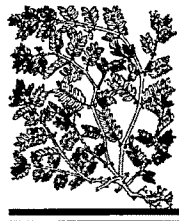
CHAPTER V

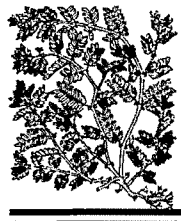
Steroidal Saponins from fruits



INTRODUCTION:

In Indian Ayurvedic medicines the fruit of *T.terrestris Linn,* have long been used for the treatment of eye trouble, edema and abdominal distention, emission and morbid lucorrhea as well as vitilago *Seth and Jagdeesh (1976). Jiangsu, New Medical College (1977)* and *Chakraborty and Neogi (1978)*. Ten spirostanol-type saponins were isolated from the fruits of *T.terrestris Linn,* growing in Jaunpur. Among them five compounds (Terrestrosin A-E) were new saponins *Wang et al. (1996)*. Here in this report the isolation and structural elucidation of six furostanol saponins are investigated.

The six new furostanol saponins as constituents of fruits of *T.terrestris Linn,* are reported: i.e. 26-O-β-D, glucophyranosyl (25R) furostane 2a, 2b, 22a, 26 tetrol-3-0-β-D glucophyranosyl (1-4)-β-D- galactophyranoside, 26-o-β-D- glucopyramosyl (25R,S) 5α furostane- 2a, 3β, 2a, 26 tetrol- 3-0-β-D galactophyranosy (1-2)-β-D. glucophyranosy (1-A) β-D glucophyranosyl (25-R.S.-. -5α- furostane 3α>22 α, 2.6 triol 3-D-β-D- galactophyramosyl (1-2)-β -D - glucophyranosyl (1-4) -β -D- galactphyranosyl (1-2)- β - D - glucopyranosly (1-4)-β -D galactophyranoside, 26-o-β-D- glucopyranosyl (25R,S)- furost - 5 ene - 3β, 22α, 2.6 triol -3-O-β-D- galactopy ranosyl (1-2)-β -D- glucopyranosyl (1-4)-β-D- galactop yranoside, 26-O-β-D- glucopyranosyl (25R, S) -5α - furost - 20 (22) - en -12 - one 3β, 26 diol-3-O-β-D-



galactopyramoside, named terrestrosin - F - K, respectively. The structures were elucidated on the basis of spectroscopic studies of the isolated compounds and their hydrolysed products.

EXPERIMENTAL:

Optical rotation: Union PM-101. NMR (ppm, *J* Hz): JEOL JNM-GX 500, TMS as int. standard. FAB MS: JEOL JMS-SX 102, direct inlet method. HPLC: a Tosoh HPLC system (pump, HLC-803 D; detector, RI-8000) equipped with D-ODS-5 column (20 mm i.d. x 25 cm YMC) and polyamine-II column (20 mm i.d. x 25 cm YMC) with flow rate of mobile phase 6 ml mur[1]. CC: Kieselgel 60 (70-230 mesh. Merck) and Li. Chroprep RP.18 (Merck). TLC: Kieselgel 60 precoated plates, F^ (Merck) and HPTLC using RP-18 precoated plates, FM (Merck) or Kieselgel 60 precoated plates (Merck) for acid hydrolysis on TLC and spots were visualized by spraying with Ehrlich's rcagentgr 10% H_2SO_4 followed by heating.

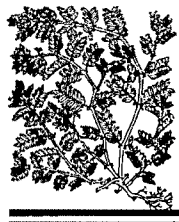
PLANT METERIAL:

Tribulus terrestris L. (Zygophyllacae) was collected in 2000 from rural areas of Junpur district and idenified by Dr. K.N. Mishra, Reader in Botany, T.D. College, Jaunpur-222002 (U.P.). A vaucher specimen is deposited in the herbarium of the college.

