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STEROIDAL SAPONINS FROM *SOLANUM UNGUICULATUM* (A.) RICH

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Key word Index: Solanum unguiculatum, Solanaceae; steroidal saponins; diosgenin; chlorogenin; diosgenin glycoside; laxogenin glycoside; isonarthogenin glycoside, spirostanol glycoside.

ABSTRACT: Seven steroidal saponins were isolated for the first time from the green berries of *Solanum unguiculatum*. Their structures were determined by spectroscopic analysis as well as hydrolysis of the glycosides into the corresponding sapogenins, diosgenin, chlorogenin, diosgenin-3-O-β-D-galactopyranoside, diosgenin-3-O- β-D-glucopyranosyl-(1 → 4)- β-D-galactopyranoside, 3β-hydroxyl-(25R) 5 α-spirostan-6-one (laxogenin) 3-O- α-L-rhamnopyranosyl-(1 → 2)- β-D-glucopyranoside, isonarthogenin-3-O- α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)- β-D-glucopyranoside and 25(S) spirost-5-en-3 β, 17 α, 27 triol-3-O-[α-L-rhamnopyranosyl-(1 → 2)] [α-L-rhamnopyranosyl (1 → 4)]- β-D-glucopyranoside.

INTRODUCTION

The occurrence of steroidal saponins in *Solanum* plants is well documented[1-16]. In our previous paper[17] we reported on the isolation and structure determination of new steroidal alkaloids from *Solanum unguiculatum* (A.) Rich. A survey of literature showed that no chemical work has been reported on the saponins from *Solanum unguiculatum* (A.) Rich. This small tree is widely distributed in Yemen, Sana'a region and its fruits are known for their

foaming properties, indicating the presence of saponins. As part of our contribution to the study of *S. unguiculatum* (A.) Rich we have now carried out a phytochemical study of the chloroform and n-butanol-soluble parts of the methanolic extract of *S. unguiculatum* (A.) Rich. This resulted in the isolation of seven saponins.

EXPERIMENTAL

GENERAL

MPs; were measured on a Fisher-Johns hot stage and are uncorr. Optical rotations were measured on a Zeiss polarimeter Model 53187 using a sodium lamp. NMR spectra were measured in $CDCl_3$ and pyridine-d_5 and recorded at 300 MHz for ^{1}HNMR and 75 MHz for ^{13}CNMR (using TMS as internal standard) on a Varian XL300 (Darmstadt, Germany). Chemical shifts are expressed in δ values (^{1}H) and ppm (^{13}C-nmr). IR spectra were recorded on a Perkin-Elmer Model 377 spectrometer in KBr discs. FAB-MS spectra were recorded with an MS 2500 high resolution spectrometer (Kratos Manchester, UK) with 70 eV, an ion source temperature of 180°C and a direct inlet using sulphur glycerol as matrix. Petroleum-ether used with bp 60-80°; chromatography silica gel (60-120 mesh); TLC: precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck). The spots were visualized by spraying with 10% H_2SO_4 solution followed by heating at 100°C for 10 minutes. Solvent system for TLC: $CHCl_3$-MeOH-EtOH-H_2O (60:20:15:6).

PLANT MATERIAL:

Green berries (1 kg) of *S. unguiculatum* were collected in August, 1991 from road and hill sides in Sana'a region (Wadi Zahr) in Yemen. The identity of the plant was confirmed by Professor M. El-Manayery, Faculty of Science, El-Azhar University, Egypt. A certified specimen has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Egypt.

EXTRACTION AND CHROMATOGRAPHY

The fresh fruits were cut into pieces and exhaustively extracted with methanol (4 L) by cold maceration. The extract was concentrated to almost dryness under reduced pressure. The residue (20 g) was suspended in water (1/2 L) and extracted with $CHCl_3$ (2 L) and then with n-BuOH (2 L). The chloroform-soluble phase was concentrated under reduced pressure, the resulting residue (7 g.) was chromatographed over silica gel column. Elution being carried out with petroleum-ether, increasing the polarity with EtOAc, early petrol-EtOAc (9:1) eluate fractions yielded compound 1 (50 mg) and petrol-EtOAc (3:7) eluate fractions yielded compound 2(100 mg).

