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STEROID SAPOGENINS FROM *TRISTAGMA UNIFLORUM*

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Key Word Index—*Tristagma uniflorum*; Liliaceae; steroid sapogenins.

Abstract—From bulbs of *Tristagma uniflorum* the known sapogenins tigogenin, neotigogenin and (20*S*,22*R*,25*S*)-5α-spirostan-3β,25-diol, as well as the new (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, were isolated and characterized by spectroscopic (IR, ^{1}H NMR, ^{13}C NMR, MS) methods.

INTRODUCTION

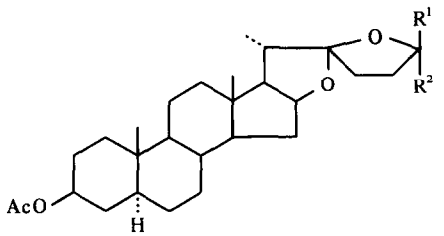
Species belonging to the family Liliaceae frequently produce steroid saponins [1], some of them being of commercial importance as potential sources for steroid hormones. In a project related to the search for sapogenins from natural sources, we have studied the sapogenins present in the bulbs of *Tristagma uniflorum* (Lindl.) Traub. and this report describes the isolation and structure elucidation of three steroid sapogenins which, to the best of our knowledge, have not previously been found in plants.

RESULTS AND DISCUSSION

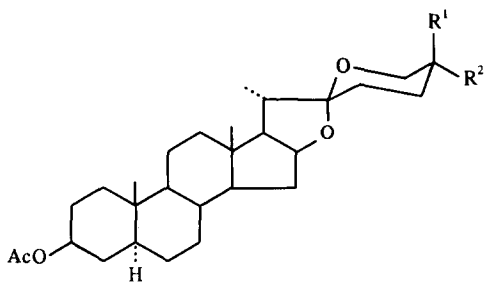
The dried vegetable material was extracted following a published procedure [2]. After acid hydrolysis the sapogenins were separated by chromatographic (column and preparative TLC) procedures leading to the isolation of tigogenin, neotigogenin and compounds **1**–**4**, the last of which had been previously found in a *Solanum* species [3].

Compound **1** was purified as its diacetyl derivative, $C_{31}H_{48}O_6$ ($[M]^+$ *m/z* 516), and showed in its mass spectrum an intense fragment at *m/z* 443 ($[M - CH_2OAc]^+$) which in addition to the common fragments from ring F having a hydroxyl group [4, 5] suggested the presence of a hydroxylated 'furanose' F-ring. This suggestion was further confirmed by its IR spectrum, which indicated the presence of a hydroxyl group at ring F [6, 7]. The ^{1}H NMR spectrum of the diacetyl derivative (Table 1) showed a singlet at δ1.35 assigned to Me-27; this chemical shift was consistent with a methyl group being attached to a 'furanose' F-ring in an α-orientation. Moreover, the downfield shift of the two protons at C-26 compared to the values obtained for similar protons of the monoacetylated compound **2** (see below) also favoured the presence of a primary hydroxyl group at C-26 (furanose series). Accordingly, compound **1** was identified as (20*S*,22*S*,25*R*)-5α-furostan-22,25-epoxy-3β,26-diol.

Compound **2** was purified as its crystalline monoacetyl derivative, $C_{29}H_{46}O_5$ ($[M]^+$ *m/z* 474), which presented in its mass spectrum a fragment at *m/z* 443 ($[M - CH_2OH]^+$) suggesting the presence of a hydroxylated 'furanose' F-ring. The IR spectrum showed absorption of a free hydroxyl group but diverged in the spiroketal region from the spectra of normal spirostanes; the absorp-



