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ORGOGENIN, A PREGNANE DERIVATIVE FROM *ORTHENTHERA VIMINEA*

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Key Word Index—*Orthenthera viminea*; Asclepiadaceae; orgogenin; steroid; pregnane.

Abstract—A new pregnane genin designated as orgogenin has been isolated from *Orthenthera viminea* and characterized to be 20-oxo-pregn-5-ene, 3β,8β,11α,12β,14β,15α-hexol.

INTRODUCTION

In several species of the Asclepiadaceae cardenolides have been reported to be present along with pregnane derivatives [1]. In our continued search for new compounds from *Orthenthera viminea*, a leafless shrub with stiff glabrous branches, a mixture of glycosides was extracted. This mixture could not be resolved satisfactorily by thin layer or paper chromatography. It was, therefore, subjected to a very mild acid hydrolysis [2] resulting in isolation of a number of genins and unhydrolysed glycosides. The sugar mixture yielded four novel oligosaccharides which we have previously identified as vimose [3], orthenthose [4], ornose [5] and digoxose [6]; the unhydrolysed glycosides afforded ornine [7] and orine [8]. We now report the structure elucidation of a novel pregnane genin designated as orgogenin isolated from the hydrolysed glycoside mixture extracted from the title plant.

RESULTS AND DISCUSSION

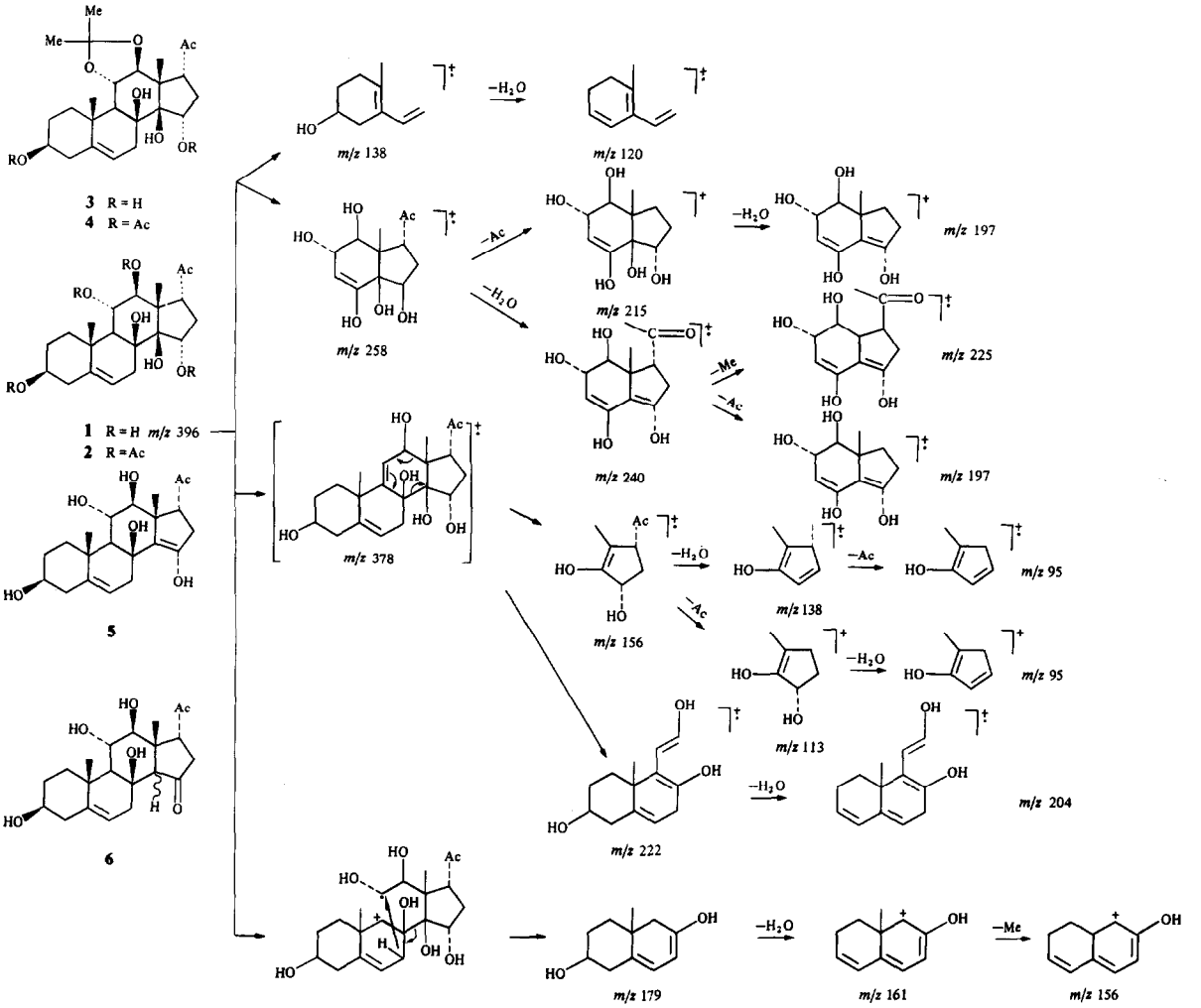
Orgogenin (**1**), mp 180–183°, $[\alpha]_D$ +27° was isolated as colourless prisms which analysed for $C_{21}H_{32}O_7$. In the infrared spectrum, the absorption maxima at 3470 cm^{-1}, 1687 cm^{-1}, 1375 cm^{-1} and 812 cm^{-1} were assigned to hydroxyl, carbonyl and methyl deformations of a methyl ketone and trisubstituted double bond respectively. The presence of a carbonyl group was further indicated by the ability of this compound to undergo facile reduction with sodium borohydride as well as its reaction with 2,4-dinitrophenylhydrazine. The nature of this carbonyl as a methyl ketone was established by a positive sodium nitroprusside test [9], the presence of a three-proton singlet at δ2.04 in its ^1H NMR spectrum and mass spectral peaks at *m/z* 353 $[M-Ac]^+$ and 43 $[Ac]^+$.

