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CISSOGENIN, A PREGNANE GENIN FROM *MARSDENIA TENACISSIMA*

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

Abstract—A new polyhydroxy pregnane designated as cissogenin has been isolated from the seeds of *Marsdenia tenacissima*. Its structure has been established as 3β,11α,12β,14β,20 *S*-pentahydroxy-pregn-5-ene.

INTRODUCTION

Marsdenia tenacissima (Wight & Arn.) is a perennial climber, fairly widespread in tropical India. In the chemical investigation of the seeds of this plant, pregnane glycosides of 2-deoxy sugars were extracted. Mild acid hydrolysis [1] of these glycosides afforded a mixture of genins and sugars which were separated and characterized [2]. We now report the structure of a new pregnane genin, cissogenin, isolated from the genin mixture by column chromatography on silica gel.

RESULTS AND DISCUSSION

Cissogenin (**1**)

Cissogenin (**1**), mp 210–214°, $[\alpha]_D^{25} - 2.34°$ (MeOH) analysed for $C_{21}H_{34}O_5$, in agreement with the highest mass ion peak in its high resolution mass spectrum at *m/e* 348.2325 ($M - H_2O$, $C_{21}H_{32}O_4$). Its IR spectrum provided evidence for hydroxyl groups (3200, 1080 and 1040 cm^{-1}) and a trisubstituted double bond (806 cm^{-1}). Its molecular formula indicated it to be a highly hydroxylated pregnane genin (C_{21}-steroid). Cissogenin, on acetylation with acetic anhydride and pyridine at 100°, gave a tetraacetate (**2**), $C_{29}H_{32}O_9$, mp 146–148° indicating the presence of four hydroxyl groups. The IR spectrum of the tetraacetate showed strong bands for acetyl groups at 1735–1718 and 1255–1220 cm^{-1} and for a hydroxyl group at 3400 cm^{-1}, possibly tertiary in nature and presumably at C-14.

The ^{1}H NMR spectrum of cissogenin tetraacetate showed three proton signals at δ 1.02(*s*), 1.11 (*d*, $J = 6$ Hz) and 1.14(*s*) which were assigned to the C-18, C-21 and C-19 methyl groups, respectively, and four singlets of three protons each at 1.93, 1.97, 1.99 and 2.04 for four acetyl groups. A triplet centred at 5.46 ($J = 10$ Hz) and a corresponding doublet at 4.83 ($J = 10$ Hz) were assigned to the carbinol methine protons at C-11 and C-12, respectively. The large value of their coupling constant (10 Hz) confirmed their *trans*-biaxial arrangement and fixed the configuration of the acetyl groups at C-11 and C-12 as α and β, respectively. Two multiplets of one proton each in the region 5.31–5.57 and 4.7–4.9 were attributed to a vinyl proton at C-6 and the carbinol methine proton at C-20, respectively. The broad multiplets of one proton each at 4.55 and 2.35 were ascribed to the 3-α and 17-α protons respectively.

The MS of cissogenin (**1**) did not show a M$^+$ peak but a significant peak appeared at *m/e* 348.2325 ($M - H_2O$, $C_{21}H_{32}O_4$). This fragment ion peak further showed the loss of water molecules and side chain (–CHOHMe) giving fragment ion peaks at *m/e* 303 (348 – CHOHMe), 285 (303 – H_2O), 267 (285 – H_2O) and 249 (267 – H_2O). The loss of four water molecules and the –CHOHMe side chain fragment further confirmed the presence of four hydroxyl groups in the tetracyclic ring and one hydroxyl group in the C-17 side chain. The appearance of fragment ions at *m/e* 120 (138 – H_2O) and 105 (120 – Me) formed by the retro Diels–Alder fission was characteristic of a Δ^5-3-ol arrangement in the molecule. The occurrence of other peaks at *m/e* 228, 192, 165 and 147, fully supported the presence of hydroxyl groups at C-11, C-12, C-14 and C-20 in this pregnane genin, which can be explained by standard degradation pathways [3], from its derived structure (**1**). Furthermore, the loss of four acetic acid molecules and one water molecule in the MS of cissogenin tetraacetate is in full agreement with this proposed structure.

