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7-KETOTAMUSGENIN, 7-KETODIOSGENIN, 25S-HYDROXY-TAMUSGENIN AND AFURIGENIN, FOUR NEW STEROIDAL SAPOGENINS FROM *TAMUS EDULIS**

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Abstract—Four new steroidal sapogenins have been isolated from the leaves and twigs of *Tamus edulis* Lowe: 7-ketotamusgenin (IIIa), 7-ketodiosgenin (IVa), 25S-hydroxytamusgenin (Va) and afurigenin (IXa) Compounds IIIa, Va and IXa each bear a carbonyl group at C_{11}. The structures of IIIa and IVa have been confirmed by synthesis from tamusgenin and diosgenin, respectively, and that of Va by transformation into tamusgenin (Ia). For afurigenin, we provisionally propose structure IXa, based on its conversion into Va

INTRODUCTION

IN PREVIOUS papers[2,3] we reported the isolation of the steroidal sapogenins tamusgenin [(25R)-spirost-5-en-3β-ol-11-one], eduligenin [(25R)-cholest-5-en-3β,26-diol-11,16,22-trione] and lowegenin [(25R)-spirost-5-en-3β,16α-diol-11-one] from the twigs and leaves of *Tamus edulis* Lowe (Dioscoreaceae). Continuing the investigations on this plant, we separated four more sapogenins. To three of them, namely 7-ketotamusgenin (IIIa), 7-ketodiosgenin (IVa) and 25S-hydroxytamusgenin (Va), we assign the structures (25R)-spirost-5-en-3β-ol-7,11-dione, (25R)-spirost-5-en-3β-ol-7-one and (25S)-spirost-5-en-3β,25-diol-11-one, respectively. For the fourth one, afurigenin (IXa), we provisionally propose the structure (20S, 22S, 25S)-furost-5-en-22,25-epoxy-3β,26-diol-11-one.

RESULTS AND DISCUSSION

7-Ketotamusgenin (IIIa), $C_{27}H_{38}O_5$ (*m/e* 442), was separated and purified by column chromatography and preparative TLC. In the u.v. it has a maximum at 237 nm (ϵ 11000), characteristic of an α,β-unsaturated ketone. Its i.r. spectrum presents the typical absorptions of OH groups and (20S, 22R, 25R)-spirostan ring. The band at 1710 cm^{-1} corresponds to a cyclohexanone, whereas those at 1675 and 1630 cm^{-1} may be assigned to an α,β-unsaturated ketone. IIIa gives a monoacetate (IIIb), the i.r. spectrum of which does not show any OH absorptions. In the NMR spectrum of this acetate (Table 1) appears a broad signal ($w_{\frac{1}{2}} = 13$ count/sec) centred at approximately 6·56† which in C_6D_6 is deshielded to 6·46 and is characteristic of the two protons at C_{26} in a (20S, 22R, 25R)-spirostan sapogenin.[4] The singlet at

* Part X in the series 'New Sources of Steroidal Sapogenins'. Compare Ref 1

† All chemical shifts are reported on the τ-scale.

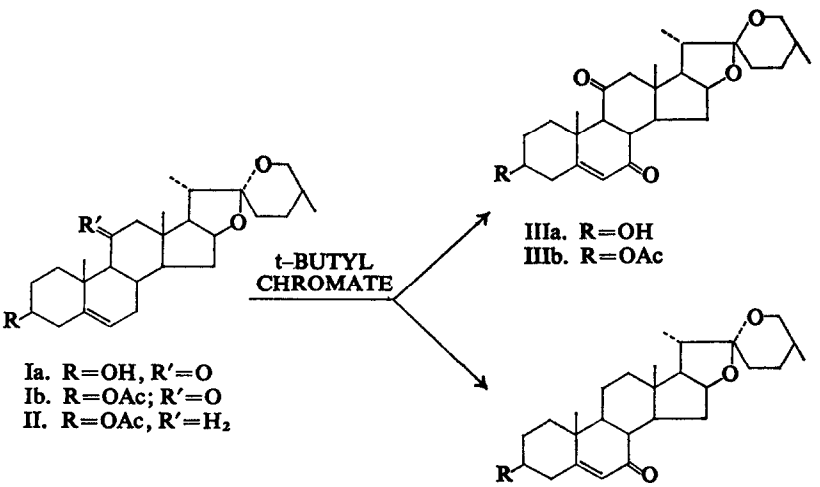
[1] R. FREIRE BARREIRA, A. G. GONZÁLEZ, J. A. SALAZAR ROCÍO and E. SUÁREZ LÓPEZ, *Anal Quim.* **66**, 415 (1970)

[2] R. FREIRE BARREIRA, A G GONZÁLEZ and E SUÁREZ LÓPEZ, *Anal. Quim* **64**, 745 (1968).