Acetylation of **1** with acetic anhydride in pyridine at 100° afforded a tetra-*O*-acetyl derivative **2**, mp 117–120°, $[\alpha]_D$ +99° and molecular formula $C_{29}H_{40}O_{11}$. The IR spectrum of **2** retained the absorption bands for free hydroxyl groups of the parent compound (3490 cm^{-1}), which are presumably tertiary in nature, in addition to the prominent absorption bands for acetyl groups (1730, 1240 cm^{-1}), methyl ketone (1690, 1315 cm^{-1}) and trisubstituted double bond (780 cm^{-1}). The preceding data suggest that four oxygens of compound **1** are in hydroxyl groups which can be acetylated; one oxygen is involved in a methyl ketone and the remaining two oxygen atoms are presumably tertiary hydroxyl groups.

In the ^1H NMR spectrum of **2** in $CDCl_3$, the signals for two tertiary methyl groups at δ1.24 and 1.14 (each 3H, *s*), a methyl ketone at 2.04 (3H, *s*), four acetyl groups at 1.98, 2.02 (each 6H, *s*) and a vinyl proton at 5.22 (1H, *m*) were observed.

For compound **1**, which is a C_{21} molecule containing two tertiary methyl groups, one methyl ketone and one double bond, the observed double bond equivalent value of six is indicative of a highly hydroxylated pregnene derivative. The property of **1** to undergo isomerisation with alkali indicated the absence of any other substituent group at C-17 except the methyl ketone. In pregnane derivatives, the Cotton effect of a 20-ketone is positive for a 17β-side chain but negative for a 17α-side chain [10]. The observed negative Cotton effect for orgogenin led to the conclusion that the acetyl group at C-17 in **1** had an α-configuration. A positive reaction of **1** with $NaIO_4$ suggested the presence of an α-diol system in the molecule, which was further supported by the formation of a isopropylidene derivative **3**. A one proton doublet at δ3.56 (J = 8 Hz) and another one proton triplet at 3.35 (J = 8 Hz) in the spectrum of **1** could be attributed to the protons at C-12 and C-11 bearing the vicinal hydroxyl groups in β- and α-configurations, respectively. A downfield shift of the carbinol methine proton signals to δ4.58 (*d*, J = 8 Hz) and 4.26 (*t*, J = 8 Hz) in the ^1H NMR spectrum of **2** further confirmed the presence of the C-12 and C-11 hydroxyl groups in the β- and α-configurations, respectively. A one proton multiplet in **1** at δ3.90 shifting to 4.82 in the corresponding acetate **2**, was attributable to the methine proton at C-3 bearing a commonly reported β-hydroxyl group [11].

