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NEW SOURCES OF STEROID SAPOGENINS—XIX[1]

20*S*-HYDROXYVESPERTILIN, A NEW STEROID LACTONE FROM *SOLANUM VESPERTILIO*

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Abstract—From stems and leaves of *Solanum vespertilio* Ait. 20*S*-hydroxyvespertilin (1c), a new steroid lactone, has been isolated and the structure confirmed by synthesis of its dihydro derivative from tigogenin. 1c proved to be identical with a dihydroxy lactone obtained by Gashchenko *et al* as by-product of the conversion of diosgenin to 16-dehydropregnenolone. The stereochemistry at C_{20} proposed by these authors is revised.

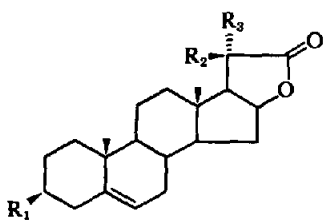
INTRODUCTION

Solanum vespertilio Ait. is a Solanacea endemic to the Canary Isles. In a previous paper[2] we reported the identification of the two new natural steroids 16-dehydropregnenolone and vespertilin (1a) in the unhydrolysed ethanolic extract of the fruits, no steroid alkaloids being detected. The present work describes the isolation of the aforesaid two steroids, β-sitosterol, diosgenin, solasodine, tomatidenol and 20*S*-hydroxyvespertilin (1c) from stems and leaves of this same plant. The structure of the last compound was established as (20*S*)-3β,16β,20-trihydroxy-pregn-5-en-20-carboxylic acid (22,16)-lactone, being isolated for the first time from Nature.

RESULTS AND DISCUSSION

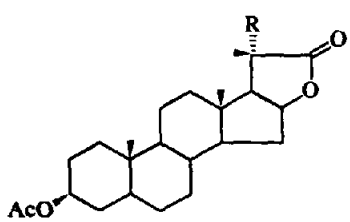
20*S*-Hydroxyvespertilin (1c), $C_{22}H_{32}O_4$, m.p. 308–310°, $[\alpha]_D$ −64°, was eluted together with the probable artefact $\Delta^{3,5}$-solasodine and a new spirostan sapogenin (now being investigated) and separated by preparative TLC of the corresponding acetates. By mild acetylation for 3 days or treatment at 100° for 1 hr with Ac_2O in pyridine 1c forms the diacetate **1d**, $C_{26}H_{36}O_6$, which in the IR lacks OH absorptions. The bands at 1780 and 1750 cm^{-1} are assigned to a γ-lactone and those at 3030, 2830 and 830 cm^{-1} to a Δ^5. The presence of this double bond and the probable location of an OH group with β configuration at C_3 is also suggested by two multiplets at 4·58† (H—C_6) and 5·25 (H—C_3) with $W_{1/2}$ = 10 and 25 Hz respectively in the NMR spectrum of **1d** (Table 1). Furthermore, the value found for the Me—C_{10} (8·96) agrees with the calculated one by the method of Zürcher (8·95).[3] Comparison of the chemical shifts of the Me groups of **1d** with those of vespertilin acetate (**1b**) shows that the signal for the Me—C_{10} is found at the same position, the Me—C_{13} is deshielded by 0·10 ppm, and the Me—C_{20} appears as a singlet at 8·20 and not as a doublet. We therefore deduce that a tertiary OH group must be situated at C_{20}. The spectral data thus suggest that 1c corresponds to 20-hydroxyvespertilin.

†τ-scale.



