THREE SPIROSTANOL GLYCOSIDES FROM THE SEEDS OF HYOSCYAMUS NIGER L.

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Abstract: Three steroidal glycosides of spirostane series have been isolated from the seeds of *Hyoscyamus niger L.* (Solanaceae). Their structures were determined on the basis of chemical evidence and extensive spectroscopic methods including one-dimensional, two-dimensional NMR and MS analysis. In the genus Hyoscyamus the given compounds have been found out for the first time.

Keywords: steroidal glycoside, NMR analysis, Hyoscyamus.

Introduction

Hyocyamus niger L., commonly known as Black herbane, is widely distributed in Europa and Asia [1]. The chief constituent of Henbane leaves is the alkaloid Hyoscyamine, together with smaller quantities of Atropine and Hyoscine, also known as Scopolamine [2,3]. Other constituents of Henbane are a glucosidal bitter principle called hyoscytricin, choline, mucilage, albumin, calcium oxalate and potassium nitrate. On incineration, the leaves yield about 12 per cent of ash. By destructive distillation, the leaves yield a very poisonous empyreumatic oil. The basic component of the seeds is about 0.5 to 0.6 per cent of alkaloid, consisting of Hyoscyamine, with a small proportion of Hyoscine. The seeds also contain about 20 per cent of fixed oil [4].

Constituents of Henbane are generally used in various pharmaceutical preparations, which possess anti-spasmodic, sedative and analgesic properties [5]. All parts of the plant are very toxic. Symptoms of poisoning include impaired vision, convulsions, coma and death from heart or respiratory failure. H. niger has also shown the presence of tyramine derivative [6], withanolides [7], lignanamides [8] and flavonoids [9].Our interest in the chemical constituents elaborated by plants of Solanaceae family prompted us to take up the phytochemical investigation of the seeds of H. niger and report the isolation and structural elucidation of steroidal glycosides.

Results and Discussion

The ^{1}H NMR spectrum of **1** showed signals for four steroidal methyl groups at δ0.89 (3H, s, Me-19), 0.84 (3H, s, Me-18), 1.02 (3H, d, H-21), 0.98 (3H, d, Me-27), two methine proton signals at δ 3.68 (1H, m, H-3) and 4.39 (1H, m, H-16) indicative of secondary alcoholic functions, two methylene proton signals at δ3.41 (1H, m, H-26a) and 3.29 (1H, m, H-26b), ascribable to a primary alcoholic function, and signals for two anomeric protons at δ 4.52 (1H, d, J = 7.5 Hz) and 5.21(1H, d, J = 7.5 Hz). On the basis of the HSQC and HMBC correlations, the aglycone moiety of compound **1** was identified as (25*R*)- 5α-spirostan-3β, 26-diol - tigogenin. The C-25 configuration was deduced to be *R* based on the difference of chemical shifts ($\Delta_{ab} = \delta_a - \delta_b$) of the geminal protons at H_2-26 (Δ_{ab} = 0.12 ppm). It has been described that Δ_{ab} is usually > 0.57 ppm in 25*S* compounds and < 0.48 in 25*R* compounds [10]. 5α configuration was deduced by HMBC correlation between the methyl signal at δ 0.89 (Me-19) and carbon resonances at δ 55.7 (C-9), 46.0 (C-5) and 37.9 (C-1). Using a combination of 1D-TOCSY and DQF-COSY spectral analysis, the sugar moiety have been identified as glucose and rhamnose. A glycosidation shift was observed for C-2_{glc} (δ 79.3). The HMBC spectrum showed key correlation peaks between the proton signal at δ 4.52 (H-1_{glc}) and the carbon resonance at δ 78.3 (C-3 of the aglycon), the proton signal at δ 5.21 (H-1_{rha}) and the carbon resonance at δ 79.3 (C-2_{glc}). Thus, the structure of compound **1** was deduced as (25*R*) - 5α - spirostan - 3β, 26-diol - 3-*O*-[α-L-rhamnopyranosyl-(1→2) - β - D-glucopyranoside].

