CHAPTER VI

Steroidal Glycoside from
T. terrestris Linn

INTRODUCTION:

The plant *Tribulus terrestris* L. (Zygophyllaceae) is used in Chinese folk medicine for impotency. Other properties, such as an antitumoral effect or an activity on the cardiovascular system have also been cited [1,2]. In this chapter we reported the isolation and structural elucidation of one known and two new steroidal saponins. The proton and carbon assignments of the isolated compounds are based on different NMR techniques (DQF-COSY, HMQC, NOESY, TOCSY and ID TOCSY) and chemical experiments.

The new hecogenin 3-0-β-D-glucopyranosside, (1→4)- βD-galactopyranoside, two new steroidal saponins were isolated from the aerial parts of *Tribulus terrestris* L. On the basis of chemical and spectroscopic evidence, especially 2D NMR spectroscopic techniques, the structures of the new saponins were established as 26-0-β-D-glucopyranosyl-3-0-[{b-D-xylopyranosyl(l→3)Àb-D-galactopyranosyl(l →2)}-β-D-glucopyranosyt (I →4)-β-D-glucopyranosyl]-5a-fiirost-20(22)-en-12-one-3β,26-diol and 26-0-β-D-glucopyranosy 1-3-0-[{β-D-xylopyranosyl(l→ 3)}À{β-D-galactopyranosyl(l →2)}-β-D-glucopyranosyl(l → 4)-β-D-glucopyranosyl}-5a-riirostan-12-one-3b,22,26-triol.

EXPERIMENTAL :

NMR spectra were measured at 400 and 600 MHz in pyridine-d_5

with TMS as int. standard. TLC: silica gel GF_{254}: CC: 200-300 mesh silica gel. MPLC: pre-packed column RP-18. MCI: gel CHP-20p.

PLANT MATERIAI:

Tribulus terresiris L. (Zygophyllaceae) was collected in12000 from rural areas of Jaunpur district and identified by Dr. K.N. Mishra, Reader in Botany, T.D. College, Jaunpur in 222002 (U.P.). A voucher specimen is deposited in the herbarium of the college.

EXTRACTION AND ISOLATION:

Air-dried and powdered aerials parts of *T. terrestiris* kg were extracted with hot H_2O. After removal of solvent by evaporation, the residue was then extracted with CH_2Cl_2, EtOAc and n-BuOH, respectively. The n-BuOH layers were then coned to dryness giving a crude saponin fr. (350 g). Part of this extract (50 g) was subjected to CC on a silica gel, eluted with $CHCl_3$-MeOH-H_20 (90:10:1-10:10:1), and were combined by the results detected on the HPTLC plate. The mixts were subjected to CC on MCI (gel CHP20p) and MPLC (pre-packed column RP-18), eluted with MeOH-H_2O mixts, yielding **1-3.**

Saponin 1. Powder from EtOH (50 mg), mp. 218-220°, $[\alpha]_D$ 3.82° (pyridine; c 0.3); IR v^{KBr} cm^{-1}: 3400 (br), 1705 (CO); FAB MS *m/z* (rel. int): 1249 $[M+K]^+$ (33). 1233 $(M+Na]^-$ (42), 1210 $[MR]^+$ (18): UV A_{max}, nm: 202, 240, 255.

Saponin 2. Powder from EtOH (40 mg), mp. 262-264°; (lit.[3]46.7° (pyridine; c 0.1); IR ν^{KBr} cm^{-1}: 3400 (br). 1705 (CO), FAB MS m/z (rel. int): 1267 $[M+ KT]^-$ (26), 1251 $[M+Na]^+$ (28); UV A_{max} . nm: 199, 240. 255.

Saponin 3. Powder from EtOH (10mg); mp. 262-264° (lit. [3] 268-270°); [a]n 150.72° (pyridine; c 0.4); IR V^{KBr}. 3410 (br); FAB MS m/z (rel. int): 777 $[M + Na]^+$ (7), 754 $[M]^+$ (28); UV A_{max} „ nm: 199. 255.

