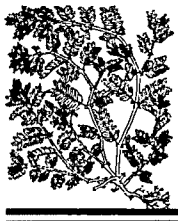
CHAPTER IV

Sapogenins



INTRODUCTION:

The plant of *T. Terreestris L.* is one of the most important ingredients of an Aurvedic (A herbal Indian prepartion of medicine) prepartion. The drugs are diuretiatorie aphrodisiac and often used in painful micturition. It induces mild hypotension with anti-acetylcholine like action on the rat intestine. The cardiac stimulamt action of *T. Terreestris L.* was reported by *Seth et al. (1976)*. The crude saponin fraction of the whole plant has been used as a cordial drug. Some chemical constituents of this plant have been reported *Wand and Lu (1989), Wang and Lu (1990), Ren et al. (1994) and Wang et al (1996)*.

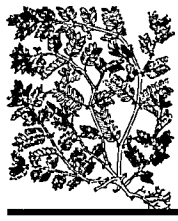
It this chapter the isolation and structural elucidation of five known and two new steroidal sapogenins are described.

EXPERIMENTAL:

I- Two Spagenins From Tribulusterrestris Linn

Plant Material:

Tribulus Terrrestris L. (Zygophyllaceae) was collected in 1999 from rural areas of Jaunpur district and identified by Dr. K.N. Mishra, Reader in Botany, T.D. College, Jaunpur-222002 (U.P.). A voucher specimen is deposited in the herbarium of the college.



EXTRACTION AND ISOLATION:

Air dried and powdered plants (10Kg) were extracted with cool 85% ethyl alcohal by evaporation, the residue was extracted with petrol, CH Cl_3 and n-Bu OH (n-Bustanol) respectively. The $CHCl_3$ layer (66g) was separted and fractionated by silica. gel chromatography with petrol- Et OH Ac (ethylactetate) (20:1; 10:1; 5:1) and $CHCl_3$ - Me OH (20:1, 10:1) to afford 1 (380mg) 2 (390mg), 3 (120mg) and 5 (67mg). The medical frs were further separted by preparations. TLC and $CHCl_3$-Eto Ac (10:1) to yield 4 (16mg) 6 (12mg) and 7 (14mg).

GENERAL:

MPS : ZMD 83-1 electric hot-stage **Uncorr:** UV: Shimadzu UV-265 FW, IR, Hitachi 275-50. NMR (þþm JH_3): Bruker AC-300 and Bruker AMX - 400, TMS as int. standara EI-MS: Variation MAT 212,, direct in let method CC: silica gel H (19-40µ, made in public Health engineering IT. BHU. TLC: Sillica gel H (10-40µ(, spot) were visualized by spraying with 10% H_2SO_4 following by heating.

RESULT AND DISCUSSION:

The material obtained after ethanolic extraction of *T. Terrestris L.* was partitioned between water and chloroform. The Chloroform fraction was further fractionated by silica-gel chromatography to afford compounds 1-7

compounds 1-4 were identified as tigogenin, lecognin, gitogenin and Leco genin.

Compound 5 was obtained as needless from chloroform. This constituent was considered to have both a saturated and an $\alpha - \beta$. unsaturated ketone based on the ultraviolet (MEON max 23.2nm) and infrared spectra.

(C = 0, 1716. 1682 cm^{-1}; C = C 1621 cm^{-1}). Comparing with hecogenone ^{1}HNMR, ^{13}C NMR and DET spectroscopic data indicated that the structure of 5 was 25R - spirostan - 4 ene - 3, 12 dione (Table 1-1 and -2). This structure was confirm by comparison of the ^{1}H NMR and ^{13}C NMR spectra with the known steroidal sapogenin, (5α-25S) - spirostan-4-ene-3, 12-deone *Domingnez et al. (1985).*

In the ^{1}H NMR spectrum of 5 $CDCl_3$, the absorption of H-26 "eq" and H-26 "ax" (δ3.49 and 3.34) was observed as a narrow doublet. Characteristic of the 25-R - configuration; the 25S - configuration produces signals at δ 3.32 and 3.93 *Dominaguez et al. (1985).* The ^{13}C NMR spectrum showed the chemical shifts of the F-ring compound 6 was obtained as needless from methanol. Its molecular formula $C_{27}H_{38}O_5$, was established by HREI-MS. The pease beak at m/z 139 and a fragment ion at m/z 126 were indicative of a lack of substitution in the *E and F rings Budzikiewiez et al. (1964).* The ^{1}H NMR spectrum of 6 showed signals attributable to the C-18 and C-19 methly groups at δ 1.09 and 1.06 the C-27 and C-21 methyls at δ 0.80 (3H, d, J=6.3 Hz) and 1.08 (3H d, J= 6.3 Hz). By comparison of the ^{1}H NMR ^{13}C NMR and DEPT

spectra with those of a series of spirostane steroids *Blunden and Cralb (1990) and Patel et al. (1994)* the structure was assiged as a 5α, 25R- sapogenin with three ketones (δ 207.3, 209.8, 211.1).

