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EDULIGENIN AND LOWEGENIN, TWO NEW STEROIDAL SAPOGENINS FROM *TAMUS EDULIS**

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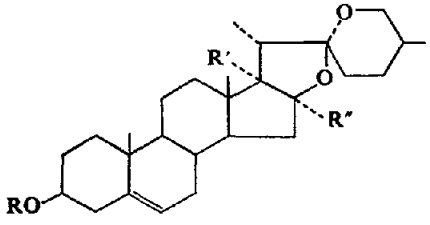
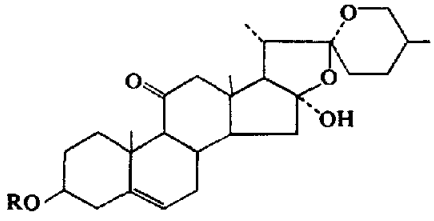
Abstract—Two new steroidal sapogenins have been isolated from the leaves and twigs of *Tamus edulis* Lowe: eduligenin (Va) and lowegenin (IIa), both having a carbonyl group at C_{11}.

INTRODUCTION

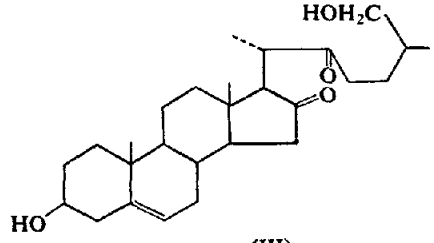
IN A PREVIOUS paper[1] we described the isolation and identification of the steroidal sapogenins diosgenin (Ia) and tamusgenin (VII), as well as β-sitosterol from the twigs of *Tamus edulis* Lowe (Dioscoreaceae). Tamusgenin is the first spirostan sapogenin found in nature with a carbonyl group at C_{11}.



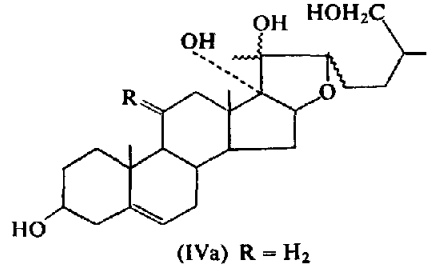
(Ia) R = R′ = R″ = H
(Ib) R = R″ = H, R′ = OH
(Ic) R = Ac, R′ = OH, R″ = H
(Id) R = R′ = H, R″ = OCH_3



(IIa) R = H
(IIb) R = Ac



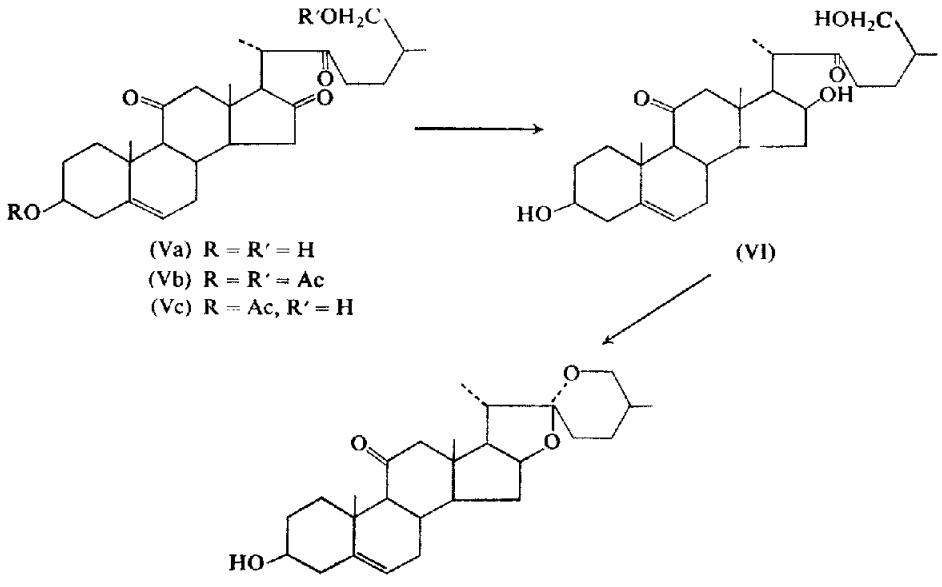
(III)



(IVa) R = H_2
(IVb) R = O

* Part VI in the series "New Sources of Steroidal Sapogenins".

