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STEROID SAPOGENINS FROM *NOTHOSCORDUM GRAMINEUM* VAR. *PHILIPPIANUM* AND *TRISTAGMA UNIFLORUM*

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Key Word Index—*Nothoscordum gramineum* var. *philippianum*; *Tristagma uniflorum*; Liliaceae; steroid sapogenins.

Abstract—From *Nothoscordum gramineum* var. *philippianum* the known sapogenins gitogenin, $\Delta^{25,27}$ gitogenin, and agigenin, in addition to the new (20*S*,22*R*)-5α-spirost-25(27)-en-2α,3β,6β-triol, (20*S*,22*S*,25*S*)-furost-5-en-22,25-epoxy-2α,3β,26-triol, and (20*S*,22*S*,25*S*)-5α-furostan-22,25-epoxy-2α,3β,6β,26-tetraol were isolated. From *Tristagma uniflorum* the new (20*S*,22*R*)-5α-spirost-5-en-3β-ol was also obtained. All compounds were characterized by spectroscopic (IR, ^{1}H NMR, ^{13}C NMR, MS) methods.

INTRODUCTION

In a previous paper [1] we reported the isolation of the steroid sapogenins tigogenin, neotigogenin, (20*S*,22*R*,25*S*)-5α-spirostan-3β,25-diol, (20*S*,22*R*,25*R*)-5α-spirostan-3β,25-diol, (20*S*,22*S*,25*S*)-5α-furostan-22,25-epoxy-3β,26-diol and (20*S*,22*S*,25*R*)-5α-furostan-22,25-epoxy-3β,26-diol from bulbs of *Tristagma uniflorum*. Continuing with the investigation on species belonging to the Liliaceae, we have isolated seven sapogenins, three of which, isolated from *Nothoscordum gramineum* var. *philippianum*, were the previously known gitogenin, $\Delta^{25,27}$ gitogenin (**5**) [2], and agigenin (**6**) [3], and the other three were, to the best of our knowledge, new substances whose structures were established as (20*S*,22*R*)-5α-spirost-25(27)-en-2α,3β,6β-triol (**2**), (20*S*,22*S*, 25*S*)-furost-5-en-22,25-epoxy-2α,3β,26-triol (**1**), and (20*S*,22*S*,25*S*)-5α-furostan-22,25-epoxy-2α,3β,6β,26-tetraol (**3**). Besides, from *Tristagma uniflorum* the not previously reported (20*S*,22*R*)-5α-spirost-25(27)-en-3β-ol (**4**) was also isolated.

RESULTS AND DISCUSSION

Dried bulbs of *Nothoscordum gramineum* var. *philippianum* were extracted following a published procedure [4]. The saponins were hydrolysed and the free sapogenins were separated by chromatographic (column and preparative TLC) methods, leading to the isolation of gitogenin, $\Delta^{25,27}$ gitogenin (**5**), agigenin (**6**), and compounds **1**–**3**.

Compound **1** was purified as its triacetyl derivative, $C_{33}H_{48}O_8$ ([M]$^+$ *m/z* 572), and showed in its mass spectrum an important fragment at *m/z* 449 ([M $-CH_2OAc]^+$) and the base peak at *m/z* 197 which in addition to the common fragments from ring F having a hydroxyl group [5, 6] suggested the presence of a hydroxylated 'furanose' F-ring. Its mass spectrum also displays prominent peaks in the high mass range at *m/z* 445, 442, 400, 398, 385 and 371 characteristic of an unsaturated dihydroxyspirostene moiety. The ^{1}H NMR spectrum of the triacetyl derivative (Table 1) showed a singlet at δ 1.17 assigned to Me-27, a value consistent with a methyl group being attached to a 'furanose' F-ring with a β-orientation [1, 7]. The downfield chemical shift values for the two protons at C-26 indicated the presence of a primary acetoxy group at this carbon. A wide doublet at δ 5.44 for the vinylic proton at C-6 and the downfield shift of the Me-19 which appears at δ 1.12 confirmed the presence of two acetoxy groups at C-2α and at C-3β in a spirost-5-en derivative. From all data, compound **1** was identified as (20*S*,22*S*,25*S*)-furost-5-en-22,25-epoxy-2α,3β,26-triol.