EXTRACTION AND ISOLATION:

The fruits were defatted with petrol (bp 60-90°). The defatted material was extracted with 80% EtQH. The extract was subjected to CC on silica gel using $CHCl_3$, Me_2CO and MeOH, successively. Crude saponin was obtained from MeOH withMe_2CO precipitation.

Crude saponin (24 g) was sepd into 7 frs over silica gel CC with CH_2Cl_2-MeOH-HA (50:10:1; 40:10:1; 30:10:1; 20:10:1; 10:10:1) and finally with MeOH. Fr. 3 (2.46 g) was further chromatographed over LiChroprep RP-18 CC using a gradient elution with 25-50% aq. McCN t6 give 11 frs. Fr. 3-1 (770 mg) was repeatedly subjected to prep. ODS-HPLC with 25% aq. McCN and prep. polyamine-HPLC with 83% aq. MeCN to give 1 (35 mg) and la (20 *mg).* Fr. 4 (4.20 g) was sepd by CC on reversed-phase silica gel, LiChroprep RP-18 using a gradient elution with 20-30% aq. MeCN and silica gel CC with CH_2Cl_2-MeOH-H_2O (30:10:1) to give la (93 mg) and 3 (199 mg) with a little impurity. Fr. 5 (6.67 g) was subjected to LiChroprep RP-18 CC using a gradient elution with 20-35% aq. MeCN to give 13 frs. Fr. 5-4 (1.25 g) was repeatedly subjected to silica gel CC with CH_2Cl_2-MeOH-H_20 (30:10:1) and ODS-MPLC with 80% aq. MeCN to give Fr. 5-4-4-1 (580 mg) which was almost pure. Part of Fr. 5-4-4-1 (230 mg) was further purified by prep. polyamine-HPLC using 79.5% aq. MeCN to give 4 (40 mg). Fr. 5-6 (1.44 g) was sepd by silica gel CC with CH_2Cl_2-MeOU-H_2O (30:10:1) to give Fr. 5-6-3 (1.17 g) which was almost pure. Part of Fr. 5-6-3 (240 mg) was further purified by prep. polyamine-HPLC



using 80% aq. MeCN to give 2 (78 mg). Fr. 5-8 (1.39 g) was repeatedly subjected to ODS-MPLC with 23% and 25% aq. MeCN, silica gel CC with CH_2Cl_2-MeOH-H_2O (30:10:1) and prep. ODS-HPLC with 26.5% and 27% aq. MeCN to give pure 3 (82 mg), 5 (10 mg), 6 (19 mg) and 6a (32 mg).

After subjecting the frs to silica gel CC, they were refluxed with 30 % Me_2CO to convert (he 22-methoxy form to the original 22-hydroxy form then proceeded as described.

Terrestrosin F(l). White powder, $[\alpha]_d^{2o}$,- 20.0° (pyri-dine; c 0.60). HR-FAB-MS (neg.) *m/z.* 935.4871 [$C_{45}H_{76}O_{20}$-H), requires 935.4852. FAB-MS (neg.) *m/z:* 935 [M-H]-, 773 [M-GIc]-, 611 [M-GIc -Gal]-. 'H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin G (2). White powder, $[\alpha]_D^{24}$ - 26.8° (pyri-dine; *c* 0.75). HR-FAB-MS (neg.) *m/z:* 1097.5440 [$C_{51}H_{86}O_{25}$-H]-, requires 1097.5379. FAB-MS (neg.) *miz:* 1097 [M-H]-, 935 [M-GIc]-, 773 [M-GIc -Gal]-, 611 [M-Glc-Gal-GIc]-. 'H NMR: Table *}; "C* NMR: Table 2.

Terrestrosin H (3). White powder, $[\alpha]_D^{24}$-*20.4*^{0} (pyridine; c 0.54). HR-FAB-MS (neg.) *m/z:* 1081.5400 [$C_{51}H_{86}$,0_{24}]-, requires 1081.5428. FAB-MS (neg.) m/z: 1081(M-H]-,919[M-Glc]-, 757[M-Glc-Gal]$^-$, 757 [M-GIc-Gal-GIc)-. 'H NMR: Table 1; ^{13}C NMR: Table 2.

