}O_6$ requires: C, 72·34; H, 9·01%). $\nu_{max}^{CS_2}$ 3030, 2830, 830 (Δ^5), 1745, 1235 (OAc), 985, 918, 900, 850 cm^{-1} (spirostan ring): NMR: see Table 1.

Sansevierigenin Va (0·50 g), m.p. 234–237° (acetone–MeOH), $[\alpha]_D$ −125° (*c*, 0·130). Mass spectrum: *m/e* 444 (2%: M^+, $C_{27}H_{40}O_5$ requires 444), 426 (17%: found 426·2766, $C_{27}H_{38}O_4$ requires 426·2768), 408 (18%: found 408·2685, $C_{27}H_{36}O_3$ requires 408·2664), 393 (6%: $M^+ - 2\,H_2O - CH_3$, found 393·2412, $C_{26}H_{33}O_3$ requires 393·2429), 390 (4%), 361 (42%: found 361·2393, $C_{22}H_{33}O_4$ requires 361·2378), 343 (72%:

found 343·2273, $C_{22}H_{31}O_3$ requires 343·2273), 325 (100%: found 325·2167, $C_{22}H_{29}O_2$ requires 325·2166), 301 (4%), 298 (9%), 287 (86%: found 287·2012, $C_{19}H_{27}O_2$ requires 287·2010), 283 (8%), 280 (5%), 269 (76%: found 269·1918, $C_{19}H_{25}O$ requires 269·1904), 251 (67%: found 251·1777, $C_{19}H_{23}$ requires 251·1799). *Acetate* (Vb), amorphous: $\nu_{max}^{CS_2}$ 3073, 870 (CH_2=), 3030, 2830, 830 (Δ^5), 1740, 1240 cm^{-1} (OAc); NMR: see Table 1.

(25R)-19-*Nor*-4-*methyl-spirost*-1,3,5-*triene* (IV) *from* IIb. To a soln of IIb (160 mg) in EtOH (80 ml) conc HCl (40 ml) was added and the mixture refluxed for 11 hr under N_2. Then it was poured into water, extracted with $CHCl_3$ and the extract washed with $NaHCO_3$aq and water. After drying over Na_2SO_4 and concentrating *in vacuo* it was purified by chromatography on a dry column eluting with benzene–light petroleum (8:2) which yielded IV (30 mg), m.p. 177–180° ($CHCl_3$–MeOH), $[\alpha]_D$ −28° (*c*, 0·130). (Found: C, 82·03; H, 9·80. Calc. for $C_{27}H_{38}O_2$: C, 82·18; H, 9·71%). $\nu_{max}^{CS_2}$ 3060, 3020, 775, 735 (aromatic ring), 980, 920, 900, 860 cm^{-1} (spirostan ring); NMR: see Table 1.

Compound IV *from* IIIa. To a soln of IIIa (165 mg) in EtOH (80 ml) conc HCl (40 ml) was added and the mixture treated as described above, giving IV (16·3 mg) which proved to be identical with the one obtained from IIb (physical constants, IR, NMR).

Ruscogenin acetate (IIIb) *from* IIb. To a soln of IIb (120 mg) in MeOH (80 ml) conc HCl (20 ml) was added and the mixture treated as mentioned above, but refluxing it for 24 hr. The resulting product was first purified by chromatography on a dry column with $CHCl_3$–acetone (95:5) as eluent and then acetylated. Preparative TLC, eluting six times with benzene–EtOAc (98:2), afforded IIIb (18 mg) and IIb (15 mg) which were identified by direct comparison with authentic samples.

25S-*Ruscogenin acetate* (IIb) *from* Ib. Neoruscogenin acetate Ib (292 mg) in EtOH (50 ml) was hydrogenated for 1 hr over 10% Pd/C (153 mg) at room temp and atm press. After filtering off the catalyst and evaporating the solvent *in vacuo*, a mixture (280 mg) of IIb and IIIb was obtained which was separated by preparative TLC eluting five times with benzene–EtOAc (98:2). Thus, IIb (150 mg) and IIIb (55 mg) were obtained in pure form (the rest of the mixture was not separated further) and identified by comparing them with authentic material.