Compound **2** was purified as its triacetyl derivative, $C_{33}H_{48}O_8$ ([M]$^+$ *m/z* 572). Its MS showed important fragment peaks in the high mass range at *m/z* 505, 502, 460, 445 and 431 characteristic of saturated trihydroxy spirostanes. The presence of the base peak at *m/z* 137 and ions at *m/z* 124 and 113 were indicative of an exocyclic methylene group attached to C-25 [7, 8]. The IR spectrum lacked the characteristic band of the spirostane ring, but showed absorptions at 3080, 925 and 880 cm^{-1} typical of an exocyclic methylene group [9]. The ^{1}H NMR spectrum of the acetyl derivative (Table 1) lacked the doublet normally observed for the Me-27 of a saturated spirostane. Moreover, the protons at C-26 appeared as an AB quartet at δ 3.84 and 4.31. The geminal protons of the methylene group were observed at δ 4.74 as a broad singlet which is characteristic of an exocyclic methylene [7]. The singlet at δ 1.12, assigned to Me-19 as well as a broad set of lines centered at δ 4.95 and at 4.98 respectively assigned to H-2β, H-3α, and to H-6α, were indicative that compound **2** has a (20*S*,22*R*)-5α-spirost-25(27)-en-2α,3β,6β-triol structure.

Compound **3** was purified as its tetraacetyl derivative ([M]$^+$ *m/z* 632) and showed in its mass spectrum the base peak at *m/z* 197 and an intense fragment at *m/z* 572 ([M–$CH_2OAc]^+$) suggesting the presence of a hydroxylated F-ring. The prominent fragment peaks in the high mass range at *m/z* 505, 502, 460, 458, 445 and 431 were characteristic of trihydroxy spirostanes. The ^{1}H NMR spectrum of the tetraacetyl derivative presented, besides

Table 1. ^{1}H NMR data for compounds **1**–**6** as their respective acetyl derivatives (100 MHz, $CDCl_3$–TMS)

H	1	2	3	4	5	6
Me-18	0.77 *s*	0.80 *s*	0.79 *s*	0.78 *s*	0.78 *s*	0.80 *s*
Me-19	1.12 *s*	1.12 *s*	1.11 *s*	0.84 *s*	0.94 *s*	1.12 *s*
Me-21	0.96 *d*, $J=7$	0.96 *d*, $J=7$	0.95 *d*, $J=7$	0.95 *d*, $J=7$	0.95 *d*, $J=7$	0.96 *d*, $J=7$
H-27	1.17 *s*	4.74 *br s*	1.17 *s*	4.75 *br. s*	4.78 *br. s*	0.79 *d*, $J=7$
Me–CO	2.01 *s*	2.01 *s*	2.01 *s*	2.03 *s*	2.03 *s*	2.00 *s*
	2.02 *s*	2.02 *s*	2.02 *s*			2.01 *s*
	2.08 *s*	2.05 *s*	2.05 *s*			2.05 *s*
			2.08 *s*			
H-26β	3.93 *d*, $J=11$	3.84 *d*, $J=12$	3.90 *d*, $J=11$	3.86 *d*, $J=12$	3.86 *d*, $J=12$	3.41 *m*
H-26α	4.19 *d*, $J=11$	4.31 *d*, $J=12$	4.20 *d*, $J=11$	4.31 *d*, $J=12$	4.30 *d*, $J=12$	
H-16	4.40 *m*	4.42 *m*	4.37 *m*	4.30 *m*	4.40 *m*	4.36 *m*
H-3α / H-2β	4.93 *m*	4.95 *m*	4.90 *m*	4.80 *m*	5.01 *m*	4.96 *m*
H-6α	5.44 *m*	4.98 *m*	4.99 *m*			5.01 *m*