For the chemical support of the structure of cissogenin, it was treated with $NaIO_4$, which gave two products, product I (**3**) and product II (**4**) in order of their decreasing mobility on TLC.

Oxidation product I (**3**)

The less polar oxidation product I had mp 154–156°, molecular formula $C_{21}H_{30}O_4$ (346.2075, M$^+$). Its IR spectrum suggested the presence of hydroxyl groups (3400 and 1060 cm^{-1}), aldehyde functions (2740 and 1715 cm^{-1}) and trisubstituted double bonds (830 and 820 cm^{-1}). The formation of a C_{21} aldehyde further supported the presence of two vicinal hydroxyl groups at the C-11 and C-12 positions.

The ^{1}H NMR spectrum of product I showed the C-19 protons at δ 0.98 (*s*), C-21 protons at 1.07 (*d*, $J = 6$ Hz), C-18 protons at 1.10 (*s*), 3α-H at 3.63–3.80 (*m*) and the C-20 proton at 3.40–3.57 (*m*), whereas the one proton signal at

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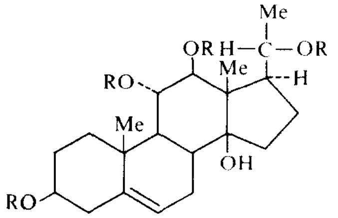

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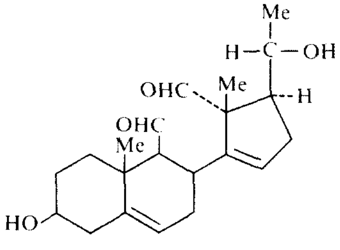
2 R = Ac



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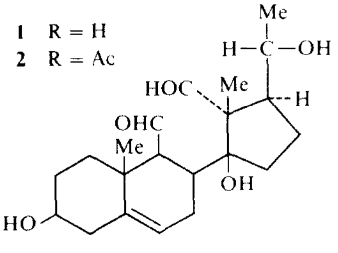

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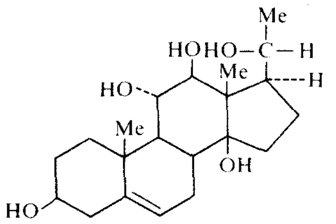

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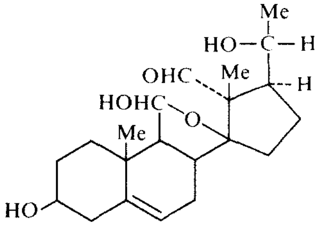

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5.34 (*m*) was the vinyl proton at C-6, also present in the original molecule. However, an additional one proton multiplet observed in the lower field region at 5.17 indicated the presence of another vinyl proton, presumably at C-15, which is anticipated to have originated by the simultaneous dehydration of the hydroxyl group at C-14 in cissogenin during oxidation. Two other prominent signals at 9.4 (*s*, 1H) and 9.29 (*d*, $J = 4.0$ Hz, 1H) were assigned to the two highly deshielded aldehyde protons expected at C-12 and C-11, respectively. The identity of the C-11 proton was confirmed by a double resonance experiment.

The MS of product I provided further evidence for the presence of two aldehyde groups and for the position of the newly created trisubstituted double bond at Δ^{14}.

Oxidation product II (**4**)

Compound **4** had mp 212–214° and high resolution measurement of its highest mass ion peak at *m/e* 315.1989 (M − Me − 2OH, $C_{20}H_{27}O_3$) led to the molecular formula $C_{21}H_{32}O_5$. Its IR spectrum also exhibited strong bands for associated hydroxyl groups (3375 and 1038 cm^{-1}), aldehydic groups (2710, 1714 and 1705 cm^{-1}) and a trisubstituted double bond (820 cm^{-1}). The ^{1}H NMR spectrum of product II was almost identical to that of oxidation product I, and contained a C-6 proton multiplet at δ 5.47, but the signal for the C-15 proton was absent. This fact, accompanied by the observation that the molecular formula of product II indicated that dehydration had not occurred, showed that possibly the C-14 hydroxyl group had remained unaffected in this product during the course of reaction. The MS of product II was in full agreement with its proposed structure.