Terrestrosin I(4). White powder, $[\alpha]_D^{24}$-17.0° (pyridine; c 0.53). HR-FAB-MS (neg.) m/z: 1095.5220 [$C_{51}H_{84}A_{25}$-H]-, requires 1095.5219. FAB-

MS (neg.) *m/z:* 1095 [M-H)-, 933 [M-GIc]-, 771 [M-Glc-(Gal, 609 [M-Glc-Gal-GIc]-. 'H NMR: (Table:1.1) ^{13}C NMR: (Table1.2).

Terrestrosin J (5). White powder, $[\alpha]_D^{25}$-42.9° (pyridine 0.79), HR-FAB-MS (neg.) m/z: 1079.5280 [$C_{51}H_{84}O_{24}$-H]-, requires 1079.5273. FAB-MS (neg.) *m/z.* 1079 [M-H]-, 917 [M-GIc]-, 755 [M-GIc-Gal]-, 593 [M-Glc-Gal-GIc]-. 'H NMR: 5.30 (1H, *br, s,* H-6), the other signals see Table 1; ^{13}C NMR: Table 2.

Terrestrosin K (6). White powder, $[\alpha]_D^{24}$ +3.1° (pyridine; c 1.31). HR-FAB-MS (neg.) *m/z:* 1077.5140 [$C_{51}H_{84}O_{24}$-H]-, requires 1077.5125. FAB-MS (neg.) w/z: 1077 [M-H]-, 915 [M-GIc]-, 753 [M-Glc-Gal]-, 591 [M-Glc-Gal-GIc]-. 'H NMR: Table l;'^ NMR: Table 2.

Acid hydrolysis of 1-6. A soln of each saponin (about 2 mg) in 2 N HCl-dioxane (1:1, 0.5 ml) was heated at 95^0 for 2 hr. The reaction mixt. was diluted with H_20 and then extracted with EtOAc. The EtOAc layer and H_2O layer was checked for identification of aglycone and sugar moieties, respectively. Aglycones were identified with TLC by comparison with authentic samples, using CH_2Cl_2-MeOH (50:1) as developing solvent and 10% H_2SO_4 as detection reagent. Saponins 1 and 2 gave gitogenin (A/0.09), 3 gave tigogenin *(Rf* 0.33), 4 and 6 gave hecogenin *(Rf* 0.20) and 5 gave diosgenin *(Rf* 0.39). Sugars were checked by TLC using CH_2Cl_2-McOH-H_2O (15:6:1) as developing solvent and TTC reagent for detection. 1-6 gave glucose *{Rf* 0.21) and galactose *(Rf* 0.17), respectively.

Enzymatic hydrolysis of 1-4. A solution of 1 (20 mg) and β-glucosidase (20 mg) in acetate buffer (5 ml, pH 5.0) was incubated at 37° overnight. The solution was extracted with n-BuOH. The n-BuOH extract was coned and subjected to prep. polyamine HPLC using 87% aq. MeCN afforded IS (5 mg). IS was identified as gitogenin 3-0-β-D-glucopyranosyl(l-4)-β-D-galactopyranoside based on the physical properties and NMR spectral data [4]. Glucose was detected by TLC as described above.

By the same procedure carried out for 1a (8 mg), 2 (23 mg), 3 (25 mg) and 4 (32 mg) yielded the corresponding prosapogenins 1 aS (2 mg), 2S (5 mg), 3S (4 mg) and 4S (6 mg), respectively, as well as glucose.

The prosapogenins 2S, 3S and 4S were identical with terrestrosin E, terrcstrosin A and tcrrestrosin C, respectively, isolated earlier from the same plant [2].