(20S,22S,25S)-*Spirost*-5-*en*-1β,3β-*diol*-23-*nitrimine diacetate* (VIa) *from* IIb. To a soln of IIb (308 mg) in glacial AcOH (6 ml) BF_3–ether complex (48%: 0·3 ml) and $NaNO_2$ (200 mg) were added in small portions and with stirring at room temp during 1½ hr. Then the mixture was poured into water, extracted with $CHCl_3$ and washed with $NaHCO_3$aq and water. After drying over Na_2SO_4 the solvent was evaporated and the residue chromatographed on a dry column (eluent: benzene–EtOAc, 95:5) affording VIa (143 mg), VIb (30 mg) and starting material (88 mg).

Compound VIa was obtained amorphous. (Found: C, 65·28; H, 7·46; N, 5·00. $C_{31}H_{44}O_8N_2$ requires: C, 65·02; H, 7·74; N, 4·89%); $\nu_{max}^{CHCl_3}$ 1740, 1250 (OAc), 1645 (C=N), 1580, 1321 (NO_2), 3030, 2830, 835 cm^{-1} (Δ^5); NMR ($CDCl_3$): 4·40 (1H, m, $W_{\frac{1}{2}}$ = 10 Hz, H—C_6), 5·77, 5·96, 6·54, 6·73 (2H, m, $W_{\frac{1}{2}}$ = 4 Hz, 2H—C_{26}), 8·85 (3H, s, 10-Me), 8·93 (3H, d, J = 6 Hz, 25-Me), 8·98 (3H, d, J = 6 Hz, 20-Me) and 9·19 (3H, s, 13-Me).

(20S,22S,25S)-*Spirost*-5-*en*-1β,3β-*diol*-23-*one diacetate* (VIb) *from* VIa. A soln of VIa (143 mg) in the minimum quantity of benzene–light petroleum (1:1) was adsorbed on a column of neutral Al_2O_3 (activity III) and eluted first with benzene–light petroleum (1:1; 50 ml) and then with benzene (200 ml), thus obtaining VIb (100 mg), amorphous; NMR ($CDCl_3$): 4·40 (1H, m, $W_{\frac{1}{2}}$ = 10 Hz, H—C_6), 5·61, 5·80, 6·48, 6·67 (2H, m, $W_{\frac{1}{2}}$ = 5 Hz, 2H—C_{26}), 8·84 (3H, s, 10-Me), 8·93 (3H, d, J = 6 Hz, 25-Me), 9·03 (3H, d, J = 7 Hz, 20-Me) and 9·21 (3H, s, 13-Me).

Saponification of VIb gave VIc, m.p. 226–228°, $[\alpha]_D$ −104° (*c*, 0·190). (Found: C, 72·74; H, 9·07. $C_{27}H_{40}O_5$ requires: C, 72·94; H, 9·07%); $\nu_{max}^{CHCl_3}$ 3605 (OH), 3030, 2830, 835 (Δ^5), 1730 cm^{-1} (C=O); mass spectrum: *m/e* 444 (0·6%: M^+), 426 (1%), 416 (74%), 361 (100%), 343 (81%), 325 (99%), 287 (88%), 269 (57%), 251 (44%).

(20S,22S,23S,25S)-*Spirost*-5-*en*-1β,3β,23-*triol* 1,3-*diacetate* (VIIa) *from* VIb. A soln of $NaBH_4$ (66 mg) in EtOH (20 ml) was added to VIb (100 mg) in EtOH (20 ml) and the mixture stirred at room temp for 1½ hr, after which it was poured into water and extracted with $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. Chromatography of the residue on a dry column eluting with benzene–EtOAc (9:1) yielded, besides (20*S*,22*S*,23*R*,25*S*)-spirost-5-en-1β,3β,23-triol 1,3-diacetate (6 mg), VIIa (60 mg), amorphous. $\nu_{max}^{CS_2}$ 3570 (OH), 1745, 1230 (OAc), 3035, 2830, 837 cm^{-1} (Δ^5); NMR ($CDCl_3$): 4·40 (1H, m, $W_{\frac{1}{2}}$ = 10 Hz, H—C_6), 5·88, 6·08, 6·69, 6·88 (2H, m, $W_{\frac{1}{2}}$ = 5 Hz, 2H—C_{26}), 6·38 (1H, m, $W_{\frac{1}{2}}$ = 12 Hz, H—C_{23}), 8·83 (3H, d, J = 6 Hz, 25-Me), 8·85 (3H, s, 10-Me), 8·98 (3H, d, J = 6 Hz, 20-Me) and 9·18 (3H, s, 13-Me).