The n-BuOH-soluble phase was concentrated under reduced pressure and the resulting residue (10 g) was chromatographed over silica gel column. Elution being carried out with chloroform then increasing the polarity with methanol. Chloroform-methanol (93:7) eluate fractions yielded compound 3 (30 mg), chloroform-methanol (90:10) eluate fractions yielded compound 4 (140 mg), chloroform-methanol (80:20) eluate fractions yielded compound 5 (100 mg); chloroform-methanol (70:30) eluate fractions yielded a mixture of 2 spots (compounds 6 and 7) (300 mg). The latter mixture were rechromatographed on silica gel column and elution was started with chloroform-methanol (80:20) increasing the polarity with methanol, fractions (60-40) yielded compound 6 (120 mg) and fractions (45-55) yielded compound 7 (120 mg).

Compound (1) eluted with petrol-EtOAc (9:1) on further preparative chromatography furnished diosgenin (50 mg) crystallizing from $CHCl_3$-petrol in colourless needles mp 205°C [lit. 206°C], $[\alpha]_D^{20}$ -126°C ($CHCl_3$; 0.096) [lit. $[\alpha]_D^{20}$ -121°]; ^{1}HNMR (300 MHz, $CDCl_3$): δ .78 (3H, d, J= 6.2 Hz, CH_3-27), 1.01 (3H, d, J=6.1 Hz, CH_3-21), 1.03 (6H, s, CH_3-18 and CH_3-19), 2.28 (3 H, m, H_2-4 and H_{equ}-7), 3.32 __ 3.51 (3H, m, H-3 and CH_2-26), 4.36-4.43 (1H, m, CH-16), 5.34 (1H, d, J=5.1 Hz, CH-6). ^{13}CNMR see Table 1.

Compound (2) eluted with petrol-EtOAc (3:7) on further preparative chromatography furnished chlorogenin (100 mg) crystallized from MeOH as fine needles mp 268-270°C, $[\alpha]_D^{20}$ -42° (MeOH; 0.4), HRFABMS (neg.) mlz 430 [M-H]$^-$, calculated for $C_{27}H_{43}O_4$, 394, 358, 316, 298, 269, 139 (base peak-1 $C_9H_{15}O_1$). IR υ_{max}^{KBr} cm^{-1} 3400, 985, 922, 900 cm^{-1}. ^{1}HNMR (300 MHz, $CDCl_3$) δ 0.77 (3 H, s, Me-18), 0.84 (3H, s, Me-19), 0.94 (3H, d, J=7 Hz, Me-21), 0.78 (3H, d, J=7Hz, Me-27), 2.15 (1H, s, C_3-β-OH), 3.35 (1H, m, C-16-α–H), 3.4 (1H, s, C-6-α–OH), 4.35 (1H, m, C-6 HOH). ^{13}CNMR see table 1.

Compound (3) eluted with $CHCl_3$-MeOH (93:7) on further column chromatography furnished diosgenin glycoside (130 mg) as powder from MeOH, FABMS (neg.) 575 [M($C_{33}H_{52}O_8$)-H]$^-$; ^{1}HNMR: 0.7 (3H, d, J=6.5 Hz, Me-27), 0.9 (3H, s, Me-19), 1.0 (3 H, s, Me-18), 1.37 (3H, d J= 7Hz, Me-21) 4.98 (1H, d, J= 8Hz, Gal. H-1), 5.29 (br, s, H-6). ^{13}CNMR see (Table 1).

Compound (4) eluted with $CHCl_3$-MeOH (90:10) on further column chromatography furnished laxogenin glycoside (140 mg) needles from MeOH, mp 247-249°(dec.), $[\alpha]_D^{20}$ -60.4°(MeOH; C 0.51), -neg. FABMS m/z 737 [M ($C_{39}H_{62}O_{13}$)-H]$^-$, 575 [M-Glc.-H]$^-$; ^{1}HNMR: δ 0.7 (3H, d, J=6 Hz, Me-27), 0.93 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.84 (3H, d, J=7 Hz, Me-21), 4.35 (1H, d, J=8 Hz, Gal. H-1), 5.29 (1H, br, s, H-6), 5.33 (1H, d, J=7.7 Hz, Glc H-1).

