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New steroidal saponins from *Agave lophantha* Schiede and their pharmacological evaluation

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The structures of one new monodesmosidic spirostanoside and one new bisdesmosidic furanostanol glycoside isolated from leaves of *Agave lophantha* Schiede have been determined by means of spectroscopic and chemical methods as (25*R*)-5β-spirostan-3β-ol-3-*O*-{β-D-apiofuranosyl(1→4)β-D-glucopyranosyl(1→3)[β-D-glucopyranosyl(1→2)]β-D-galactopyranoside} and 26-*O*-β-D-glucopyranosyl(25*R*)-5β-furost-20(22)-ene-3β,26-diol-3-*O*-{β-D-xylopyranosyl(1→3)-[β-D-glucopyranosyl(1→2)]β-D-galactopyranoside}, respectively. The ^{1}H and ^{13}C NMR resonances of the two compounds were assigned by NMR (^{1}H, ^{13}C, HOHAHA, ^{1}H-^{1}H COSY, HMQC, HMBC, NOE difference) studies. The pharmacological activities of the saponin containing fraction are discussed.

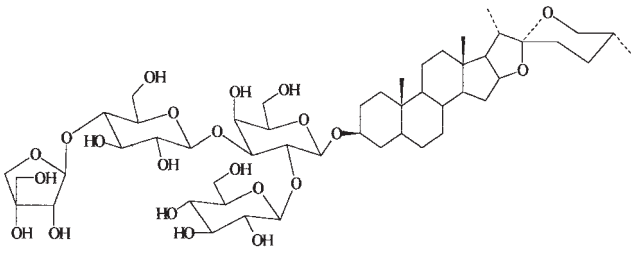
1. Introduction

Agave plants are annual and perennial herbs distributed in tropical and subtropical regions. A survey of the literature showed that several *Agave* species have already been chemically studied and found to contain steroidal saponins [1–3]. As a part of our project on investigating steroidal saponins and their pharmacological properties, we studied their content in *A. lophantha* Schiede. In this paper, we present the isolation and structural elucidation of two steroidal saponins **1** and **2** from the saponin fraction of *A. lophantha* and its analgesic, anti-inflammatory and anti-ulcer activities.

2. Investigations, results and discussion

2.1. Chemistry

The methanolic extract of the leaves of *Agave lophantha* was concentrated then diluted with acetone to afford a crude saponin mixture. After fractionation using VLC, the saccharides contained in the mixture were removed by passage through a porous ion-exchange resin (Diaion HP-20) column. The obtained material was repeatedly separated and purified using CC and HPLC to yield two new saponins **1** and **2**. The ^{1}H and ^{13}C NMR data of **1** and **2** are shown in Tables 1 and 2.



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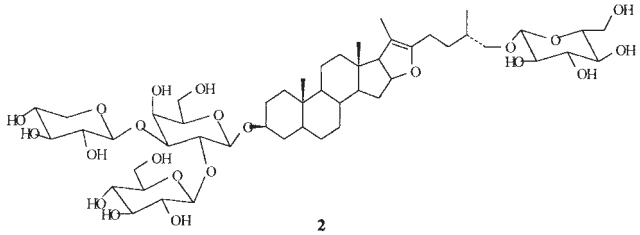

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Compound **1** was assigned the molecular formula $C_{50}H_{82}O_{22}$ by ^{13}C NMR data and positive-ion FAB MS which showed a quasi-molecular ion peak $[M + Na]^+$ at m/z 1057. Fragment ions at m/z 925 and 763, arising from sequential loss of a pentose and a hexose, were observed. The ^{1}H NMR spectrum of **1** exhibited two-proton singlet signals at δ 0.83 and 0.97 indicating the presence of two angular methyl groups as well as two-proton doublets signals at δ 1.15 (J = 7.0 Hz) and 0.71 (J = 5.7 Hz) assignable to two secondary methyl groups. The structure of **1**, based upon a spirostanol derivative, was suggested by the above ^{1}H NMR data and by a quaternary carbon

