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A NOVEL POLYHYDROXY PREGNANE ESTER FROM *ORTHENTHERA VIMINEA*

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

Abstract—A new pregnane ester named, therogenin has been isolated from *Orthenthera viminea* and characterized as 12β, 20*S*-di-*O*-cinnamoyl-3β,5α,6β,8β,14β,17β-hexahydroxy pregnane.

INTRODUCTION

In a recent chemical investigation of dried twigs of *Orthenthera viminea*, the presence of α-amyrin acetate, β-amyrin, β-sitosterol, penupogenin, sarcostin, sarcogenin [1] and structure elucidation of novel oligosaccharides [2–5] and pregnane ester glycosides [6–8] have been reported. In the present communication, the structure of a new pregnane ester, therogenin (**1**) isolated from this plant is reported.

RESULTS AND DISCUSSION

Therogenin (**1**), $C_{39}H_{48}O_{10}$, responded positively to Liebermann–Burchardt reaction [9], suggesting it to be a steroidal compound. Its property of undergoing alkaline hydrolysis affording deacylated product **2**, $C_{21}H_{36}O_8$, a pregnane genin (C_{21}-steroid) indicated the presence of an ester function(s) in the molecule. To identify the acid component of the ester function as a methyl ester, **1** was subjected to methanolysis by Zemplen method [10, 11] and the product analysed by mass spectrum which in the lower mass region contained diagnostic ion peaks at *m/z* 162, 131 and 103 confirming the presence of cinnamate ester function(s). Determination of the number of cinnamoyl groups in **1** came from the results (TLC) of its very mild alkaline hydrolysis at room temperature which afforded partially and completely hydrolysed products. After three days the reaction mixture exhibited two new spots; the polar being identical in mobility with fully deacylated product **2**, also obtained by complete alkaline hydrolysis of **1** in an alternative experiment, whereas the second spot of intermediate mobility between completely deacylated product **2** and starting material **1** was presumably the partially deacylated monocinnamoyl derivative. In seven days **1** was fully converted into **2**. Furthermore, the ^{13}C NMR and ^{1}H NMR spectral data also provided evidence for the presence of two cinnamoyl groups in **1**.

The formula difference $C_{18}H_{12}O_2$ of **1** and **2** suggested **1** to be a di-*O*-cinnamoyl ester of the pregnane genin $C_{21}H_{36}O_8$. The pregnane moiety in **1** was also evident from its ^{1}H NMR spectrum at 400 MHz which consisted of the expected two tertiary methyl groups (δ 1.24, 1.20) of steroid moiety, whereas the doublet (δ 1.28, $J=6$ Hz) for a secondary methyl group indicated this pregnane genin to possess a C-17 hydroxy ethyl chain.

The ^{1}H NMR spectral data of **1** not only provided evidence for the presence of two cinnamoyl groups in the molecule but also helped in fixing their positions. The lower field one proton quartet at δ4.90 ($J=6$ Hz) and another one proton double doublet at δ4.75 ($J=10$ and 4 Hz) were assigned to C-20 and C-12 methine protons, respectively bearing two cinnamoyl groups. Acetylation of **1** afforded di-*O*-acetyl derivative **3**, characterized from its ^{1}H NMR spectrum.

The preceding data suggested that out of ten oxygen atoms in **1**, two are acetylable hydroxyl groups, four are involved in two cinnamoyl groups and the remaining four oxygen atoms in this pregnane derivative are therefore presumably unacetylable tertiary hydroxyl groups.

Although **1** reacted with $NaIO_4$, its acetylated product **3** was inert to this reagent. Out of two acetylable secondary hydroxyl groups, one could be the common pregnane C-3 hydroxyl group and the other acetylable hydroxyl group was inferred to be a part of the vicinal diol system of the molecule.

The structure of **1** was largely elicited from its high resolution mass spectrum which not only showed that six oxygens of this di-*O*-cinnamoyl pregnane derivative are present as hydroxyl groups but also helped in assigning their positions at C-3, C-5, C-6, C-8, C-14 and C-17. Although the mass spectrum did not record the $[M]^+$ ion but the highest fragment ion peak at *m/z* 380.2197 ($C_{21}H_{32}O_6$) could be interpreted as $[M-2\ PhCH=CHCOOH]^+$ followed by the losses of expected six water molecules giving ion peaks at *m/z* 362 $[380-H_2O]^+$, 344 $[380-2H_2O]^+$, 326 $[380-3H_2O]^+$, 308 $[380-4H_2O]^+$, 290 $[380-5H_2O]^+$ and 272 $[380-6H_2O]^+$ thus confirming the presence of six hydroxyl groups in **1**. The mass spectrum also contained the fragment ion peaks, originated by the radical ion cleavage of C-9 and C-11 bond which gave the fragment ion peak at *m/z* 213 ($C_{11}H_{17}O_4$) consisting of rings A and B. It was accompanied by the peak at *m/z* 195 ($C_{11}H_{15}O_3$) corresponding to the loss of a water molecule $[213-H_2O]^+$. The ion peak at *m/z* 213 ascertained the position of hydroxyl groups at C-3, C-5, C-6 and C-8. Although the second fragment of this C-9 and C-11 bond