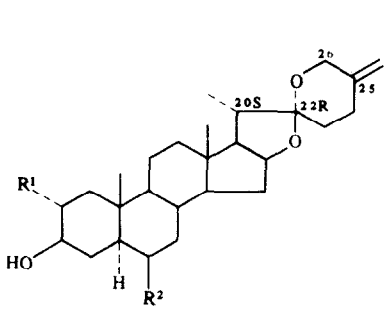
singlets for Me-18 and Me-19 at δ0.79 and δ1.11 respectively, a further singlet at δ1.17 assigned to Me-27. The resonance value of this methyl group confirmed a 'furanose' structure with the methyl group in the β-orientation. The observed downfield shift of the two protons at C-26 confirmed the presence of a primary acetoxy group at this carbon. The broad set of signals between δ4.60–5.20 assigned to H-2β and H-3α, and that at δ4.99 assigned to H-6α, corroborated the presence of 2α, 3β, and 6β acetoxy groups in a spirostane structure. Hence, compound **3** was identified as (20*S*,22*S*,25*S*)-5α-furostan-22,25-epoxy-2α,3β,6β,26-tetraol.

Compound **4**, isolated from *Tristagma uniflorum* as previously described [1], was purified as its monoacetyl derivative, $C_{29}H_{44}O_4$ ([M]$^+$ *m/z* 456). The presence of the base peak at *m/z* 137 and important fragments at *m/z* 124 and 113 were indicative of an exocyclic methylene group at C-25. Moreover, ions in the high mass range at *m/z* 445, 442, 400, 398, 385 and 371 characterized a saturated monohydroxy spirostane. The IR spectrum showed absorptions at 3080, 925 and 880 cm^{-1} which were also characteristic of a spirostane ring. The ^{1}H NMR spectrum of the monoacetyl derivative showed a downfield shift of the signal from the methylene group at C-26 which appears as an AB quartet at δ3.86 and 4.31; besides, the spectrum showed two vinylic protons at δ4.75 as a broad singlet which accounted for the protons at C-27. The signals at δ0.78, 0.84 and 2.03 respectively assigned to Me-18, Me-19 and Me-acetoxy group indicated a monacetoxy spirostane derivative. Hence, compound **4** is the (20*S*,22*R*)-5α-spirost-25(27)-en-3β-ol.

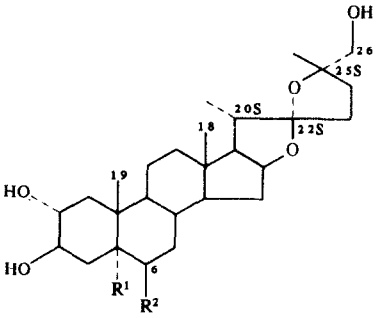
The ^{13}C NMR spectra of the sapogenins, as their acetyl derivatives, are shown in Table 2. The assignments were done by comparison with spectra reported in literature [10, 11], by calculations using chemical shift considerations, and by 'attached proton test' experiments. Some of the isolated sapogenins are likely to be artefacts formed during the hydrolysis treatment.

EXPERIMENTAL

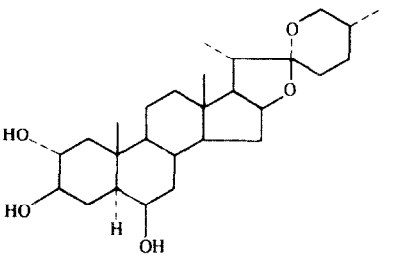
Nothoscordum gramineum var. *philippianum* was collected near Carmen de Patagones (Argentina) and *Tristagma uniflorum* near Bahía Blanca (Argentina). Voucher specimens have been deposited at the Herbarium of the Department of Agricultural Sciences of Universidad Nacional del Sur under N° BB-3786 and N° BB-1637 respectively.