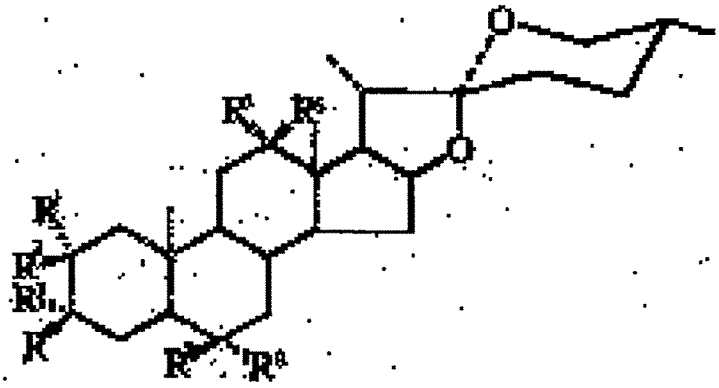
The structure of 6 was determined through the application of 2-dimensional (2D) NMR techniques including HMQC and HMBC, HMQC and HMBC spectra showed that the signal at δ 211.1 was related to 18-CH (δ 1.09m S) which indicated 12-keto group. The other two ketones (δ 209.8, 207.3) were related to the proton (H-5) at δ 2.61 which was correlation with C-10 (δ 40.6) and C-19 (δ 12.3). The chemical shift of C-5 (δ 56.9) showed a 10.7% þþm upfield shift compared to the corresponding value for hecogenone (δ 46.2,C-5) (Table: 4.1).

The two ketones were located close to C-5 and that one of them was in orthroposition to C-5. The HMQC and HMBC studies show that the ketone (a δ 207.3) was related to an unequal CH_2 group (δ 2.09, 2:51m), which was correlated with C-8 (δ 36.3). C-9 (δ 54.0) and C-14 (δ 55.1).

The other ketone (at δ 209.08) was related to two CH_2 goup (δ -39;2.38) and one of these (δ 2.58) was correlated with the ketone at 207.3. Thus the structure of 6 was established as (5α , 25R)- spirostan-3,6.12-trione. This structure was cofirmed by comparison of the ^{13}C NMR spectrum with those of chlorogenone *Carabot at al. (1991)* and hecogenone (Table: 4.2).

Compound 7 was obtained as a white powder Its moleenlar formula, $C_{27}H_{36}O_5$ was established by HREI-MS. It also had the spirostane structure. Its ^{1}H NMR and ^{13}C NMR spectra indicated the presence of three ketones and an unsaturated double bond. 2D NMR experiments including $^{1}H^{13}C$ COSY and HMBE were used to assing the hydrogen atoms attached to each carbon, to identify adjacent coupled protons and to examine long range coupled protons. Similary the ketone at δ 210.6 was related to 18-CH_3 (δ 1.13,S), C-5 (δ 158.4), a ketone (δ 200.1) and a CH_2 group (δ 33.7). These data suggested that the unsaturated double bond was either $\Delta^{4.5}$ or $\Delta^{5.6.}$ From the ultraviolet spectrum (Me OH max 24.4nm) and the chemical shifts of two ketones (δ 198.4 and δ 2001), it can be deduced that the two petones have conjugated with unsaturated double bond. Thus either a 4-ene-3, 6-dione group or a 5 ene-4, 7- dione group was possible.

From the HMBC measurement, the two ketones (δ 200.1,198.4) were long range coupled with two CH_2 groups and the two CH_2 groups had no relationship. This proved the presence of a 4-3n3-3 6- dione group. This structure was cofirmed from the infrared spectrum (C=O, 1700, 1710 cm^{-1}, C=C, 1620cm^{-1}) and by comparison of the ^{13}C specturm with 25R- spirostan - 4 - ene-3, 12 dione. All the above data indentified 7 as 25 R-spirosta-4-ene-3,6,12-trione.