Table 1: ^{1}H and ^{13}C chemical shifts of the aglycone part of compounds 1 and 2 in pyridine-d_5

	1		2	
	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	30.7	1.43, 1.80	30.8	
2	26.9	1.47, 1.87	26.9	1.50, 1.90
3	75.2	4.30	75.2	4.34
4	30.8	1.80, 1.80	30.8	
5	36.7	2.20	36.7	2.22
6	27.1	1.18, 1.86	26.9	1.20, 1.85
7	26.8	0.97, 1.29	26.9	0.98, 1.27
8	35.6	1.51	35.3	1.47
9	40.4	1.30	40.3	1.31
10	35.3		35.3	
11	21.2	1.12, 1.32	21.4	1.18, 1.34
12	40.4	1.09, 1.69	40.3	1.17, 1.74 (brd, 10.1)
13	41.0		43.9	
14	56.5	1.08	54.8	0.86
15	32.2	1.41, 2.03	34.5	1.46, 2.11
16	81.3	4.61	84.6	4.82
17	63.2	1.85	64.8	2.49 (d, 10.4)
18	16.6	0.83 (s)	14.4	0.72 (s)
19	24.0	0.97 (s)	24.0	0.98 (s)
20	42.1	1.97	103.6	
21	15.0	1.15 (d, 7.0)	11.8	1.64 (s)
22	109.2		152.4	
23	31.9	1.65, 1.73	23.7	2.23, 2.23
24	29.3	1.58, 1.58	31.5	1.48, 1.85
25	30.9	1.60	33.5	1.96
26	66.9	3.52 (t, 11.1), 3.59 (dd, 11.1, 3.3)	75.0	3.64, 3.95
27	17.3	0.71 (d, 5.7)	17.4	1.04 (d, 6.3)

Table 2: ^{1}H and ^{13}C chemical shifts of the sugar moities of compounds 1 and 2 in pyridine-d_5

	1		2	
	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
Galactose				
1	102.2	4.84 (d, 7.8)	102.2	4.91 (d, 7.6)
2	78.0	4.79 (t, 9.0)	77.9	4.82 (t, 8.2)
3	84.0	4.31 (dd, 9.0, 3.2)	84.3	4.29 (dd, 8.8, 2.9)
4	70.0	4.71 (d, 3.2)	69.9	4.78
5	76.4	3.95	76.4	4.04
6	62.4	4.26	62.2	4.38
6′		4.36		4.43
Glucose I				
1	104.5	5.55 (d, 7.8)	104.5	5.56 (d, 7.8)
2	76.5	4.03 (t, 8.3)	76.4	4.04
3	78.5	4.20 (t, 8.3)	78.7	4.20 (t, 9.1)
4	72.7	4.16 (8.3)	72.8	4.16 (t, 9.1)
5	77.9	3.83 (m)	77.7	3.78 (m)
6	63.6	4.37 (dd, 12.6, 5.0)	63.6	4.34
6′		4.48 (dd, 12.6, 3.8)		4.46 (dd, 12.0, 3.3)
Glucose II			*Xylose*	
1	105.1	5.33 (d, 7.9)	1 106.3	5.23 (d, 7.6)
2	75.2	3.93 (t, 8.2)	2 75.2	3.94 (t, 8.0)
3	76.5	4.16 (t, 8.2)	3 78.5	4.10 (t, 8.9)
4	78.9	4.27	4 71.1	4.14
5	76.9	3.78 (m)	5 67.3	3.61 (t, 11.3)
6	61.3	4.22	5′	4.20 (d, 11.0)
6′	–			
Apiose			*Glucose II*	
1	111.0	5.99 (d, 3.1)	1 104.9	4.83 (d, 7.8)
2	77.5	4.76 (d, 3.1)	2 75.2	4.02
3	80.2		3 78.7	4.23
4	75.3	4.74 (d, 9.2)	4 71.8	4.21 (t, 8.0)
5	65.1	4.16 (br. s)	5 78.6	3.95 (m)
			6 62.9	4.39 (dd, 12.0, 4.3)
			6′	4.55 (dd, 12.0, 2.5)