Compound (5) eluted with $CHCl_3$-MeOH (80:20) on further chromatography furnished compound-5 as amorphous powder $[\alpha]_D^{25}$ -90 (MeOH, C 0. 04), positive FABMS m/z 777 [M+K]$^+$, 762 [M+Na+H]$^+$, 721 [M-OH]$^+$, UV λ_{max} MeOH 284; IR υ_{max}^{KBr} cm^{-1}; 3440 (OH), 2940, 2870 (CH), 1705 (C=O), 1455, 1375, 1260, 1175, 1045, 975, 915, 895, 800, 700 [(25R)-spiroacetal intensity 915 < 895]. ^{1}HNMR (300 MHz, pyridine-d_5): δ 4.53 (1H, qu-like, J=7 Hz, H-16), 3.96 (1H, m, H-3), 3.59 (1H, dd, J=10.6, 3.6 Hz, H-26a), 3.49 (1H, dd, J=10.6 Hz, H-26b), 2.37 (1H, dd, J=12.7, 4.1 Hz, H-7 equ), 2.16 (1H dd, J=12.6, 2.4 Hz, H-5), 2(1H, dd, J=12.7, 12.7 Hz, H-7ax), 1.15 (3H, d, J=6.9 Hz,

Me-21), 0.79 (3H, s, Me-18), 0.7 (3H, d, J=5.8 Hz, Me-27), 0.64 (3H, s, Me-19), 4.93 (1H, d, J=7.8 Hz, H-1′), 3.88 (1H, dd, J=9.3, 7.8 Hz, H-2′), 4.22 (overlapping H-3′), 4.45 (1H, dd, J=9.3, 9.3 Hz H-4′), 3.96 (1H, m, H-5′), 4.83 (1H, br, d, J=10.4, H-6′a), 4.68 (1H, dd, J=10.8, 3.6 Hz, H-6′b), 6.33 (1H, br s, H-1″)m 4.76 (1H, br d, J=3.4 Hz, H-2″), 4.64 (1H, dd, J=9.5, 3.4 Hz, H-3″), 4.42(1H, dd, J=9.5, 9.5 Hz, H-4″), 4.96 (1H, d qu, J=9.5, 6.2 Hz, H-5″).

Compound (6) eluted with $CHCl_3$-MeOH (70:30) on further chromatography furnished compound 6 as amorphous powder, $[\alpha]_D^{30}$ -76°(MeOH; C 0.08), positive FABMS mlz 885 $[M+H]^+$; IR υ_{max}^{KBr} cm^{-1} 3400(OH), 2923 and 2857 (CH), 1458, 1250, 1094, 1055, 985, 840. ^{1}HNMR (pyridine-d_5): δ 6.38 1H, br, s, H-1″), 5.84 (1H, br, s, H-1‴), 5.33 (1H, br, d, J=4.4 Hz, H-6), 4.94 (1H, d, J=7 Hz, H-1′)1.77 (3H, d, J=6Hz, H-6″) 1.6 (3H, d, J=6Hz, H-6‴) 1.16 (3H, d, J=7Hz, Me-21), 1.06 (3H, s, Me-19), 0.85 (3H, s, Me-18).

Compound (7) eluted with, $CHCl_3$-MeOH (70:30) on further column chromatography furnished compound 7 as amorphous powder with mp 300°C(dec.), $[\alpha]_D^{30}$ -90° (pyridine; C 0.98), IR υ_{max}^{KBr} cm^{-1} 3400 (OH), 1637 (C=CH), 1040 (C-O-C), 993 (25-S-27-hydroxyl spriostanol), 838, 812 (Δ^5). FABMS m/z 923 $(M+Na)^+$, 907 $(M+Li)^+$, ^{1}HNMR: δ 0.96 (3H, s, Me-18), 1.08 (3H, s, Me-19); 1.22 (3H, d, J=7 Hz, Me-21), 2.25(1H, qu, J=7Hz, H-20), 2.71(1H, m, H-25), 3.6(1H, dd, J=11.9, 7Hz, H-27a), 3.7 (1H, dd, J=12, 1 Hz, H-27b), 3.8 (1H, dd, J=14, 12Hz, $H_{26\alpha\beta}$, 26_α/25), 2.68 (1H, dd, J = 14, 4 Hz, $H_{26\alpha\beta}$, H-26_β/25), 5.3 (1H, m, H-5), 4.9(1H, d, J=6Hz, Glc. H-1), 5.82 (1H, br, s, 4-Rha. H-1), 6.36 (1H, br s, 2-Rha. H-1). ^{13}CNMR (Table 1)