[1] R. FREIRE BARREIRA, A. G. GONZÁLEZ and E. SUÁREZ, *Anal. Real Soc. Fis. Quím.* **64B**, 745 (1968).



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

(VI)

(VII)

The present paper gives the results of a reinvestigation of the same plant. In addition to the compounds above, we have isolated two new sapogenins, eduligenin (Va) (25R-cholest-5-en-3β,26-diol-11,16,22-trione) and lowegenin (IIa) (25R-spirost-5-en-3β,16α-diol-11-one).

RESULTS AND DISCUSSION

Following the technique of Takeda *et al.*,[2] we obtained a mixture of steroids which, when separated and purified by preparative column and thin-layer chromatography, gave β-sitosterol, diosgenin, tamusgenin, eduligenin and lowegenin. The first three compounds were identified by comparing their constants and i.r. spectra with those of authentic samples.

The elemental analysis of eduligenin (Va) is consistent with the empirical formula $C_{27}H_{40}O_5$. Its i.r. spectrum lacks the characteristic absorptions of the spirostan ring, but shows bands at 3610, 1050 (OH), 3030, 2830, 1675 (unsaturation), 1745 (cyclopentanone), and 1710 cm^{-1} (cyclohexanone or C=O in open chain). Va gives a diacetate (Vb), whose i.r. spectrum does not show any hydroxyl absorptions.

TABLE 1. τ-VALUES IN $CDCl_3$ (IN PARENTHESES: J-VALUES)

Compound	H—C_6	H—C_3	2H—C_{26}	Me_{19}	Me_{21}	Me_{27}	Me_{18}
Eduligenin (Va)	4·63 m	6·54 m	6·54 d (6)	8·78 s	8·97 d (7)	9·06 d (6)	9·26 s
Acetate (Vb)	4·63 m	5·45 m	6·06 d (6)	8·77 s	8·97 d (6)	9·05 d (6)	9·26 s

In the NMR spectra of Va and Vb (see Table 1), there is a signal at τ 4·63 (1H) which is characteristic of vinylic protons. The molecule therefore contains a tertiary double bond >C=CH—. The NMR spectrum of Vb shows a multiplet ($w\frac{1}{2} = 24$ counts/sec) centred

[2] K. TAKEDA, T. OKANISHI, H. MINATO and A. SHIMAOKA, *Chem. Pharm. Bull.* **12**, 779 (1964).

approximately at τ5·45 and corresponding to a proton situated at the same carbon as the acetate group. Two singlets at τ 7·96 and 7·99 are assigned to the CH_3 of the two acetate groups. In the spectrum of the alcohol, there is a doublet centred at τ 6·54 (2H; $J = 6$ counts/sec) which on acetylation is displaced to τ 6·06, thus indicating the presence of a primary hydroxyl group. In the region τ 7–8 there appear several peaks characteristic of allylic protons and protons in α position to carbonyl groups. The chemical shifts observed in the spectrum of eduligenin for the Me_{18} and Me_{19} (τ 9·26 and 8·78, respectively) are in accord with those calculated by the method of Zürcher[3] ($\tau_{\text{kryptogenin}} + \tau_{\text{tamusgenin}} - \tau_{\text{diosgenin}}$) for an 11-keto-kryptogenin (9·24 and 8·77, respectively).

All these spectroscopic data indicate that eduligenin has the structure of 11-keto-kryptogenin. This was confirmed by selective reduction of the carbonyl group at C_{16}, with $NaBH_4$ in isopropyl alcohol,[4] giving compound VI. By subsequent acid treatment of VI an acetal is formed between the hydroxyl groups at C_{16} and C_{26} and the carbonyl group at C_{22}, thus yielding tamusgenin (VII).

