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STEROIDAL SAPONINS FROM A CULTIVATED FORM OF *AGAVE SISALANA*

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Key Word Index—*Agave sisalana*, Agavaceae; steroidal saponin; dongnoside C, D, E.

Abstract—Three new steroidal saponins, dongnosides C–E, were isolated from the methanol extracts of the fermented residues of leave-juices of *Agave sisalana* form Dong No 1. On the basis of chemical and spectral evidence, the structures of dongnosides E, D and C were determined as tigogenin-3-*O*-β-D-xylopyranosyl(1→2)[β-D-glucopyranosyl(1→3)]β-D-glucopyranosyl(1→4)β-D-galactopyranoside, tigogenin-3-*O*-β-D-xylopyranosyl(1→3)β-D-xylopyranosyl(1→2)[β-D-glucopyranosyl (1→3)]β-D-glucopyranosyl(1→4)β-D-galactopyranoside and tigogenin-3-*O*-α-L-rhamnopyranosyl (1→4)β-D-xylopyranosyl(1→2)[β-D-glucopyranosyl (1→3)]β-D-glucopyranosyl(1→4)β-D-galactopyranoside, respectively.

INTRODUCTION

Agave sisalana is a very important resource of hard fibre and steroidal material, which was introduced from abroad and has been cultivated in the south of China. Several sapogenins have been isolated from Dong No 1, a cultivated form of this plant [1, 2]. In the present paper, we report the isolation and structure elucidation of three new steroidal saponins, dongnoside E(**1**), D(**2**) and C(**3**) from this plant.

RESULTS AND DISCUSSION

The methanol extract of fermented residues of leaf-juices were subjected to repeated column chromatography and preparative TLC on silica gel to afford three saponins, all of which were positive in the Liebermann–Burchard reaction, but negative to the Ehrlich reagent [3]. They were predicted to be glycosides of a (25*R*)-spirostanol steroid based on the characteristic absorption band in the IR spectra [4].

On mineral acid hydrolysis, all three saponins yielded a common aglycone. By comparing mp, mmp, optical rotation, IR, EIMS, R_f value on TLC and NMR spectra, the aglycone was determined to be tigogenin.

Saponin **1** was hydrolysed with acid to yield D-galactose (Gal), D-glucose (Glc) and D-xylose (Xyl). The negative ion FAB mass spectrum of **1** exhibited a molecular ion peak at *m/z* 1033 $[M-H]^-$ and fragment ions at *m/z* 901 [1033 − pentose]$^-$, 871 [1033 − hexose]$^-$, 739 [1033 − pentose − hexose]$^-$ and 577 [1033 − pentose − 2 hexose]$^-$. The ^{1}H NMR spectrum of **1** (C_5D_5N + CF_3COOH) exhibited four anomeric proton signals at δ4.85 (1H, *d*, *J* = 7.3 Hz), 5.12 (1H, *d*, *J* = 7.8 Hz), 5.18 (1H, *d*, *J* = 7.8 Hz) and 5.50 (1H, *d*, *J* = 7.3 Hz), respectively. EIMS of acetylated **1** showed fragment ions at *m/z* 835 [(hexose–hexose–pentose) $Ac_9]^+$, 331 [(terminal hexose) $Ac_4]^+$ and 259 [(terminal pentose) $Ac_3]^+$. These data indicated **1** to contain 1 mol of Xyl and 3 mol of hexose (Glc and Gal).

When saponin **1** was partially hydrolysed with dilute hydrochloric acid in ethanol, three prosapogenins, **4**–**6**, were obtained. From acid hydrolysis test on TLC [5], both **4** and **5** gave Glc and Gal, **6** only gave Gal.

The positive ion FAB mass spectrum of **6** showed a molecular ion peak at *m/z* 579 $[M+H]^+$ and fragment ions at *m/z* 417 $[M-Gal]^+$, suggesting that **6** should be a tigogenin-3-*O*-β-D-galactopyranoside.

^{13}C NMR spectra of **1**, **4** and **5** (Table 1) showed a common downfield shift (+6.9 ppm) for C-3 of the aglycone moeity comparing with tigogenin [6], therefore the sugar chain must be attached at the C-3 position of the aglycone. Prosapogenin **5** exhibited peaks at *m/z* 763 $[M+Na]^+$ and 740 $[M]^+$ in FDMS and showed the signals of a terminal Glc and a inner Gal besides the aglycone in the ^{13}C NMR spectrum (Table 2). According to the glycosylation shift effect [7], the downfield shift (+9.6 ppm) of C-4 of Gal suggested that the Glc should attach to C-4 of the Gal. It had the same chemical shifts for the sugar moiety as **7** which is a saponin of diosgenin isolated from *Aspitistra elatior* [8] Thus, compound **5** was established as tigogenin-3-*O*-β-D-glucopyranosyl (1→4)β-D-galactopyranoside.

