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SARCOGENIN, A PREGNANE DERIVATIVE FROM *PERGULARIA PALLIDA* AND *SARCOSTEMMA BREVISTIGMA*

NAVEEN K. KHARE, RAJ KUMAR, MAHESHWARI P. KHARE and ANAKSHI KHARE

Department of Chemistry, Lucknow University, Lucknow, India

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Key Word Index—*Pergularia pallida*; *Sarcostemma brevistigma*; Asclepiadaceae; sarcogenin; steroid; pregnane; 3β,8β,11α,12β,14β,17β-hexahydroxypregn-5-en-20-one.

Abstract—A new pregnane genin, designated as sarcogenin has been isolated from *Pergularia pallida* and *Sarcostemma brevistigma* and characterized as 3β,8β,11α,12β,14β,17β-hexahydroxypregn-5-en-20-one.

INTRODUCTION

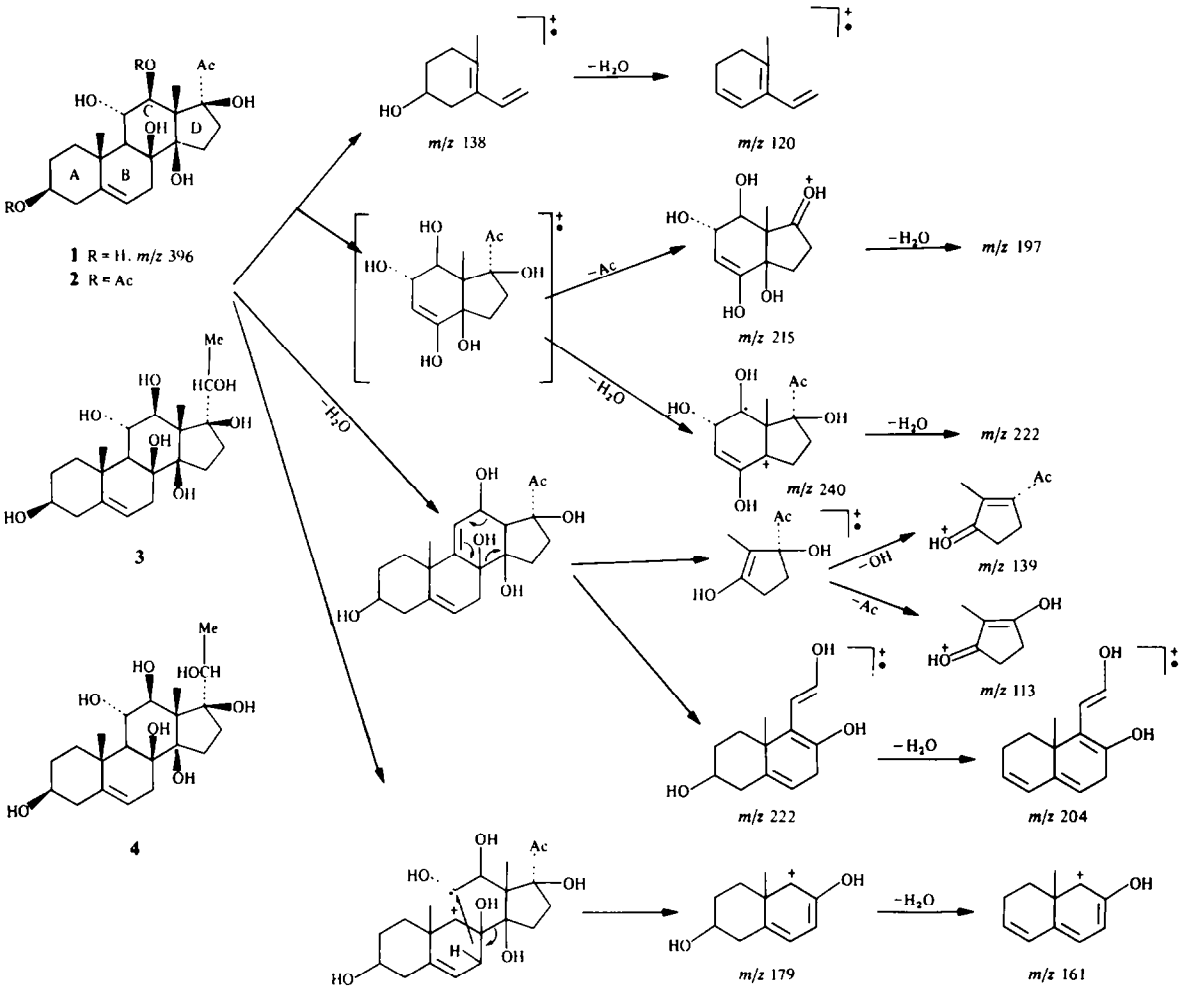
A recent chemical investigation of dried twigs of *Pergularia pallida* revealed the presence of α-amyrin, β-amyrin, sitosterol, drevogenin B, cissogenin [1] and the structures were elucidated of two new pregnane ester glycosides, pallidine and pallidinine [2]. In the present communication, the structure is reported of a new pregnane genin, sarcogenin (**1**) isolated from the chloroform extract of this plant and also isolated earlier from *Sarcostemma brevistigma* in our laboratory [3].