2 R^1 = OH; R^2 = OH
4 R^1 = H; R^2 = H
5 R^1 = OH; R^2 = H



1 R^1, R^2 = Δ^5
3 R^1 = H; R^2 = OH



6

Table 2. ^{13}C NMR data for compounds **1–6** as their respective acetyl derivatives (25.2 MHz, $CDCl_3$)

C	1	2	3	4	5	6
1	42.3	43.4	43.4	36.6	42.3	43.4
2	71.4	71.3	71.4	27.4	71.8	71.3
3	74.4	74.1	74.1	73.6	74.5	74.1
4	38.0	29.6	29.7	34.0	27.5	29.6
5	137.3	45.6	45.6	44.5	44.1	45.6
6	123.2	72.0	72.0	28.5	29.6	71.9
7	31.9	36.3	36.3	32.1	31.8	36.3
8	31.7	29.9	29.9	35.0	34.3	29.9
9	49.8	53.6	53.6	54.1	53.9	53.6
10	36.3	37.0	37.0	35.5	37.1	37.0
11	20.9	20.9	20.9	21.0	21.1	20.9
12	39.5	39.4	39.5	39.9	39.7	39.6
13	40.3	40.6	40.6	40.4	40.5	40.5
14	56.1	55.5	55.4	56.2	56.0	55.5
15	31.9	31.6	31.6	31.7	31.6	31.6
16	80.5	80.8	80.4	81.0	81.0	80.5
17	61.6	62.1	61.5	62.2	62.1	62.0
18	16.1	16.5	16.3	16.5	16.4	16.5
19	20.0	15.9	15.9	12.2	13.0	15.9
20	38.2	41.5	38.2	41.5	41.5	41.6
21	14.6	14.5	14.5	14.5	14.5	14.5
22	119.9	109.1	119.8	109.2	109.2	109.1
23	32.7	28.5	32.7	28.5	28.5	31.3
24	32.9	32.8	32.8	32.8	32.7	28.7
25	82.2	143.4	82.3	143.5	143.4	30.2
26	70.3	64.8	70.2	64.8	64.8	66.7
27	23.8	108.5	23.8	108.4	108.5	17.1
$\underline{Me}CO_2R$	21.1	21.1	21.1	21.4	21.1	21.1
	21.1	21.1	21.1			21.1
		21.3	21.3			21.3
$Me\underline{C}O_2R$	170.1	170.2	170.2	170.4	170.3	170.2
	170.2		170.8			

Isolation of sapogenins. (1) From *N. gramineum* var. *philippianum.* Dried and powdered bulb (580 g) was extracted and the extract was hydrolysed as described in ref. [5] yielding raw aglycones (1.86 g). This product was chromatographed on a silica gel 60 (70–230 mesh) column. The fractions were monitored by TLC on silica gel ($CHCl_3$–Me_2CO, 19:1 and 12:1). Elution of the column with $CHCl_3$–MeOH (49:1) afforded a product which after acetylation was separated by prep. TLC ($AgNO_3$–silica gel, $CHCl_3$–Me_2CO, 49:1, two developments) and chracterized as gitogenin and $\Delta^{25,27}$ gitogenin by comparison (IR, mp, ^1H NMR) with authentic standards. Elution of the original column with $CHCl_3$–MeOH (24:1) yielded a crystalline mixture that was acetylated and separated by prep. TLC ($AgNO_3$–silica gel, $CHCl_3$–MeOH, 49:1, two developments) to give compound **1**. Elution with $CHCl_3$–MeOH (16:1) afforded a crystalline product which, after acetylation, was separated by CC ($AgNO_3$–silica gel, hexane–$CHCl_3$, 1:4) and prep. TLC ($AgNO_3$–silica gel, $CHCl_3$–Me_2CO, 49:1, two developments) to give agigenin (**6**) and compounds **2** and **3**. (ii) From *Tristagma uniflorum.* This was performed as described in ref. [1] leading to the isolation of tigogenin, neotigogenin and compound **4**.