[3] R FREIRE BARREIRA, A G GONZÁLEZ, J. A. SALAZAR ROCÍO and E. SUÁREZ LÓPEZ, *Phytochem* **9**, 1641 (1970).

[4] D. H WILLIAMS and N S BHACCA, *Tetrahedron* **21**, 1641 (1965)

4·25 (1H) may be attributed to a proton of the type $\diagdown C{=}CH{-}C{=}O$.[5] The singlet for the Me_{18} at 9·25 coincides with the corresponding value observed in tamusgenin acetate (Ib). This together with a singlet at 7·69 (2H-C_{12} for 11-ketosteroids) indicates C_{11} as probable position for one of the keto groups. The chemical shifts for the Me_{19} (8·59) and Me_{18} (9·25) in IIIa are in accord with those calculated by the method of Zürcher[6] for 7-keto tamusgenin (8·58 and 9·23, respectively).*



Ia. R=OH, R′=O
Ib. R=OAc; R′=O
II. R=OAc, R′=H_2

IIIa. R=OH
IIIb. R=OAc

IVa. R=OH
IVb. R=OAc

The structure of IIIa was confirmed by selectively oxidizing tamusgenin acetate (Ib) at C_7 with *tert*-butyl chromate in CCl_4,[7] obtaining IIIb; saponification yielded IIIa which proved to be identical in all respects with our compound.

7-Ketodiosgenin (IVa), $C_{27}H_{40}O_4$, obtained by preparative column chromatography, shows a u.v. maximum at 238 nm (ϵ 16000) assignable to an α,β-unsaturated ketone. In the i.r. it presents the characteristic absorptions of OH groups and (20S, 22R, 25R)-spirostan ring as well as two bands at 1675 and 1635 cm^{-1} corresponding to an α,β-unsaturated ketone. It gives a monoacetate (IVb) whose i.r. spectrum lacks the OH absorptions. The NMR spectrum of IVa (Table 1) shows a singlet at 4·27 (1H) attributed to a proton of the type $\diagdown C{=}CH{-}C{=}O$.[5] A multiplet at 5·45 (H-C_{16}; $w_{\frac{1}{2}} = 30$ counts/sec) together with a signal centred at 6·56 (2H-C_{26}; $w_{\frac{1}{2}} = 12$ counts/sec)[4] confirm the presence of a (20S, 22R, 25R)-spirostan ring. Two singlets at 8·78 and 9·21 are assigned to the angular Me_{19} and Me_{18},

* $\tau_{\text{tigogenin}} + x_{\Delta^5\text{-7-one}} + \tau_{\text{tamusgenin}} - \tau_{\text{diosgenin}}$. The contributions x of a Δ^5-7-one to the chemical shifts of the Me_{18} and Me_{19} are taken from Ref 5.

[5] N. S. BHACCA and D. H. WILLIAMS, *Application of NMR Spectroscopy in Organic Chemistry*, Holden-Day, San Francisco (1964).
[6] R. ZURCHER, *Helv. Chim. Acta* **46**, 2054 (1963).
[7] C. W. MARSHALL and R. E. RAY, *J. Am. Chem. Soc.* **79**, 6310 (1957).

TABLE 1 τ-VALUES (IN PARENTHESES: *J*-VALUES, IN BRACKETS: $w_{\frac{1}{2}}$)

