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TENASOGENIN, A PREGNANE ESTER FROM *MARSDENIA TENACISSIMA*

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Key Word Index—*Marsdenia tenacissima*; Asclepiadaceae; tenasogenin; steroid; polyhydroxy pregnane ester.

Abstract—A new polyhydroxy pregnane ester named tenasogenin was isolated from the seeds of *Marsdenia tenacissima*. On the basis of chemical and spectroscopic evidence and identification of its hydrolysis products, its structure has been established as 11α-*O*-β,β-dimethylacryloyl-3β,12β,14β,20*R*-tetrahydroxypregn-5-ene.

INTRODUCTION

In the chemical investigation of the seeds of *Marsdenia tenacissima* (Wight & Arn.), the pregnane glycosides of 2-deoxy sugars were extracted. Mild acid hydrolysis [1] of these glycosides gave a mixture of genins and sugars which were separated and characterized [2]. This paper describes the structural elucidation of a novel pregnane ester, substance F (= tenasogenin) which was isolated from the seeds of *M. tenacissima*.

RESULTS AND DISCUSSION

Tenasogenin (1)

Tenasogenin (**1**), mp 180–183°, $[\alpha]_D^{25}$ + 9.63° (MeOH) analysed for $C_{26}H_{40}O_6 \cdot \frac{1}{2}H_2O$. High resolution MS measurement of the highest mass ion peak at *m/e* 430.2705 ($M^+ - H_2O$, $C_{26}H_{38}O_5$) confirmed the molecular formula as $C_{26}H_{40}O_6$. Its IR spectrum exhibited strong absorption bands for hydroxyl groups (3470 and 1055 cm^{-1}), an α,β-unsaturated acid ester group (1679 and 1274 cm^{-1}) and for a trisubstituted double bond at 812 cm^{-1}.

Tenasogenin, on acetylation at room temperature, yielded tri-*O*-acetyl-tenasogenin (**2**) $C_{32}H_{46}O_9$, mp 172–175° indicating that tenasogenin contained three acetylable hydroxyl groups. The IR spectrum of tenasogenin acetate showed strong bands for acetyl group(s) (1733, 1728, 1721, 1282, 1240 and 1230 cm^{-1}) and a hydroxyl group absorption band at 3520 cm^{-1}, which could be tertiary in nature, presumably at C-14.

Alkaline hydrolysis of tenasogenin afforded a deacyl compound (**3**) and an acid which were isolated.

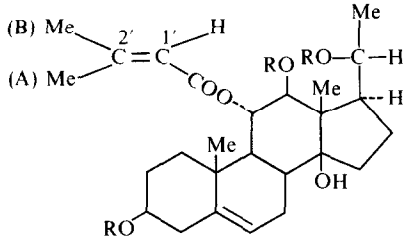
Deacyl component from tenasogenin

The deacyl component (**3**), mp 217–219°, colourless granules from acetone ether, $[\alpha]_D^{25}$ − 7.38°, analysed for $C_{21}H_{34}O_5 \cdot \frac{1}{2}H_2O$. Its IR spectrum provided evidence for the presence of hydroxyl group(s) and a trisubstituted double bond in the molecule, but an absorption band in the carbonyl region was absent.

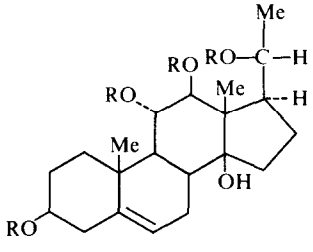
Acetylation of this product with acetic anhydride and pyridine at 100° gave a tetraacetate (**4**), $C_{29}H_{42}O_9$, mp 172–174°. Its IR spectrum still contained a band in the hydroxyl region at 3476 cm^{-1}, besides the absorption bands for acetyl groups, which indicated that the fifth oxygen atom of **3** was present as a non-acetylable hydroxyl group, possibly tertiary in nature.

The ability of tenasogenin to give a tri-*O*-acetyl derivative and its deacyl product to form a tetra-*O*-acetyl derivative suggested tenasogenin to be a monoester.

The ^{1}H NMR spectrum of the tetraacetate of **3** exhibited 18-Me at δ 1.01 (*s*), 21-Me at 1.08 (*d*, *J* = 6 Hz), 19-Me at 1.08 (*s*), 17α-H at 2.29 (*m*), 3α-H at 4.53 (*m*), 20-H at



1 R = H
2 R = Ac



3 R = H
4 R = Ac

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4.65–4.97 (*m*) and a vinyl proton at C-6 at 5.45 (*m*). A triplet centred at 5.30 ($J = 9.5$ Hz) and the corresponding doublet at 4.75 ($J = 9.5$ Hz) were assigned to the 11β-H and 12α-H, respectively. Evidence for the presence of hydroxyl groups at C-11 and C-12 was forthcoming from the $NaIO_4$ oxidation of **3**. The presence of a tertiary hydroxy group was confirmed by the appearance of a one proton signal at δ 1.58 which disappeared after deuteration.