R^3=H unless otherwise stated

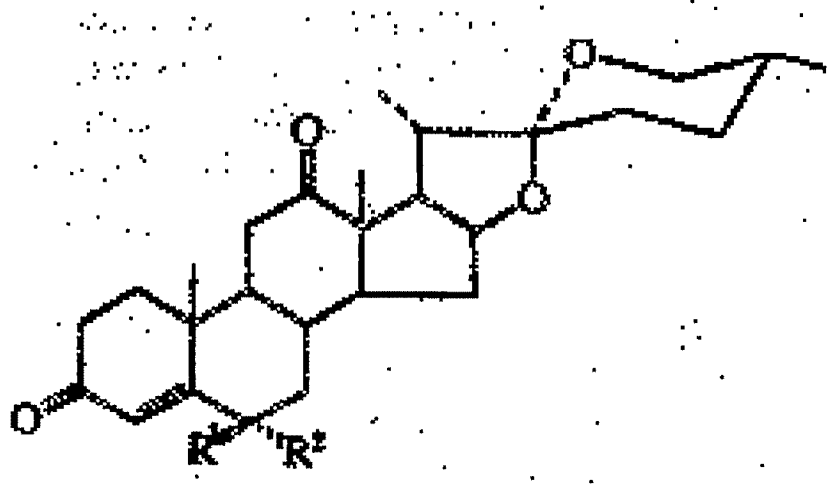
1 R^4 = OH

2 R^4 = OH, R^3R^4 = O

3 R^1 = R^4 = OH

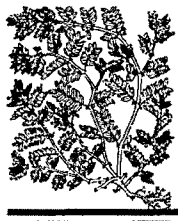
4 R^3R^4 = R^5R^6 = O

6 R^3R^4 = R^5R^6 = R^7R^8 = O



5 R^1 = R^2 = H

7 R^1R^2 = O



RESULTS:

25R-Spriostan-4-ene-3, 12-dione (5). White neeles from CH_3OH- mp 2.56-257°C.

Liebermann-Burchard Redction showed positive UV. Me OH max 238.2nm: IR (KBr) νmax cm^{-1}); 1718,1682 (C=O), 1624 (C= C), 982, 924, 902, 863 (924<902), E-CO) (13), 367 (16), 354 (49), 312 (100), 283 (8), 269 (25), 139 (85), 126 (48), ^{1}H NMR: Table 1; ^{13}C NMR: (Table: 4.2.)

" 5α,25R-Spirostan-3,6,i2-triom (€). White needless from CHsOH. mp 267-269°. IR (ν^{KBr}_{max} cm^{-1}): 1710, 1714 (C=0), 980, 920, 900, 860 (920 < 900). EIMS (probe) 70 eV, *m/z:* 442.2917 ((Mr$^+$ calc. for $C_{27}H_{38}O_5$. (442.2719) (21), 414 [M-CO] (8). 383 [$C_{24}H_{31}O_4^+$] (12), 370 [$C_{23}H_{30}N_4$] (28), 355 [$C_{23}H_{31}O_4^+$-] (15), 328 [$C_{21}H_{28}O_3$] (27), 299 [$C_{19}H_{23}O_4^+$] (6), 285 [$C_{19}H_{25}O_2$] (33). 139(100), 126(39). 115 (77). ^{1}NMR: Table 1; ^{13}C NMR: (Table 4.2).

25R-Spirostwi-4-eHe-3,6,l2-tione (7). White powder, *mp* 264-266°. UVλmax: 240.4 nm. IR (ν^{KBr}_{max} cm^{-1}): 1700, 1710 (C=0), 1620 (C=C), 980, 920, 900, 862 (920 < 900). EIMS (probe) 70 eV, *m/z:* 440.2571 (IM]$^+$, calc. for [$C_{27}H_{36}O_4^+$] , (440.2563) (77), 412 [M-CO] (20), 398 [M-42] (4), 381 [$C_{27}H_{28}O_4^+$] (31), 368 [$C_{23}H_{28}O_4^+$] (82), 353 [$C_{24}H_{29}O_4^+$] (36), 326 [$C_{21}H_{26}O_3^+$] (100), 312 [$C_{24}H_{29}O_4^+$] (34), 297 [$C_{19}H_{31}O_3^+$] (15), 283 [$C_{19}H_{23}O_2$] (41), 139 (63); 126 (11), 115 (47), 69 [C_4H_5O] (19). ^{1}H NMR: (Table 4.1) ^{13}C NMR: (Table: 4. 2).