Compound	Solvent	H—C_6	H—C_{16} H—C_3	2H—C_{26}	2H—C_{12}	Me_{19}	Me_{21}	Me_{27}	Me_{18}
7-Keto tamusgenin (IIIa)	$CDCl_3$	4 27 s	~5 40 m [24]	6 56 m [15]	7 69 s	8·59 s	9 05 d (6)	9 22 d (6)	9·25 s
Acetate (IIIb)	$CDCl_3$	4·25 s	~5 40 m [24]	6 56 m [13]	7 69 s	8 59 s	9 05 d (6)	9 32 d (7)	9 25 s
Acetate (IIIb)	C_6D_6	4·37 s	~5 30 m [27]	6·46 m [13]		8 87 s	8 96 d (6)	9 35 d (6)	9 38 s
7-Keto diosgenin (IVa)	$CDCl_3$	4 27 s	~5 45 m [30]	6 56 m [12]		8 78 s	9 03 d (6)	9 21 d (6)	9 21 s
Acetate (IVb)	$CDCl_3$	4 27 s	~5 45 m [27]	6 56 m [12]		8 78 s	9 03 d (6)	9 20 d (6)	9 21 s
Acetate (IVb)	C_6D_6	4 30 s	~5 25 m [24]	6 43 m [12]		9 25 s	8 79 d (6)	9·35 d (6)	9 25 s
25S-Hydroxy tamusgenin (Va)	$CDCl_3$	4 63 m [12]	~5 50 m [24]	6·24 d (12) 6 73 d (12)	7 67 s	8 77 s	8 99 d (6)	8 88 s	9 25 s
Acetate (Vb)	$CDCl_3$	4 60 m [12]	~5·50 m [24]	6 25 d (12) 6 75 d (12)	7 69 s	8 77 s	8·99 d (6)	8 89 s	9·25 s
Afurigenin (IXa)	$CDCl_3$	4 62 m [12]	~5 40 m [24]	6·43 d (12) 6 68 d (12)	7 68 s	8 79 s	9 03 d (6)	8 84 s	9 25 s

respectively; their chemical shifts agree with the values calculated by the method of Zürcher[6] for 7-ketodiosgenin (8·79 and 9·22, respectively).*

The structure of IVa was confirmed by selectively oxidizing diosgenin acetate (II) as mentioned above,[7] obtaining 7-ketodiosgenin acetate (IVb) whose physical and spectroscopical constants coincide with those of our compound. It has already been synthesized by Marker and Turner[8] from diosgenin acetate, but this is the first time it has been isolated from a plant.

The contributions of an 11-one and a Δ^5-7-one to the chemical shifts of the two protons at C_{26}, the Me_{19}, Me_{21} and Me_{18} are given in Table 2. In the same Table we show the solvent shifts ($CDCl_3 \rightarrow C_6D_6$) of these groups due to the presence of an 11-one, a Δ^5-7-one and a Δ^5-7,11-dione, the values being the expected ones[4,9] considering the location of the groups cited relative to these substituents.

TABLE 2. EFFECT OF SUBSTITUENTS ON THE CHEMICAL SHIFT OF THE PROTONS INDICATED AND THEIR SOLVENT SHIFT (τ-SCALE)

Substituent	Solvent	$2H-C_{26}$	Me_{19}	Me_{21}	Me_{18}
11-One*	$CDCl_3$	−0 02	−0 19	+0 02	+0 03
	C_6D_6	+0·01	−0 38	+0 16	+0 14
Δ^5-7-One†	$CDCl_3$	0 00	−0 38	−0 02	−0 03
	C_6D_6	−0 02	−0 08	−0 03	+0 05
11-One‡	$CDCl_3 \rightarrow C_6D_6$	+0 03	−0 19	+0 14	+0 11
Δ^5-7-One§	$CDCl_3 \rightarrow C_6D_6$	−0 02	+0 30	−0 01	+0·08
Δ^5-7,11-Dione¶	$CDCl_3 \rightarrow C_6D_6$	+0 01	+0 11	+0 14	+0 17