The proposed structure for cissogenin (**1**) has already been given for drevogenin D, reported earlier by Reichstein *et al.* [4, 5]. However, cissogenin and its tetraacetate were different from the authentic samples of drevogenin D and its tetraacetate by TLC analysis. This conclusion was also supported from the observed differences in the mp, rotation and IR spectra of cissogenin and its tetraacetate with those of drevogenin D and its tetraacetate [4]. It was therefore concluded, and supported by the virtually identical MS of the two, that cissogenin was presumably a stereoisomer of drevogenin D. Moreover, oxidation [6] of cissogenin and drevogenin D afforded identical products.

The result that $NaIO_4$ oxidation of drevogenin D (**5**) afforded only one product (TLC) which could be the hemiacetal (**6**) reported earlier [4], whereas $NaIO_4$ oxidation of cissogenin afforded two products (**3** and **4**), neither being a hemiacetal, led us to presume that cissogenin is a C-20 configurational isomer of drevogenin D. This conclusion was substantiated by the selective oxidation of cissogenin and drevogenin D with *N*-bromosuccinimide [7], where again identical products were obtained. As *N*-bromosuccinimide can oxidize only axial and aliphatic secondary hydroxyl groups, it was evident that oxidation of cissogenin and drevogenin D afforded only C-20 keto products, which, being identical, confirmed that the two differed in the configuration of the hydroxyl group at C-20 only.

The absolute configuration of the C-20 hydroxyl group in drevogenin D was not fully established earlier, although the *R* configuration was anticipated by Reichstein *et al.* [8] based upon the formation of drevogenin D by $NaBH_4$ reduction of drevogenin P. This is substantiated from the recent proposed mechanisms of $NaBH_4$ reduction of C-20 keto pregnanes by Hayashi and Mitsuhashi [9]. It was thus concluded that drevogenin D has the 20*R* configuration.

As cissogenin (**1**) has been found to be the C-20 isomer of drevogenin D, it is concluded that the structure of cissogenin is $3\beta,11\alpha,12\beta,14\beta,20S$-pentahydroxy-pregn-5-ene (**1**).

EXPERIMENTAL

Mps were determined on a Boetius micro-melting point apparatus and are uncorr.; the ^1H NMR spectra were recorded at 90 MHz; Si gel G was used for TLC.

Cissogenin (**1**). Mp 210–224° (Me_2CO–Et_2O), $[\alpha]_D^{25}$ – 2.34° (*c* 1.06, MeOH). (Found: C, 68.52; H, 9.38. $C_{21}H_{34}O_5$ requires: C, 68.85; H, 9.29%). Colour in Liebermann–Burchard test, pink; with 50% aq. H_2SO_4, violet blue; with 20% $SbCl_3$ in $CHCl_3$, violet blue. IR ν_{max}^{KBr} cm^{-1}: 3200, 1367, 1250, 1184, 1126, 1080, 1060, 1040, 992, 968, 880 and 806. MS *m/e* (rel. int.) M^+ (not observed), 348.2325 (23.13, M – H_2O, $C_{21}H_{32}O_4$), 330.2195 (26.41, 348 – H_2O, $C_{21}H_{30}O_3$), 312 (11.5, 330 – H_2O), 303.1973 (34.67, 348 – MeCHOH, $C_{19}H_{27}O_3$), 285.1853 (70.63; 303 – H_2O, $C_{19}H_{25}O_2$), 267.1743 (34.79, 285 – H_2O, $C_{19}H_{23}O$), 249.1653 (25.66, 267 – H_2O, $C_{19}H_{21}$), 228 (4, RDA at Δ^5); 192 (7,228 – $2H_2O$), 165 (17,228 – CHOHMe – H_2O); 148.0903 (21.46, 165 – OH, $C_{10}H_{12}O$), 147 (46.23, 165 – H_2O), 145.0991 (79.13, $C_{11}H_{13}^+$), 137.0952 (27.45, $C_9H_{13}O$), 120.0915 (26.10, C_9H_{11}), 119.0841 (42.44, 137 – H_2O, C_9H_{11}), 105.0704 (64.26, 120 – Me, C_8H_9), 81.0680 (100, C_6H_9).