Partial hydrolysis of saponin 1. Saponin 1 (50 mg) was heated on a boiling-water bath with 1 N HC1 in 50% n-BuOH for 6 hr. After cooling, the reaction mixt. was neutralized with NaHCO, and extracted with *n*-BuOH. The n-BuOH frs were combined and coned to dryness. The residue was chromatographed on silica gel, eluted with $CHCl_3$-MeOH-H_2O (14:6:1), then subjected to MPLC (pre-packed column RP-18) to give PsI.

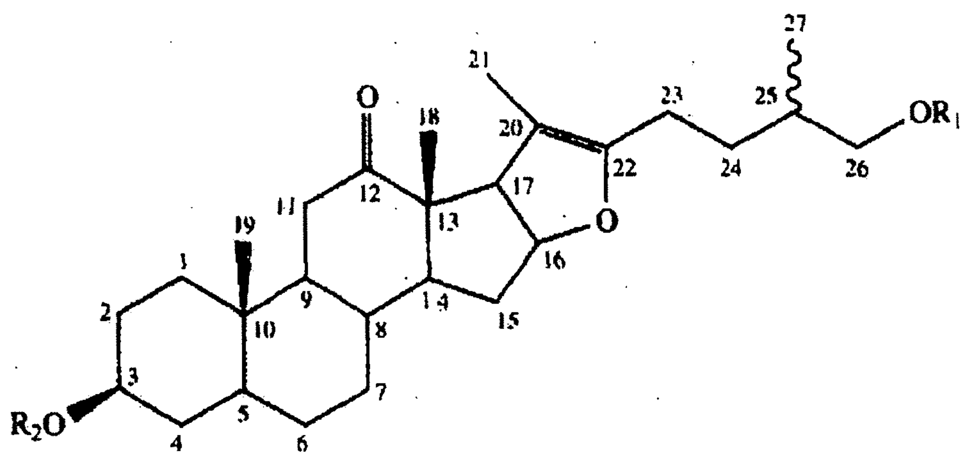
RESULTS AND DISCUSSION:

An ethanolic extract of the aerial parts of the plant, subjected to repeated chromatographic purifications, gave three steroidal glycosides **1-3,** which produced a yellow colour after spraying the TLC plate with H_2SO_4 followed by heating.

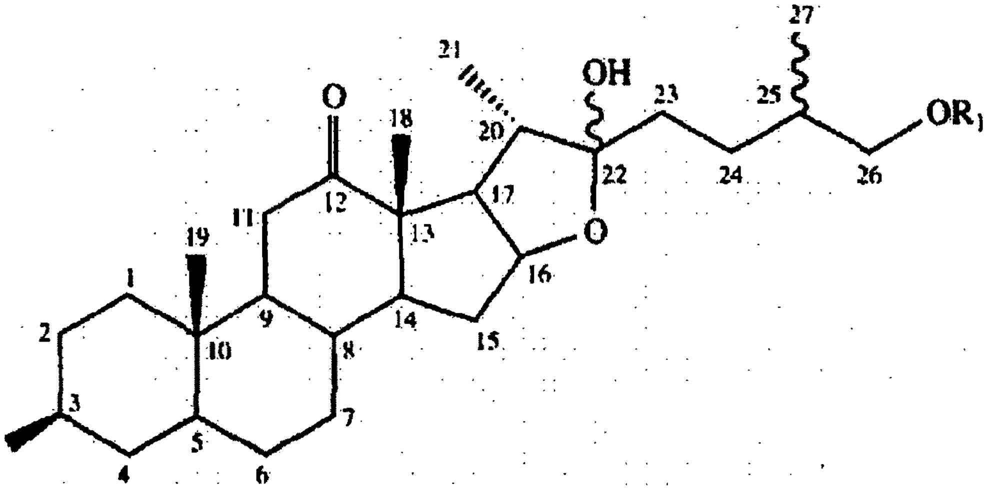
Saponin 3 exhibited molecular ion peaks at *m/z* 777 $[M + Na]^+$ and 754 $[M]^+$ in the FAB mass spectrum. The ^1H and ^{13}C NMR spectra indicated the presence of a terminal β-D-glucopyranosyl unit and an inner *β-o*-galactopyranosyi unit [3] [anomeric carbons: 102.43 and 107.10; anomeric protons: 4.86 (1H, *d*, *J* = 7.6 Hz) and 5.29 (1H, *d*, .7=7.8 Hz)]. From DQF-COSY, HMQC and HMBC, the chemical shift of protons and carbons were definitely assigned. The ^{13}C NMR spectrum showed C-20 and C-27 signals at δ 42.58 and 17.26, characteristic of a *25R* configuration [4]. Long-range correlation peaks were detected between the signals of C-l of glucose and H-4 of galactose, C-l of galactosc and H-3 of aglycone as well as C-l and H-17, H-1 1 and H-18. By comparison of the ^{13}C NMR spectrum with those of reported steroidal sapogcnins [5], the structure of 3 was established as hecogenin 3-0-β-D-glucopyranosyl(1 → 4)-galactopyranoside.