Galactose = β-D-galactopyranose
Glucose = β-D-glucopyranose
Apiose = β-D-apiofuranose
Xylose = β-D-xylopyranose

signal at δ 109.2 in the ^{13}C NMR spectrum [2]. The spirostanol character was further supported by the presence of peaks at m/z 418 and 399 in the FAB-MS which were indicative to a saturated monohydroxy spirostane nucleus. The IR spectrum of **1** added additional confirmation by displaying absorptions at 980, 920, 900, 863 cm^{-1} with lower intensity peak at 920 cm^{-1} than 900 cm^{-1} indicating that **1** belongs to the 25*R* series of spirostanes [4]. The equatorial orientation of the C-27 methyl was further verified by the axial-axial coupling of H-26$_{ax}$ (δ 3.52) and H-25$_{ax}$ (δ 1.60) $J_{26ax,25ax} = 11.1$ Hz in the ^{1}H NMR and the lower field resonance of C-27 (δ 17.3) as compared to the ^{13}C NMR chemical shift of (25*S*)-spirostanes (δ 16.0–16.5) [2]. The proton and carbon resonances, due to the sterol part of **1**, were assigned by different homonuclear and heteronuclear correlation experiments (^{1}H-^{1}H COSY, HMQC, HMBC). Comparison of these assignments with the chemical shift values reported in the literature for structurally related compounds [2, 5], allowed identification of the sterol nucleus as (25*R*)-5β-spirostan-3β-o1 (smilagenin). Acid hydrolysis of **1** gave the aglycone whose NMR data were in good agreement with those reported in the literature for smilagenin [2]. The glycosidic nature of **1** was shown by strong IR absorptions at 3420 and 1050 cm^{-1} together with ^{1}H and ^{13}C NMR resonances of four anomeric functions identified in the HMQC spectrum [^{1}H: 4.84, 5.33, 5.55, 5.99; ^{13}C: 102.2, 105.1, 104.5, 111.0]. The combined use of ^{1}H-^{1}H COSY and HOHAHA experiments allowed grouping of the proton signals arising from each spin system corresponding to a monosaccharide residue. The anomenic proton at δ 5.55 (d, J = 7.8 Hz) was grouped, in sequence, with four oxymethine and one oxymethylene groups, thus suggesting a hexose unit in the pyranose form. The sugar moiety was identified as β-glucose I on the basis of large couplings observed for all the oxymethine protons, implying their axial positions. On the basis of the same arguments as those for the characterization of the previous sugar unit, the anomeric proton at δ 5.33 (d, J = 7.9 Hz) was attributed to a second β-glucopyranose unit II. From the anomeric proton at δ 4.84 (d, J = 7.8 Hz) we could identify another hexose sequence extending to an exomethylene residue via four oxymethine protons. The relative stereochemistry of the monosaccharide unit was elucidated by the analysis of coupling constants. The sugar unit was characterized by large axial-axial couplings between H-1/H-2, H-2/H-3 while the axial-equatorial relation H-3/H-4 was deduced by the small coupling constant between these protons. A NOE difference experiment provided us with dicisive indication about the axial configuration of H-5 from the observed NOE at H-5 on irradiating H-1. Considering the above information, this monosaccharide unit was identified as β-galactopyranose. The HMQC spectrum served to correlate the assigned proton signals of the three sugar residues to the carbon signals and the deduced ^{13}C chemical shifts enabled a final identification of the sugar residues. After subtraction of the ^{13}C resonances due to the three assigned pyranose units from those of the tetrasaccharide moiety, the remaining resonances appeared at δ 111.0, 77.5, 80.2, 75.3, 65.1 were consistent with the presence of an β-apiofuranose moiety [6]. The identity of the sugar units was further clarified by acid hydrolysis of **1**. The released sugar components were converted to thiazolidine derivatives and analyzed by GC which revealed the presence of D-glucose, D-galactose and D-apiose. The β-glucopyranose I and the β-apiofuranose were shown to be terminal units by the absence of any glycosylation shift for their carbon resonances which implied that the tetrasaccharide moiety had branched sequence. The glycosylation shifts were observed for C-2 and C-3 of the β-galactopyranose and C-4 of the β-glucopyranose II. The positions of the sugar residues were unambiguously defined by HMBC experiment. A cross peak due to longe-range correlation between the aglycone C-3 (δ 75.2) and H-1 (δ 4.84) of the β-galactopyranose unit, suggested that this hexose unit was directly attached to the aglycone moiety. Further long-range correlations were between C-2 (δ 78.0) of the β-galactopyranose and H-1 (δ 5.55) of the β-glucopyranose I, C-3 (δ 84.0) of the β-galactopyranose and H-1 (δ 5.33) of the β-glucopyranose II. For the remaining sugar β-apiofuranose, $^3J_{C,H}$ correlation was observed between its C-1 (δ 111.0) and H-4 (δ 4.27) of the β-glucopyranose II. The same conclusion with regard to the sugar sequence was also drawn from the NOE difference experiment. When H-1 (δ 4.84) of the β-galactopyranose was irradiated, H-3 (δ 4.30) of the aglycone responded. Analogously, on irradiating H-1 (δ 5.55) of the β-glucopyranose I and H-1 (δ 5.33) of the β-glucopyranose II, NOE's were observed at H-2 (δ 4.79) and H-3 (δ 4.31) of the β-galactopyranose, respectively. Also, NOE was seen at H-4 signal (δ 4.27) of the β-glucopyranose II, on irradiating H-1 of the β-apiofuranose. All the above data identified **1** as (25*R*)-5β-spirostan-3β-ol-3-