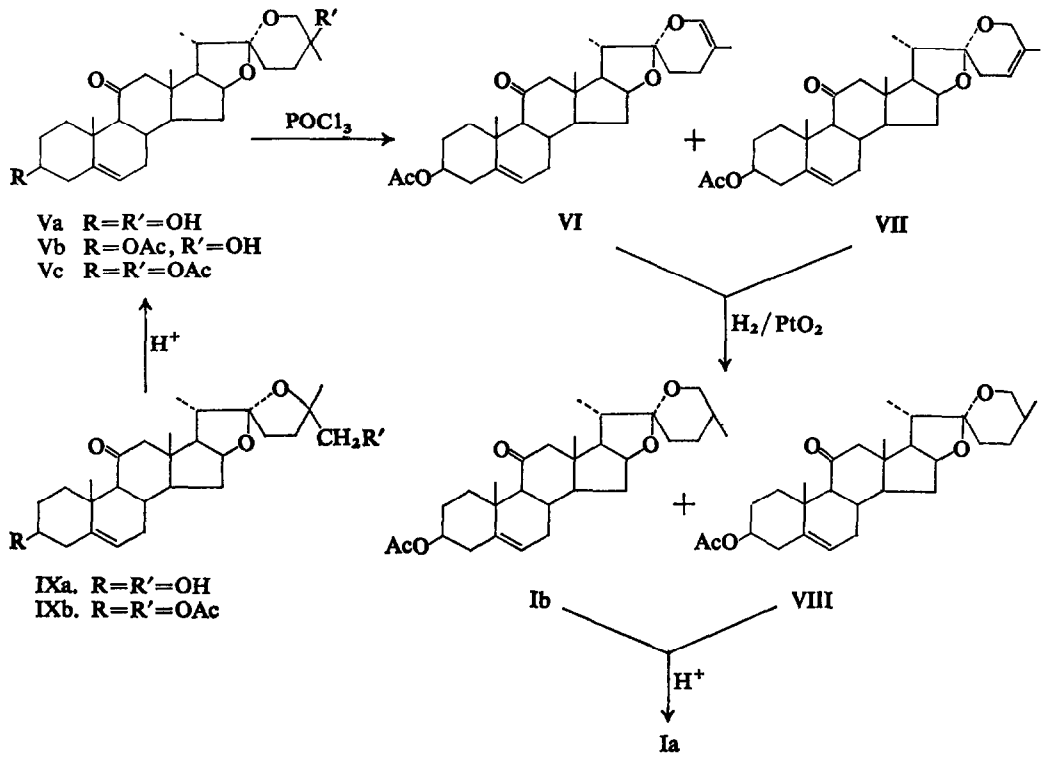
* $\tau_{\text{tamusgenin acetate}} - \tau_{\text{diosgenin acetate}}$
† $\tau_{\text{7-ketodiosgenin acetate}} - \tau_{\text{tigogenin acetate}}$
‡ $\Delta_{\text{tamusgenin acetate}} - \Delta_{\text{diosgenin acetate}}$ $(\Delta = \tau_{C_6D_6} - \tau_{CDCl_3})$
§ $\Delta_{\text{7-ketodiosgenin acetate}} - \Delta_{\text{tigogenin acetate}}$
¶ $\Delta_{\text{7-ketotamusgenin acetate}} - \Delta_{\text{tigogenin acetate}}$

25S-Hydroxytamusgenin (Va), $C_{27}H_{40}O_5$ (*m/e* 444), isolated by column chromatography, shows the typical i.r. absorptions of OH groups. The band at 1710 cm^{-1} may be assigned to a cyclohexanone and those at 980, 940, 910, 890 and 850 cm^{-1} to the spirostan ring. The absorption at 940 cm^{-1} has not been observed in any of the spirostan sapogenins studied by us and might be related with the spirostan ring bearing an axial OH at C_{25}.[10] The band at 890 is more intense than that at 910 cm^{-1}, thus indicating probably that the Me_{27} is equatorial.[10] Mild acetylation of Va at room temperature yielded the 3-monoacetate (Vb) whose i.r. spectrum presents absorptions at 3595 (OH) and 1740 cm^{-1} (OAc). Strong acetylation at 100° gave the 3,25-diacetate (Vc) whose i.r. spectrum is free of OH absorptions thus indicating that Va has two hydroxyl groups, one of them tertiary.

In the NMR spectrum of Va (Table 1) appears a signal at 4·63 (1H; $w_{\frac{1}{2}} = 12$ count/sec) corresponding to a vinylic proton and another at 5·50 (1H; $w_{\frac{1}{2}} = 24$ count/sec) assignable

* $\tau_{\text{7-ketotamusgenin}} - \tau_{\text{tamusgenin}} + \tau_{\text{diosgenin}}$

[8] R. E. MARKER and D. L. TURNER, *J. Am. Chem. Soc.* **63**, 767 (1941).
[9] D. H. WILLIAMS and N. S. BHACCA, *Tetrahedron* **21**, 2021 (1965).
[10] K. TAKEDA, H. MINATO, A. SHIMAOKA and Y. MATSUI, *J. Chem. Soc.* 4815 (1963).



to the proton at C_{16}.[4] In the region 8·70–9·30 three singlets (each 3H) and one doublet (3H) are observed, suggesting that the tertiary OH must be situated at C_{20} or C_{25}. Two doublets centred at 6·24 and 6·73 are attributed to the two protons at C_{26}. The mass spectrum of Va shows peaks at *m/e* 131 and 155 corresponding to fragments which contain ring F, and are 16 units higher than those expected for spirostan sapogenins without substituents in this ring.[11] Hence we conclude that the tertiary OH must be situated at C_{25}.