1

	R_1	R_2	R_3
a	OH	H	Me
b	OAc	H	Me
c	OH	Me	OH
d	OAc	Me	OAc



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a	R = OH
b	R = OAc

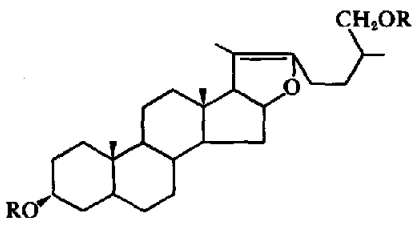
Table 1. Chemical shifts in $CDCl_3$ (60 MHz, τ-scale)[a]

Compound	2H—C_{21}	H—C_6	H—C_3	Me—C_{20}	Me—C_{10}	Me—C_{13}
Vespertilin		4·62	5·40	8·70	8·97	9·24
acetate (**1b**)[2]		m[10]	m[25]	d(7)	s	s
20*S*-Hydroxy-		4·58	6·30	8·16	8·95	9·09
vespertilin (**1c**)[b]		m[10]	m[25]	s	s	s
Diacetate (**1d**)		4·58	5·25	8·20	8·96	9·14
		m[10]	m[25]	s	s	s
5-Dihydro-17,20-dehydro-			5·25	8·15	9·17	9·20
vespertilin acetate (**5**)			m[25]	s	s	s
5-Dihydro-20,21-dehydro-	3·62 m[4]		5·25		9·17	9·17
vespertilin acetate (**6**)	4·38 m[4]		m[25]		s	s

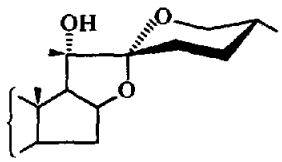
[a]Coupling constants J in parentheses, $W_{1/2}$ in brackets (both in Hz).
[b]In d_5-pyridine.

In order to determine the structure by chemical methods tigogenin was treated with Ac_2O and $MeNH_2 \cdot HCl$, obtaining pseudotigogenin acetate (**3b**). Subsequent saponification and reaction of the resulting compound **3a** with *m*-chloroperbenzoic acid afforded the less hindered 20,22 α-epoxide which *in situ* undergoes acid-catalysed opening followed by recyclization of ring F to give 20*S*-hydroxytigogenin (**4a**),[4] in the NMR spectrum of which the Me—C_{20} appears as a singlet at 8·61. Oxidation of the acetate **4b** with conc HNO_3 yielded the γ-lactone **2a**, $C_{24}H_{36}O_5$, which in the IR exhibits bands at 1780 and 1750 cm^{-1}. That **2a** possesses an OH group at position 20 was proved by treating it with $SOCl_2$, obtaining the expected dehydration compounds **5** and **6** of empirical formula $C_{24}H_{34}O_4$. The NMR spectrum of the former shows a singlet at 8·15 (Me—C_{20}) which is not present in that of the latter, appearing instead as two multiplets at 3·62 and 4·38 (=CH_2). In the IR both **5** and **6** show an α,β-unsaturated γ-lactone band (1770 and 1760 cm^{-1} respectively) and lack OH absorptions. Strong acetylation of **2a** afforded **2b**, the physical and spectroscopic data of which are identical with those of the hydrogenation product of 20*S*-hydroxyvespertilin acetate (**1d**). Hence, **1c** is assigned the structure of (20*S*)-3β,16β,20-trihydroxy-pregn-5-en-20-carboxylic acid (22,16)-lactone.

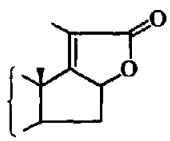
Recently, Gashchenko *et al*[5] during the conversion of diosgenin into 16-dehydropregnenolone obtained a by-product whose physical and spectral data are identical to those of our natural compound **1c** and for which they propose the structure of (20*R*)-3β,16β,20-trihydroxy-pregn-5-en-20-carboxylic acid (22,16)-lactone. The reasons why they give the stereochemistry of C_{20} as *R*, instead of *S* as shown above, are as follows: first, by reduction of the lactone CO with LAH they obtain a diol (**7**), the *cis* configuration of which they prove by



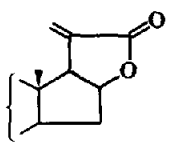
3
a: R = H
b: R = Ac



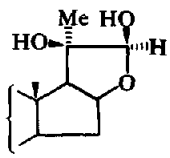
4
a: 3β-OH
b: 3β-OAc



5
3β-OAc



6
3β—OAc



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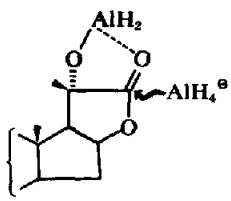