O-{β-D-apiofuranosyl(1→4)β-D-glucopyranosyl(1→3)[β-D-glucopyranosyl (1→2)]β-D-galactopyranoside}.

Compound **2** exhibited a molecular formula ($C_{50}H_{82}O_{22}$) identical to that of **1**, based on ^{13}C NMR data and posstive-ion FAB MS. It was proposed to be a 20(22)-ene-furostanol saponin in the 26-*O*-glycosidic form based on the absence of spiroketal absorption in the IR spectrum, strong IR absorptions at 3429 cm^{-1} and 1000–1100 cm^{-1} (glycosidic bond), conversion to spirostanol type during acid hydrolysis and NMR analysis [δ_H 1.64 (Me-21), s; δ_C 103.6 (C-20) and 152.4 (C-22)] [9, 10]. The ^1H and ^{13}C NMR shifts of the furostane skeleton of **2** were assigned on the combined use of ^1H-^1H COSY, HMQC and HMBC experiments. The ^1H and ^{13}C shifts of A and B rings (C-1 – C-12 and C-19) were very similar to those of **1**, while shifts of C, D and E rings (C-13 – C-22) were almost identical to the corresponding data reported in the literature for the structurally related 20(22)-ene furostanol saponins [9, 10]. The shifts of C-23 – C-26 and their attached protons were unambiguously defined by employing 2D NMR techniques particularly the HMBC which provided confirmative evidence to the deduced shift values. The observed $^2J_{H,C}$ and $^3J_{H,C}$ correlations were between, H-23 a,b and C-20, C-22, C-24; H-24a,b and C-22, C-23, C-25, C-26, C-27; H-25 and C-23, C-24, C-26, C-27; H-26 a,b and C-24, C-25, C-27, C-1 of β-glucopyranose II; H_3-27 and C-24, C-25, C-26. The resonances due to H-25 and H_2-26 were unresolved in the ^1H NMR spectrum of **2**, so $^3J_{25,26ax}$ and $^3J_{25,26eq}$ couplings could not be extracted. However, the equatorial geometry of Me-27 (25*R*) could be deduced from the acid hydrolysis of **2** which afforded the (25*R*) spirostane aglycone smilagenin. Based on the above conclusions, the structure of sterol part of **2** was established as (25*R*)-5β-furost-20(22)-ene-3β,26-diol. The aqueous hydrolysate proved by GC analysis to contain D-glucose, D-xylose and D-galactose. The tetrasaccharide nature of **2** was determined from its ^1H [δ 4.83, d, J = 7.8Hz; δ 4.91, d, J = 7.6 Hz; δ 5.56, d, J = 7.8 Hz and δ 5.23, d, J = 7.6 Hz] and ^{13}C [δ 104.9, 102.2, 104.5, 106.3] NMR data. By application of the structural approach employed in case of **1**, the HOHAHA experiment coupled with ^1H-^1H COSY spectrum allowed sequential assignments of the proton resonances for the glycosidic residues. Multiplet patterns and measurements of coupling constants confirmed the presence of