Dehydration of the 3-monoacetate (Vb) with $POCl_3$ in pyridine[12] yielded VI and VII whose i.r. spectra lack the absorptions of the tertiary OH group. The NMR spectrum of VI presents a singlet at 3·93 (1H, H-C_{26}) and another one at 8·43 (3H, Me_{27}), whereas that of VII shows a multiplet at 4·64 (2H, H-C_{24}, H-C_6) and a singlet at 8·38 (3H, Me_{27}).[12] Hydrogenation of VI, as well as of VII, gave a mixture of Ib and VIII the i.r. spectrum of which shows the characteristic bands of the spirostan ring (985, 920, 900 cm^{-1}),[13] the absorption at 900 cm^{-1} being slightly weaker than that at 920 cm^{-1}, as is expected for a mixture of the 25S and 25R isomers. All these results confirm that the tertiary OH is located on C_{25}. Prolonged acid treatment of the mixture of Ib and VIII yielded tamusgenin (Ia) which upon acetylation gave Ib.

The i.r. spectrum of a dilute solution of Va presents absorptions at 3615 and 3595 cm^{-1} corresponding to the two hydroxyl groups at C_3, and C_{25}, respectively. This indicates that the OH at C_{25} forms a H-bond with the O atom between C_{22} and C_{26}, which is possible

[11] C. Djerassi, *Monatsh. Chem.* **93**, 1033 (1962).
[12] K. Takeda, T. Okanishi, H. Minato and A. Shimaoka, *Tetrahedron* **19**, 759 (1963).
[13] R. N. Jones, E. Katzenellenbogen and K. Dobriner, *J. Am. Chem. Soc.* **75**, 158 (1953).

only if the hydroxyl is axial. Based on the stretching frequency shift of the free tertiary axial OH, whose theoretical value is 3618 cm^{-1}, to 3595 cm^{-1} (experimental value for Va) we calculate a H-bond of 2·5 Å,[14] in agreement with the length (2·4 Å) measured direct on a Dreiding model. Hence, 25S-hydroxytamusgenin (Va) must be (25S)-spirost-5-en-3β,25-diol-11-one.

Afurigenin (IXa), $C_{27}H_{40}O_5$ (*m/e* 444), was separated by column chromatography and preparative TLC. Its i.r. spectrum, besides the OH absorptions, shows a band at 1710 cm^{-1} which may be assigned to a cyclohexanone, the region 1000–800 cm^{-1} being very different from that expected for a spirostan ring. Mild acetylation of IXa gives the diacetate IXb whose i r. spectrum lacks the OH frequencies. This means that IXa has two unhindered hydroxyl groups. The mass spectrum of IXa presents a signal at *m/e* 413 assignable to a fragment $[M—CH_2OH]^+$. The peaks at *m/e* 131 and 155 indicate the presence of an additional O atom in ring F.[11] The NMR spectrum (Table 1) shows a broad signal at 5 40 (1H, $w_{\frac{1}{2}} = 24$ count/sec) which may be attributed to the proton at C_{16}.[4] Two singlets at 8·79 and 9·25 correspond to the Me_{19} and Me_{18}, respectively. Instead of the two doublets of the methyl groups at C_{20} and C_{25} appear a doublet at 9·03 (3H) and a singlet at 8·84 (3H); since the mass spectral analysis situates one of the two OH groups in ring F, the doublet is assigned to the methyl group at C_{20} and the singlet to that at C_{25}, the latter C atom bearing a tertiary OH if ring F is hexacyclic, or a CH_2OH group if it is pentacyclic. The former case, however, does not agree with the chemical behaviour of afurigenin, which evidently lacks a hindered OH. On the other hand, a pentacyclic ring F would be in accord with the fact that dilute acids easily transform IXa into Va.[15]

The i.r. spectrum of a dilute solution of IXa presents absorptions at 3615 and 3440 cm^{-1} corresponding to the OH groups at C_3, and C_{26}, respectively. This indicates that the OH at C_{26} must form a H-bond with the O atom of ring E. Considering the stretching frequency shift of the free primary OH, whose theoretical value is 3640 cm^{-1}, to 3440 cm^{-1} (experimental value for IXa) we calculate a H-bond of 0·9 Å.[14] Direct measurement of this bond on a Dreiding model with configuration 25S yields 1·3 Å. We therefore provisionally propose for afurigenin (IXa) the structure (20S, 22S, 25S)-furost-5-en-22,25-epoxy-3β,26-diol-11-one. The synthesis of this compound is in progress.