ACID HYDROLYSIS OF THE GLYCOSIDES

Acid hydrolysis of compounds 3 – 7: A solution of each saponin (about 5 mg) in 2NHCl-dioxane-H_2O (1:1 ml) was heated separately for 6 hr. The reaction

mixture was blown to dryness with a N_2 stream, the residue was checked respectively. For identification of aglycone and sugar moieties. Aglycones were identified using TLC and comparison with authentic samples using ($CHCl_3$-MeOH, 12:1) as developer and 10% H_2SO_4 for detection. Saponins 3 and 4 gave diosgenin (R_f 0.77), saponin 5 gave laxogenin (R_f 0.56), saponin 6 gave isonarthogenin (R_f 0.5) and compound 7 gave isonarthagenin. -17-OH (R_f 0.53). Sugars were checked with TLC carried out on silica gel F_{254} plate using n-BuOH-PrOH-H_2O (10:5:4) as developer and anisaldehyde – H_2SO_4 for detection. Saponin 3 gave Gal. (R_f 0.24), saponin 4 gave Gal. (R_f 0.24) and GLc. (R_f 0.29), while saponin 5-7 gave Glc. (R_f 0.29) and Rha. (R_f 0.57).

RESULTS AND DISCUSSION

The residue remaining from methanolic extract of the green berries of *S. unguiculatum* was partitioned between chloroform and H_2O then between n-butanol and H_2O. A series of chromatographic separations of the chloroform-soluble phase and n-butanol-soluble phase furnished compounds 1,2 and 3 – 7 respectively.

Compound 1 crystallized from chloroform-petrol mixture as colourless needles with mp. 205°C, $[\alpha]_D$ –126°($CHCl_3$). The ^{1}HNMR (300 MHz, $CDCl_3$) spectrum suggested the presence of two secondary methyl groups at δ 0.78 (d, J=6.2Hz) and 1.01 (d, J=6.1 Hz), two tertiary methyl groups as singlet at δ 1.03 and an olefinic proton appearing at 5.34 as doublet (J=5.1 Hz)[18]. A comparison of the carbon shifts for 1 with those of diosgenin[19-20] led to the assignment of all the carbon shifts and elucidation of the structure and stereochemistry of 1 as diosgenin (Table 1). The ^{13}CNMR spectrum (75 MHz, $CDCl_3$) of 1 showed the presence of signals appearing at δ 140.7, 121.3 are attributed to C-5 and C-6 respectively[21], the observed close similarity of the shifts for C-23, C-24, C-25, C-26 and C-27 in compound 1 with those in diosgenin are diagnostic of the equatorial orientation of the C-25 methyl in the 22 α-O-spirostane skeleton and

thus settled the 22R and 25R configuration of compound 1. Diosgenin is thus shown to be (25R)-22α-O-spirost-5-ene-3β-ol (1).

Compound 2 showed molecular ion peak at m/z 430 $[M-H]^-$ corresponding to the molecular formula $C_{27}H_{43}O_4$ with prominent peaks at m/z 139 and 115 indicative of spiroketal moiety[22]. The IR spectrum showed prominent hydroxyl band, as well as the characteristic bands in the finger print zone attributed to the spiroketal moiety. Analysis of the IR spectrum indicated that 2 was a 25R spirostan because the 900 cm^{-1} band was more intense than 922 cm^{-1} band[23-24]. The ^{13}C-nmr spectrum confirmed this conclusion. The F-ring carbons of compound 2 exhibited resonances at 109.2 ppm (C-22), 31.5 (C-23), 28.8 (C-24), 30.2 (C-25), 66.8 (C-26) and 16.2 (C-27). The carbons carrying hydroxyls appeared at 71.7 and 69.2 ppm. Thus, compound 2 was identified as chlorogenin by comparison of its mp. and physical properties with the experimental values reported by Wall et al.[25]

Compound 3 showed a molecular ion peak at m/z 575 $[M\ (C_{33}H_{52}O_8)-H]^-$ in the negative FABmass spectrum. Upon acid hydrolysis, 3 yielded 1 as aglycone and galactose. In the ^{1}HNMR spectrum of 3, one anomeric proton signal was observed at δ 4.98 (1H, d, J=8Hz) diagnostic for the β-configuration of the sugar moiety. It was observed that the glycosylation at the hydroxyl group of C-3 of the aglycone resulted in a downfield shift (6.3 ppm) of (C-3)[26] Therefore, the structure of 3 was elucidated to be diosgenin-3-O-β-D-galactopyranoside.