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the fact that it forms an acetonide. Second, they affirm that the said diol has β configuration because attack of the reduction reagent must take place from the α side of the molecule. However, it is well known[6] that the reduction mechanism of α-hydroxy-carbonyls with LAH cannot be explained by directly applying the rule of asymmetric induction, but that one must consider the steric hindrance caused by the formation of a salt, which in our case would correspond to formula 8 and the reduction of which would give a *cis*-diol no matter the stereochemistry at C_{20}. Therefore, the reasons exposed by Gashchenko *et al*[5] are not valid for determining the stereochemistry at C_{20}.

EXPERIMENTAL

M.ps determined on a Kofler block are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter in $CHCl_3$ unless otherwise stated and IR spectra on a Perkin-Elmer 237 spectrometer. NMR spectra were taken with a Perkin-Elmer R-10 instrument (60 MHz) using TMS as internal reference and mass spectra with a Hitachi Perkin-Elmer RMU-7. The chromatographic adsorbents were Merck products. Column chromatography was performed on silica gel 0·2–0·5 mm and dry column chromatography on silica gel 0·063–0·2 mm, the spray reagent for TLC being H_2SO_4—HOAc—H_2O (4:80:16). Recrystallization solvent was MeOH unless otherwise indicated. Light petroleum refers to fractions of b.p. 40–60°.

Isolation of the steroids. The air-dried stems and leaves of *Solanum vespertilio* (32 kg) collected near San Andrés (Tenerife) were finely cut and extracted with EtOH in a soxhlet. After filtering the cold ethanolic extract it was concentrated *in vacuo*, diluted with an equal volume of water and degreased with benzene in a liquid–liquid extractor. Then conc HCl was added till it was 2N. After refluxing for 3 hr it was poured into water, neutralized with $NaHCO_3$ and filtered. The ppt was extracted several times with boiling $CHCl_3$. Evaporation of the solvent afforded the crude mixture of steroids (320 g) which was chromatographed on a column. Elution with benzene-EtOAc (9:1) gave a mixture of β-sitosterol and diosgenin, separated by dry column chromatography. With benzene-EtOAc (85:15) a mixture of 16-dehydropregnenolone and vespertilin (**1a**) was obtained which was separated on a dry column. Benzene-EtOAc (7:3) eluted a mixture of **1c**, $\Delta^{3,5}$-solasodine and an unknown steroid, being investigated, which was acetylated and separated by preparative TLC (benzene-EtOAc 95:5). Finally, with benzene-EtOAc (1:1) a mixture of solasodine and tomatidenol was obtained which was separated on a dry column.

β-Sitosterol (0·5 g), m.p. 134–137°, $[\alpha]_D$ −34° (*c*, 0·16). Identified with an authentic sample (TLC, IR spectrum superimposable).

Diosgenin (5 g), m.p. 203–205°, $[\alpha]_D$ −117° (*c*, 0·17). Identified with an authentic sample (TLC, IR spectrum superimposable).

16-*Dehydropregnenolone* (0·5 g), m.p. 210·5–212°, $[\alpha]_D$ −31° (*c*, 0·15). Identified with an authentic sample (TLC, IR spectrum superimposable).

Vespertilin **1a** (0·8 g), m.p. 223·5–225° (EtOAc-light petroleum), $[\alpha]_D$ −98° (*c*, 0·18). Identified with an authentic sample isolated from the fruits of the same plant (m.m.p., TLC, IR, NMR spectra superimposable).[2]

Solasodine (30 g), m.p. 202–204°, $[\alpha]_D$ −107° (*c*, 0·30). (Found: C, 78·20; H, 10·36; N, 3·29. Calc. for $C_{27}H_{43}O_2N$: C, 78·40; H, 10·48; N, 3·39%); IR and NMR data identical with those given in [7,8].