EXPERIMENTAL

M ps were taken with a Kofler block and are uncorrected The optical activities were determined in $CHCl_3$ and the u.v. spectra in EtOH. If not otherwise stated, the acetates were prepared with Ac_2O in pyridine, leaving the mixture at room temp. overnight. Dry column chromatography was carried out on silica gel (0·05–0 2 mm) and TLC on silica gel, the spray reagent being H_2SO_4–HOAc–H_2O (4 80 16). Solvent used for recrystallizing compounds was acetone–petroleum (40–80°) unless otherwise indicated

Isolation of the Sapogenins

To obtain the crude sapogenins, the dry plant material (33 kg) was treated according to the technique described by Takeda *et al.*[16] (compare Ref 3) After separating β-sitosterol, diosgenin, tamusgenin, lowegenin and eduligenin from the extract (57 g), the resinous product was purified by dry column chromatography, eluting with benzene–EtOAc (7 3) and crystallized from acetone/H_2O The semicrystalline mixture (600 mg) thus obtained was separated in 3 fractions by chromatography using benzene–EtOAc (75 25) as eluent Crystallization of *fraction A*, which by TLC consisted of one compound only, yielded 7-ketodiosgenin (IVa, 110 mg). TLC of the acetylated *fraction B*, continuously eluting for 10 hr, revealed it to be a mixture of two

[14] L P. Kuhn, *J. Am. Chem Soc.* **74**, 2492 (1952), **76**, 4323 (1954).
[15] R Tschesche and K. H. Richert, *Tetrahedron* **20**, 387 (1964).
[16] K Takeda, T Okanishi, H. Minato and A. Shimaoka, *Chem Pharm. Bull.* **12**, 779 (1964)

compounds They were separated by preparative TLC (0·5 mm) eluting 5 times with $CHCl_3$–benzene (7 3) Subsequent saponification and crystallization gave afurigenin (IXa; 30 mg) and 7-ketotamusgenin (IIIa; 150 mg). Crystallization of *fraction C* yielded 25S-hydroxytamusgenin (Va; 45 mg).

7-Ketotamusgenin (IIIa)

M p 250–253°, $[\alpha]_D$-131° (conc 0 140%) Found: C, 73·03, H, 8 79. $C_{27}H_{38}O_5$ required· C, 73·27; H, 8·65% *m/e* 442. λ_{max} 237 nm, ϵ 11000 (α,β-unsaturated ketone) $\nu_{max}^{CHCl_3}$ 3600, 1055 (OH), 3020, 2835, 1630, 845 (unsaturation), 1710 (cyclohexanone), 1675 (α,β-unsaturated ketone), 985, 920, 900, 865 cm^{-1} (spirostan ring). NMR: see Table 1.

Acetate (IIIb), m p. 191–194° (amorphous), $[\alpha]_D$-133° (conc 0 090%) Found. C, 72 14, H, 8·50. $C_{29}H_{40}O_6$ required: C, 71 87; H, 8 32%. $\nu_{max}^{CS_2}$ 3020, 2835, 1630, 845 (unsaturation), 1745 (OAc), 1715 (cyclohexanone), 1680 (α,β-unsaturated ketone), 985, 920, 900, 865 cm^{-1} (spirostan ring). NMR: see Table 1.

7-Ketodiosgenin (IVa)

M p 215–220°, $[\alpha]_D$-170° (conc. 0·110%). Found. C, 75 42, H, 9 37 $C_{27}H_{40}O_4$ required C, 75 66, H, 9 41%. λ_{max} 238 nm, ϵ 16000 (α,β-unsaturated ketone) $\nu_{max}^{CHCl_3}$ 3600, 1050 (OH), 3025, 2830, 1635, 845 (unsaturation), 1675 (α,β-unsaturated ketone), 985, 920, 900, 865 cm^{-1} (spirostan ring) NMR· see Table 1.