To ascertain the number of the hydroxyl groups in orgogenin (**1**), an acetyl derivative **4** of its monoisopropylidene derivative **3** was prepared (mp 153–155° and $[\alpha]_D$ +36°, $C_{28}H_{40}O_9$). The ^1H NMR spectrum of **4** at 80 MHz contained a 9H singlet at δ2.01 attributable to the two acetyl and one methyl ketone in the molecule, and a 12H singlet at δ1.25 was attributed to four methyl groups indicating **4** to be a di-*O*-acetyl-monoisopropylidene orgogenin. The number of acetyl groups in **4** was also ascertained by its very mild alkaline hydrolysis [3] at room temperature. The reaction was followed by



Scheme 1.

TLC which in 72 hr exhibited three spots. The fastest spot (R_f 0.7) was identical in mobility with the starting material **4** whereas the slowest spot (R_f 0.25) was identical with **3**. The intermediate mobility spot (R_f 0.50) was attributable to the partially deacetylated derivative. The hydrolysis was complete in 5 days resulting in only one spot for the fully deacylated product **3**. The latter could also be obtained by the complete alkaline hydrolysis of **4** in an alternative reaction [12, 13].

That six of the seven oxygen atoms in this Δ^5 pregnene-20-one molecule (**1**) are present as hydroxyl groups was also shown by its mass spectrum which further helped in fixing the positions of these groups. Although the mass spectrum did not record the $[M]^+$ ion, the other prominent ions in the higher mass region were of great value. The fragment ion peak at *m/z* 353 $[M-CH_3CO]^+$ was observed to lose the expected six water molecules yielding ion peaks at *m/z* 335 $[353-H_2O]^+$, 317 $[353-2H_2O]^+$, 299 $[353-3H_2O]^+$, 282 $[353-4H_2O]^+$, 266 $[281-Me]^+$, and 230 $[353-6H_2O-Me]^+$ thus confirming the presence of six hydroxyl groups and a methyl ketone in **1**. The prominent ion peaks at *m/z* 138 and 120 are attributable to the Δ^5-3-ol arrangement [14] in the molecule giving rise to these fragments by the characteristic retro-Diels–Alder fission at Δ^5. The fragmentation pattern of the ion peak at *m/z* 258 involved the loss of five water molecules and one acetyl unit resulting in the formation of peaks at *m/z* 240 $[258-H_2O]^+$, 222 $[258-2H_2O]^+$, 215 $[258-Ac]^+$, 197 $[258-3H_2O-Ac]^+$, 179 $[258-4H_2O-Ac]^+$ and 161 $[258-5H_2O-Ac]^+$. This was obviously the second fragment of retro-Diels–Alder fission involving at least five hydroxyl groups in rings C and D, suggesting the position of at least four of its hydroxyl groups at C-11, C-12, C-8 and C-14. This also accounts for its two tertiary hydroxyl groups. The fifth hydroxyl group must, therefore, be in ring D at the C-15 or C-16 position in order to be secondary in nature. On the basis of the ubiquitous occurrence of the β-configuration of the C-14 hydroxyl group in natural pregnanes [1], the C-14 hydroxyl group of orgogenin was also assigned a β-configuration. The presence of a hydroxyl group at C-11 was also derived from prominent fragment ions at *m/z* 156, 138 and 113 resulting from the loss of water and CH_3CO from the retro-Diels–Alder fragmentation at the $\Delta^{9,11}$ double bond created by the loss of C-11 hydroxyl group [15]. This further resulted in another series of ions at *m/z* 222 and 204 lending support to fixing the position of hydroxyl groups at C-3, C-8 and

C-12. The common fragment ion peak at m/z 179 reported for pregnanes containing hydroxyl groups in rings A and B at C-3 and C-8 yielded a species at m/z 161 by the loss of a water molecule further supporting the position of these hydroxyl groups. In view of the usual *trans*-fusion of rings B and C in steroids, it is probable that the C-8 hydroxyl group in orgogenin is in the β-configuration.

Chemical support for the presence of a hydroxyl group at C-15 came from the UV spectrum of 15-keto-orgogenin (**6**) ($[\alpha]_D$ +72°), obtained by *p*-toluenesulphonic acid treatment of **1** in benzene [16]. It is considered to have been formed through the intermediate Δ^{15}-anhydro-orgogenin (**5**). The UV spectrum showed a two-fold increase in the carbonyl absorbance of **6** when compared with that of **1**. The configuration of the C-15 hydroxyl group could be defined from the coupling constant of the 15β-methine proton in the ^{1}H NMR spectrum of **2** which appeared as a double doublet at δ5.46 (J = 8 and 4 Hz), suggesting an α-configuration for the C-15 hydroxyl group [17].