Tomatidenol (0·5 g), m.p. 235–239°, $[\alpha]_D$ −45° (*c*, 0·18). (Found: C, 78·37; H, 10·29; N, 3·19. Calc. for $C_{27}H_{43}O_2N$: C, 78·40; H, 10·48; N, 3·39%); NMR data identical with those given.[8]

$\Delta^{3,5}$-*Solasodine* (0·1 g), m.p. 176–178°, $[\alpha]_D$ −180° (*c*, 0·19). (Found: C, 81·26; H, 10·58; N, 3·74. Calc. for $C_{27}H_{41}ON$: C, 81·97; H, 10·45; N, 3·54%); IR and NMR data identical with those given in [9].

20S-*Hydroxyvespertilin* **1c** (50 mg), m.p. 308–310°, $[\alpha]_D$ −64° (*c*, 0·13; pyridine) [lit.[5] m.p. 309–310°, $[\alpha]_D$ −68° (pyridine)]. (Found: C, 73·33; H, 8·94. $C_{22}H_{32}O_4$ requires: C, 73·30; H, 8·95%); ν_{max}^{KBr} 3380 (OH), 3030, 2830, 840 (Δ^5), 1780, 1750 cm^{-1} (γ-lactone). NMR: see Table 1; MS: *m/e* (%) 360 (M^+; 27), 342 (M^+—H_2O; 42), 327 (38), 301 (20), 275 (35). *Diacetate* **1d**, prepared with Ac_2O in pyridine at room temp for 3 days or at 100° for 1 hr. M.p. 244–246°, $[\alpha]_D$ −91° (*c*, 0·20). (Found: C, 69·96; H, 7·95. $C_{28}H_{36}O_6$ requires: C, 70·24; H, 8·16%); ν_{max}^{KBr} 3030, 2830, 830 (Δ^5), 1780, 1750 (γ-lactone), 1730, 1260 cm^{-1} (OAc); NMR: see Table 1.

Pseudotigogenin acetate **3b**. A soln of tigogenin (4 g) in pyridine (12 ml) containing Ac_2O (8 ml) and $MeNH_2$·HCl (1·4 g) was refluxed for 3½ hr. After adding water the soln was extracted with $CHCl_3$, washed with $NaHCO_3$ aq and water and the solvent evaporated *in vacuo*. Dry column chromatography of the residue (benzene-EtOAc 97:3) afforded **3b**, m.p. 65–70° (lit.[10] 68–70°).

Pseudotigogenin **3a**. **3b** was saponified with 2% KOH in MeOH at room temp for 12 hr. Usual work-up gave **3a** (2 g) which was unstable and therefore used without further purification.

20S-*Hydroxytigogenin* **4a**. To a soln of **3a** (2 g) in CH_2Cl_2 (150 ml) *m*-chloroperbenzoic acid (3·5 g) was added and the mixture kept at room temp in the dark for 15 hr. It was washed with $NaHSO_3$ aq, $NaHCO_3$ aq and water and concentrated. Dry column chromatography of the residue (benzene-EtOAc 1:1) gave **4a** (0·9 g), m.p. 219–221°, $[\alpha]_D$ −61° (*c*, 0·18). (Found: C, 74·67; H, 10·43. Calc. for $C_{27}H_{44}O_4$: C, 74·96; H, 10·25%); $\nu_{max}^{CHCl_3}$ 3610 (OH), 985, 960, 920, 900, 860 cm^{-1} (25*R*-spiroketal); NMR ($CDCl_3$): 8·61 (3H, s, Me—C_{20}), 9·06 (3H, s, Me—C_{13}), 9·18 (3H, s, Me—C_{10}), 9·21 (3H, d, *J* 6 Hz, Me—C_{25}). 3-*Acetate* **4b**, prepared as usual, m.p. 235–238°, $[\alpha]_D$ −66° (*c*, 0·19) (lit.[4] m.p. 234–236°, $[\alpha]_D$ −71°). (Found: C, 73·13; H, 9·92. Calc. for $C_{29}H_{46}O_5$: C, 73·38; H, 9·77%); $\nu_{max}^{CHCl_3}$ 3500 (OH), 1730, 1260 cm^{-1} (OAc); NMR ($CDCl_3$): 7·96 (3H, s, OAc), 8·61 (3H, s, Me—C_{20}), 9·05 (3H, s, Me—C_{13}), 9·16 (3H, s, Me—C_{10}), 9·21 (3H, d, *J* 6 Hz, Me—C_{25}).