	R^1	R^2
1	CH_2OAc	Me
2	Me	CH_2OH
2a	Me	CH_2OAc



	R^1	R^2
3	Me	OH
4	OH	Me

Table 1. ^{1}H NMR data for compounds 1–4 (100 MHz, $CDCl_3$, TMS)*

H	1	2	2a	3	4
Me-18	0.76 *s*	0.78 *s*	0.74 *s*	0.77 *s*	0.78 *s*
Me-19	0.84 *s*	0.84 *s*	0.82 *s*	0.84 *s*	0.83 *s*
Me-21	0.95 *d*, $J=7$	0.97 *d*, $J=7$	0.94 *d*, $J=7$	0.96 *d*, $J=7$	1.01 *d*, $J=7$
Me-27	1.35 *s*	1.17 *s*	1.17 *s*	1.30 *s*	1.11 *s*
Me-CO	2.03 *s*	2.03 *s*	2.05 *s*	2.02 *s*	2.02 *s*
	2.07 *s*		2.08 *s*		
H-26β	3.81 *d*, $J=11$	3.39 *m*	3.81 *d*, $J=11$	3.22 *dd*, $J=11$; 3	3.24 *dd*, $J=12$; 3
H-26α	3.99 *d*, $J=11$		4.16 *d*, $J=11$	3.60 *d*, $J=11$	3.75 *d*, $J=12$
H-16	4.48 *m*	4.50 *m*	4.38 *m*	4.40 *q*, $J=7.5$	4.39 *q*, $J=8$
H-3	4.72 *m*	4.68 *m*	4.68 *m*	4.70 *m*	4.68 *m*

*Coupling constants (J) in Hz.

tions below 1000 cm^{-1} indicated that the free hydroxyl group was located at the F-ring [6, 7]. This structure was confirmed by the mass spectrum, which in addition to the fragment at m/z 443, presented further F-ring fragments at m/z 155 and 137 pointing to a hydroxylated F-ring [4, 8]. The ^{1}H NMR spectrum presented, besides singlets for Me-18 and Me-19, a further singlet at δ1.17 assigned to Me-27. Comparison of the resonance of this Me-27 with spirostanes having a hydroxyl group attached to ring F confirmed a 'furanose' structure for this ring with the methyl group in the β-orientation [7, 9]. Acetylation of the monoacetyl derivative afforded a diacetylated compound (**2a**) whose spectroscopic properties indicated that it had the same configuration at C-25 as nuatigenin [10]. Hence, compound 2 was identified as (20*S*,22*S*,25*S*)-5α-furostan-22,25-epoxy-3β,26-diol.

Compound **3**, $C_{29}H_{46}O_5$ as monoacetate, had the same spectroscopic properties as compound **4** except that the resonance of the methyl protons at C-27 in the ^{1}H NMR spectrum appeared at δ1.30 [11, 12] and those of the spiroketal side-chain carbon atoms (C-24, C-25, C-26 and C-27) in the ^{13}C NMR spectrum indicated an opposite configuration of C-25 [12]. Therefore, **3** is (20*S*,22*R*,25*R*)-5α-spirostan-3β,25-diol.

The ^{13}C NMR spectra of the four sapogenins, as their acetyl derivatives, are shown in Table 2. The indicated assignments were done by comparison with spectra reported in the literature [12], by calculations using chemical shift considerations, and by the use of compiled data [13].

Some of the sapogenins are likely to be artefacts formed during the hydrolysis process.

EXPERIMENTAL

T. uniflorum was collected near Bahía Blanca (Argentina) and a voucher specimen has been deposited at the Herbarium of the Department of Agricultural Sciences of Universidad Nacional del Sur under No. BB 1637.

Isolation of sapogenins. Dried and powdered bulbs (1.35 kg) were extracted and the extract was hydrolysed as described in ref. [2], yielding 2.88 g of raw aglycones. This product was chromatographed on a neutral alumina column. The fractions were monitored by TLC on silica gel ($CHCl_3$–Me_2CO, 19:1). Elution with toluene–$CHCl_3$ (99:1) afforded a crystalline product (two spots on TLC), which after acetylation was separated by CC ($AgNO_3$–silica gel, hexane–toluene, 1:1) and prep. TLC (silica gel, $CHCl_3$–toluene, 9:1) and characterized as tigogenin and neotigogenin by comparison (IR, mp, ^{1}H NMR) with authentic standards. Elution of the original column with $CHCl_3$–MeOH (19:1) yielded a crystalline mixture that was acetylated and separated by prep. TLC (silica gel, $CHCl_3$–Me_2CO, 19:1) to give compounds **1**–**4**.

(20S,22S,25R)-5α-*Furostan*-22,25-*epoxy*-3β,26-*diol diacetate* (**1**) was recrystallized from MeOH, mp 191–193°; IR ν_{max}^{KBr} cm^{-1}: 1740, 1730 (C=O), 1240, 1230 (ester), 995, 950, 925, 865, 835 (spiroketal chain); MS m/z (rel. int.): 516 $[M]^+$ (2), 501 $[M-Me]^+$ (2), 443 $[M-CH_2OAc]^+$ (83), 344 (39), 329 (16), 315 (43), 197 (100), 185 (22), 155 (11), 137 (31).