On the basis of the ^{1}H NMR spectral analysis it was evident that product **3** contained 3β,11α,12β,20-hydroxy groups and a tertiary hydroxy group presumably at C-14 in β-orientation (like all other naturally occurring pregnanes). The structure of **3** was thus deduced to be that of drevogenin-D [3, 4]. A comparison of the mp, specific rotation and IR spectrum of **3** and mp, IR and ^{1}H NMR spectrum of **4** with those of drevogenin D [3] and its acetate confirmed this conclusion.

Reichstein *et al.* [5] had anticipated the *R* configuration for the C-20 hydroxyl group in drevogenin D from its formation by the $NaBH_4$ reduction of drevogenin P. This could be substantiated from the recent proposed mechanisms of $NaBH_4$ reduction of C-20 keto pregnanes by Hayashi and Mitsuhashi [6]. On the basis of these facts, it was concluded that drevogenin D has the C-20*R* configuration.

Acid from tenasogenin

The acid obtained from the hydrolysis failed to crystallize. Identification of the deacyl product **3** of tenasogenin as drevogenin D led to the formula $C_5H_8O_2$ for the α,β-unsaturated acid. This was supported by the high resolution MS of tenasogenin which contained a peak corresponding to $M^+ - C_5H_8O_2$ and complimentary peaks for $C_4H_7CO^+$ and $C_4H_7^+$. This acid could therefore be tiglic or β,β-dimethylacrylic acid. Tenasogenin when heated with aniline afforded an acid anilide [7], which differed from tiglic acid anilide (TLC). Tenasogenin must therefore be a β,β-dimethyl-acrylic acid ester of drevogenin D. This was substantiated by the analysis of the ^{1}H NMR spectrum of tenasogenin acetate.

The ^{1}H NMR spectrum of tenasogenin acetate (**2**) included three proton signals at δ 0.91 (*s*), 1.05 (*d*, $J = 6$ Hz) and 1.11 (*s*) for C-19, C-21 and C-18 Me groups, respectively and singlets at 1.9 and 2.1 for vinyl 2′B and 2′A methyl groups [8], respectively. The three acetyl groups appeared as singlets at 1.95, 2.01 and 2.1. A one proton triplet centred at 5.30 ($J = 9.5$ Hz) and the supplementary doublet at 4.74 ($J = 9.5$ Hz) corresponded to 11β-H and 12α-H, respectively. Multiplets of one proton each at 2.32, 4.50 and 4.65–4.90 were evidently the protons for 17α-H, 3α-H and 20-H, respectively. The low field two proton multiplet in the region 5.4–5.64 was attributed to the C-6 vinyl proton and the vinyl proton at C-1′ of the acid component.

The absence of a vicinal diol system in tenasogenin inferred from its negative $NaIO_4$ reaction and the generation of a vicinal diol in the deacyl component (**3**) obtained by alkaline hydrolysis of tenasogenin indicated the presence of an ester group at C-11 or C-12 in the drevogenin D moiety.

The position of the ester group in tenasogenin was finally assigned to C-11 from the fragments at *m/e* 206 and at 142 which were interpreted to originate by the *retro*-Diels–Alder fission at $\Delta^{9,11}$ in the fragment ion at *m/e* 348, which is formed by the elimination of β,β-dimethylacrylic acid from the molecular ion.

Many prominent peaks in the MS of tenasogenin (**1**) and its acetate (**2**) can be explained by standard fragmentation pathways [9] which fully support its proposed structure.

On the basis of the above chemical and spectroscopic evidence, the structure of tenasogenin has thus been established as **1**.

EXPERIMENTAL

Mps were determined on a Boetius micro-melting point apparatus and are uncorr.; ^{1}H NMR spectra were recorded on a 90 MHz spectrometer with TMS as internal standard; Si gel G was used for TLC.