Prosapogenin **4** showed peaks at *m/z* 903 $[M+H]^+$ and 902 $[M]^+$ upon FDMS and only added signals originating from a Glc unit more than **5** in the ^{13}C NMR spectrum. The chemical shifts of the inner Glc showed a downfield shift (+9.6 ppm) for C-3, suggesting the terminal Glc should attach to C-3 of the inner Glc. Therefore, the structure of **4** was deduced as tigogenin-3-*O*-β-D-glucopyranosyl(1→3)β-D-glucopyranosyl(1→4)β-D-galactopyranoside.

Saponin **1** only had additional signals of a terminal Xyl more than **4** in the ^{13}C NMR spectrum The chemical shifts of the inner Glc showed a downfield shift (+4.4 ppm) for C-2, suggesting that **1** should consist of a

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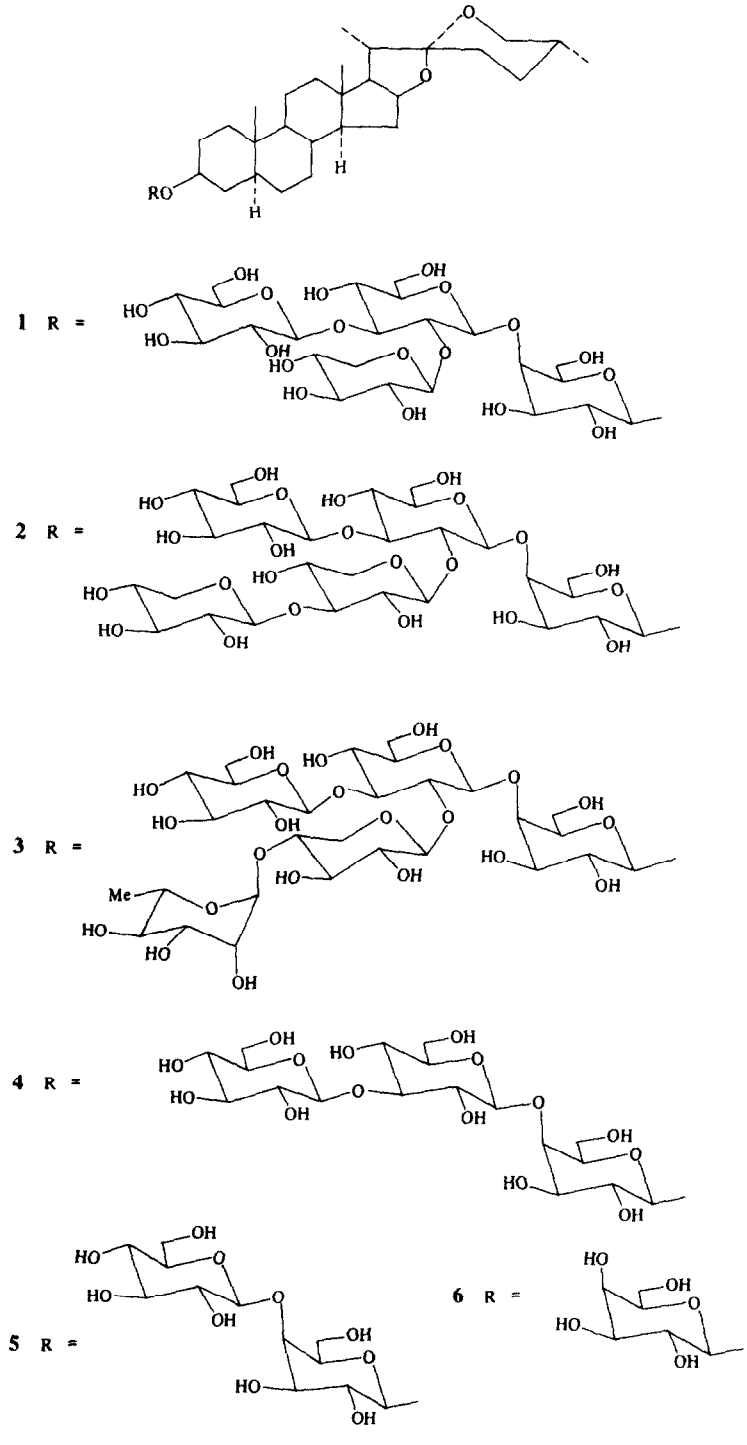

branch sugar moiety and the terminal Xyl should attach to C-2 of the inner Glc. On the basis of the above findings, the structure of **1** was concluded to be tigogenin-3-*O*-*β*-D-xylopyranosyl(1→2)[*β*-D-glucopyranosyl(1→3)]*β*-D-glucopyranosyl(1→4)*β*-D-galactopyranoside.