Tetraacetyl-cissogenin (**2**). Cissogenin (**1**) (50 mg) on acetylation with pyridine (1 ml) and Ac_2O (0.8 ml) at 100° for 8 hr afforded tetraacetyl-cissogenin (**2**) which crystallized as colourless prisms (27 mg) from Me_2CO–petrol, mp 146–148°. (Found: C, 65.20; H, 7.40. $C_{29}H_{42}O_9$ requires: C, 65.17; H, 7.86%.) Colour in tetranitromethane test, yellow. IR ν_{max}^{KBr} cm^{-1}: 3440, 1735–1718, 1363, 1255–1200, 1075, 1042, 1028, 897, 850 and 808. ^1H NMR ($CDCl_3$): δ 1.02 (3H, *s*, C-18), 1.11 (3H, *d*, $J = 6$ Hz, C-21), 1.14 (3H, *s*, C-19), 1.93 (3H, *s*, OCOMe), 1.97 (3H, *s*, OCOMe), 1.99 (3H, *s*, OCOMe), 2.04 (3H, *s*, OCOMe), 2.35 (1H, *m*, C-17α), 4.55 (1H, *m*, C-3α), 4.83 (1H, *d*, $J = 10$ Hz, C-12α), 4.7–4.9 (1H, *m*, C-20), 5.46 (1H, *t*, $J = 10$ Hz, C-11β), 5.31–5.57 (1H, *m*, C-6). MS *m/e* (rel. int.) M^+ (not observed), 456.2510 (1.22, M^+ – HOAc – H_2O, $C_{27}H_{36}O_6$), 396.2284 (1.70, 456 – HOAc, $C_{25}H_{32}O_4$), 354.2226 (1.86, M^+ – HOAc, $C_{23}H_{30}O_3$), 336 (8.6, 396 – HOAc or 354 – H_2O), 294 (2.5, 354 – HOAc), 276.1863 (43.25, 336 – HOAc, $C_{21}H_{24}$), 261.1622 (13.22, 276 – Me, $C_{20}H_{21}$), 145 (7); 120 (5, C_9H_{12}), 105.0774 (11.60, 120 – Me, C_8H_9).

$NaIO_4$ *oxidation of cissogenin*. Cissogenin (**1**) (100 mg) was dissolved in MeOH (9.5 ml). To it a soln of $NaIO_4$ (295 mg) in H_2O (4 ml) was added and the reaction mixture was allowed to stand at room temp. for 4 hr. After the usual work-up the crude product (85 mg) was obtained. On TLC ($CHCl_3$–MeOH, 92:8) it exhibited two products designated product I (**3**) and product II (**4**) of higher mobility than starting material. Chromatography of crude product on Si gel (10 g) using C_6H_6–EtOAc (90:10) as eluant yielded polar crystalline product I (20 mg) and product II (22 mg).

Oxidation product I (**3**). Mp 154–156° (Me_2CO–Et_2O–petrol) as colourless granules. Molecular composition by high resolution MS was found to be $C_{21}H_{30}O_4$. IR ν_{max}^{KBr} cm^{-1}: 3400, 2740, 1715, 1370, 1330, 1280, 1098, 1078, 1060, 890, 850, 830 and 820. ^1H NMR ($CDCl_3$): δ 0.98 (3H, *s*, C-19), 1.07 (3H, *d*, $J = 6$ Hz, C-21), 1.10 (3H, *s*, C-18); 3.40–3.57 (1H, *m*, C-20), 3.63–3.80 (1H, *m*, C-3α), 5.17 (1H, *m*, C-15), 5.34 (1H, *m*, C-6), 9.29 (1H, *d*, $J = 4$ Hz, C-11), 9.4 (1H, *s*, C-12). MS *m/e* (rel.int.): 346.2075 (0.03, M^+, $C_{21}H_{30}O_4$), 329.2019 (0.03, M^+ – OH, $C_{21}H_{29}O_3$), 328.1949 (0.05, M^+ – H_2O, $C_{21}H_{28}O_3$), 314.1817 (0.08, M^+ – OH – Me, $C_{20}H_{26}O_3$), 302 (0.08, M^+ – CHO – Me), 301.1799 (0.25, M^+ – CHOHMe, $C_{19}H_{25}O_3$), 300.1998 (0.17, M^+ – OH – CHO; $C_{20}H_{28}O_2$), 273 (14.97, 302 – CHO), 255 (6.6, 273 – H_2O), 237 (3, 255 – H_2O), 145 (15.23, $C_{11}H_{13}^+$), 120 (5, RDA at Δ^5-H_2O), 105.0693 (18.49, 120 – Me, C_8H_9), 81.0713 (100, C_6H_9).