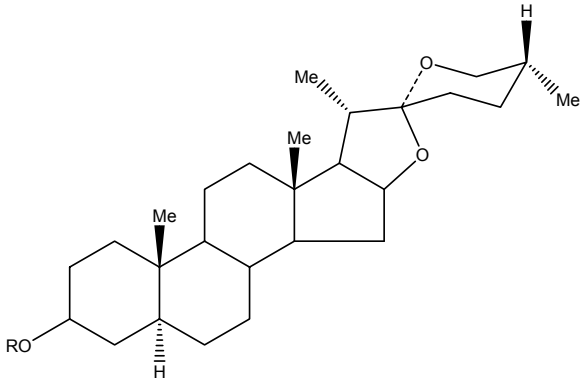
Compound **2**, in the positive ESIMS, showed a major ion peak at *m/z* 723[M + Na]$^+$ and significant fragments at *m/z* 579 [M + Na - 146]$^+$ attributable to the loss of a pentose or a rhamnose unit. The molecular formula of **2** was unequivocally established to be $C_{39}H_{62}O_{12}$ by HR-MALDI-MS (*m/z* 725.392 [M + Na]$^+$). The ^{1}H NMR spectrum of **2** showed signals for four steroidal methyl groups at δ 0.79 (s, 3H-18), 0.79(d, 3H-27), 0.79(d, 3H-21) and 1.03(s, 3H-19), two methine proton signals at δ 3.38 (t, H-26a) and 3.47 (dd, H-26b) ascribable to a primary alcoholic functions, two methylene proton signals at δ 3.49 (1H-3) and 4.41 (1H-16) indicative of secondary alcoholic function and one double bond δ 5.35 (broad d, 1H-6). On the basis of the HSQC and HMBC correlations, the aglycon moiety was identified as (25*R*)–spirost-5-ene-3β, 26-diol – diosgenin. The NMR data of sugar moiety was identical of compound **1.** The structure of compound **2** was assigned as (25*R*)-spirost 5-ene-3β,26-diol 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyde]. Compound **1** and **2** have been previously reported in literature [11].

Table 1. ^{13}C NMR spectral data (600MHz, CD_3OD) of saponins (1-3) compounds carbon

	1	**2**	**3**
1	38.0	38.3	37.9
2	30.6	32.6	30.4
3	78.3	79.4	79.0
4	34.9	40.9	34.9
5	46.2	141.8	45.8
6	32.9	122.8	32.9
7	40.8	32.6	34.6
8	36.6	31.2	36.0
9	55.6	51.6	55.2
10	36.8	37.8	36.5
11	21.6	21.4	31.0
12	40.9	40.7	40.5
13	41.2	41.4	41.8
14	57.7	57.6	57.5
15	32.4	32.5	32.3
16	82.0	82.3	82.1
17	63.7	63.4	63.7
18	16.6	16.9	16.7
19	12.8	14.7	12.5
20	43.1	42.8	42.6
21	14.3	18.0	14.2
22	110.5	110.7	110.5
23	32.5	32.5	32.2
24	29.5	29.7	29.1
25	31.1	31.1	31.2
26	67.7	67.8	67.7
27	16.6	19.8	16.7
1'	100.2	100.2	102.4
2'	79.3	79.3	72.9
3'	78.9	78.9	75.2
4'	71.4	71.4	80.3
5'	77.4	77.4	79.1
6'	62.6	62.6	60.6
1"	101.8	101.8	104.8
2"	71.9	71.9	84.8
3"	72.2	72.2	78.0
4"	73.5	73.5	77.9
5"	69.5	69.5	71.6
6"	18.8	18.8	63.0
1"'	106.1		106.1
2"'	76.1		76.1
3"'	75.2		75.2
4"'	73.5		73.5
5"'	70.5		70.5
6"'	61.4		61.4