Compound 4, demonstrated a molecular formula of $C_{39}H_{62}O_{13}$ determined from the negative FABMS. Upon acid hydrolysis 4 yielded 1 as aglycone with galactose and glucose in ratio 1:1 as sugar components. In the ^{1}HNMR spectrum of 4 two anomeric proton signals were observed at δ 4.35 (1H, d, J=8Hz) and 5.33 (1H, d,J=7.7 Hz), diagnostic of the β-configuration of the two sugars. Comparison of ^{13}CNMR spectrum of 4 with that of 3 indicated that the terminal glucose was attached at C-4 of the inner galactose. The ^{13}CNMR spectrum of 4 indicted that it was a 3-O-diglycoside of 1, for the signal of C-3 was shifted

downfield at 77.7 where as the signals at C-2 and C-4 were displaced upfield at 30 and 39.1 respectively. Furthermore, a typical signal at 80 arising from the glycosylation at the hydroxyl of C-4′ of galactose was confirmed. Based on the above evidence, the structure of 4 was proved to be diosgenin-3-O-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside, identical to Funkioside C isolated from *Funkia ovata*[27].

Compound 5 was assigned the molecular ion peaks at m/z 777$[M+K]^+$, 762$[M+Na+H]^+$ and 721$[M-H]^-$ corresponding to the molecular formula $C_{39}H_{62}O_{13}$. The IR spectrum indicated the existence of a hydroxyl group [3440 cm^{-1}], a carbonyl group [1705 cm^{-1}] and a (25R)-spiroacetal moiety (975, 915, 895 and 860 cm^{-1}), intensity 915 < 895[23,24]. The existence of a carbonyl group was indicated by the UV (λ_{max} 284 nm) and ^{13}CNMR (at 209.6) spectra. The ^{1}HNMR spectrum exhibited signals for two tertiary methyl protons at δ 0.79 and 0.64, two secondary methyl protons at δ 1.15 (d, J= 6.9 Hz) and 0.7 (d, J=5.8 Hz) and two anomeric protons at 4.93 (d, J= 7.8 Hz) and 6.33 (br.s). The above data suggested 5 to be a (25R)-spirostanol disaccharide. On acid hydrolysis of 5 (2N hydrochloric acid in dioxane-H_2O 1:1), 5 was hydrolysed to give D-glucose and L-rhamnose as the carbohydrate moieties and an aglycone identified as 3β-hydroxyl-(25R)-5α-spirostan-6-one (Laxogenin) by its physical and spectral data[28]. The structure of the disaccharide was determined by comparison of the carbon assignments with those of reference methyl glycosides[29] indicated the presence of terminal α-L-rhamnose unit and an inner β-D-glucopyranosyl unit joined at C-2. Accordingly, the structure of 5 was formulated as (25R)-3β-5α-spirostan-6-one-3-O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside.

Compound 6 is a major constituent $[\alpha]_D^{20}$ −76 (MeOH). The post. FABMS showed a molecular ion peak at m/z 885 $(M+H)^+$, constituent with the molecular formula $C_{45}H_{72}O_{17}$. The ^{1}HNMR spectrum exhibited three secondary methyl proton signals at δ 1.77 (3H, d, J=6.2Hz), 1.63 (3H, d, J=6.2 Hz), 1.16 (3H, d, J=6.9 Hz), two tertiary methyl proton signals at δ 1.06 and 0.85 and three anomeric proton signals at δ 6.38 (br,s), 5.84 (br,s) and 4.94 (d, J=7.4 Hz). The

signals at δ 1.77 and 1.63 were due to 6-deoxyhexoses. The ^{13}CNMR spectrum showed 45 carbon resonances. The ^{1}H and ^{13}CNMR spectra of 6 indicated that the C-27 was modified as hydroxyl-methyl. Acid hydrolysis of 6 gave D-glucose, L-rhamnose and (25S)-spirost-5-en-3β, 27-diol (isonarthogenin)[30,31]. Thus, the structure of 6 was characterized as isonarthogenein-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside.