On the basis of the above chemical and spectroscopic evidence the structure of orgogenin (**1**) is, therefore probably 20-oxo-pregn-5-ene-3β,8β,11α,12β,14β,15α-hexol.

EXPERIMENTAL

Mps were determined on a Boetius micromelting point apparatus and are uncorr. The CD spectrum was taken in EtOH on an SA-JOBIN-YVON dichrograph III. ^{1}H NMR spectra ($CDCl_3$) were recorded on at 200 MHz (Varian XL200) and 80 MHz (Varian CFT-20) with TMS as internal standard. MS were recorded with an AEI-MS-30 mass spectrometer. $[\alpha]_D$ were measured in a 1 dm tube with a Jasco DIP 180 automatic polarimeter.

Extraction and isolation. Shade dried powdered twigs (10 kg) of *O. viminea* were extracted with aq. EtOH and the concentrate was fractionated with organic solvents of different polarities [5]. The $CHCl_3$–EtOH (4:1 and 3:2) soluble extracts were combined and hydrolysed with 25 mM H_2SO_4 in 50% MeOH which afforded a mixture of sugars, genins and partially hydrolysed glycosides. Repeated column chromatography of this genin mixture over silica gel gave crystalline orgogenin (**1**) (41 mg).

Orgogenin (**1**). Mp 180–183° (MeOH–Et_2O), $[\alpha]_D^{25}$ +27° (*c* 0.12, MeOH) (Found C, 63.25; H, 8.13. Calc. for $C_{21}H_{32}O_7$; C, 63.63; H, 8.08%.) It gave a positive colour test with 2,4-dinitrophenyl hydrazine, tetranitromethane and sodium nitroprusside. It reacted with $NaIO_4$ and also underwent reduction with $NaBH_4$. IR ν_{max}^{KBr} cm^{-1}: 3470 (b), 3000 (m), 1687 (s), 1375 (s), 1275 (m), 1150 (m), 1040 (s), 910 (w), 868 (w) and 812 (m), $[\alpha]_{290}$ −38748.02, UV λ_{max}^{EtOH} nm (log ε): 288 (2.10). ^{1}H NMR (80 MHz): δ5.28 (1H, *m*, H-6), 3.90 (1H, *m*, H-3), 3.56 (1H, *d*, J = 8 Hz, H-12), 3.35 (1H, *t*, J = 8 Hz, H-11), 2.04 (3H, *s*, COMe), 1.25 (6H, *s*, 18-Me and 19-Me). MS m/z (rel. int.): $[M]^+$ (not observed), 353 (20), 335 (18), 317 (23), 299 (8), 281 (12), 266 (8), 258 (6), 241 (2), 240 (8), 238 (3), 233 (10), 230 (15), 225 (12), 222 (5), 215 (12), 204 (7), 198 (15), 197 (18), 179 (18), 161 (22), 139 (30), 138 (42), 120 (45), 113 (60), 105 (40), 97 (52), 95 (60), 81 (65), 69 (55), 55 (76) and 43 (100).

Isomerization of **1**. To a soln of **1** (1 mg) in MeOH (0.25 ml) was added a soln of 5% methanolic KOH (0.25 ml) and the mixture was kept at room temp. After 2 hr the reaction mixture showed complete consumption of **1** (R_f 0.6) and two more polar spots (R_f 0.5 and 0.40) (TLC 90:10, $CHCl_3$–MeOH).

$NaIO_4$ *oxidation of* **1**. To a soln of **1** (2 mg) in MeOH (0.2 ml) was added a soln of $NaIO_4$ (6 mg) in H_2O (0.1 ml) and the mixture was kept for 4 hr at room temp, diluted with H_2O (0.4 ml) and evaporated under red. pres. By cochromatography, the residue showed complete consumption of orgogenin (**1**) (TLC $CHCl_3$–MeOH, 90:10).

$NaBH_4$ *reduction of* **1**. Compound **1** (3 mg) was dissolved in MeOH (1.2 ml) and $NaBH_4$ (3 mg) was added and the mixture was kept for 2 hr at room temp. By cochromatography the mixture showed complete consumption of **1** (TLC $CHCl_3$–MeOH, 90:10).