Oxidation product II (**4**). Mp 212–214° (Me_2CO–petrol) as colourless granules. IR ν_{max}^{KBr} cm^{-1}: 3375, 2710, 1714, 1705, 1360, 1320, 1278, 1260, 1070, 1038, 846, 820 and 800. ^1H NMR ($CDCl_3$): δ 1.07 (3H, *s*, C-19), 1.13 (3H, *s*, C-18), 1.16 (3H, *d*, $J = 6$ Hz, C-21), 3.60–3.84 (2H, *m*, C-3α and C-20), 5.47 (1H, *m*, C-6), 9.36 (1H, *d*, $J = 4$ Hz, C-11), 9.43 (1H, s, C-12). MS *m/e* (rel.int.): (M^+ not observed), 315.1989 (0.35; M^+ – Me – 2OH, $C_{20}H_{27}O_3$), 313 (0.3, M^+ – Me – $2H_2O$), 301.1788 (0.57, M^+ – CHOHMe – H_2O, $C_{19}H_{25}O_3$), 297.1906 (11.22, 315 – H_2O, $C_{20}H_{25}O_2$), 290.1866 (12.05, M^+ – CHOHMe – CHO, $C_{18}H_{26}O_3$), 289.1782 (45.05, 290 – H, $C_{18}H_{25}O_3$), 272.1762 (28.97, 290 – H_2O, $C_{18}H_{24}O_2$), 271.1698 (65.38, 289 – H_2O, $C_{18}H_{23}O_2$), 254.1653 (12.79, 272 – H_2O, $C_{18}H_{22}O$), 253.1600 (50.80, 271 – H_2O. $C_{18}H_{21}O$), 243.1702 (19.92, 272 – CHO, $C_{17}H_{33}O$), 235 (18,289 – $3H_2O$), 225 (30, 243 – H_2O or 254 – CHO), 120 (6, RDA at Δ^5-H_2O), 105 (120 – Me).

Oxidation of cissogenin (**1**) *and drevogenin D* (**5**). To a cold (10–15°) soln of cissogenin (**1**) (3 mg) in Me_2CO (0.4 ml), CrO_3 reagent (0.05 ml) [CrO_3 (1.34 g) dissolved in conc H_2SO_4 (1.15 ml) and diluted with H_2O to 5 ml] was added rapidly with stirring in an atmosphere of N_2. After 10 min, the reaction mixture was diluted with H_2O (0.4 ml), neutralized with $NaHCO_3$ soln and extracted with Et_2O (4 × 1 ml). The organic layer was washed with H_2O, dried (Na_2SO_4) and evapd to dryness. The amorphous residue (1 mg) showed one spot of higher mobility than cissogenin on TLC ($CHCl_3$–MeOH, 92:8, developed 2 ×). Similarly, drevogenin D (**5**) (3 mg) was also oxidized with CrO_3 reagent which afforded an amorphous oxidation product (1 mg) exhibiting a single spot on TLC. Both the oxidation products of cissogenin and drevogenin D were found to be identical by co-chromatography on TLC in the solvent system $CHCl_3$–MeOH (92:8) developed 2 × and C_6H_6–EtOAc, (70:30) developed 3 ×.

N-*Bromosuccinimide oxidation of cissogenin and drevogenin D.* Cissogenin (**1**) (2 mg) in Me_2CO (0.2 ml) and H_2O (0.02 ml) was treated with *N*-bromosuccinimide (1.2 mg) and HOAc (0.002 ml) and shaken at room temp. After standing overnight the soln was diluted and extracted with Et_2O (4 × 1 ml) and the extract was washed with $NaHCO_3$ (1 × 1 ml) and H_2O (1 × 1 ml), dried and concd to obtain an amorphous oxidation product. Similarly, drevogenin D (**5**) (2 mg) was also oxidized with *N*-bromosuccinimide which afforded an amorphous oxidation product. The oxidation products of cissogenin and of drevogenin D had identical mobilities on TLC ($CHCl_3$–MeOH, 90:10, developed 2 ×).

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