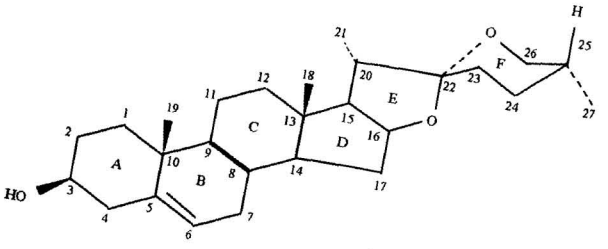
Compound 7 gave a positive Liebermann-Burchard test and Molisch reactions but did not respond to the Ehrlich reagent. It possessed broad absorption IR bands at 3400 and 1040 cm^{-1} indicating a glycosidic nature. The positive FABmass spectrum gave quasi molelcular ion peak at m/z 923 $(M+Na)^+$ and 907 $(M+Li)^+$ suggesting that MW is 900. The molecular formula of 7 was estimated as $C_{45}H_{72}O_{18}$ by ^{13}CNMR (Table 1) and FABMS spectrometry. On acid hydrolysis, 7 afforded D-glucose and L-rhamnose as sugar moieties identified by TLC with authentic samples. The ^{1}HNMR spectrum of 7 exhibited three anomeric protons of sugar components at δ 4.91 (d, J=6Hz), 5.82 (brs.) and 6.36 (brs.). Its ^{13}CNMR spectrum exhibited three anomeric carbon resonances at 102.9, 102 and 100.3. Therefore, one glucosyl and two rhamnosyl units must be contained in saponin 7. Comparison of ^{13}CNMR data of the sugar moieties in 7 with methyl β-D-glucopyranoside indicated that the C-2 and C-4 of the glucosyl moiety were shifted downfield to 78 and 78 from 74.8 and 70.3 respectively[32]. The significant glycosylation shifts clearly showed that two terminal rhamnopyranoses were linked to the C-2 and C-4 positions of the inner glucopyranose[32]. The anomeric proton signal of the glucose of 7 at 4.9 (d, J=6.2 Hz) showed that the glucosyl band of 7 had the β-configuration, however, on the basis of the coupling constant of anomeric proton of rhamnose in the ^{1}HNMR spectrum an α and β nature could not be deduced, but α-rhamnose could be identified from its ^{13}CNMR chemical shift with C-5 of α- and β-rhamnose appearing at δ 69.4 and 73.5 respectively[32]. The C-5 signals of two rhamnose units in compound 7 were at 69.4 and 70.3 respectively, thus indicating that the two rhamnose units have the α -configuration.

Also, ^{13}CNMR spectrum of 7 exhibited a resonance at δ90 of a quaternary carbon bearing an oxygen atom. In its ^{1}HNMR spectrum, the appearance of a quartet at δ2.29 (J=7.2 Hz) due to H-20 methine proton which only coupled to the 21-Me protons, suggested the occurrence of an α-hydroxyl groups at C-17 position. In addition, the ^{13}CNMR chemical shifts of the carbons of A, B, C, D and E rings of 7 were in accord with those of pennogenin glycoside[33] indicating that the aglycone of 7 possessed 17α hydroxyl-Δ^5-spirostanol skeleton. The IR spectrum of 7 lacked the characteristic normal F-ring spirostene bands at 980, 920, 900 and 880 cm^{-1} [23] indicating ring F substitution. By comparison of the ^{13}CNMR spectrum of 7 with that of pennogenin glycoside[33], C-27 of 7 shifted downfield to δ64.4 from 17.2, thus, a hydroxyl group must be located at C-27 and this was also confirmed by the lack of secondary methyl protons of δ<1 in its ^{1}HNMR spectrum. The C-25 configuration of 7 was deduced to be S from its ^{1}HNMR spectrum. The strong band at 993 cm^{-1} in the IR spectrum corresponded to that at 995 cm^{-1} in isonarthogenin[34] suggested the presence of hydroxymethyl on C-25 and revealed its 25 S-spirostanol behaviour. In the ^{1}HNMR spectrum, the coupling constant of H-25 in the axial position and hydroxymethyl in the equatorial position[31]. In the ^{13}CNMR spectrum of 7 the chemical shift of C-3 at δ 78.2 revealed that the compound was the 3-O-glycoside[20]. Thus 7 is 25(S) spirost-5-en-3β, 17α, 27-triol-3-O-[α-L-rhamnopyranosyl-(1 → 2)] [α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