Table 2. ^{13}C NMR data for compounds 1–4 (25.2 MHz, $CDCl_3$)

C	1	2	3	4
1	36.6	36.9	36.7	36.7
2	27.4	28.1	27.5	27.4
3	73.6	73.8	73.6	73.5
4	33.3	33.5	34.0	33.9
5	44.5	44.6	44.6	44.6
6	28.4	28.1	28.5	28.4
7	31.7	32.1	32.2	32.1
8	35.0	34.9	35.7	35.1
9	54.1	54.2	54.2	54.2
10	35.5	35.8	35.6	35.5
11	21.4	21.4	21.4	21.4
12	38.2	38.4	39.9	39.9
13	39.9	40.1	40.9	40.6
14	56.1	56.2	56.2	56.2
15	32.1	32.4*	31.6	31.6
16	80.6	80.6	81.1	81.3
17	61.9	61.9	62.0	61.9
18	16.3	16.4	16.5	16.4
19	12.2	12.3	12.2	12.2
20	40.6	40.6	41.0	41.5
21	14.6	14.8	14.3	14.4
22	120.0	119.9	108.7	108.9
23	33.3	32.5*	23.9	24.6
24	33.9	34.1	34.9	32.7
25	81.9	83.9	67.4	66.6
26	69.6	69.6	69.1	68.9
27	25.9	25.0	29.7	27.0

*Values may be interchanged.

(20S,22S,25S)-5α-*Furostan*-22,25-*epoxy*-3β,26-*diol*-3-*monoacetate* (**2**). Needles from MeOH, mp 206–208°; IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1740 (C=O), 1240 (ester), 990, 970, 955, 915, 895, 875 (spiroketal chain); MS *m/z* (rel. int.): 474 $[M]^+$ (2), 443 $[M-CH_2OH]^+$ (14), 386 (29), 344 (14), 315 (49), 255 (18), 155 (100), 137 (9).

Acetylation (Ac_2O–C_5H_5N) of **2** gave the diacetyl derivative **2a** as colourless needles, mp 183–186° (MeOH). The IR spectrum showed the disappearance of the free hydroxyl group band.

(20S,22R,25R)-5α-*Spirostan*-3β,25-*diol monoacetate* (**3**) was crystallized from MeOH, mp 211–213°; IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1730 (C=O), 1250 (ester), 1030, 990, 925, 890, 850 (spirostan); MS *m/z* (rel. int.): 474 $[M]^+$ (1), 443 $[M-CH_2OH]^+$ (70), 389 (7), 386 (79), 344 (16), 329 (13), 315 (68), 255 (30), 155 (100).

(20S,22R,25S)-5α-*Spirostan*-3β,25-*diol monoacetate* (**4**), mp 230–232° (MeOH) (lit. [3] mp 240°). IR ν_{max}^{KBr} cm^{-1}: 3580 (OH), 1730 (C=O), 1240 (ester), 970, 955, 940, 905, 880, 850 (spirostan); MS *m/z* (rel. int.): 474 $[M]^+$ (3), 443 $[M-CH_2OH]^+$ (12), 389 (9), 386 (99), 344 (15), 329 (9), 315 (30), 255 (13), 155 (100). Its ^1H NMR and ^{13}C NMR spectra (see Tables) resembled those of 5α,6-dihydroisonuatigenin (isocaelagenin) [3, 10].

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DISTRIBUTION OF ANTHRAQUINONE PIGMENTS IN *RUMEX* SPECIES OF KENYA

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Key Word Index—*Rumex*; Polygonaceae; anthraquinones; chrysaphanol; emodin; physcion; nepodin; distribution.

Abstract—A correspondence between pigment structures and leaf morphology has been found in Kenyan *Rumex* species.

The genus *Rumex* is represented in Kenya by five species [1, 2]. Of the Kenyan species listed in Table 1, only *R. abyssinicus* has attracted chemical study on two occasions previously. In 1962, it was reported to contain chrysophanic acid (chrysophanol) [3]. More recently work in our laboratories has revealed the existence of chrysophanol, physcion and emodin [4]. We now investigated all five Kenyan species including *R. abyssinicus* which was collected in another locality than the plants formerly [4] analysed in order to characterize their pigments. Results are presented in Table 1.

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EXPERIMENTAL

Plant materials. The plants were collected from different parts of the high altitude areas of Kenya and authenticated at the