Saponin **2** yielded Gal, Glc and Xyl by hydrolysis with acid, and yielded compounds **1**, **4**–**6** as prosapogenin by partial hydrolysis with dilute hydrochloric acid. The negative ion FAB mass spectrum exhibited a molecular ion peak at *m/z* 1165 $[M-H]^-$ and fragment ions at *m/z* 1033 $[1165-Xyl]^-$, 901 $[1165-2Xyl]^-$, 871 $[1165-Xyl-Glc]^-$, 739 $[1165-2Xyl-Glc]^-$ and 577 $[1165-2Xyl-2Glc]^-$. The ^1H NMR spectrum of **2** $[C_5D_5N+CF_3COOH]$ exhibited the presence of five anomeric proton signals at 4.86 (1H, *d*, *J* = 7.4 Hz), 5.06 (1H, *d*, *J* = 7.4 Hz), 5.10 (1H, *d*, *J* = 7.8 Hz), 5.14 (1H, *d*, *J* = 7.8 Hz), 5.54 (1H, *d*, *J* = 6.4 Hz). EIMS of the acetylated **2** showed fragment ions at *m/z* 331 $[(\text{terminal Glc})Ac_4]^+$ and 259

Table 1. ^{13}C NMR chemical shifts of aglycone moiety (C_5D_5N) of saponins 1–5 and reference compound tigogenin (400 MHz)

C	Tigogenin	1	2	3	4	5
1	37.5	37.2	37.2	37.2	37.1	37.1
2	32.5	30.6	30.6	30.6	30 6	30.5
3	70.6	77.5	77.5	77 4	77.5	77 1
4	39.3	35.3	35.3	35.3	35.3	35.2
5	45.2	44.8	44.8	44 8	44.8	44.5
6	29.1	29.0	28.9	29.0	28.9	28.8
7	32 5	32 4	32 4	32.4	32.4	32.3
8	35.4	34.9	34.8	34.9	34 8	34.7
9	54.6	54.5	54.5	54.5	54.5	54 3
10	35.9	35.8	35.8	35.8	35.8	35.7
11	21.1	21 3	21.3	21.3	21 3	21.2
12	40.3	40.2	40.2	40.2	40.2	40.0
13	40.8	40.8	40.8	40.8	40.8	40.7
14	57.6	56.5	56.5	56.5	56.5	56.4
15	32.1	32 1	32.1	32 1	32.1	32.1
16	81.1	81.2	81.1	81 2	81.1	81.1
17	63.1	63.1	63.0	63.1	63.0	63.0
18	16.7	16.6	16.6	16.6	16.6	16.6
19	12.5	12 3	12.3	12.3	12.3	12.2
20	42.0	42.0	42.0	42.0	42.0	41 9
21	15.0	15.0	14.9	15.0	15.0	14.9
22	109.2	109.2	109.2	109.2	109.2	109.1
23	31.9	31.7	31.8	31 7	31.8	31.7
24	29.3	29.3	29.2	29.3	29.2	29.2
25	30.6	29 9	29 9	29.9	29.9	29.9
26	66.9	66 9	66.9	66 9	66.9	66.9
27	17 3	17.3	17.3	17.3	17.3	17,3

[(terminal Xyl)Ac_3]$^+$. Comparison of the ^{13}C NMR chemical shifts of **2** with **1** showed the only difference to be that **2** contained added signals of a Xyl. Based on the glycosylation shifts, the downfield shift (+8.2 ppm) of the C-3 of the inner Xyl indicated that the terminal Xyl should attach to the C-3 position of the inner Xyl. These results led to the structure of saponin **2** being assigned as tigogenin-3-*O*-*β*-D-xylopyranosyl(1→3)*β*-D-xylopyranosyl(1→2)[*β*-D-glucopyranosyl(1→3)]*β*-D-glucopyranosyl (1→4)*β*-D-galactopyranoside.