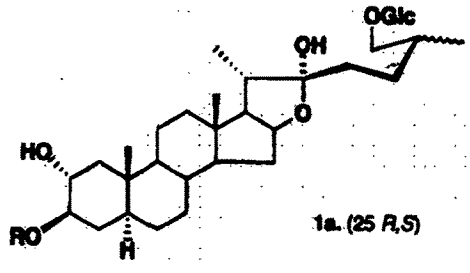
RESULTS AND DISCUSSION :

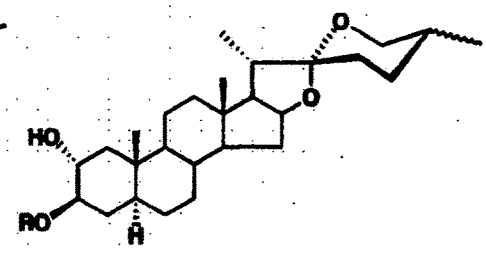
The crude saponin fraction of *T. terrestris was* subjected to re-peated silica gel, reversed phase RP-18 column chromatography and prepara-tive HPLC to afford compounds 1-6. Alt die compounds 1^6 were easily de-duced to be furostanol saponins on the basis of the colour reaction with Ehrlich's spray reagent on.

TLC *Kiyosawa et al. (1968)* and their C-22<x-configurations were confirmed based on 2D ROE spectrum of compound 3. On add hydrolysis,

they yielded glucose and galactose as sugar residues.

Compound 3 gave a red colour with Ehriich's reagent. Its molecular formula was determined as C51 H86 O24 from the high-resolution negative FAB-mass spectrometry. The 'H NMR spectrum of 3 displayed four doublet signals of anomeric protons at δ5 4.75, 4.85, 5.05 and 5.08 with 7.6, 7.0, 7.6 and 7.5 Hz coupling constants, respectively, diagnostic of the β-D-configuration for all four sugars. All the "C NMR signals of the sugar moieties of 3 were identical with those ofterrestrosin A *Wang et al. (1996)*, except for a set of additional signals corresponding to a β-D-glucopyranosyl unit. On enzymatic hydrolysis with β-glucosidase, 3 afforded terrestrosin A (3S) and D-glucose indicating 3 is the 26-0-(β-D-glucopyranoside) of spirostanol form saponin



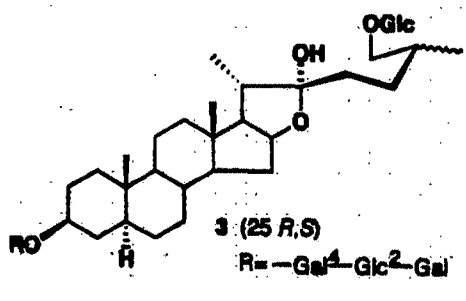


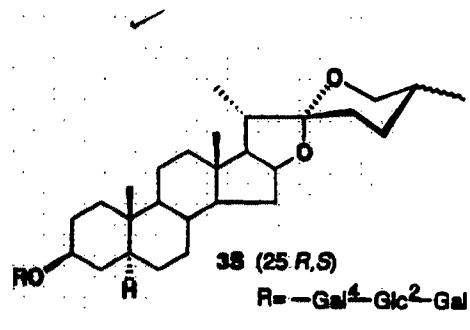
1 (25fl) 1a.(25fl,S)

R.-Oal^-Gte

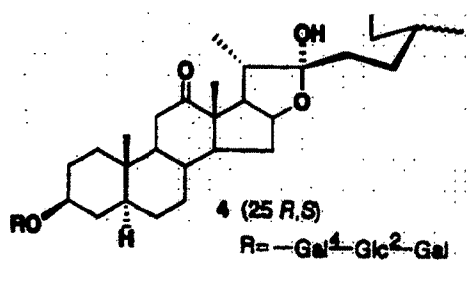
2 (25 fl,S)