The FAB mass spectrum of 1 showed fragment peaks at *m/z* 1249 $[M+K]^+$, 1233 $[M+Na]^+$ and 1210 $[M^+]$. Its IR spectrum showed a broad band at 3400cm^{-1} for hydroxyl groups and a strong band at 1705cm^{-1} for a six-membered ring carbonyl. The ^1H NMR spectrum of 1 showed signals attributable to the C-l 8 and C-l 9 methyl groups at 0.90 and 0.63, the C-27 and C-2) methyls at 1.00 (3H, *d*, *J* = 6.6 Hz) and 1.72 (3H, *s),* indicating the absence of protons at C-20, and five anomeric proton signals at δ5.45 (1H, *d*, *J* =8.0 Hz), 5.15 (1H. *d*, 7=8.0Hz), 5.04 (1H, *d*, *J* = 7.2 Hz), 4.87 (1H, *d*, *J* = *7.2* Hz) and 4.81 (1H, *d*, *J* = 8.0 Hz), representative of the β-configurations of the five sugars [6]. The ^{13}C NMR spectrum of 1 exhibited the signals of a 5α steroidal saponin [3] (C-5: 43.61; C-9: 55.21; C-19: 11.96), a carbonyl signal at δ 212.47 and two signals in the olefinic carbon region at δ 102.83 and 152.80, which could be assigned to C-12, C-20 and C-22, respectively. These assignments can be confirmed through long-range couplings in the HMBC spectrum.

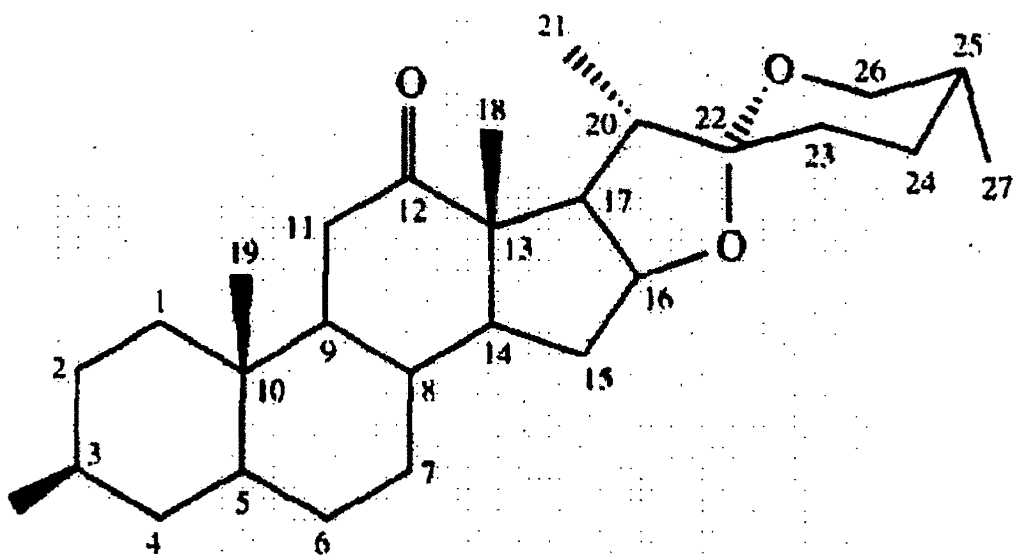
The techniques of DQF-COSY, 2D total correlation spectroscopy (TOCSY and HOHAHA), HMQC, HMBC and NOESY appeared to be very useful for the assignment of the ^{1}H and ^{13}C NMR signals ofcarbohydrates. However, in some cases, especially where there are little differences between the chemical shifts of protons, it seemed to be efficient to apply a few selected ID TOCSY experiments which can be performed normally in less than I hour. In general, we can obtain a complete ^{1}HNMR subspectrum with high digital resolution and a complete assignment of all ^{1}H signals of the selected moiety. Moreover, the linkage of the different moieties and hence the assignment of the obtained subspectra to the different carbohydrate rings can be very efficiently derived by HMBC, NOESY or a few selected ID nuclear Overhauser effect (NOE) experiments [7]. Our strategy for the assignment of the ^{1}H signals of carbohydrates of 1 is demonstrated in Table 1, which was obtained from ID TOCSY with increasing mixing time. A spin system was observed the different moieties and hence the assignment of the obtained subspectra to the different carbohydrate rings can be very efficiently derived by HMBC, NOESY or a few selected ID nuclear Overhauser effect (NOE) experiments [71. Our strategy for the assignment of the 'H signals of carbohydrates of 1 is demonstrated in Table 1, which was obtained from ID TOCSY with increasing mixing time. A spin system was observed.