RESULTS AND DISCUSSION

Sarcogenin (**1**) mp 105–108°, $[\alpha]_D$ +49° has the molecular formula, $C_{21}H_{32}O_7$. The IR spectrum showed hydroxyl groups (3420 cm^{-1}), a trisubstituted double bond (800 cm^{-1}) and a methyl keto function (1690 cm^{-1}) accompanied by a methyl deformation band (1364 cm^{-1}). The presence of the carbonyl group was also shown by its reduction [4] with sodium borohydride and formation of 2,4-dinitrophenyl hydrazone. Its nature as a methyl keto group was shown by the characteristic colour reaction with sodium nitroprusside [5] and the prominent mass fragment ion peaks at *m/z* 336 $[M-Ac-OH]^+$ and 43 $[Ac]^+$. The ^1H NMR spectrum of compound **1** at 400 MHz in $CDCl_3$ consisted of the signals for two tertiary methyl groups (δ1.18, 1.28), a methyl keto group (δ2.38) and a vinylic proton (δ5.38). The double bond equivalent value of six, derived from the molecular formula, indicated compound **1** to be a highly hydroxylated pregnane genin (C_{21} steroid). As compound **1** did not isomerize with methanolic potassium hydroxide, it indicated the presence of a substituent, presumably a hydroxyl group, at C-17. Its property to react with sodium periodate suggested the presence of vicinal diol system in the molecule. A doublet at δ3.77 (J = 8 Hz) and a double doublet at δ3.69 (J = 8 and 6 Hz) of one proton each in the ^1H NMR spectrum of compound **1**, were attributable to the methine protons of these vicinal hydroxyl groups at the C-12 and C-11 positions in the β- and α-configurations, respectively.

Acetylation of compound **1** afforded an amorphous compound **2**, $[\alpha]_D$ +17°. The ^1H NMR spectrum of compound **2** in $CDCl_3$ at 90 MHz consisted of signals of two acetyl groups and a methyl keto group at δ1.92, 2.02 and 2.22, respectively. The lower field chemical shifts of one proton methine multiplet at δ4.48 and a doublet at 4.40 (J = 8 Hz) could be assigned to the C-3 and C-12 positions bearing the β-acetoxy groups [6]. The unaffected higher field double doublet at δ3.46 indicated that the hindered C-11 hydroxyl group was not acetylated under the present experimental conditions. The resistance of compound **2** to sodium periodate oxidation is in conformity to the conclusion that at least one of the vicinal hydroxyl groups underwent esterification which could be most probably at C-12.