two β-glucopyranose, one β-galactopyranose and one β-xylopyranose units. The assignments were further supported by NOE studies which verified the position of H-3 and H-5 resonances in each spin system. From the HMQC spectrum which correlated the proton resonances with those of the corresponding carbons, the two β-glucopyranose and the β-xylopyranose were terminal units. Glycosylation shifts were observed for C-2 and C-3 of β-galactopyranose. The interglycosidic linkages were deduced from the HMBC spectrum and confirmed by NOE studies. The observed HMBC correlations were between H-1 (δ 4.91) of the β-galactopyranose and C-3 (δ 75.2) of the aglycone, H-1 (δ 5.56) of the β-glucopyranose I and C-2 (δ 77.9) of the β-galactopyranose, H-1 (δ 5.23) of the β-xylopyranose and C-3 (δ 84.3) of the β-galactopyranose. Further correlation was observed between H-1 (δ 4.83) of the β-glucopyranose II and C-26 (δ 75.0) of the aglycone. The NOE difference experiment confirmed the above observations and showed close spatial proximity of β-galactopyranose H-1 (δ 4.91) to aglycone H-3 (δ 4.34), of β-glucopyranose I H-1 (δ 5.56) to β-galactopyranose H-2 (δ 4.82), of β-xylopyranose H-1 (δ 5.23) to β-galactopyranose H-3 (δ 4.29), of β-glucopyranose II H-1 (δ 4.83) to H-26 (δ 3.95) of the aglycone. Thus, the structure of **2** was elucidated as 26-*O*-β-D-glucopysanosyl(25*R*)-5β-furost-20(22)-ene-3β,26-diol-3-*O*-{β-D-xylopyranosyl(1→3)[β-D-glucopyranosyl(1→2)]-β-D-galactopyranoside}.

The anomeric configurations of D-glucose, D-galactose and D-xylose were determined to be all β from each $^3J_{H\text{-}1,H\text{-}2}$ value. The β-anomeric configuration of D-apiose was evident from ^{13}C NMR data [6].

2.2. Pharmacology

We studied the acute toxicity, analgesic and anti-inflammatory activities as well as the anti-ulcer effect of the saponin fraction of *A. lophantha*. The results are summar-

Table 3: Analgesic activity of the saponin fraction of *Agave lophantha* Schiede in mice

Treatment	Dose (mg/kg)	Reaction time in minutes after treatment					
		0	10	20	30	60	120
Control	–	14.6 ±0.57	14.7 ±0.49	17.0 ±0.9	17.2 ±0.78	17.5 ±0.66	18.0 ±0.61
SF	100	13.8* ±1.10	16.7* ±0.90	22.8*** ±1.31	23.5*** ±0.61	27.0*** ±0.90	28.5*** ±0.78
Paracetamol	50	14.9 ±0.76	19.7 ±1.1	25.4 ±0.86	25.7 ±1.52	26.3 ±1.35	25.7 ±1.27

*P < 0.05 *** P < 0.001 Values are percent of control ±SE; n = 6 Vs control; Student's T test.
SF = saponin fraction, SE = standard error

Table 4: Anti-inflammatory and anti-ulcer of the saponin fraction of *Agave lophantha* Schiede