1. R_1 = β-D-Glc R_2 = β-D-Glc^4- β-D-Glc^3 -β -D-Xy^1



2. R_1 = β-D-Glc R_2 = β-D-Glc^4- β-D-Glc^3 -β -D-Xy^1



3. R_1 = β-D-Glc R_2 = β-D-Glc^4- β-D-Glc^3 -β -D-Xy^1

TABLE 6.1

[1]H NMR SUBSPECTRUM OF SUGAR UNIT 1 EXPERIMENTS (SAPONIN 1)

Selected ^1H signal (annomeric proton)	Result of ID TOCSY	Mixing time (ms)
H11	H12,H13,H14(s)	30.45,70.130
H21	H22, H23, H24, H25	30,45,70, 130
H31	H32, H33	30,45,70
H41	H42, H43, H44, H45	30,45,70.130
H51	H52, H53, H54, H55	30,45,70.130

for galactopyranose (sugar 1), because $J_{4.5}$ = 0.0 Hz. The signals of protons of carbohydrates were assigned by DQF-COSY and HMQC. The connection of the sugar components was carried out by analysing the NOEs using NOESY measurements. All anomeric protons (except for sugar 5) apparently showed strong NOEs across the interglycosidic oxygen bridge to the hydrogen of the subsequent sugar unit or to H-3 of aglycone (Table 2). Unfortunately, sugar 5 had no cross-peaks, but in the HMBC spectrum, the carbon (C-26 of aglycone) at δ 74.78 is correlated with the proton (H-51 of sugar 5) at δ 4.81, and the carbonyl carbon (C-3 of aglycone) at δ 76.99 is correlated with the proton (H-

41 of sugar 4) at δ4.87. Extensive signals of the HMBC experiment are given in Table 2. Thus, the complete sequence of the sugar moieties was known. On the basis of the information of ^{1}H signals, the ^{13}C signals could be assigned by HMQC experiments (Table 3).

Acid hydrolysis of 1 yielded β-D-glucose, β-D xylose, δ-D-galactose (TLC) and prosapogenin PsI. The FAB mass spectrum of Ps 1 showed a peak due to [M^+] at *m/z* 592. The ^{13}C NMR spectra showed that PsI has the same aglycone as **1,** but only one sugar unit, glucose (based on the coupling constant of the sugar). From the HMBC measurement, the anomeric proton of glucose (δ 4.81) was long-range coupled with C-26 (δ 78.31) of the aglycone. Based on the above data, the structures of PsI and 1 were established as 26-0-β-D-glucopyranosyl-5a-furosto-20(22)-en-12-one-3β.26-diol and 26-0-β-D-glucdpyranosyl-3-0-[{β-D-xylopyranosyH I $\rightarrow$ 3)} ℵ' β - D - galactopyranosyl(1$\rightarrow$2)} - β-D- glucopyranosyl (1 $\rightarrow$4)-β-D-glucopyranosyl1-5α-furost-20(22)-en-12-one-3δ,26-diol.