Acetylation of **1**. Compound **1** (12 mg) on acetylation with pyridine (1.1 ml) and Ac_2O (0.18 ml) at 100° for 4 hr afforded **2** as an amorphous residue (12 mg) which on crystallisation with MeOH–Et_2O gave colourless needles (9 mg) mp 117–120°, $[\alpha]_D^{25}$ +99° (*c* 0.12, MeOH); (Found: C, 61.39; H, 7.26 Calc. for $C_{29}H_{40}O_{11}$; C, 61.70; H, 7.09%.) IR ν_{max}^{KBr} cm^{-1}: 3490 (b), 2890 (m), 1730 (m), 1690 (m), 1385 (s), 1375 (m), 1240 (m), 1020 (m), 780 (m). ^{1}H NMR (200 MHz): δ5.46 (1H, *dd*, J = 8 and 4 Hz, H-15), 5.22 (1H, *m*, H-6), 4.82 (1H, *m*, H-3), 4.58 (1H, *d*, J = 8 Hz, H-12), 4.26 (1H, *t*, J = 8 Hz, H-11), 2.04 (3H, *s*, COMe), 2.02 (6H, *s*, 2 × OAc), 1.98 (6H, *s*, 2 × OAc), 1.24 (3H, *s*, 18-Me) and 1.14 (3H, *s*, 19-Me).

Isopropylidene derivative (**3**) *of* **1**. Substance **1** (5 mg) was taken in dry Me_2CO (2.5 ml) and a drop of conc. H_2SO_4 (0.01 ml) was added. The reaction mixture was kept overnight. Na_2CO_3 (0.25 g) was added for neutralisation after 5 hr. The mixture was filtered and evaporated to dryness yielding the isopropylidene derivatives of higher mobility **3** (5 mg) (TLC, $CHCl_3$–MeOH, 98:2).

Acetylation of **3**. A soln of isopropylidene derivative **3** (4 mg) in pyridine (0.2 ml) and Ac_2O (0.2 ml) was refluxed at 100° for 2 hr. The pyridine and excess of Ac_2O was then removed under red. pres. The viscous residue taken in $CHCl_3$ (5 ml) was washed in sequence with ice cold 2 N $NaHCO_3$ (2 × 1 ml) and with H_2O (2 × 1 ml). The washed organic layer was dried over dry Na_2SO_4, filtered and evaporated to dryness yielding crystalline **4** (4 mg), mp 153–155°, $[\alpha]_D^{25}$ +36° (*c* 0.10; MeOH); (Found: C, 64.32; H, 8.36. Calc. for $C_{28}H_{40}O_9$; C, 64.61; H, 8.08%.) ^{1}H NMR (80 Mz): δ2.01 (9H, *s*, 2 × OAc and COMe), 1.25 (12H, *s*, 4 × Me).

Deacetylation of **4**. To a soln of **4** (1 mg) in MeOH (0.3 ml) was added NaOMe (0.3 ml), and the mixture was kept at room temp. After 10 min it showed one spot (TLC $CHCl_3$–MeOH, 90:10), which had the same mobility as **3**.

Very mild hydrolysis of **4** *with alkali*. To a soln of **4** (1 mg) in MeOH (0.5 ml) was added 0.5% KOH (0.5 ml) in 99.5% aq. MeOH, and the soln was kept at room temp. After 72 hr it showed three spots (TLC $CHCl_3$–MeOH, 99:1). The spot of R_f 0.7 and the spot with R_f 0.25 were identical in mobility to those of **4** and **3**, respectively. The spot of intermediate mobility (R_f 0.50) was presumably the partially deacetylated mono-*O*-acetyl-isopropylidine derivative. After 5 days, the hydrolysate showed only one spot, which had the same mobility as **3**.

Dehydration of **1**. To a soln of **1** (5 mg) in C_6H_6 a crystal of *p*-toluenesulphonic acid was added and the mixture refluxed. The reaction mixture was monitored on TLC. After 1 hr the reaction showed complete consumption of **1** and exhibited only one spot of higher mobility (TLC $CHCl_3$–MeOH, 99:1). The reaction mixture was made neutral by the addition of TRA-400 (OH) resin, the suspension was filtered, and the filtrate was evaporated to dryness yielding an amorphous residue **6** (1.5 mg), $[\alpha]_D^{25}$ +72° (*c* 0.10; MeOH); UV λ_{max}^{EtOH} nm (log ε): 288 (4.10).

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