Treatment	Dose (mg/kg)	Anti-inflammatory activity		% Ulcer inhibition
		Carrageenin Paw oedema (%inhibition ± SE)	Carrageenin induced pleurisy (ïnhibition ± SE)	
SF	100	69.62 ± 0.03***	54.0 ± 0.03***	43.55***
	200			58.67***
Diclofenac sodium	50	82.0 ± 0.04	70.0 ± 0.05	
Mucogel	0.25 ml			97.0

***P < 0.000 Values are percent of controls ±SE; n = 6 Vs control; Student's T test.
SF. = saponin fraction, SE = standard error

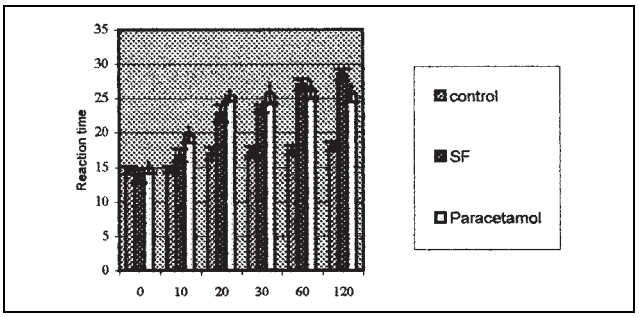

Fig. 1: Analgesic activity of the saponin fraction of *Agave Iophantha* Schiede in mice

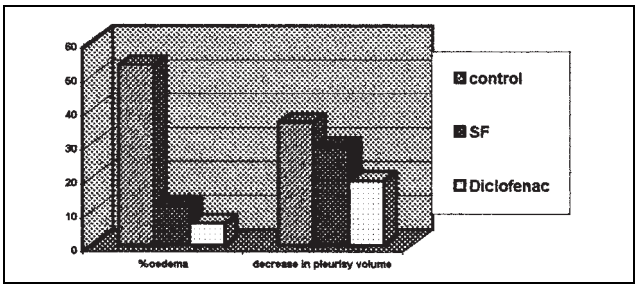

Fig. 2: The antiinflammatory activity of saponin fraction of *Agave Iophantha* Schiede in rats

ized in Tables 3 and 4 and Figs. 1 and 2. The saponin fraction (SF) in dose up to 800 mg/kg failed to produce any mortalities in mice either po or ip.

The results of analgesic activity showed that the SF exhibited a marked analgesic activity at dose of 100 mg/kg, on applying the hot plate test. The reaction time was significantly longer than both that of the control and the paracetamol.

In the rat paw oedema test, the SF was found to cause a significant inhibition (69.6%) of the oedema weight at a dose of 100 mg/kg po which is less than that produced by diclofenac sodium (82%). In the carrageenin induced pleurisy test, reduction in the volume of the exudates was observed for the SF at dose of 100 mg/kg ip. The percentage of reduction was 54% compared to 70% for the reference drug.

The results of the ulceroprotective action proved that the SF possessed a significant protection against lesions in rats and at both doses 100 mg/kg and 200 mg/kg op.

3. Experimental

3.1. Chemistry

3.1.1. Equipment

Optical rotations were measured with JASCO DIP-1000 digital polarimeter. JEOL JMS-SX 102 was used for positive mode FAB-MS. ^{1}H and ^{13}C NMR spectra were obtained with a JEOL-α-4000 FT NMR spectrometer (^{1}H-400 MHz; ^{13}C 100 MHz, in pyridine-d_5, at 35 °C) and chemical shifts were given in ppm with TMS as int. standard. HPLC was carried on JASCO system 800 and GC analysis was performed with G-3000 gas chromatograph. The IR spectra were obtained with JASCO FT/IR – 5MP.

3.1.2. Plant material, extraction and isolation

The leaves of *A. lophantha* were collected from EL-Orman public garden, Giza, Egypt in June 1998 and identified by Mrs Theraes Labib, Head of specialist for plant identification.