Purification of lowegenin (IIa) by column chromatography on alumina or silica gel showed that it was partially transformed into eduligenin. Separation was achieved by preparative TLC on deactivated silica gel. IIa is isomeric with eduligenin. In the i.r. it shows bands at 3605, 3525 (OH), 3030, 2835, 1670, 830 (unsaturation) and 1710 cm^{-1} (cyclohexanone). There also appear the characteristic absorptions of the spirostan ring 20S, 22R, 25R,[5] which is confirmed by a multiplet at τ 6·45 in the NMR spectrum, assigned to the two protons at C_{26}[6] (see Table 2). Another multiplet at τ 4·64 (1H) may be associated with a vinyl proton. The fact that the spectrum lacks the characteristic signal of the proton at C_{16}, which in spirostan sapogenins appears centred at τ 5·45,[6] indicates that this proton either is not present or is affected by a near substituent. The chemical shift for the Me_{19} is the same as that found in the spectra of tamusgenin and eduligenin, thus suggesting the positions Δ^5 and 11 for the double bond and the carbonyl group, respectively. A singlet at τ 7·64 (2H) would correspond to the two protons at C_{12}.

IIa yields a monoacetate (IIb) whose i.r. spectrum still shows a hydroxyl absorption at 3545 cm^{-1}, indicating that an associated tertiary hydroxyl group is present. On changing the solvent from chloroform to benzene, the solvent shift for the Me_{19} due to the presence of a carbonyl group in the molecule is –0·19 ppm.* The negative value of this shift can only be explained if the carbonyl group is situated at C_{11}.[7]

Treatment of lowegenin (IIa) with acid or with silica gel gives eduligenin (Va) together with some starting material. This indicates that the tertiary hydroxyl group can only be at positions C_{16} or C_{17}. Marker[8] isolated a spirostan sapogenin with a hydroxyl at C_{17}, called pennogenin (Ib), from the roots of *Trillium erectum* L. By acid treatment of Ib the spirostan ring is opened, giving kryptogenin (III).[9] However, unlike lowegenin, pennogenin is not altered at all by the presence of silica gel. This suggests the more labile position C_{16} for the tertiary hydroxyl group in IIa. In the NMR spectra of pennogenin (Ib) and its monoacetate

* $(\tau_{C_5D_5} - \tau_{CDCl_3})_{\text{lowegenin ac.}} - (\tau_{C_5D_5} - \tau_{CDCl_3})_{\text{diosgenin ac.}} = -0{\cdot}05 - 0{\cdot}14 = -0{\cdot}19$ ppm.

[3] R. F. ZURCHER, *Helv. Chim. Acta* **46**, 2054 (1963).
[4] F. C. UHLE, *J. Am. Chem. Soc.* **83**, 1460 (1961).
[5] R. N. JONES, E. KATZENELLENBOGEN and K. DOBRINER, *J. Am. Chem. Soc.* **75**, 158 (1953).
[6] D. H. WILLIAMS and N. S. BHACCA, *Tetrahedron* **21**, 1641 (1965).
[7] N. S. BHACCA and D. H. WILLIAMS, *Tetrahedron Letters* 3127 (1964).
[8] R. E. MARKER, R. B. WAGNER, P. R. ULSHAFER, E. L. WITTBECKER, D. P. J. GOLDSMITH and C. H. RUOF, *J. Am. Chem. Soc.* **65**, 1199 (1943).
[9] R. E. MARKER, R. B. WAGNER, P. R. ULSHAFER, E. L. WITTBECKER, D. P. J. GOLDSMITH and C. H. RUOF, *J. Am. Chem. Soc.* **69**, 2167 (1947).

TABLE 2. τ VALUES IN $CDCl_3$ AND C_6D_6 (IN PARENTHESES: J-VALUES)

Compound	Solvent	H—C_6	H—C_3	2H—C_{26}	2H—C_{12}	Me_{19}	Me_{21}	Me_{27}	Me_{18}
Lowegenin (IIa)	$CDCl_3$	4·64 m	6·45 m	6·45 d (6)	7·64 s	8·78 s	8·99 d (6)	9·17 d (6)	9·29 s
Acetate (IIb)	$CDCl_3$	4·64 m	5·44 m	6·45 d (5)	7·65 s	8·78 s	9·01 d (6)	9·20 d (7)	9·31 s
Acetate (IIb)	C_6D_6	4·81 m	5·20 m	6·46 m	7·69 d (13) 7·97 d (13)	8·73 s	9·02 d (6)	—	9·42 s

(Ic) the signal due to the proton at C_{16} appears as a triplet centred at τ 6·00. This assignment is confirmed by the spectrum of bethogenin (Id) which lacks this triplet. The fact that the spectra of IIa and IIb do not show it either, eliminates position C_{17} for the tertiary hydroxyl group. Hence, lowegenin contains a hemiacetal hydroxyl at C_{16}.