Acid hydrolysis of saponin **3** yielded Gal, Glc, Xyl and L-rhamnose(Rham). On partial hydrolysis of **3** with dilute hydrochloric acid, four prosapogenins **1** and **4–6** were also obtained. The negative ion FAB mass spectrum exhibited a molecular ion peak at *m/z* 1179 [M−H]$^-$ and fragment ions at *m/z* 1033 [1179−Rham]$^-$, 1017 [1179−Glc]$^-$, 901 [1179−Rham−Xyl]$^-$, 871 [1179−Rham−Glc]$^-$, 739 [1179−Rham−Xyl−Glc]$^-$ and 577 [1179−Rham−Xyl−2Glc]$^-$. The ^1H NMR spectrum of **3** (C_5D_5N+CF_3COOH) showed the presence of five anomeric proton signals at *δ*4.88(1H, *d*, *J*=7.6 Hz), 5.11(1H, *d*, *J*=8.0 Hz), 5.22 (1H, *d*, *J*=8.0 Hz), 5.45(1H, *br s*) and 5.52 (1H, *d*, *J*=7.8 Hz). EIMS of acetylated **3** exhibited fragment ions at *m/z* 489 [(Rham−Xyl)Ac_5]$^+$, 331 [(terminal Glc)Ac_4]$^+$ and 273 [(terminal Rham)Ac_3]$^+$. Comparing the ^{13}C NMR chemical shifts of **3** with **1** showed that the difference between them added signals of 1 mol of terminal Rham in **3** and the chemical shift of C-4 of Xyl was shifted downfield (+5.4 ppm). This suggested that Rham should be attached to the C-4 position of Xyl. To further prove the structure of **3**, 2D-NMR spectra were also carried out to verify the assignments of the chemical shifts of the linkage positions. From ^1H–^1H and ^{13}C–^1H COSY spectra, H-4 of Gal, H-2 and H-3 of the inner Glc and H-4 of Xyl were assigned. In the long range ^1H–^1H COSY spectrum, by correlating the anomeric proton signals with the proton signals of the linkage position, the interglycosidic linkage of saponin **3** was further confirmed. Based on these results, the structure **3** was elucidated as tigogenin-3-*O*-*α*-L-rhamnopyranosyl(1→4)*β*-D-xylopyranosyl(1→2)[*β*-D-glucopyranosyl(1→3)] *β*-D-glucopyranosyl(1→4)*β*-D-galactopyranoside.

It is noteworthy that all of the saponins isolated from this plant contain tigogenin as a common aglycone, a Gal as the inner sugar and a Glc linked to C-3 of the inner Glc but a Xyl branched to C-2 of the same inner Glc, this may have significance for the chemotaxonomy of the *Agave* genus.

EXPERIMENTAL

Mps: uncorr. NMR spectra: TMS as int. standard. The EI-, FD-, and FAB-MS were recorded on 7070 EQ, ZAB-HS, 7070HF mass spectrometers. Solvent systems for TLC and CC: S1, $CHCl_3$–MeOH–H_2O (14.6:1 homogeneous); S2, $CHCl_3$–MeOH–H_2O (7.3·1 lower phase); S3, $CHCl_3$–MeOH (50:1 homogeneous); S4, $CHCl_3$–MeOH–H_2O (7:3:1) 9 ml+HOAc 1 ml. TLC spots were detected by spraying with 10% H_2SO_4 followed by heating.

Table 2. ^{13}C NMR chemical shifts of sugar moiety (C_5D_5N) of saponins **1–5** and reference compound **7** (400 MHz)

C-3 sugar	**1**	**2**	**3**	**4**	**5**	**7** [8]
Gal-1	102 3	102 5	102 5	102 3	102.4	103.0
2	73.1	73 0	73 1	73 2	73 4	73 5
3	75.0	74 9	74 8	75 0	75 2	75 4
4	81 3	80 5	81 1	78 9	80.0	79 8
5	76 2	75 4	76.0	76.7	76 0	75 9
6	60 5	60 7	60 6	60 4	61 0	61 0
Glc-1	104 9	103 7	104 6	105 1	107 1	107 0
2	79 9	79 4	79 7	75 5	75.4	75 2
3	86 6	87 0	86 8	86 0	78 4	78 4
4	70 4	70 3	70 4	70 2	72 2	72 4
5	77 6	77 7	77 6	78 1	78 7	78 7
6	62 4	62 2	62 4	61.5	63 0	63 1
Glc-1	104.9	104.5	104 6	106 8		
2	75 3	75 2	75.3	75 6		
3	78.7	78 2	78 5	78 9		
4	70.9	70 6	71 1	71 7		
5	77.2	77 3	77 7	78 4		
6	62 9	62.8	62 9	62 9		
Xyl-1	105 1	104 7	104 8			
2	75 5	74.9	75 5			
3	78 6	86 8	75.1			
4	70 6	69 2	76 0			
5	67 3	66 9	64 0			
Xyl-1		105 8				
2		75 1				
3		78 0				
4		70 6				
5		67 1				
Rha-1			99 7			
2			72 4			
3			72 3			
4			73 8			
5			69 9			
6			18 5			