The air dried leaves (500 g) were exhaustively extracted with MeOH. The combined extract was concentrated under vacuum and diluted with Me_2CO to precipitate the crude saponin mixture (11.5 g). The mixture was chromatographed on silica gel (TLC grade) using VLC and eluted with $CHCl_3$ then $CHCl_3$–MeOH. The saponin containing fractions eluted with chloroform : methanol 70–30 and 80–20 (7.9 g) were combined and passed through a porous polymer gel column (Mitsubishi Diaion HP-20), eluted with H_2O then MeOH. A part (1 g) of the methanolic eluate (3.2 g) was subjected to silica gel FL-60B column, eluted with $CHCl_3$–MeOH–H_2O mixture (70 : 28 : 2) to give 14 fractions (a-n), 30 ml each. Fractions (f-n) showing similar TLC profiles (Si-gel 60, $CHCl_3$–MeOH–H_2O (67 : 30 : 3) were pooled and the material was repeatedly fractionated on HPLC columns [Develosil ODS, 5 × 50 cm, 20–34% CH_3CN; PhA-5, 2 × 25 cm, 22.5% CH_3CN; 6.5 ml/min.; UV, 195 nm] to give **1** (25 mg) and **2** (15 mg).

*3.1.3. Saponin **1***

Amorphous powder $[\alpha]_D^{23}$ −49.5° (c = 1.28, MeOH); IR ν_{max}^{KBr} cm^{-1} 3420, 2930, 1050, 980, 920, 900, 863; FABMS (m/z) 1057 $[C_{50}H_{82}O_{22} + Na]^+$, 925, 877, 791, 763, 418, 399; ^{1}H and ^{13}C NMR see Tables 1 and 2.

*3.1.4. Saponin **2***

Amorphous powder $[\alpha]_D^{23}$ −14.3° (c = 0.89, MeOH); IR ν_{max}^{KBr} cm^{-1} 3429, 2924, 1076; FABMS (m/z) 1057 $[C_{50}H_{82}O_{22} + Na]^+$, 1057, 924, 713, 583, 495, 397, 357, 255; ^{1}H and ^{13}C NMR see Tables 1 and 2.

3.1.5. General method for acid hydrolysis [11]

Each saponin (7 mg) was heated in dioxane-2N HCl (1 : 1) (0.18 ml) at 95 °C for 5 min. The reaction mixture was diluted with H_2O and extracted with EtOAc. The extract was washed with water then evaporated to give smilagenin (2 mg), identified by NMR data. The aq. layer was passed through an Amberlite IRA-60E column (6 × 50 mm) and the eluate was evaporated to dryness. The residue was dissolved in pyridine (0.18 ml) and D-cysteine methyl ester (9 mg) was added to the solution and the reaction mixture was stirred for 30 min at 65 °C. To the reaction mixture, hexamethyl disilazane (0.045 ml) and trimethyl silyl chloride (0.045 ml) were added and the mixture was stirred for 30 min at 65 °C. The supernatant of the mixture was then analysed by GC. Conditions: column, supelco SPB-TM1 (0.25 mm × 27 m); column temperature 215 °C; carrier gas, N2; retention times, D-apiose (11.9 min), L-apiose (10.9 min), D-xylose (12.6 min), L-xylose (11.7 min), D-glucose (22.5 min), L-glucose (21.5 min), D-galactose (24.7 min), L-galactose (23.1 min). From saponins **1** and **2**, D-apiose, D-xylose, D-glucose and D-galactose were detected.

3.2. Pharmacology

3.2.1. Materials and methods

Adult male albino rats weighing 120–150 g and adults albino mice of both sexes weighing 18–20 g were used. Drugs like carrageenin, paracetamol, diclofenac sodium and mucogel were administered orally (po) or intraperitoneally (ip). The tested saponin fraction, obtained after Si-gel VLC and HP-20 CC, was suspended in distilled water or saline and the control animals received the same amount of the vehicle.

3.2.2. Analgesic activity [12]

Analgesia was assayed by means of the hot plate test in groups each of six mice. One group was used as control received distilled water, the second and the third groups were given orally the tested fraction (100 mg/kg) and the paracetamol (50 mg/kg) respectively. The animal was dropped gently upon a hot plate at 55 °C. The reaction time was measured before and 10, 20, 30, 60 and 120 min after administration of the tested fraction.