Marker[8] considers kryptogenin (III) to be formed during the acid hydrolysis of nolonin, a glycoside present in the original plant. For its aglycone nologenin he proposes structure IVa (Δ^5-25D-furosten-3β-17α,20ξ,26-tetrol). In the same way, eduligenin and lowegenin might derive from the acid hydrolysis of a possible 11-keto-nologenin (Δ^5-25D-furosten-3β,17α,20ξ,26-tetrol-11-one; IVb).

EXPERIMENTAL

The melting points were taken with a Kofler block and are uncorrected. The optical activities were determined in $CHCl_3$.

Isolation of the Sapogenins

The leaves and twigs (33 kg) of *Tamus edulis* Lowe, collected in October on the Monte de las Mercedes (Tenerife), were dried in a shadowy place, finely cut and extracted several times with EtOH in a soxhlet. The alcoholic extracts were combined, filtered and concentrated by vacuum distillation. The wax was eliminated by liquid–liquid extraction with benzene saturated with H_2O. Then the extract was hydrolysed with 4 N H_2SO_4 for 6 hr, whereupon it was poured into H_2O and neutralized with $NaHCO_3$. The precipitate formed was filtered off, dissolved in $CHCl_3$, and washed first with 10 per cent aq. KOH solution and then with H_2O. The $CHCl_3$ solution, which contains the crude sapogenins, was concentrated under vacuum giving a brown residue (115 g; yield: 0·35 per cent).

This residue was percolated through silica gel (Merck, 0·2–0·5 mm; 950 g), giving a mixture (57 g) of resinous products. They were chromatographed on silica gel (0·2–0·5 mm; 2 kg) and eluted with benzene, mixtures of benzene/$CHCl_3$, $CHCl_3$, and $CHCl_3$/MeOH (99:1). The fractions were monitored by TLC on silica gel, using H_2SO_4/HOAc/H_2O (16:4:80) as spray reagent.

Eduligenin (Va)

Elution with $CHCl_3$ and $CHCl_3$/MeOH (99:1) yielded a resinous product (7 g) which, after several crystallizations from EtOAc/petrol. ether, gave eduligenin (600 mg), m.p. 233–237°, $[\alpha]_D$ − 139° (*c* 0·83 per cent). Found: C, 72·74; H, 9·05. $C_{27}H_{40}O_5$ required: C, 72·94; H, 9·07 per cent. $\nu_{max}^{CHCl_3}$ 3610, 1050 (OH), 2830, 1675 (unsaturation), 1745 (cyclopentanone) and 1710 cm^{-1} (cyclohexanone or C=O in open chain). NMR: see Table 1.

By acetylation with Ac_2O/pyridine at room temp. a diacetate (Vb) was obtained. Crystallized from EtOAc/petrol. ether, it showed m.p. 187–189° and $[\alpha]_D$ − 125° (*c* 0·9 per cent). Found: C, 70·32; H, 8·57. $C_{31}H_{44}O_7$ required: C, 70·43; H, 8·39 per cent. $\nu_{max}^{CS_2}$ 3030, 2830, 1670, 815 (unsaturation), 1745 (OAc), and 1710 cm^{-1} (cyclohexanone or C=O in open chain). NMR: see Table 1.

Lowegenin (IIa)

As shown by TLC, the mother liquors from the crystallization of eduligenin mainly consist of two compounds, eduligenin and lowegenin. Their separation was achieved by preparative TLC on silica gel G layers (Merck; thickness 0·5 mm), revealing and deactivating with H_2O, the silica gel being extracted with $CHCl_3$ saturated with H_2O. Lowegenin crystallized from acetone/petrol. ether, m.p. 223–226°, $[\alpha]_D$ −55° (*c* 1·04 per cent). Found: C, 72·75; H, 9·13. $C_{27}H_{40}O_5$ required: C, 72·94; H, 9·07 per cent. $\nu_{max}^{CHCl_3}$ 3605, 3525 (OH), 3030, 2835, 1670, 830 (unsaturation), 1710 (cyclohexanone) and 980, 925, 900, 865 cm^{-1} (spirostan ring). NMR: see Table 2.