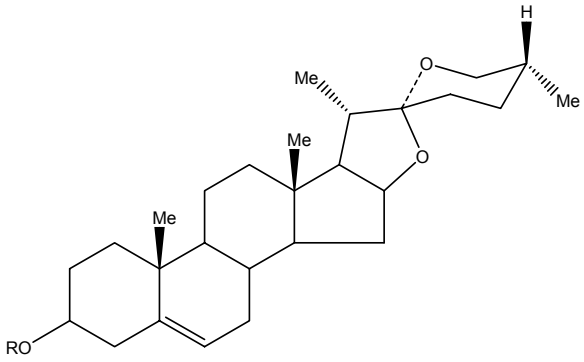
The ^1H NMR spectrum of **3** showed signals for four steroidal methyl groups at δ 0.89 (3H, s, Me-19), 0.82 (3H, s, Me-18), 1.00 (3H, d, H-21), 1.12 (3H, d, Me-27). The HMBC correlation of methyl groups clearly showed that the aglycon moiety was similar of compound **1** was identified as (25*R*)- 5α - spirostan-3β, 26-diol. One primary alcoholic function at δ 67.7 (C-26), suggesting the occurrence of a glycoside spirostanol skeleton. The ^1H NMR spectrum showed signals for three anomeric protons at δ 4.39 (1H, d, *J* = 7.5 Hz), 4.56 (1H, d, *J* = 7.5 Hz), and 4.69 (1H, d, *J* = 7.5 Hz). It was evident from the ^1H and ^{13}C NMR data that the sugar chain at C-3 of **3** consisted of three sugar units. The chemical shifts of all the individual protons of the three sugar units were ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C chemical shifts of their relative attached carbons could be assigned unambiguously from the HSQC spectrum. These data showed the presence of one β-galactopyranosyl unit (δ 4.40) and two β-glucopyranosyl unit (δ 4.56 and 4.69). A glycosidation shifts were observed for C-4_{gal} (δ 80.3) and for C-2_{glc}(δ 85.9). The HMBC spectrum showed key correlation peaks between the proton signal at δ 4.40 (H-1_{gal}) and the

carbon resonance at δ 79.2 (C-3 of the aglycon), the proton signal at δ 4.56 (H-1_{glc}) and the carbon resonance at δ 80.3 (C-4_{gal}), the proton signal at δ 4.69 (H-1_{glcI}) and the carbon resonance at δ 85.9 (C-2_{glc}). The proton signal at δ 4.26 (H-1_{glcII}) and the carbon resonance at δ 75.9 (C-26 of the aglycon). Thus, the structure of compound **3** was deduced as (25*R*) - 5α- spirostan - 3β, 26-diol 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl -(1→4)-*O*-β-D-galactopyranoside], which has been previously isolated from Solanum plants [12].



1: R=Rha(1-2)Glc

3: R=Glc(1-2)Glc(1-4)Gal



2: R=Rha(1-2)Glc

Conclusion

Three steroidal glycosides of spirostane series have been isolated for the first time from the seeds of *Hyoscyamus niger L*. During the investigation their structures have been determined as (25*R*) - 5α - spirostan - 3β, 26-diol - 3-*O*-[α-L-rhamnopyranosyl-(1→2) - β - D-glucopyranoside] for compound **1**, (25*R*)-spirost 5-ene-3β,26-diol 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyde] for compound **2**, and (25*R*) - 5α- spirostan - 3β, 26-diol 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl -(1→4)-*O*-β-D-galactopyranoside] for compound **3**. The given compounds have been earlier described in the literature.

Experimental

Plant Material has been collected in the scientific research field of the Institute of Scientific Researches and Technological Constructions for Tobacco and Tobacco Products of Moldova in November 2001 year. The voucher specimen has been deposited in the Laboratory of Selection

500 g of dry seeds were extracted three times in 50°C with n-buthanol saturated with water. After evaporation of n-butanol water extract was purified with chloroform and finally was crystallised in acetone. The residue was dried in vacuum at 40°C and the sum of steroidal saponins has been obtained in yellow powder in the yield 3,7%. 5g of extract has been chromatographied on silica gel column (30-500mm, 60-100μm, Merck). The column was washed with system chloroform-methanol-water (8:2:0→20:10:1) and 4-4ml fractions were collected. Fractions showing identical characteristics [TLC, silica gel, chloroform-methanol (4:1)] were combined. Obtained fractions were further separated on a C_{18} column (7,8x300mm, LiChroprep RP18, 25-40μm, X Terra Waters) using a MeOH/H_2O (60-80% MeOH) gradient. Three single compounds were obtained.