Tenasogenin (**1**). Colourless rhombs from Me_2CO–petrol, mp 180–183°; $[\alpha]_D^{25}$ + 9.63° (*c* 1.23, MeOH). (Found: C, 68.55; H, 9.03. $C_{26}H_{40}O_6 \cdot \frac{1}{2}H_2O$ requires: C, 68.27; H, 8.97%.) Colour with 20% $SbCl_3$ in $CHCl_3$, pink; with 50% aq H_2SO_4, bright pink; Liebermann–Burchard test, pink. IR ν_{max}^{KBr} cm^{-1}: 3470, 2940, 1687, 1679, 1463, 1375, 1274, 1156, 1078, 1055, 1030 and 812. MS *m/e* (rel. int.): M^+ (not observed), 430.2705 (0.52, $M^+ - H_2O$, $C_{26}H_{38}O_5$), 412.2743 (0.94, $M^+ - 2H_2O$, $C_{26}H_{36}O_4$), 394.2456 (0.25, $M^+ - 3H_2O$, $C_{26}H_{34}O_3$), 349.2328 (0.32, $M^+ - C_5H_7O_2$, $C_{21}H_{33}O_4$), 348.2294 (1.64, $M^+ - C_5H_8O_2$, $C_{21}H_{32}O_4$), 330 (11.0, 348 – H_2O), 312.2099 (31.11, 330 – H_2O, $C_{21}H_{28}O_2$), 297 (5.0, 312 – Me), 294 (3.0, 394 – $C_5H_8O_2$), 279 (12.0, 297 – H_2O), 210 (2.5, RDA at Δ^5), 206 (7.5, RDA at $\Delta^{9,11}$), 192.1158 (28.15, 210 – H_2O, $C_{12}H_{16}O_2$), 188 (6.0, 206 – H_2O), 145 (24.0, $C_{11}H_{13}^+$), 142 (3.0, 2nd fragment of RDA at $\Delta^{9,11}$), 120 (16.0, 138 – H_2O), 105 (23.0, 120 – Me), 83.0493 (100, $C_5H_7O^+$), 55 (66.0, $C_4H_7^+$).

Tri-O-acetyl-tenasogenin (**2**). To a soln of tenasogenin (**1**) (50 mg) in pyridine (1 ml), Ac_2O (0.8 ml) was added and the reaction mixture was left overnight at room temp. Usual work-up yielded amorphous residue (70 mg), which on CC over Si gel (7 g) using C_6H_6–EtOAc (95:5) as eluant yielded pure tenasogenin acetate (**2**) (45 mg). It was crystallized from C_6H_6–*n*-C_6H_{12} as colourless needles (35 mg), mp 172–175°. (Found: C, 65.86; H, 7.88. $C_{32}H_{46}O_9 \cdot \frac{1}{2}H_2O$ requires: C, 65.86; H, 8.06%.) Colour in tetranitromethane test, yellow. IR ν_{max}^{KBr} cm^{-1}: 3520, 1733, 1728, 1721, 1460, 1360, 1282, 1240, 1230, 1185, 1065, 1024 and 808. ^{1}H NMR ($CDCl_3$): δ 0.91 (3H, *s*, 19 – Me), 1.05 (3H, *d*, $J = 6$ Hz, 21 – Me), 1.11 (3H, *s*, 18 – Me), 1.9 (3H, *s*, 2′(B)–Me), 1.95 (3H, *s*, OCOMe), 2.01 (3H, *s*, OCOMe), 2.1 (6H, *s*, OCOMe + 2′(A)–Me), 2.32 (1H, *m*, 17α-H), 4.50 (1H, *s*, 3α-H), 4.74 (1H, *d*, $J = 9.5$ Hz, 12α-H), 4.65–4.90 (1H, *m*, 20-H), 5.30 (1H, *t*, $J = 9.5$ Hz, 11β-H), 5.4–5.64 (2H, *m*, $>C^6{=}C^{6'}\!<_{H}$ + $>C^{2'}{=}{}^{1'}C\!<_{H}^{COO}$). MS *m/e* (rel. int.): M^+ (not observed), 514.3109 (0.67, $M^+ - HOAc$, $C_{30}H_{42}O_7$), 414.2484 (0.20, 514 – $C_5H_8O_2$, $C_{25}H_{34}O_5$), 396 (0.81, 414 – H_2O), 354 (0.61, 414 – HOAc), 336 (2.7, 354 – H_2O or 396 – HOAc), 294.1990 (7.19, 354 – HOAc, $C_{21}H_{26}O$), 276.1859 (8.95, 294 – H_2O or 336 – HOAc, $C_{21}H_{24}$), 261.1639 (7.35, 276 – Me, $C_{20}H_{21}$), 145.0974 (7.11, $C_{11}H_{13}^+$), 120 (3.1, C_9H_{12}), 105.0747 (6.32, 120 – Me, C_8H_9), 85.0644 (7.69, $C_5H_9O^+$).