fission, expected at *m/z* 463 was not observed, but its subsequent fragment ions were recorded at *m/z* 167 ($C_{10}H_{15}O_2$) [463 − 2 PhCH=CHCOOH]$^+$ and *m/z* 149 ($C_{10}H_{13}O$) [167 − H_2O]$^+$, indicated two of its hydroxyl groups at C-14, C-17 and two cinnamoyl groups at C-12 and C-20. Similarly, the mass ion peak, originated by the cleavage of C-9 and C-10 bond, at *m/z* 171 is visualized to undergo further fragmentation giving an ion at *m/z* 153 ($C_9H_{13}O_2$) [171 − H_2O]$^+$. Again, the second fragment of this fission expected at *m/z* 505 could not be observed but its subsequent fragment ions recorded at *m/z* 209 ($C_{12}H_{17}O_3$) [505 − 2 PhCH=CHOOH]$^+$ and *m/z* 191 [209 − H_2O]$^+$ further supported the positions of hydroxyl groups at C-3, C-5, C-6, C-8, C-14 and C-17.

The structure of therogenin (**1**) was derived by comparing the mobility of its deacylated product, **2**, with the synthetic products (*cis* and *trans* diols) obtained by *cis* hydroxylation of sarcostin (**4**) with OsO_4 [12], and the trans diol *viz.* 5α,6β-dihydroxy sarcostin obtained by the opening of 5,6α-oxide of sarcostin with perchloric acid [13]. The two *cis*-hydroxylated products of sarcostin presumably 5α,6α- and 5β,6β-diols thus obtained, differed in properties from **2**, whereas the trans hydroxylated product, i.e. glycosarcostin [14] was found to be identical (TLC, mp, mmp, $[\alpha]_D$) with **2**.

A more direct chemical support for **1** being the 12,20-di-*O*-cinnamoyl derivative of glycosarcostin was obtained through the epoxidation of ornogenin (**5**) [6] (12,20-di-*O*-cinnamoyl sarcostin) with *m*-chloroperbenzoic acid [13] which led to the formation of a 5,6α-oxide and the acid catalysed opening of this epoxide with perchloric acid afforded the trans 5α,6β-diol identical in mobility with therogenin. Thus, it could be concluded that **1** was 12β,20*S*-di-*O*-cinnamoyl 5α,6β-dihydroxy sarcostin, which was in full agreement with its ^{1}H NMR spectral data at 400 MHz (see Experimental).

On the basis of the above chemical and spectroscopic evidence, the structure of therogenin (**1**) has thus been established as 12β,20*S*-di-*O*-cinnamoyl-3β,5α,6β,8β,14β, 17β-hexahydroxy pregnane.

EXPERIMENTAL

Mps: uncorr; ^{1}H NMR, ^{13}C NMR: 400 MHz (Bruker), $CDCl_3$, TMS as int. standard.

Extraction and isolation. Shade-dried powdered twigs (10 kg) of *O. viminea* were extracted and fractionated, as reported earlier [4]. The residue from $CHCl_3$–EtOH extracts was hydrolysed with 25 mM H_2SO_4 in 50% MeOH. Repeated CC of the genin mixture over silica gel gave crystalline therogenin (40 mg).