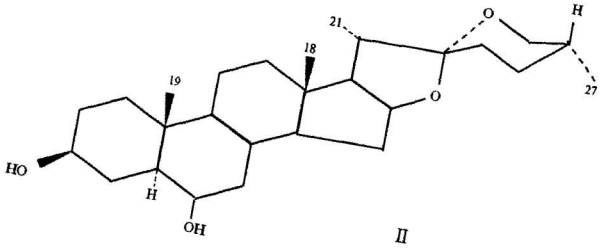
Table 1. ^{13}CNMR chemical shifts of compounds from 1 – 7..

C	1	2	3	4	5	6	7
1	37.1	37.3	37	37	36.7	37.5	37.5
2	31.2	29.6	30	30	37.4	30.2	30
3	71.6	71.7	77.7	78	77	78.2	77.8
4	42.1	30.8	39.2	39.1	27	39	39
5	140.7	51.8	140.8	140.8	56.4	140	140
6	121.3	69.3	121.4	121.4	209.6	122	122
7	32	41.6	31.8	31.8	46.7	32.4	32.1
8	31.2	33.9	30.9	31	37.4	31.7	32.4
9	50	54	52.3	52.3	53.7	50.4	50
10	36.5	37	37.6	37.6	40.8	37.2	37
11	21	21	21.1	21	21.5	21	21
12	40	40	39.9	40	39.6	39	32
13	40.5	41	40.8	41	41.1	40.5	44
14	57	56	56	56	56.5	56	53
15	32	32	31.6	31.7	31.8	32	32
16	81	80.6	81.1	81.1	80	81	90
17	62	62.2	62.9	62.9	62.8	63	90
18	16.5	16	16.4	15.9	16.5	16.4	17
19	19.5	14	19.4	18.8	13.3	19.4	19.4
20	42	42.1	42	42.7	42	42.1	45
21	14.6	14.3	15	13.9	15	15.1	9.7
22	110	109	109.3	109.4	109.3	109	110
23	31.5	31.5	31.8	32	31.8	31.6	32
24	29	29	29.3	29.6	29.4	24	24
25	30.3	30	30.6	30.6	30.7	39	39
26	66.8	66.8	66.9	67	66.7	64.1	64
27	17.1	16.9	17.3	173	17.2	64.5	65
1′			103.2	102.9	99.6	100.4	100.3
2′			72.7	73.4	79.8	78.8	78
3′			75.5	75.4	78.4	76.9	77
4′			70.4	80	72	78	78
5′			77	76	78.2	77.9	77.6
6′			62.6	61	62.9	61.4	61
1″				107.1	102.2	102	102
2″				75.2	72.6	72.5	72
3″				78.7	72.9	72.8	72.7
4″				72.3	74.2	74.2	73.9
5″				78.4	69.5	69.2	69
6″				63.2	18.7	18.7	18.6
1‴						103	102.9
2‴						72.6	72
3‴						72.9	72.8
4‴						74	74
5‴						70	70
6‴						18	18.5

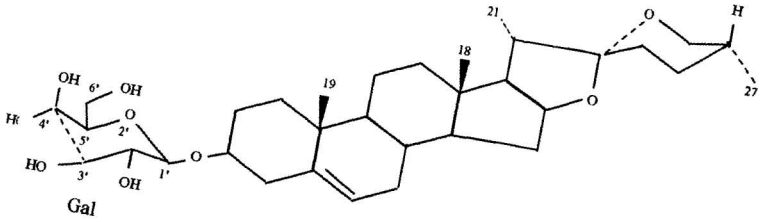
Spectra for compounds 1, 2 were done in $CDCl_3$
Spectra for compounds 3-7 were done in pyridine d_5