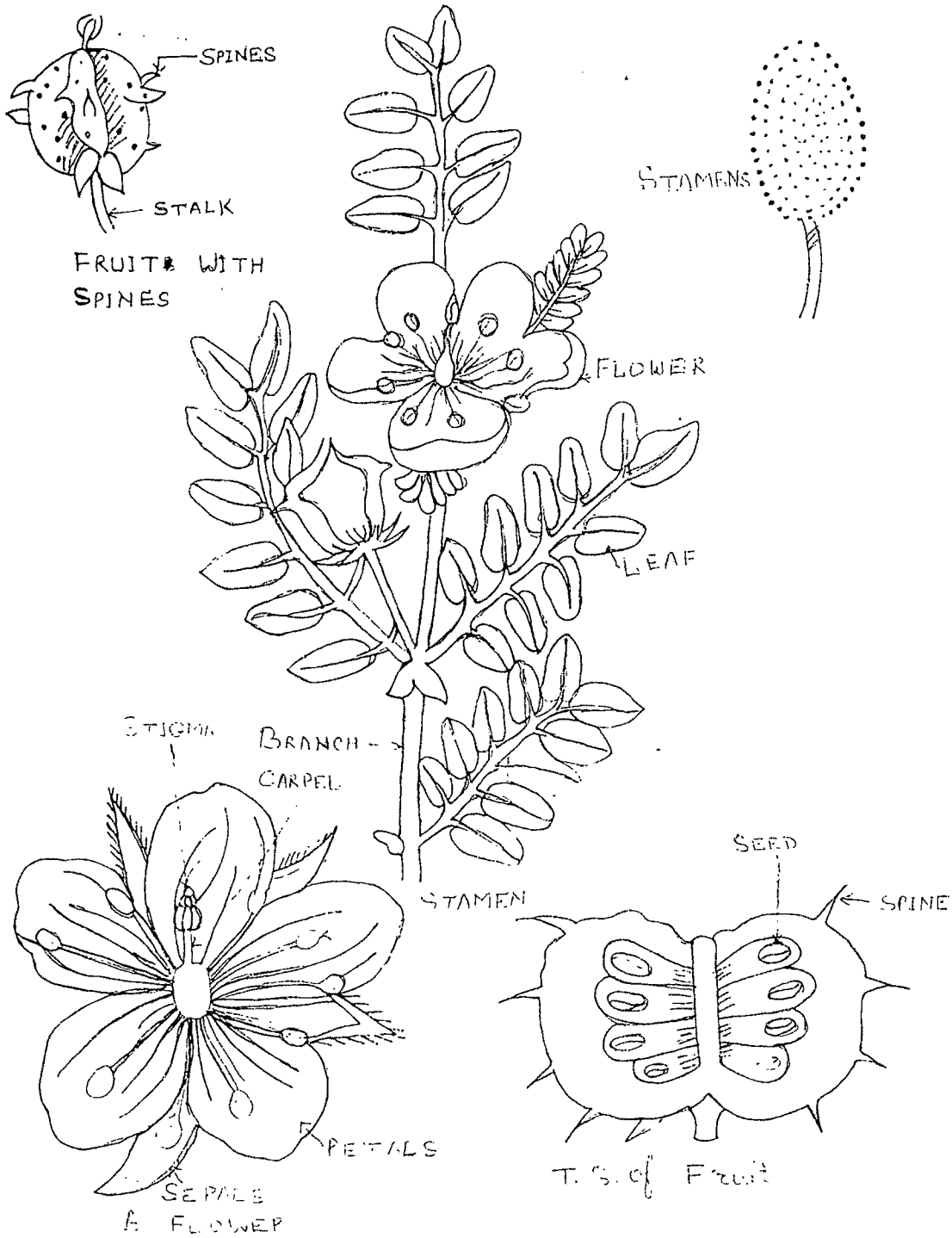


Fig 2.1 TRIBULUS TERRESTRIS LINN

TABLE 4.1 ^{1}H NMR SPECTRAL DATA OF COMPOUNDS 5-7 IN $CDCl_3$,

Compound	Me-18	Me-19	Me-21	Me-27	H-16		H-26	Others
5	1.11s	1.28s	1.07d (7.0)	0.80d (6.3)	4.36m	3.34t	3.49dd	5.78 {s, H-4)
6	1.09s	1.06s	1.08d (6.3)	0.80d (6.3)	4.38m	3.34t	3.49dd	2.09:2.51 {m:H.7} 2.58 {H-4} 2.39 {H-2} 2.61 {H-5} 1.83 {H-9} 1.71 {H-14} 2.55 {H-17}
7	1.13s	1.28s	1.08d (7.0)	0.80d (6.3)	4.38m	3.34t	3.49dd	6.26 {s;H-4} 2.12m:2.82dd {H-7} 2.54 (m,H-2} 1.87 {H-9} 1.64 {m;H-14} 2.58 {m;H-17}