Therogenin (**1**). Mp 125–128° (MeOH), $[\alpha]_D^{25}$ +128.4° (MeOH, *c* 0.12) (Found C, 69.38; H, 7.21; $C_{39}H_{48}O_{10}$ requires C, 69.23; H, 7.10%). It reacted with $NaIO_4$ and hydrolysed with alkali. ^{1}H NMR: δ7.95–7.90 (4H, *m*, Ar-H), 7.76 (1H, *d*, *J* = 16 Hz), 7.40 (1H, *d*, *J* = 16 Hz), 7.36–7.30 (6H, *m*, Ar-H), 6.42 (1H, *d*, *J* = 16 Hz), 6.08 (1H, *d*, *J* = 16 Hz), 4.90 (1H, *q*, *J* = 6 Hz, H-20), 4.75 (1H, *dd*, *J* = 10 and 4 Hz, H-12), 3.72 (1H, *m*, H-3), 3.56 (1H, *m*, H-6), 1.28 (3H, *d*, *J* = 6 Hz, 21-Me), 1.24 (3H, *s*, 18-Me), 1.20 (3H, *s*, 19-Me). ^{13}C NMR: δ78.07 (*d*, C-3), 74.03 (*s*, C-5), 67.58 (*d*, C-6), 78.15 (*s*, C-8), 74.73 (*d*, C-12), 87.85 (*s*, C-14), 87.97 (*s*, C-17), 71.01 (*d*, C-20), 166.52 (*s*, C-1′), 166.15 (*s*, C-1″), 119.02 (*d*, C-2′), 117.66 (*d*, C-2″) 146.12 (*d*, C-3′), 144.26 (*d*, C-3″), 134.22 (*s*, C-4′), 132.90 (*s*, C-4″), 128.93 (*d*, 2C-5′), 128.90 (*d*, 2C-5″), 128.40 (*d*, 2C-6′), 128.32 (*d*, 2C-6″), 130.04 (*d*, C-7′), 129.81 (*d*, C-7″). MS *m/z* (rel. int.): [M]$^+$ (not observed), 380.2199 (6.22) [M − 2 PhCH=CHCO$_2$H]$^+$ ($C_{21}H_{32}O_6$), 362. 2115 (8.49) [380 − H_2O]$^+$ ($C_{21}H_{30}O_5$), 344.1987 (9.21) [390 − 2H_2O]$^+$ ($C_{21}H_{28}O_4$), 326.1882 (6.40) [380 − 3H_2O]$^+$ ($C_{21}H_{26}O_3$), 308.1775 (2.45) [380 − 4H_2O]$^+$ ($C_{21}H_{24}O_2$), 290.1669 (0.91) [380 − 5H_2O]$^+$ ($C_{21}H_{22}O$), 272.1567 (0.96) [380 − 6H_2O]$^+$ ($C_{21}H_{20}$), 213.1154 (0.61) ($C_{11}H_{17}O_4$), 209.1200 (1.83) ($C_{12}H_{17}O_3$), 195.1042 (1.93) ($C_{11}H_{15}O_3$), 191.1068 (3.31) ($C_{12}H_{15}O_2$), 171.1070 (4.39) ($C_9H_{15}O_3$), 167.1057 (2.88) ($C_{10}H_{15}O_2$), 153.0914 (13.63) ($C_9H_{13}O_2$), 149.0961 (7.94) ($C_{10}H_{13}O$), 148.0520 (26.35) ($C_9H_8O_2$), 131.0497 (100) (C_9H_7O) and 103.0550 (36.86) (C_8H_7).

Alkaline hydrolysis of compound **1**. Compound **1** (5 mg) was dissolved in 5% methanolic KOH (1 ml) and refluxed for 1 hr. Usual work-up [12] yielded **2** (2 mg) which crystallized from MeOH–H_2O, mp 265–270°, $[\alpha]_D^{25}$ +21.5° (MeOH, *c* 0.07) (found C, 60.74; H, 8.70; $C_{21}H_{36}O_8$ requires C, 60.58; H. 8.65%).

Methanolysis of **1** by the Zemplen method. To a soln of **1** (2 mg) in absolute MeOH (0.5 ml) was added $NaOCH_3$ (0.05 ml), and mixture was kept at room temp. After 15 min, it was neutralized with IR 120 H resin and filtered, MeOH was removed under red. pres. and yielded residue (1.4 mg), which gave ion peaks at *m/z* 162, 131 and 103 in the lower mass region of its MS.

Very mild alkaline hydrolysis of compound **1**. Substance **1** (1 mg) was dissolved in MeOH (0.1 ml) to which $KHCO_3$ (2 mg) in H_2O (0.1 ml) was added and the reaction was monitored by TLC. After three days the reaction mixture showed the formation of three spots. The fastest spot (R_f 0.60) was identical in mobility with starting material **1**, whereas the slowest spot (R_f 0.20) was identical with fully deacylated product **2** and the intermediate mobility spot (R_f 0.50) was attributable to the partially deacylated derivative. The hydrolysis was complete in seven days resulting in only one spot for product **2**.

Acetylation of compound **1**. A soln of **1** (10 mg) in pyridine (0.5 ml) and Ac_2O (0.4 ml) was heated at 100° for 4 hr. Usual work-up yielded di-*O*-acetyl therogenin (**3**) (6.2 mg).