3.2.3. Anti-inflammatory activity

3.2.3.1. Carrageenin induced rat foot-paw oedema [13]

Groups of 6 rats were used. The tested fraction and diclofenac sodium were given orally at doses of 100 mg/kg and 50 mg/kg respectively. 1 h later, 0.05 ml of 1% carrageenin solution was injected into the sub-plantar tissue of the right hind paw. The volume of the oedema was measured prior to the injection of carrageenin and 4 h later: the increase in volume of the paw 4 h after the injection of carrageenin was adopted as a measure of oedema. Swelling in treated animals was calculated as percentage inhibition in comparison with controls injected by an equal volume of saline into the other hind paw.

3.2.3.2. Carrageenin induced pleurisy in rats [14]

Groups of 6 rats were tested. The tested fraction was orally given a dose of 100 mg/kg. 1 h later, animals were intrapleurally injected between the 8th and 9th rib with 0.9 ml of 2% carrageenin in saline. After 3 h, rats were bled to death and the volume of pleural exudates was measured. Diclofenac sodium was administered in the same way and the results were compared to the control.

3.2.4. Anti-ulcer activity

Four groups of 6 rats were fasted for 18 h. The first group served as control receiving oral dose of distilled water. The two other groups received the test

fraction at two different concentrations (100 and 200 mg/kg) and the fourth group received mucogel. After 30 min absolute ethanol (1.5 ml/kg) was orally given and 1 h later animals were decapitated, the stomach was removed and opened along the greater curvature then examined with a magnifying lens for the presence of lesions or erosions. The ulcer mean number recorded in each group was calculated using the following formula:

$$100 - \frac{100 \times \text{mean numbers of test lesions}}{\text{mean numbers of control lesions}} = \% \text{ inhibition}$$

References

1 Mahato, S. B.; Ganguly, A. N.; Sahu, N. P.: Phytochemistry **21**, 959 (1982)
2 Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S.: Phytochemistry **24**, 2479 (1985)
3 Blunden, G.; Patel, A. V.; Grabb, T. A.: J. Nat. Prod. **49**, 687 (1986)
4 Wall, M. E.; Mcclemmen, M. L.; Klumpp, M. E.: Anal. Chem. **24**, 1337 (1952)
5 Eggret, H.; Djerassi, C.: Tetrahedron Lett. **42**, 3635 (1975)
6 Kitagawa, I.; Sakagamt, M.; Hashiuchi, F.; Zhou, J. L.; Yoshikawa, M.; Ren, J.: Chem. Pharm. Bull. **37**, 551 (1989)
7 Watt, J. M.; Breyer-Brandwijk, M. G.: Medicinal and Poisonous Plants of Eastern and Southern Africa. Edinburgh: E. S. Livingstone 1962
8 Tschesche, R.; Tjoa, B. T.; Wulff, G.; Noronha, R. V.: Tetrahedron **49**, 5141 (1968)
9 Mimaki, Y.; Takaashi, Y.; Kuroda, M.; Sashida, Y.: Phytochemistry **45**, 1229 (1997)
10 Sang, S.; Lao, A.; Wang, H.; Chen, Z.: Phytochemistry **52**, 1611 (1999)
11 Hara, S.; Okabe, H.; Mihashi, K.: Chem. Pharm. Bull. **34**, 1843 (1986)
12 Janssen, P. A. J.; Jageneau, A.: J. Pharm. Pharmacol. **9**, 381 (1957)
13 Winter, C. A.; Risley, E. A.; Nuss, G. W.: Pro. Soc. Exp. Biol. Med., **3**, 1544 (1962)
14 Correll, T.; Hasselman, G.: Pharmacol. Toxicol. **53**, 288 (1983)
15 Peskar, B. M.; Lsnugs, K.; Hoppe, M.; Peskar, B. A.: Prostaglandin **31**, 283 (1986)

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