1. HRMS, *m/z* 725.321 [calculated for $C_{39}H_{64}O_{12}$ $(M)^+$]; 579.7 $[M-146]^+$; ^{1}H NMR (aglycon) δ 4.41 (1H, m, H-16), 3.77 (1H, m, H-3), 3.47 (1H, m, H-26a), 3.34 (1H, m, H-26b), 0.88 (3H, s, Me-19), 0.82 (3H, s, Me-18), 1.00 (3H, d, H-21), 1.12 (3H, d, Me-27). (sugars) 4.52 (d, *J*=7.5 Hz, H-1Glc), 3.48 (dd, *J*=7.5 and 9.0 Hz, H-2Glc), 3.37 (dd, *J*=9.0 and

9.0 Hz, H-3Glc), 3.29 (dd, *J*=9.0 and 9.0 Hz, H-4Glc), 3.27 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5Glc), 3.67 (dd, *J*=4.5 and 11.5 Hz, H-6aGlc), 3.88 (dd, *J*=2.5 and 11.5 Hz, H-6bGlc). 5.21 (d, H-1Rha), 3.95 (dd, H-2Rha), 3.67 (dd, H-3Rha), 3.42 (dd, H-4Rha), 4.16 (m, H-5Rha), 1.26 (d, H-6Rha). For ^{13}C NMR see Table 1.

2. HRMS, *m/z* 723.547 [calculated for $C_{39}H_{62}O_{12}$ $(M)^+$]; 577.5 $[M-146]^+$; ^{1}H NMR (aglycon) δ 4.41 (1H, m, H-16), 3.93 (1H, m, H-3), 3.47 (1H, m, H-26a), 3.34 (1H, m, H-26b), 1.02 (3H, s, Me-19), 0.84 (3H, s, Me-18), 0.82 (3H, d, H-21), 1.02 (3H, d, Me-27). (sugars) 4.52 (d, *J*=7.5 Hz, H-1Glc), 3.48 (dd, *J*=7.5 and 9.0 Hz, H-2Glc), 3.37 (dd, *J*=9.0 and 9.0 Hz, H-3Glc), 3.29 (dd, *J*=9.0 and 9.0 Hz, H-4Glc), 3.27 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5Glc), 3.67 (dd, *J*=4.5 and 11.5 Hz, H-6aGlc), 3.88 (dd, *J*=2.5 and 11.5 Hz, H-6bGlc). 5.21 (d, H-1Rha), 3.95 (dd, H-2Rha), 3.67 (dd, H-3Rha), 3. 42 (dd, H-4Rha), 4.16 (m, H-5Rha), 1.26 (d, H-6Rha). For ^{13}C NMR see Table 1.

3. HRMS, *m/z* 903.443 [calculated for $C_{45}H_{74}O_{18}$ $(M)^+$]; 741.6 $[M-162]^+$**;** 579 $[M-2x162]^+$**;** ^{1}H NMR (aglycon) δ 4.40 (1H, m, H-16), 3.69 (1H, m, H-3), 3.47 (1H, m, H-26a), 3.34 (1H, m, H-26b), 0.89 (3H, s, Me-19), 0.82 (3H, s, Me-18), 0.99 (3H, d, H-21), 1.27(3H, d, Me-27). (sugars) δ4.39 (d, *J*=7.4 Hz, H-1Gal), 3.60 (dd, *J*=7.4 and 9.0 Hz, H-2Gal), 3.53 (dd, *J*=4.0 and 9.0 Hz, H-3Gal), 4.02 (dd, *J*=2.5 and 4.0 Hz, H-4Gal), 3.69 (ddd, *J*=2.5, 2.5 and 4.5 Hz, H-5Gal), 3.62 (dd, *J*=4.5 and 12.0 Hz, H-6aGal), 3.95 (dd, *J*=2.5 and 12.0 Hz, H-6bGal), 4.56 (d, *J*=7.5 Hz, H-1Glc), 3.55 (dd, *J*=7.5 and 9.0 Hz, H-2Glc), 3.60 (dd, *J*=9.0 and 9.0 Hz, H-3Glc), 3.35 (dd, *J*=9.0 and 9.0 Hz, H-4Glc), 3.25 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5Glc), 3.60 (dd, *J*=4.5 and 11.5 Hz, H-6aGlc), 3.93 (dd, *J*=2.5 and 11.5 Hz, H-6bGlc), 4.69 (d, *J*=7.5 Hz, H-1GlcI), 3.29 (dd, *J*=7.5 and 9.0 Hz, H-2GlcI), 3.36 (dd, *J*=9.0 and 9.0 Hz, H-3GlcI), 3.40 (dd, *J*=9.0 and 9.0 Hz, H-4GlcI), 3.42 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5GlcI), 3.69 (dd, *J*=4.5 and 11.5 Hz, H-6aGlcI), 3.82 (dd, *J*=2.5 and 11.5 Hz, H-6bGlcI). For ^{13}C NMR see Table 1.

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