Acetate (IVb), m p 197–198 5° (from MeOH), $[\alpha]_D$-170° (conc. 0 110%) Found: C, 74 28; H, 9·03 $C_{29}H_{42}O_5$ required: C, 74 01; H, 8 99%. λ_{max} 234 nm, ϵ 16400 (α,β-unsaturated ketone) $\nu_{max}^{CS_2}$ 3025, 2830, 1635, 845 (unsaturation), 1745 (OAc), 1675 (α,β-unsaturated ketone), 985, 920, 900, 865 cm^{-1} (spirostan ring). NMR: see Table 1.

25S-Hydroxytamusgenin (Va)

M p. 245–247°, $[\alpha]_D$-76° (conc 0 150%). Found C, 72 64, H, 8 99 $C_{27}H_{40}O_5$ required. C, 72 94, H, 9·07%. *m/e* 444. $\nu_{max}^{CHCl_3}$ 3600, 1050, 1040 (OH), 2830, 1675, 830 (unsaturation), 1710 (cyclohexanone), 980, 940, 910, 890, 850 cm^{-1} (spirostan ring), upon dilution $\nu_{max}^{CCl_4}$ 3615, 3595 cm^{-1} (OH). NMR: see Table 1

Monoacetate (Vb), m p. 210–213°, $[\alpha]_D$-87° (conc. 0·104%). Found. C, 71·27; H, 8 89. $C_{29}H_{42}O_6$ required: C, 71 58; H, 8 70% $\nu_{max}^{CS_2}$ 3595, 1035 (OH), 3030, 2830, 1670, 830 (unsaturation), 1740 (OAc), 1710 (cyclohexanone), 980, 940, 910, 890, 850 cm^{-1} (spirostan ring) NMR see Table 1

Strong acetylation of Va with Ac_2O in pyridine at 100° gave the diacetate (Vc), m p 210–212° $\nu_{max}^{CS_2}$ 3030, 2830, 1670, 830 (unsaturation), 1740 (OAc), 1710 cm^{-1} (cyclohexanone)

Afurigenin (IXa)

M p 240–243°, $[\alpha]_D$-50° (conc. 0 116%) Found: C, 73 03; H, 9 07. $C_{27}H_{40}O_5$ required: C, 72 94, H, 9·07%. *m/e* 444. $\nu_{max}^{CHCl_3}$ 3600, 3420, 1050 (OH), 2830, 1670, 830 (unsaturation), 1710 cm^{-1} (cyclohexanone); upon dilution· $\nu_{max}^{CCl_4}$ 3615, 3440 cm^{-1} (OH). NMR: see Table 1.

Diacetate (IXb), m p 147–151° Found: C, 70 39; H, 8 43 $C_{31}H_{44}O_7$ required C, 70 43, H, 8 39% $\nu_{max}^{CS_2}$ 3030, 2830, 1670, 830 (unsaturation), 1740 (OAc), 1710 cm^{-1} (cyclohexanone).

7-Ketotamusgenin Acetate (IIIb) from Tamusgenin Acetate (Ib)

To Ib (456 mg) dissolved in CCl_4 (4 ml) were added HOAc (1 ml) and Ac_2O (0 25 ml). Then, with stirring and during 45 min at 55–60° was added a solution of HOAc (1 ml), Ac_2O (0 25 ml) and 5 6 ml of a solution of *tert*-butyl chromate in CCl_4 (prepared as follows· 1·36 g CrO_3 were dissolved in 4 ml *tert*-butyl alcohol at 0° and diluted with 12 ml CCl_4, then washed well with H_2O and dried over Na_2SO_4[7]). Stirring was continued for 11 hr at 65°, the mixture cooled to 20°, and the excess of *tert*-butyl chromate reduced by adding during 1 hr a 10% aqueous solution of oxalic acid (10 ml) in small portions The reaction mixture was poured into water (200 ml) and extracted with $CHCl_3$ After washing the organic layer 3 times with H_2O and drying over Na_2SO_4, the solvent was evaporated and the residue (460 mg) chromatographed Elution with benzene–EtOAc (9:1) yielded, besides starting material (360 mg), IIIb (76 mg) whose i.r spectrum was superimposable with that of the acetate of our compound Saponification with 1% methanolic KOH gave 7-ketotasmugenin (IIIa), m p 250–254°, $[\alpha]_D$-136° (conc 0 130%) Found. C, 73 08; H, 8 73. Calc. for $C_{27}H_{38}O_5$· C, 73 27, H, 8 65% λ_{max} 237 nm, ϵ 10800 I.r spectrum superimposable with that of our compound.