Acetylation of IIa with Ac_2O/pyridine at room temp. yielded a monoacetate (IIb) which crystallized from acetone/petrol. ether, m.p. 200–203°, $[\alpha]_D$ −62° (*c* 0·11 per cent). Found: C, 71·45; H, 8·51. $C_{29}H_{42}O_6$ required: C, 71·58; H, 8·70 per cent. $\nu_{max}^{CS_2}$ 3545 (tertiary OH), 1745 (OAc), 1715 (cyclohexanone), and 985, 925, 900, 865 cm^{-1} (spirostan ring). Upon dilution: $\nu_{max}^{CCl_4}$ 3545 cm^{-1} (associated tertiary OH). NMR: see Table 2.

Preparation of Tamusgenin (VII) from Eduligenin (Va)

To a suspension of eduligenin (60 mg) in isopropyl alcohol (25 ml) was added $NaBH_4$ (29 mg) with stirring. The mixture was stirred for 24 hr, whereupon it was acidified with HCl and heated for 2 min. The alcoholic solution was poured into water, extracted with ether, washed with H_2O and dried over Na_2SO_4. The residue obtained after evaporating the solvent was chromatographed on silica gel (0·05–0·2 mm; 15 g). Elution with

benzene/EtOAc (9:1) yielded a product which crystallized from EtOAc/petrol. ether gave tamusgenin (25 mg; yield: 42 per cent), m.p. 182–184°, $[\alpha]_D$ −77° (*c* 0·85 per cent). Found: C, 75·78; H, 9·57. Calc. for $C_{27}H_{40}O_4$: C, 75·66; H, 9·41 per cent. I.r. spectrum was superimposable with that of an authentic sample of tamusgenin. Acetylation with Ac_2O/pyridine at room temp. gave a monoacetate, m.p. 208–214° (from MeOH), $[\alpha]_D$ −78° (*c* 0·83 per cent), the i.r. spectrum of which was superimposable with that of an authentic sample of tamusgenin acetate.

Preparation of Eduligenin (Va) from Lowegenin (IIa)

To a solution of lowegenin (50 mg) in EtOH (50 ml), conc. H_2SO_4 was added until the solution was 4 N. After refluxing for 2 hr, it was poured into water, extracted with $CHCl_3$, washed with saturated $NaHCO_3$ and with H_2O and dried over Na_2SO_4. Evaporation of the solvent yielded a residue which by TLC ($CHCl_3$/acetone, 9:1) was shown to consist of two compounds. Their separation was obtained by preparative TLC on silica gel G layers (thickness 0·5 mm), eluting with $CHCl_3$/acetone (9:1), deactivating with H_2O and extracting with $CHCl_3$ saturated with H_2O. One compound was identified as lowegenin by its i.r. spectrum and the other as eduligenin, m.p. 232–235° (from acetone/petrol. ether), $[\alpha]_D$ −135° (*c* 1·34 per cent). Found: C, 72·67; H, 9·02. Calc. for $C_{27}H_{40}O_5$: C, 72·94; H, 9·07 per cent. I.r. spectrum was superimposable with that of an authentic sample of eduligenin.

Preparation of Eduligenin Diacetate (Vb) from Lowegenin Acetate (IIb)

To a solution of lowegenin acetate (70 mg) in $CHCl_3$, silica gel was added and the mixture left at room temp. overnight. After adding acetone, the silica gel was filtered off and washed with $CHCl_3$/acetone (1:1). Evaporation of the solvent gave a residue consisting of two compounds (TLC), whose separation again was achieved by preparative TLC, thus giving lowegenin acetate identified by its i.r. spectrum, and compound Vc (28 mg) showing $\nu_{max}^{CHCl_3}$ 3610 (OH) and 1740 cm^{-1} (OAc). Acetylation of Vc with Ac_2O/pyridine at room temp. gave eduligenin diacetate, m.p. 187–189° (from acetone/petrol. ether), $[\alpha]_D$ −120° (*c* 0·12 per cent). Found: C, 70·53; H, 8·38. Calc. for $C_{31}H_{44}O_7$: C, 70·43; H, 8·39 per cent. I.r. spectrum was superimposable with that of an authentic sample of eduligenin diacetate.

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