^{1}H NMR: δ7.96–7.90 (4H, *m*, Ar-H), 7.64 (1H, *d*, *J* = 16 Hz), 7.44 (1H, *d*, *J* = 16 Hz), 7.42–7.32 (6H, *m*, Ar-H), 6.42 (1H, *d*, *J* = 16 Hz), 6.06 (1H, *d*, *J* = 16 Hz), 4.88 (1H, *q*, *J* = 6 Hz, H-20), 4.76 (1H, *dd*, *J* = 10 and 4 Hz, H-12), 4.74 (1H, *m*, H-3), 4.66 (1H, *m*, H-6), 2.04 (6H, *s*, 2 OAc), 1.30 (3H, *d*, *J* = 6 Hz, 21-Me), 1.28 (3H, *s*, 18-Me), 1.24 (3H, *s*, 19-Me). MS *m/z* (rel. int.): [M]$^+$ (not observed) 464.2386 (1.60) [M − 2 PhCH=CHCO$_2$H]$^+$ ($C_{25}H_{36}O_8$), 404.2202 (13.04) [464 − AcOH]$^+$ ($C_{23}H_{32}O_6$), 344.1982 (1.36) [404 − AcOH]$^+$ ($C_{21}H_{28}O_4$), 326.1877 (4.83) [344 − H_2O]$^+$ ($C_{21}H_{26}O_3$), 308.1766 (6.15) [344 − 2H_2O]$^+$ ($C_{21}H_{24}O_2$), 290.1669 (2.33) [344 − 3H_2O]$^+$ ($C_{21}H_{22}O$), 272.1567 (1.09 [344 − 4H_2O]$^+$ ($C_{21}H_{20}$), 148.0516 (22.68) ($C_9H_8O_2$), 131.0496 (100.00) (C_9H_7O), 103.0548 (40.05) (C_8H_7) and 60.0233 (12.62) ($C_2H_4O_2$).

$NaIO_4$ *oxidation of compound* **1**. Compound **1** (2 mg) in MeOH (0.2 ml) was oxidized with $NaIO_4$ (6 mg) and kept at room temp. for 4 hr which after the usual work-up [15] yielded one spot of lower R_f value than **1**.

cis-*Hydroxylated products of sarcostin.* To 20 mg tri-*O*-acetyl sarcostin in 5 ml absolute Et_2O added a soln of 15 mg OsO_4 in 0.5 ml absolute Et_2O dropwise in 5 min and left for 36 hr at room temp. It was then diluted with MeOH, H_2S passed slowly for 10 min and after 2 hr standing, filtered. The filtrate was evapd yielding residue (22 mg) which was hydrolysed by alkali as in alkaline hydrolysis of **1** affording residue (11 mg) containing two major products (TLC). These products were separated on a silica gel column having mp 170–172° (MeOH) and 235–240° (MeOH–H_2O). These two products were found to be different in properties from completely deacylated product **2** obtained from **1**.

trans-*Hydroxylated product of sarcostin.* A soln of *m*-chloro-

perbenzoic acid (10 mg) in CH_2Cl_2 (1 ml) was added during 1 hr to a stirred soln of sarcostin **4** (15 mg) in CH_2Cl_2) (1 ml) under cooling with ice H_2O. Usual work-up [13] yielded 5,6α-oxide of sarcostin. For obtaining *trans*-hydroxylated product of sarcostin, a soln of 7% $HClO_4$ (0.1 ml) was added to a stirred soln of sarcostin 5,6α-oxide (10 mg) in THF (1 ml) at room temp. during 3 hr. After the usual work up [13] it yielded 5α,6β-dihydroxy sarcostin, i.e. glycosarcostin [14] (5.7 mg), mp 270–274° ($MeOH–H_2O$), $[\alpha]_D^{25}$ +22.5° (MeOH, *c* 0.11), which was found to be identical (TLC, mp, mmp, $[\alpha]_D$) with **2**.

trans-*Hydroxylated product of Ornogenin.* A soln of *m*-chloroperbenzoic acid (3 mg) in CH_2Cl_2 (0.5 ml) was added during 1 hr to a ice-cooled soln of ornogenin (**5**) (5 mg) in CH_2Cl_2) (0.5 ml) under stirring. Usual work-up [13] yielded ornogenin 5,6α-oxide (4.2 mg). A soln of 7% $HClO_4$ (0.1 ml) was added to a stirred soln of this epoxide (2.8 mg) in THF (0.3 ml) at room temp. during 3 hr. After the usual work-up [13] it yielded glycoornogenin, found to be identical in properties with therogenin (**1**).

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