Table 2. ^{13}C NMR Chemical shift (δ) of Compound 4-7 and 25 S Spirostan-4-ene-3, 12-dione in $CDCl_3$

^{13}C Chemical Shift (δ)	Commpound 4	25S*	5	6	7
1	37.6	35.3	35.3	37.4	35.2
2	37.7	32.9	32.3	36.9	33.7
3	210.6	198.6	198.6	209.8	198.4
4	44.4	124.7	124.7	37.1	126.8
5.	46.2	168.5	168.4	56.9	158.4
6.	28.6	33.7	33.6	107.3	200.1
7	31.4	31.1	31.1	45.8	45.9
8	34.3	34.4	34.4	36.3	32.7
9	54.9	54.6	54.5	54.0	51.7
10	36.2	38.7	38.7	40.6	39.3
11	37.7	37.1	37.1	37.6	36.7
12	212.7	211.9	211.9	211.1	210.6
13	55.1	54.8	54.8	55.1	54.8
14	55.5	54.8	54.8	55.1	55.1
15	31.2	31.1	31.4	31.4	31.4
16	79.1	79.1	78.9	78.8	78.7
17	53.6	53.3	53.5	53.7	53.5

18	16.0	15.9	15.9	16.0	15.9
19	11.1	16.9	16.8	12.3	17.4
20	42.2	42.7	42.2	42.3	42.3
21	13.2	13.0	13.2	13.2	13.2
22	109.3	109.7	109.2	109.3	109.3
23	31.2	25.8	31.2	31.0	31.0
24	28.8	26.0	28.7	28.7	28.8
25	30.2	27.0	30.1	30.2	30.2;
26	66.9	65.2	66.9	67.0	67.0
27	17.1	16.0	17.1	17.1	17.1

Results:

25R- Spirostan - 4 - ene - 3, 12 - dione (5). White needles from CH_3OH - mp 256-257^{0}C.

Liebermann-Burchard reaction:

Showed positive UV: MeOH max 238.2 nm: IR $Vmax^{KBr}$ Cm^{-1}; 1718, 1682 (C=O), 1624 (C=C), 982, 924, 902, 863 (924<902), EIMS (Probe) 70ev, m/z: 426 $[M]^+$ (42), 398 [M-CO] (13), 367 (16), 354 (49), 312 (100), 283 (8), 269 (25), 139 (85), 126 (48) ^{1}H NMR: Table1; ^{13}C NMR:Table2.

Sα R-Spirostan-3,6,12-*lrioHe (6).* White needle from CH_3OH. mp 267-269°. **1R** (V^{KBr}_{max} cm^{-1}): 1710, 1714 (C=0), 980, 920, 900, 860 (920 < 900). EIMS (probe) 70 eV, *m/z:* 442.2917 ([Mr, calc. for $C_{27}H_{38}0_{5+}$, 442.2719) (21), 414 [M-CO] (8), 383 [$C_{24}H_{31}O_4$] (12), 370 [$C_{24}H_{30}O_4$] (28), 355 [$C_{24}H_{31}$-CO] (15), 328 [$C_{21}H_{28}O_3$] (27), 299 [$C_{19}H_{23}0_3$] (6), 285 ($C_{19}H_{25}O_2$] (33), 139 (100), 126 (39), 115 (77). ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

25R-Spirostan-4-ene-3,6,l2-lrione (7). White powder, mp 264-266[3]. UV: λ^{MeOH}_{max} 240.4 nm. 1R (V^{KBr}_{max} cm^{-1}): 1700, 1710 (C==0), 1620 (C==C), 980, 920, 900, 862 (920 < 900). EIMS (probe) 70 eV, *m/z:* 440.2571 ([M]$^+$, calc. for [$C_{27}H_{29}O_4^+$] 440.2563) (77), 412 [M-CO]$^+$ (20), 398 [M-42] (4), 381 [$C_{24}H_{29}O_4^+$] (31), 368[$C_{24}H_{29}O_4^+$] (82), 353 [$C_{24}H_{29}O_4^+$] (36), 326 [$C_{24}H_{29}O_4^+$] (100), 312 [$C_{24}H_{29}O_4^+$] (34), 297 [$C_{24}H_{29}O_4^+$] (15), 283 [$C_{24}H_{29}O_4^+$] (41), 139 (63), 126 (11), 115 (47), 69 [$C_{24}H_{29}O_4^+$] (19). ^{1}H NMR: Table I; "C NMR: Table 2.