Extraction and isolation of saponins. The dried fermentations of leaf-juice of *Agave sisalana* from Dong No 1 (3 kg) were defatted with hot petrol The defatted powders were extracted with hot MeOH until the extracts were colourless The extracts were concd *in vacuo* and the residues (774 g) were subjected to CC on 100–200 mesh silica gel with solvent S1, separating several fractions The high polarity fractions were repeatedly subjected to low pressure CC on 10–40 μ silica gel H with solvent S2, followed by prep TLC on 10–40 μ silica gel G with solvent S1 to afford saponin **1** (110 mg), **2** (1 25 g) and **3** (186 mg) as white powder from MeOH

Saponin **1** A white powder from MeOH Mp 250–253°(dec) $[\alpha]_D^{26}$ −58 82° (pyridine, *c* 0 034) IR ν_{max}^{KBr} cm^{-1}. 3440–3460, 1050–1080 (OH), 985, 920 < 900, 870 (25*R* spirostanol). ^1H NMR ($C_5D_5N + CF_3COOH$, 400 MHz) δ0.63 (3H, *s*, Me), 0 70 (3H, *d*, *J* = 7 8 Hz, Me), 0 81 (3H, *s*, Me), 1.12 (3H, *d*, *J* = 7 3 Hz, Me), 4 85 (1H, *d*, *J* = 7 3 Hz, anomeric H), 5 12 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5 18 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5 50 (1H, *d*, *J* = 7 3 Hz, anomeric H) Anal calcd for $C_{50}H_{82}O_{22}$ $4H_2O$ C, 55 15, H, 8.09 Found C, 55 23, 55 15, H, 7 47, 7.44. FABMS *m/z*. 1033 [M − H]$^-$, 901 [1033 − Xyl]$^-$, 871 [1033 − Glc]$^-$, 739 [1033 − Xyl − Glc]$^-$, 577 [1033 − Xyl − 2Glc]$^-$

Saponin **2** A white powder from MeOH Mp 257–260° (dec.). $[\alpha]_D^{26}$ −45 45° (pyridine *c* 0 099) IR ν_{max}^{KBr} cm^{-1} 3380–3420, 1040–1070 (OH), 985, 925 < 900, 870 (25*R* spirostanol). ^1H NMR ($C_5D_5N + CF_3COOH$ 400 MHz) δ0 64 (3H, *s*, Me), 0 71 (3H, *d*, *J* = 4 7 Hz, Me), 0 83 (3H, *s*, Me), 1 14 (3H, *d*, *J* = 7 6 Hz, Me), 4.86 (1H, *d*, *J* = 7.4 Hz, anomeric H), 5 06 (1H, *d*, *J* = 7.4 Hz, anomeric H), 5 10 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5 14 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5.54 (1H, *d*, *J* = 6 4 Hz, anomeric H) Anal calcd for $C_{55}H_{90}O_{26}$ $2H_2O$ C, 55 09, H, 7 85 Found C, 55 23, 55 15; H, 7 47, 7 44 FABMS *m/z* 1165 [M − H]$^-$, 1033 [1165 − Xyl]$^-$, 901 [1165 − 2Xyl]$^-$, 871 [1165 − Xyl − Glc]$^-$, 739 [1165 − 2Xyl − Glc]$^-$, 577 [1165 − 2Xyl − 2Glc]$^-$