Saponification of VIIa gave VIIc, m.p. 196–197° (acetone–light petroleum), $[\alpha]_D$ −119° (*c*, 0·098). (Found: C, 72·43; H, 9·37. $C_{27}H_{42}O_5$ requires: C, 72·61; H, 9·48%).

Acetylation of VIIa afforded VIIb, amorphous: $\nu_{max}^{CS_2}$ 1745, 1235 (OAc), 3030, 2830, 833 cm^{-1} (Δ^5): NMR ($CDCl_3$): 4·40 (1H, m, $W_{\frac{1}{2}}$ = 10 Hz, H—C_6), 5·50 (4H, m, $W_{\frac{1}{2}}$ = 28 Hz, H—C_1, H—C_3, H—C_{16}, H—C_{23}), 5·96, 6·15, 6·65, 6·85 (2H, m, $W_{\frac{1}{2}}$ = 5 Hz, 2H—C_{26}), 7·99 (9H, s, 3 OAc), 8·82 (3H, d, J = 6 Hz, 25-Me), 8·84 (3H, s, 10-Me), 9·01 (3H, d, J = 7 Hz, 20-Me) and 9·18 (3H, s, 13-Me).

(20S,22S,25R)-*Spirost*-5-*en*-1β,3β-*diol*-23-*nitrimine diacetate* (VIIIa) *from* IIIb. Ruscogenin acetate IIIb (340 mg) was treated in the same way as described above for the preparation of VIa. Due to the fact that during the dry column chromatography the 23-nitrimine VIIIa was hydrolyzed in great part to the 23-ketone VIIIb, only 28 mg of VIIIa were obtained; $\nu_{max}^{CHCl_3}$ 1646 (C=N), 1577, 1320 (NO_2), 3030, 2830, 835 cm^{-1} (Δ^5); NMR ($CDCl_3$): 4·40 (1H, m, $W_{\frac{1}{2}}$ = 10 Hz, H—C_6), 6·40 (2H, m, $W_{\frac{1}{2}}$ = 10 Hz, 2H—C_{26}), 8·84 (3H, s, 10-Me), 8·98 (3H, d, J = 6 Hz, 20-Me), 9·08 (3H, d, J = 6 Hz, 25-Me) and 9·18 (3H, s, 13-Me). The later fractions eluted consisted of mixtures of VIIIa and VIIIb (184 mg), and of starting material (20 mg).

(20S,22S,25R)-*Spirost*-5-*en*-1β,3β-*diol*-23-*one diacetate* (VIIIb) *from* VIIIa. The mixture of VIIIa and VIIIb (184 mg) obtained in the above chromatography was hydrolyzed by adsorption on alumina in the same manner as mentioned for the preparation of VIb, resulting in amorphous VIIIb (123 mg): NMR ($CDCl_3$): 4·40 (1H, m, $W_{\frac{1}{2}}$ = 10 Hz, H—C_6), 6·40 (2H, m, $W_{\frac{1}{2}}$ = 10 Hz, 2H—C_{26}), 8·84 (3H, s, 10-Me), 9·05 (6H, d, J = 6 Hz, 20-Me, 25-Me), 9·20 (3H, s, 13-Me).