The structure of compound **1** was largely elicited from the mass spectrum. Although its mass spectrum did not record the molecular ion, other prominent ions in the higher mass region were of great value. The ion peak at *m/z* 361 corresponding to $[M-H_2O-OH]^+$, which underwent further fragmentation yielding ion peaks at *m/z* 343 $[361-H_2O]^+$, 325 $[343-H_2O]^+$, 307 $[325-H_2O]^+$, 310 $[325-Me]^+$, 292 $[307-Me]^+$ and 274 $[292-H_2O]^+$ indicated the presence of at least six hydroxyl groups in the molecule. The fragment ion peaks at *m/z* 336 $[M-COMe-OH]^+$, 318 $[336-H_2O]^+$, 300 $[336-2H_2O]^+$, 282 $[336-3H_2O]^+$, 264 $[336-4H_2O]^+$ and 246 $[336-5H_2O]^+$ further supported the fact that six of the seven oxygen atoms in compound **1** are present as hydroxyl groups whereas the seventh oxygen is involved in a methyl keto chain. The retro-Diels–Alder fission of the Δ^5-system giving fragment ion peaks at *m/z* 138 and 120 were characteristic of a Δ^5-3-ol arrangement [7] in compound **1**. Although the second fragment of this retro-Diels–Alder fission expected at *m/z* 258 was not observed, its subsequent fragment ions were recorded at *m/z* 240 $[258-H_2O]^+$, 222 $[258-2H_2O]^+$, 215 $[258-Ac]^+$ and 197 $[215-H_2O]^+$ suggesting the positions of three of its hydroxyl groups at C-8, C-14 and C-17. In view of the *trans*-fusion of rings B and C in steroids, it is evident that the C-8 hydroxyl group in the pregnane derivative, sarcogenin, is in the β-configuration. It is further reported that C-14 and C-17 hydroxyl groups in pregnane derivatives are always in the β-configuration [8] leading to the conclusion that the two carbon chain at C-17 of compound **1** is in the α-configuration which is also



Scheme 1.

in conformity to its negative Cotton effect [9]. The position of a hydroxyl group at C-11 was derived from prominent fragment ions resulting from the retro-Diels–Alder fission of the double bond created by the typical loss of a water molecule involving the C-11 hydroxyl group and the C-9 proton [10]. This gave a fragment ion at m/z 378 which, although not observed, underwent characteristic retro-Diels–Alder fission at the $\Delta^{9,11}$-position giving ultimate fragments at m/z 139, 113 and the other series of ions at m/z 222 and 204 supporting once again the position of the 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 and thus further supporting the position of these hydroxyl groups.

The chemical proof of the structure of sarcogenin (1) came from its reduction [4] with sodium borohydride giving two products: compound 3 (major, R_f 0.40) mp 210–216° (methanol–ether), $[\alpha]_D$ +43.63°; recrystallized from acetone, mp 219–222° and compound 4 (minor, R_f 0.45) mp 185–188° (methanol–ether), $[\alpha]_D$ +60°. As expected from the proposed structure of compound 1, one of them could be stephanol [11]. Compound 3 was characterized as stephanol (mp and $[\alpha]_D$) (lit. mp 222–225° (acetone), $[\alpha]_D$ +37.5°) whereas the other product 4 could, therefore, be its C-20 enantiomer.

Mitsuhashi *et al.* [9] proved that the stereochemistry of the C-20 carbinol obtained by sodium borohydride reduction of the corresponding ketone is related to the configuration of the C-17 methyl keto chain and that an α-alcohol (*S*-configuration) is formed from the reduction of the ketone in a C-17 α-side chain. Therefore, it can be concluded that the major product stephanol (3) obtained from sodium borohydride reduction of sarcogenin must have the *S*-configuration at C-20. This is hitherto unreported. Therefore the minor reduction product (4) is concluded to have a C-20 *R*-configuration which has not been isolated from plants.

On the basis of the above chemical and spectroscopic evidence the structures of sarcogenin (1) and stephanol (3) are, therefore, 3β,8β,11α,12β,14β,17β-hexahydroxy-pregn-5-en-20-one and pregn-5-en-3β,8β,11α,12β,14β,17β,20*S*-heptaol, respectively.

EXPERIMENTAL

The general procedures were the same as those reported recently [12] except ^{1}H NMR: 400 MHz and 90 MHz, $CDCl_3$, TMS was the int. standard.

Extraction and isolation. The extraction of twigs of *P. pallida* (25 kg) was described recently [2]. Repeated CC of the $CHCl_3$ extract (10 g) over silica gel using $CHCl_3$–MeOH (96:4) as eluent afforded sarcogenin (1, 40 mg).