FAB mass spectrometry of saponin 2 showed molecular ion peaks at m/z 1267 $[M + K]^+$. and 1251 $[M + Na]^{\cdot}$. By comparison with **I,** the ^{1}H NMR spectrum of 2 exhibited a signal at δ 51.52 (3H, *d,* 7=6.4 Hz), whereas the signal at δ 1.72 (3H, s) corresponding to (H-21) was absent. The ^{13}C NMR spectrum showed signals at δ 41.44, and 110.5, whereas the signals at δ 102.83 and 152.80 (C-20, C-22) were not evident, indicating that the structure of 2 has a 22-hydroxyl-5α-furostanol moiety. DQF-COSY, HMQC, HMBC and ID TOCSY experiments showed that the signals due to the sugar moieties were identical with those of 1. Thus, the structure of 2 was established as 26-0-β-D-glucopyranosyl-3-0-[{β-D-xylopyranosyl(l → 3)} {β-D-galactopyranosyK 1→ 2)}-β-D-glueopyranosyl(1 → 4)-β- D - glucopyranosyi] - 5α - furostan -12 - one - 3 β,22,26-triol.

TABLE 6.2

ASSIGNMENT OF ANOMERIC PROTONS BY NMBC AND NOESY EXPERIMENTS (SAPONIN 1)

Anomeric proton		**NMBC**	**NOE**
Hll	C22 (sugar 2)	H22	
H21	C44 (sugar 4)	H44	
H31	C23 (sugar 2)	H23	
H41	C3 (aglycone)	H3	
H51	C26 (aglycone)	—	