(20S,22S,25S)-*Furost-5-en*-22,25-*epoxy*-2α,3β,26-*triol triacetate* (**1**). This was recrystallized from MeOH, mp 187–190°; IR ν^{KBr} cm^{-1}: 1730 (C=O), 1235 (ester), 960, 915, 870; MS *m/z* (rel. int.): 572 $[M]^+$ (2), 512 (5), 499 (92), 400 (8), 371 (10), 385 (5), 340 (7), 280 (6), 197 (100), 184 (16), 173 (10), 137 (23).

(20S,22R)-5α-*Spirost*-25(27)-*en*-2α,3β,6β-*triol triacetate* (**2**). This was recrystallized from MeOH, mp 125–130°; IR ν^{KBr} cm^{-1}: 3080 (=CH_2), 1730 (C=O), 1240, 1235 (ester), 980, 960, 938, 925, 900 (spiroketal chain), 880 (=CH_2); MS *m/z* (rel. int.): 572 $[M]^+$ (12), 505 (5), 502 (6), 445 (5), 431 (5), 400 (12), 385 (6), 371 (6), 340 (54), 325 (6), 280 (66), 265 (5), 251 (7), 137 (100), 124 (5), 122 (5), 113 (10).

(20S,22S,25S)-*Furostan*-22,25,-*epoxy*-2α,3β,6β,26-*tetraol tetraacetate* (**3**). Was recrystallized from MeOH, mp 214–218°; IR ν^{KBr} cm^{-1}: 1735 (C=O), 1240, 1233 (ester), 999, 955, 930, 918, 899, 870 (spiroketal chain); MS *m/z* (rel. int.): 632 $[M]^+$ (10), 572 (10), 559 (75), 460 (7), 435 (5), 400 (7), 371 (15), 280 (12), 251 (10), 197 (100), 184 (97), 173 (9), 137 (81), 124 (10), 122 (6).

(20S,22R)-5α-*Spirost*-25(27)-*en*-3β-*ol monoacetate* (**4**). Was recrystallized from Me_2CO–MeOH (1:9); mp 182–185°; IR ν^{KBr} cm^{-1}: 3080 (=CH_2), 1720 (C=O), 1235 (ester), 978, 955, 925, 898 (spiroketal chain), 870 (=CH_2); MS *m/z* (rel. int.): 456 $[M]^+$ (5), 441 (10), 386 (14), 344 (44), 329 (24), 315 (50), 284 (12), 269 (16), 255 (21), 137 (100), 122 (33), 113 (13).

$\Delta^{25,27}$*Gitogenin diacetate* (**5**). Was recrystallized from MeOH; mp 210–213° Lit. [5] mp 218–220°; IR ν^{KBr} cm^{-1}: 3080 (=CH_2), 1740 (C=O), 1250, 1230 (ester), 975, 950, 925, 900 (spiroketal chain), 880 (=CH_2); MS *m/z* (rel. int.): 514 $[M]^+$ (5), 499 (3), 444 (11), 402 (25), 387 (22), 373 (24), 342 (22), 253 (12), 137 (100), 124 (8), 122 (21), 113 (25).

(20S,22R,25R)-5α-*Spirostane*-2α,3β,6β-*triol triacetate* (*acetylagigenin*) (**6**). was recrystallized from MeOH; mp 128–134°, Lit. [3] mp 126–130°; IR ν^{KBr} cm^{-1}: 1740 (C=O), 1245, 1235, 1225 (ester), 982, 920, 900, 860 (spiroketal chain); MS *m/z* (rel. int.): 574 $[M]^+$ (12), 559 (2), 502 (80), 460 (10), 431 (10), 400 (70), 371 (12), 340 (72), 280 (70), 251 (10), 139 (100), 126 (12), 115 (63).

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