Methanolic KOH hydrolysis of tenasogenin. Tenasogenin (**1**) (150 mg) was dissolved in 5% methanolic KOH (12 ml) and kept overnight at room temp. H_2O (2.5 ml) was added and MeOH was removed under red. pres. The aq. concentrate was extracted with $CHCl_3$–EtOH (3:1, 5 × 5 ml). The organic layer was washed with H_2O (3 × 4 ml), dried (Na_2SO_4), filtered and evapd to dryness giving an amorphous residue (100 mg) of the deacyl component. The aq. layer and the first water washing were mixed together, acidified to pH 5 with 2 N HCl and again extracted with $CHCl_3$

(5 × 5 ml). After usual work-up amorphous acid (20 mg) was obtained.

Deacyl component (**3**). Colourless prisms (80 mg) from a mixture of Me_2CO–Et_2O, mp 217–219°, which, however, had mp 208–221° when crystallized from MeOH–Et_2O, $[\alpha]_D^{25}$ – 7.38° (*c* 1.03, MeOH). (Found: C, 67.62; H, 9.14. $C_{21}H_{34}O_5 \cdot \frac{1}{2}H_2O$ requires: C, 67.20; H, 9.33%.) IR ν_{max}^{KBr} cm^{-1}: 3290, 1360, 1100, 1076, 1045, 1033, 944, 870 and 800.

Acid component. The amorphous residue (20 mg) failed to crystallize. It gave a yellow colour in the spot test with bromocresol green and effervescence with $NaHCO_3$.

Tetra-O-acetyl derivative of deacyl component. Compound **3** (50 mg) on acetylation with pyridine (1 ml) and Ac_2O (0.8 ml) at 100° for 3 hr afforded amorphous residue (52 mg) of genin tetraacetate, which on CC over Si gel (5 g) using C_6H_6–EtOAc (90:10) as eluant yielded pure genin retraacetate (**4**) (32 mg), crystallized from Me_2CO–Et_2O as colourless granules, mp 172–174°. Colour in the tetranitromethane test, yellow. (Found: C, 64.68; H, 7.76. $C_{29}H_{42}O_9$ requires: C, 65.17; H, 7.86%.) IR ν_{max}^{KBr} cm^{-1}: 3476, 2960, 1734, 1717, 1690, 1685, 1370, 1355, 1260, 1235, 1218, 1056, 1032, 1020, 834 and 808.[1] HNMR ($CDCl_3$): δ 1.01 (3H, *s*, 18 – Me), 1.08 (3H, *d*, J = 6 Hz); 1.08 (3H, *s*, 19 – CH_3), 1.58 (1H, *s*, OH), 1.91 (3H, *s*, OCOMe), 1.94 (3H, *s*, OCOMe), 1.96 (3H, *s*, OCOMe), 2.02 (3H, *s*, OCOMe), 2.29 (1H, *m*, 17α-H), 4.53 (1H, *m*, 3α-H), 4.75 (1H, *d*, J = 9.5 Hz, 12α-H); 4.65–4.97 (1H, *m*, 20-H); 5.30 (1H, *t*, J = 9.5 Hz, 11β-H); 5.45 (1H, C-6-H).

$NaIO_4$ *oxidation of deacyl component.* Deacyl product **3** (2 mg) was dissolved in MeOH (0.2 ml), a soln of $NaIO_4$ (6 mg) in H_2O (0.1 ml) was added, and the mixture kept at room temp for 4 hr. After usual work-up an amorphous residue (1.6 mg) was obtained which showed the formation of a new spot on TLC (C_6H_6–EtOAc, 20:80) with complete disappearance of the starting material spot.

$NaIO_4$ *oxidation of tenasogenin.* Tenasogenin (**1**) (2 mg) was oxidized with $NaIO_4$ (6 mg) by the usual method affording amorphous residue (1.2 mg), which showed no change in mobility on TLC ($CHCl_3$–MeOH, 88:12).

Anilide formation from tenasogenin. Tenasogenin (**1**) (20 mg) was mixed with freshly distilled aniline (0.5 ml) and the reaction mixture was refluxed for 2 hr in an oil bath at 210°. After cooling, the reaction mixture was dissolved in Et_2O (50 ml) and washed successively with 2 N HCl (3 × 5 ml), 2 N Na_2CO_3 (3 × 5 ml) and H_2O (2 × 5 ml). After usual work-up, an amorphous residue (28 mg) of acid anilide was obtained. The mobility of this amorphous acid anilide was compared with tiglic acid anilide on TLC (C_6H_{12}–*iso*-PrOH, 98:2) using chlorine–toluidine [10] as spray reagent which was found to be different.

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