7-Ketodiosgenin Acetate (IVb) from Diosgenin Acetate (II)

II (350 mg) was treated as mentioned above Chromatography of the residue obtained, on elution with benzene–EtOAc (93 7) yielded, besides starting material (110 mg), IVb (95 mg), m p 197–198° (from MeOH), $[\alpha]_D$-175° (conc 0 110%). Found. C, 74 19; H, 9 12 Calc for $C_{29}H_{42}O_5$· C, 74·01; H, 8 99% λ_{max} 234 nm, ϵ 16000. I.r spectrum superimposable with that of the acetate of our compound Saponification with 1% methanolic KOH gave 7-ketodiosgenin (IVa), m p. 218–221°, $[\alpha]_D$-169° (conc. 0 100%). Found. C, 75 41, H, 9 47 Calc for $C_{27}H_{40}O_4$ C, 75 66, H, 9·41% I r. spectrum superimposable with that of our compound

Dehydration of the 3-Monoacetate (Vb) of 25S-Hydroxytamusgenin

$POCl_3$ (0·06 ml) was added at 0° to a solution of Vb (40 mg) in pyridine (1 ml) and the mixture left at room temp for 30 min and afterwards at 65° for 2 hr. It was then poured into water, extracted with ether and successively washed with 2N H_2SO_4, H_2O, saturated aqueous $NaHCO_3$ and H_2O. After drying over Na_2SO_4 and evaporating the solvent, the syrupy residue (38 mg) was chromatographed Elution with benzene–EtOAc (93:7) yielded first VI (15 mg), m p 208–210°, $[\alpha]_D$-127° (conc. 0·098%), $\nu_{max}^{CS_2}$ 3070, 3030, 2830, 2740, 1680, 830 cm^{-1} (unsaturation), NMR · 3·93 (1H, s, H-C_{26}), 8·43 (3H, s, Me_{27}); and afterwards VII (11 mg), m.p. 212–215°, $[\alpha]_D$-103° (conc 0 120%), $\nu_{max}^{CS_2}$ 3030, 2730, 1670, 830 cm^{-1} (unsaturation), NMR: 4·64 (2H, m, H-C_{24}, H-C_6), 8·38 (3H, s, Me_{27}).

Hydrogenation of VI and VII

A solution of VI (15 mg) in absolute EtOH (15 ml) with PtO_2 (8 mg) was hydrogenated for 90 min. After filtering off the catalyst and evaporating the solvent a mixture of Ib and VIII (14 mg) was obtained, $\nu_{max}^{CS_2}$ 985, 920, 900 cm^{-1} (spirostan ring), the absorption at 900 being slightly weaker than that at 920 cm^{-1}, thus indicating that the hydrogenated product must be a mixture of the 25S and 25R isomers

VII (11 mg) was treated as described above, yielding a mixture of Ib and VIII (10 mg) whose i.r. spectrum was superimposable with that of the hydrogenation product of VI

Tamusgenin (Ia) from the Mixture of Ib + VIII

The mixture of Ib + VIII (23 mg) in absolute EtOH (40 ml) and conc. HCl (15 ml) was refluxed in N_2 for 70 hr, whereupon it was poured into water and extracted with $CHCl_3$. The extract was successively washed with H_2O, saturated aq. $NaHCO_3$, and H_2O, dried over Na_2SO_4, and the solvent evaporated. Chromatography of the syrupy residue (20 mg), eluting with $CHCl_3$–acetone (95·5) yielded Ia, m.p 189–192°. Acetylation of Ia gave tamusgenin acetate (Ib), m p. 211–216° (from MeOH), $[\alpha]_D$-78° (conc. 0·110%). Found: C, 74·32; H, 9 20. Calc. for $C_{29}H_{42}O_5$: C, 74 01; H, 8 99%. I r. spectra of Ia and Ib superimposable with those of authentic samples of tamusgenin and its acetate.

25S-Hydroxytamusgenin (Va) from Afurigenin (IXa)

IXa (20 mg) in 4% ethanolic HCl (50 ml) was left at room temp. for 3 hr, upon which it was poured into water and extracted with $CHCl_3$ The organic layer was washed with H_2O, dilute aq. $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concentrated. The crystalline product (20 mg), after purification on a dry column eluting with benzene–EtOAc (1 1), yielded Va (16 mg) which was identified by comparison with an authentic sample (TLC, i r. spectrum)

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