Sarcogenin (1). Mp 105–108° (Me_2CO–petrol), $[\alpha]_D^{25}$ +49° (MeOH, *c* 0.11); (Found: C, 63.80; H, 8.12. $C_{21}H_{32}O_7$ requires C, 63.63; H, 8.08%). It gave positive colour tests with 2,4-dinitrophenyl hydrazine, tetranitromethane and sodium nitroprusside. It reacted with $NaIO_4$ and also underwent reduction with $NaBH_4$. IR spectrum ν_{max}^{KBr} cm^{-1}: 3420 (br), 2905 (m), 1700 (s), 1690 (s), 1442 (w), 1365 (s), 1285 (m), 1250 (s), 1158 (m), 1030 (s), 948 (m), 890 (w), 862 (w), 848 (w) and 800 (m). $[\alpha]_{290}$ – 38748.02. ^1H NMR (400 MHz): δ5.38 (1H, *m*, H-6), 3.77 (1H, *d*, *J* = 8 Hz, H-12), 3.69 (1H, *dd*, *J* = 8 and 6 Hz, H-11), 3.56 (1H, *m*, H-3), 2.38 (3H, *s*, Ac), 1.28 (3H, *s*, 18-Me) and 1.18 (3H, *s*, 19-Me). MS *m/z* (rel. int.): $[M]^+$ (not observed), 361 (1.1), 343 (2.0), 336 (26), 327 (0.8), 325 (1.8), 318 (72), 310 (1.2), 307 (0.7), 300 (61), 292 (0.8), 282 (58), 274 (2.3), 264 (21), 246 (8), 240 (10), 222 (14), 215 (8), 204 (1.4), 197 (20), 179 (12), 161 (30), 139 (1.2), 138 (45), 120 (48), 113 (79), 97 (50), 83 (62), 69 (66), 55 (92) and 43 (100).

$NaIO_4$ *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. After the usual work-up [12] it yielded one spot of lower R_f value than compound 1.

Acetylation of compound 1. Compound 1 (12 mg) on acetylation with pyridine (1.1 ml) and Ac_2O (0.18 ml) at room temp. for 4 hr afforded amorphous 2 (12 mg), $[\alpha]_D^{25}$ +17° (MeOH, *c* 0.13). ^1H NMR (90 MHz): δ5.32 (1H, *m*, H-6), 4.48 (1H, *m*, H-3), 4.40 (1H, *d*, *J* = 8 Hz, H-12), 3.46 (1H, *dd*, *J* = 8 and 6 Hz, H-11), 2.22 (3H, *s*, Ac), 2.02 (3H, *s*, OAc), 1.92 (3H, *s*, OAc), 1.41 (3H, *s*, 18-Me) and 1.15 (3H, *s*, 19-Me).

$NaBH_4$ *reduction of compound* 1. Substance 1 (12 mg) in MeOH (1.2 ml) was reduced with $NaBH_4$ (12 mg). After keeping it at room temp. for 2 hr the pH was brought to 8–9 by adding 6% alcoholic AcOH, kept for 15 hr at room temp. and excess $NaBH_4$ was decomposed with AcOH. H_2O (13.0 ml) was added and the mixture was distilled to remove MeOH. The aq. concentrate was extracted with 3 × 2 ml $CHCl_3$–MeOH (9:1) and the organic layer washed with H_2O (0.5 ml), dried, and evapd yielding residue containing two products (TLC). These products were separated on a silica gel column affording 3 (major, R_f 0.40), mp 210–216° (MeOH–Et_2O), $[\alpha]_D^{25}$ +43.63° (MeOH, *c* 0.22); recrystallized from Me_2CO, mp 219–222° and 4 (minor, R_f 0.45), mp 185–188° (MeOH–Et_2O), $[\alpha]_D^{25}$ +60° (MeOH, *c* 0.14). Compound 3 was found to be stephanol (mp and $[\alpha]_D$) while the more polar product 4 is its C-20 enantiomer. Unfortunately an authentic sample of stephanol could not be obtained for direct comparison.

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