Saponification of VIIIb gave VIIIc, m.p. 240–245°, $[\alpha]_D$ −92° (*c*, 0·180). (Found: C, 73·07: H, 9·07. $C_{27}H_{40}O_5$ requires: C, 72·94: H, 9·07%); $\nu_{max}^{CHCl_3}$ 3605 (OH), 3030, 2830, 835 (Δ^5), 1730 cm^{-1} (C=O); mass spectrum: *m/e* 444 (1·4%; M$^+$), 426 (1·4%), 416 (88%), 361 (84%), 343 (77%), 325 (100%), 287 (100%), 219 (56%), 251 (43%).

(20S,22S,23S,25R)-*Spirost*-5-*en*-1β,3β,23-*triol* 1,3-*diacetate* (IXa) *from* VIIIb. VIIIb (100 mg) was treated with $NaBH_4$ as indicated for the preparation of VIIa, affording amorphous (20*S*,22*S*,23*R*,25*R*)-spirost-5-en-1β,3β,23-triol 1,3-diacetate (10 mg) and IXa (50 mg): $\nu_{max}^{CS_2}$ 3570 (OH), 1745, 1235 (OAc), 3030, 2825, 835 cm^{-1} (Δ^5): NMR ($CDCl_3$): 4·40 (1H, m, $W_{\frac{1}{2}}$ = 10 Hz, H—C_6), 6·60 (3H, m, $W_{\frac{1}{2}}$ = 11 Hz, H—C_{23}, 2H—C_{26}), 8·84 (3H, s, 10-Me), 9·05 (3H, d, J = 7 Hz, 20-Me), 9·18 (3H, s, 13-Me) and 9·18 (3H, d, J = 6 Hz, 25-Me).

Saponification of IXa gave IXc, m.p. 249–252°, $[\alpha]_D$ −100° (*c*, 0·128). (Found: C, 72·36; H, 9·37. $C_{27}H_{42}O_5$ requires: C, 72·61: H, 9·48%).

Acetylation of IXa yielded amorphous IXb: ν_{max}^{KBr} 1745, 1240 (OAc), 3030, 2830, 835 cm^{-1} (Δ^5): NMR ($CDCl_3$): 4·41 (1H, m, $W_{\frac{1}{2}}$ = 9 Hz, H—C_6), 5·40 (4H, m, $W_{\frac{1}{2}}$ = 30 Hz, H—C_1, H—C_3, H—C_{16}, H—C_{23}), 6·60 (2H, m, $W_{\frac{1}{2}}$ = 12 Hz, 2H—C_{26}), 7·98 (9H, s, 3 OAc), 8·84 (3H, s, 10-Me), 9·06 (3H, d, J = 6 Hz, 20-Me), 9·16 (3H, d, J = 6 Hz, 25-Me) and 9·17 (3H, s, 13-Me).

(20S,22S,23S,25S)-*Spirost*-5-*en*-1β,3β,23-*triol triacetate* (VIIb) *and* (20S,22S,23S,25R)-*spirost*-5-*en*-1β,3β,23-*triol triacetate* (IXb) *from sansevierigenin acetate* (Vb). Sansevierigenin acetate Vb (30 mg) in abs EtOH (7 ml) was hydrogenated over 10% Pd/C (17 mg) for 1½ hr at room temp and atm press. After filtering and evaporating *in vacuo*, a mixture of VIIb and IXb (29 mg) was obtained which was separated by preparative TLC eluting four times with benzene–EtOAc (98:2). The resulting pure VIIb (20 mg) and IXb (9 mg) were identified by comparison with the previously prepared compounds (IR spectra superimposable).

VIIb was saponified to give VIIc, m.p. 197–199° (acetone–light petroleum), $[\alpha]_D$ −113° (*c*, 0·040): mass spectrum: *m/e* 446 (6%; M$^+$), 428 (15%; M$^+$ —H_2O), 410 (5%; M$^+$ —2 H_2O), 392 (1%; M$^+$ —3 H_2O).

Acknowledgement—The authors thank Dr. C. Pascual (Universität Basel) and Dr. W. Vetter (Hoffmann-La Roche, Basel) for the mass spectra. Two of us (R.F. and J.A.S.) thank the Ministerio de Educación y Ciencia for a fellowship "Formación de Personal Investigador". This work was performed within the Investigation Programme concerted with the Ministerio de Educación y Ciencia.

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