I

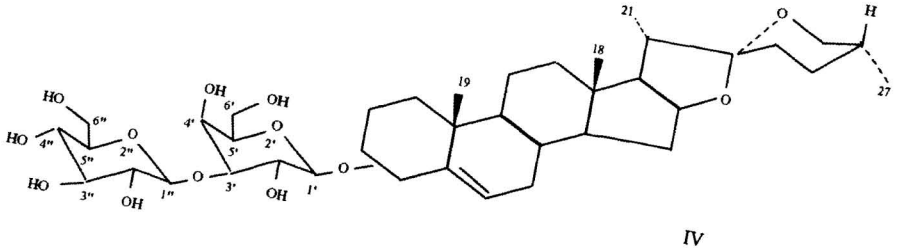


II

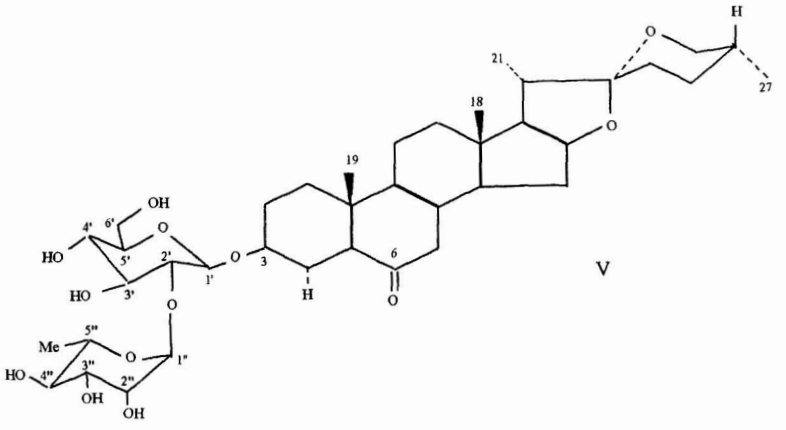


Gal

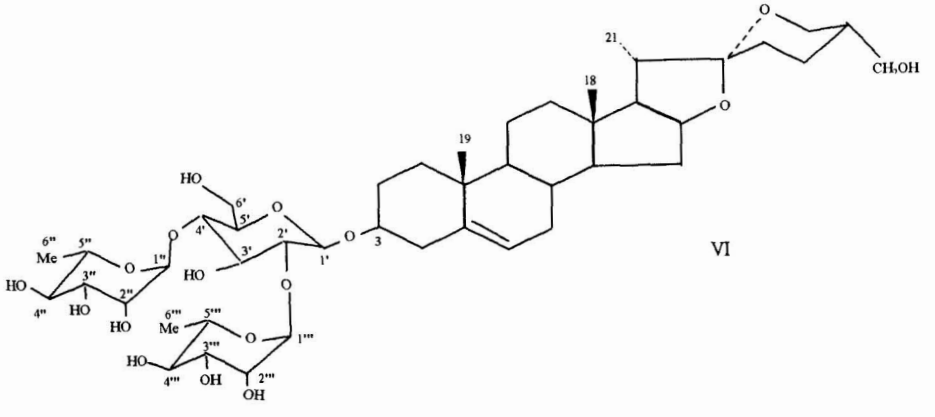
III



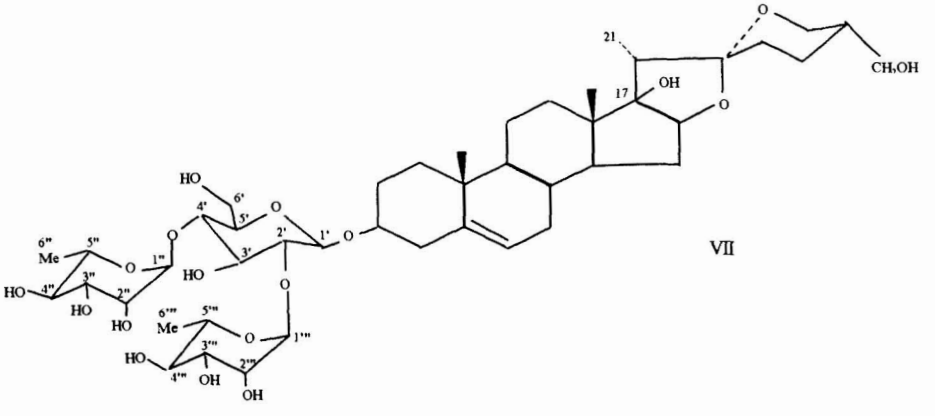
IV



V



VI



VII

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