Saponin **3** A white powder from MeOH, mp 236–239°(dec.) $[\alpha]_D^{20}$ −62 00° (pyridine, *c* 0.05) IR ν_{max}^{KBr} cm^{-1} 3380–3420, 1040–1070 (OH), 985, 925 < 900, 870 (25*R* spirostanol) ^1H NMR ($C_5D_5N + CF_3COOH$ 400 MHz) δ0 63 (3H, *s*, Me), 0 70 (3H, *d*, *J* = 5 1 Hz, Me), 0 82 (3H, *s*, Me), 1 13 (3H, *d*, *J* = 7.3 Hz, Me), 1.65 (3H, *d*, *J* = 6.6 Hz, Me), 4 88 (1H, *d*, *J* = 7.6 Hz, anomeric H), 5 11 (1H, *d*, *J* = 8 0 Hz, anomeric H), 5 22 (1H, *d*, *J* = 8 0 Hz, anomeric H), 5 45 (1H, *br s*, anomeric H), 5 52 (1H, *d*, *J* = 7 8 Hz, anomeric H) Anal calcd for $C_{36}H_{92}O_{26}$ $4H_2O$ C, 53 67, H, 7 99. Found C, 53 32, 52 28, H, 7 39, 7 44. FABMS *m/z* 1179 [M − H]$^-$, 1033 [1179 − Rham]$^-$, 1017 [1179 − Glc]$^-$, 901 [1179 − Rham − Xyl]$^-$, 871 [1179–Rham–Glc], 739 [1179–Rham-Xyl − Glc], 577 (1179 − Rham − Xyl − 2Glc]

Acid hydrolysis of saponins A soln of each saponin (50 mg) in

1 M H_2SO–50% EtOH (5 ml) was refluxed on a water bath for 4 hr. The reaction mixt. was dild with H_2O (10 ml) and filtered. The ppt. was recrystallized from MeOH to give colourless needles, mp 206–208°. $[\alpha]_D^{30}$ −51.65° (pyridine, *c* 0.091). IR ν_{max}^{KBr} cm^{-1}: 3520, 1050, 1040(OH), 980, 960, 920<900, 865(25*R* spirostanol). EIMS *m/z*. 416 $[M]^+$, 401, 357, 344, 302, 287, 273, 139(100%), 115, 69. Anal. calcd for $C_{27}H_{44}O_3$ H_2O: C, 74.65; H, 10.60. Found: C, 74.98, 74 97; H, 10.73, 10.70 The aq. layer was neutralized with solid $BaCO_3$, filtered, evapd to dryness *in vacuo*, then the residue was examined on TLC with solvent S4. Saponins and prosapogenins were acid hydrolysed with HCl vapour on the TLC plate (70° water bath for 30 min), followed by developing with solvent S4 used for identifying the sugar.

Acetylation of saponins Each saponin(30 mg) was dissolved in pyridine (1 ml) and Ac_2O(2 ml) The soln was allowed to stand 2 days at room temp. The reaction mixt was poured into ice-water and the ppt. was collected by filtration, dissolved in MeOH and filtered, then solvent removed to give a white powder. **1-Ac** $[\alpha]_D^{22}$ −32.69° (MeOH; *c* 0.052). IR ν_{max}^{KBr} cm^{-1} 1760 (C=O), 1230 (C–O–C), 980, 920<900, 840. EIMS *m/z* 835, 331, 259, 169 (100%), 139, 109, 97, 69. **2-Ac**: $[\alpha]_D^{22}$ −36.94° (MeOH, *c* 0.0785). IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O), 1225(C–O–C), 980, 920<900, 840. EIMS *m/z*: 331, 259, 139, 97, 84, 69 (100%) **3-Ac**: $[\alpha]_D^{22}$ −51 39 (MeOH; *c* 0.072). IR ν_{max}^{KBr} cm^{-1}· 1750 (C=O), 1225 (C–O–C), 980, 920<900, 840. EIMS *m/z*: 489, 331, 273, 139 (100%), 109, 97, 69, 60.

Partial hydrolysis of saponins. Compounds **1** (100 mg), **2** (1 g), **3** (100 mg) were respectively heated on a boiling water bath with 0.5 M HCl in 50% EtOH for 20 min After cooling, the reaction mixture was extracted with *n*-BuOH which was satd with H_2O and the *n*-BuOH layer was evapd to dryness *in vacuo* The residues were chromatographed on silica gel with solvent S2. Compound **1** afforded **4–6** and tigogenin. Compounds **2** and **3** afforded **1**, **4–6** and tigogenin, respectively. FABMS of **6** *m/z*: 579 $[M+H]^+$, 417 $[579-Glc]^+$. FDMS of **5** *m/z*: 763 $[M+Na]^+$, 740 $[M]^+$. FDMS of **4** *m/z*: 903 $[M+H]^+$, 902 $[M]^+$. EIMS of peracetate: **4-Ac**, 331, 259; **5-Ac**, 619, 331, **6-Ac**, 331.

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