5-*Dihydro*-20S-*hydroxyvespertilin diacetate* (**2b**) *from* **4b**. To a soln of **4b** (250 mg) in $CHCl_3$ (3 ml) and ether (12 ml) conc HNO_3 (5·5 ml) was added slowly during 1 hr and the reaction kept at room temp for 3 hr. Usual work-up gave a residue which chromatographed on a dry column (benzene-EtOAc 85:15) yielded **2a** (140 mg), m.p. 282–284°, $[\alpha]$ −54° (*c*, 0·27). (Found: C, 70·90; H, 9·22. $C_{24}H_{36}O_5$ requires: C, 71·26; H, 8·97%); $\nu_{max}^{CHCl_3}$ 3580 (OH), 1780, 1750 (γ-lactone), 1740, 1260 cm^{-1} (OAc); NMR ($CDCl_3$): 7·97 (3H, s, OAc), 8·41 (3H, s, Me—C_{20}), 9·17 (3H, s, Me—C_{10}), 9·20 (3H, s, Me—C_{13}). *Diacetate* **2b**, by refluxing **2a** with Ac_2O in pyridine for ½ hr, m.p. 261–262°, $[\alpha]_D$ −26° (*c*, 0·17). (Found: C, 69·98; H, 8·80.

$C_{26}H_{38}O_6$ requires: C, 69·93; H, 8·58%); $\nu_{max}^{CHCl_3}$ 1780, 1750 (γ-lactone), 1730, 1260 cm^{-1} (OAc). NMR ($CDCl_3$): 7·95 (6H, s, OAc), 8·21 (3H, s, Me—C_{20}), 9·17 (3H, s, Me—C_{10}), 9·20 (3H, s, Me—C_{13}).

Compound **2b** *from* **1d**. A soln of **1d** (9 mg) in glacial HOAc (3 ml) was hydrogenated over 10% Pd/C (15 mg) at room temp and atm press for 6 hr. After filtering and usual work-up the residue was chromatographed on a dry column (benzene-EtOAc 95:5) affording **2b** which was shown to be identical with the synthetic material obtained above (m.m.p., TLC, IR, NMR spectra superimposable).

*5-Dihydro-*17,20-*dehydrovespertilin* (**5**) *and 5-dihydro-*20,21-*dehydrovespertilin* (**6**) *acetates*. To a soln of **2a** (60 mg) in pyridine (1 ml) $SOCl_2$ (3 drops) was added and the mixture stirred at room temp for 1 hr. After usual work-up preparative TLC (benzene-EtOAc 97:3) of the residue gave **5** (19 mg) and **6** (30 mg). **5**, m.p. 228–230°, $[\alpha]_D$ −43° (*c*, 0·16). (Found: C, 74·37; H, 8·91. $C_{24}H_{34}O_4$ requires: C, 74·58; H, 8·87%); $\nu_{max}^{CHCl_3}$ 1770 (α,β-unsaturated γ-lactone), 1730, 1260 cm^{-1} (OAc); NMR: see Table 1. **6**, m.p. 225–227°, $[\alpha]_D$ −96° (*c*, 0·13). (Found: C, 74·26; H, 8·98. $C_{24}H_{34}O_4$ requires: C, 74·58; H, 8·87%); $\nu_{max}^{CHCl_3}$ 1760 (α,β-unsaturated γ-lactone), 1730, 1260 cm^{-1} (OAc); NMR: see Table 1.

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