Table 6.3 ^{1}H and ^{13}C NMR data for saponins

	1		2		3		PS1	
C	^{13}C(δ)	^{1}H(δ)	^{13}C(δ)	^{1}H(δ)	C(δ)	^{1}H(δ)	^{13}C(δ)	^{1}H(δ)
1	36.35	0.75	36.49	0.70	36.55	0.70	36.74	0.70
		1.30		1.21		1.25		1.36
2	29.38	1.55	29.62	1.40	29.69	1.45	31.21	1.55
		1.95		2.01				1.80
3	76.99	3.85	77.28	3.90	76.78	3.84	70.16	3.77
4	34.38	1.32	34.57	1.45	34.26	1.25	33.98	1.40
		1.80		1.65		1.76		1.75
5	43.61	0.85	44.22	0.82	44.31	0.80	44.75	0.90
6	28.25	1.15	28.46	1.10	28.53	1.10	28.49	1.19
7	31.50	0.77	31.55	0.71	31.73	1.35	31.71	0.85
		1.50		1.52				1.55
8	33.76	1.63	34.13	1.79	34.54	1.60	33.61	1.70
9	55.21	0.87	55.32	1.89	55.43	0.85	55.50	0.95
10	35.93	—	36.13	—	36.21	—	36.13	—
11	37.83	2.20	37.88	2.20	37.93	2.20	38.11	2.29
		2.30		2.35		2.35		2.39
12	212.47	—	213.06	—	210.8	—	213.19	—
13	57.22	—	55.70	—	55.30	—	62.63	—
14	53.84	1.12	55.48	1.25	55.48		56.10	1.16
15	33.45	1.55	31.56	1.51	31.64	1.50	31.87	1.60
		2.12		1.98		2.06		2.25
16	82.63	4.69	79.56	4.90	79.64	4.45	82.83	4.70
17	55.89	3.38	55.68	2.88	54.23	2.75	54.13	3.39
	d (*J* = 10.2 Hz)						*d* (*J* = 10.4 Hz)	
18	13.81	0.90 *s*	16.14	1.10 *s*	16.04	1.05 *s*	14.01	0.91 *s*
19	11.96	0.63 *s*	11.57	0.62 *s*	11.50	0.62 *s*	11.48	0.78 *s*
20	102.83	—	41.44	2.17	42.58	1.86	101.0	—
21	11.96	1.72 *s*	15.18	1.52	13.88	1.34	11.73	1.72 *s*
			d (*J* = 6.4 Hz)		*d* (*J* = 6.9 Hz)			
22	152.80	—	110.5	—	109.27	—	152.92	—
23	23.36	2.20	34.45	1.82	31.73	1.65	23.51	2.25
24	31.07	1.42	28.27	2.00	29.16	1.95	33.29	1.43
		1.79						1.80
25	33.15	1.90	24.13	1.90	29.95	1.55	33.61	1.90
26	74.78	3.60	74.90	3.57	66.89	3.45	78.31	3.58
		3.87		3.90		3.55		3.95
27	16.94	1.00	17.32	0.92	17.26	0.65	17.11	0.98
	d (*J* = 6.6 Hz)		*d* (*J* = 6.5 Hz)		*d* (*J* = 6.8 Hz)		*d* (*J* = 6.0 Hz)	
Glu (sugar 5, 26-O-)								
1	104.53	4.81	104.80	4.79	107.10	5.29	104.66	4.81
	d (*J* = 8.0 Hz)		*d* (*J* = 7.7 Hz)		*d* (*J* = 7.8 Hz)		*d* (*J* = 7.7 Hz)	
2	74.83	4.01	75.19	4.04	75.94	4.45	74.99	4.02
3	78.25	4.20	78.48	4.22	78.71	4.22	78.39	4.25
4	71.41	4.18	71.56	3.97	72.26	4.05	71.53	4.20
5	78.11	3.92	78.49	4.01	78.50	4.06	78.39	3.94
6	62.24	4.59	62.68	4.39	63.09	4.10	62.63	4.35
		4.37		4.66		4.60	*dd* (*J* = 5.2, 12 Hz)	
								4.55
Gal (sugar 1)								
1	104.95	5.45	105.25	5.45	102.43	4.86	*dd* (*J* = 2.4, 12 Hz)	
	d (*J* = 8.0 Hz)		*d* (*J* = 7.7 Hz)		*d* (*J* = 7.6 Hz)			
2	73.41	4.57	73.67	4.60	73.06	4.35		
3	73.66	3.88	73.88	3.90	75.22	4.25		
4	70.05	4.25	70.19	4.22	80.80	4.78		
5	75.05	4.01	77.29	4.01	75.45	4.05		
6	60.19	4.63	60.43	4.17	61.03	4.25		
		4.15		4.68		4.65		

Table6.3 (continued)

	1		2		3		PS1	
C	$^{13}C(\delta)$	$^{1}H(\delta)$	$^{13}C(\delta)$	$^{1}H(\delta)$	$^{13}C(\delta)$	$^{1}H(\delta)$	$^{13}C(\delta)$	$^{1}H(\delta)$
Glu (sugar 2)								
1	105.17	5.15	105.43	5.15				
		d (J = 8.0 Hz)		d (J = 7.6 Hz)				
2	80.70	4.51	85.01	4.51				
3	85.31	4.11	85.36	4.11				
4	70.36	3.74	70.63	3.75				
5	77.26	3.85	77.41	3.88				
6	62.60	4.00	62.96	4.02				
		4.49		4.49				
Xyl (sugar 3)								
1	104.47	5.04	104.67	5.10				
		d (J = 7.2 Hz)		d (J = 7.2 Hz)				
2	74.83	3.91	74.16	3.92				
3	78.11	3.96	78.38	4.20				
4	70.05	4.04	70.48	4.07				
5	66.80	3.55	67.11	3.58				
		4.17		4.19				
Glu (sugar 4, 3-O-)								
1	101.97			4.85				
		d (J = 7.2 Hz)		d (J = 7.2 Hz)				
2	72.69	4.32	72.96	4.28				
3	75.43	4.08	75.01	4.09				
4	75.06	4.54	79.48	4.55				
5	75.06	4.01	74.85					
6	62.54	4.35	62.28	4.56				
		4.53		4.49				