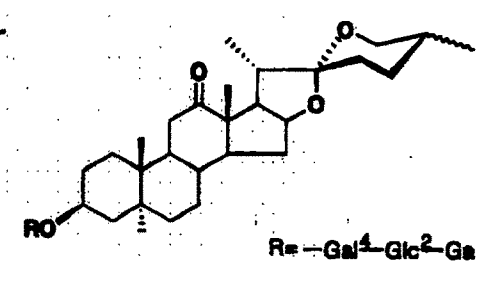
R«_QalA-Qlc2-Qal





OGte

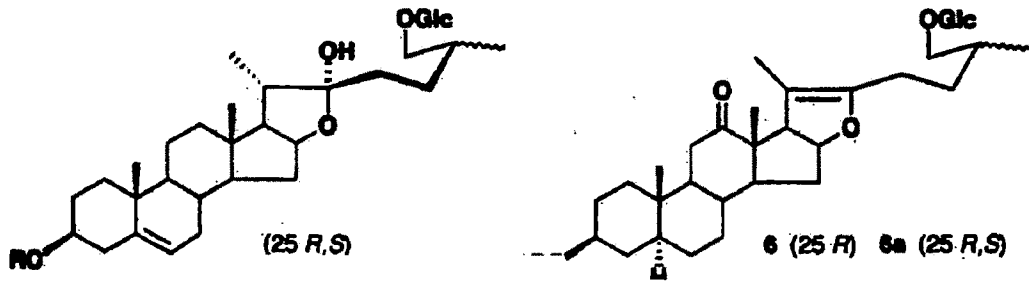




48 (25ff,S) H
Rc-GalQkGri

5 *{aSR.S)*

Bft (25fl) (25fl,^



B=-GrtA-Qlc2-Qa| ro R«-Q,|d-Qic2-Gal Scheme.

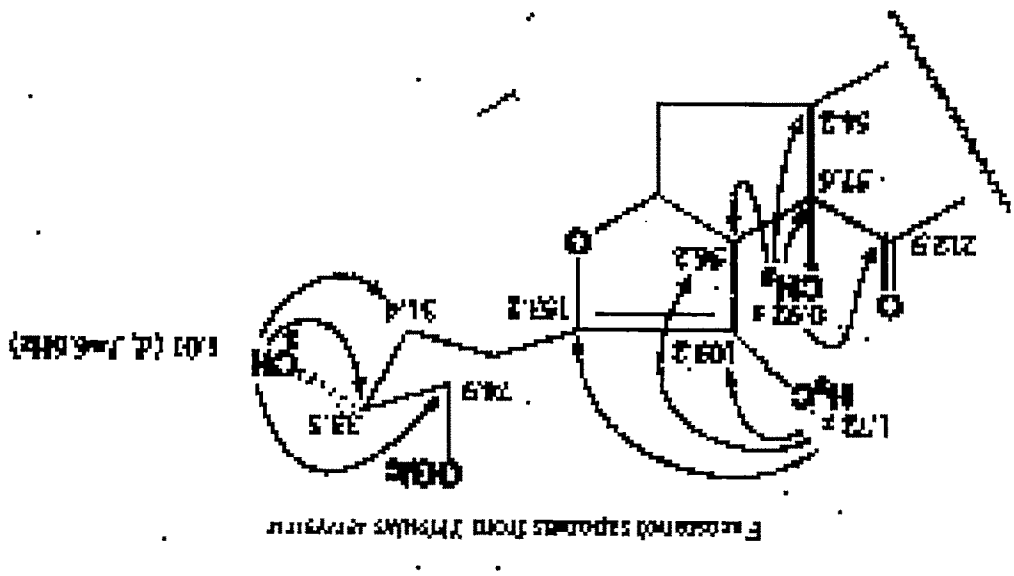
1. Chemical formulae of compounds 1-6 and prosapogenins IS, 2S, 3S and 4S.

(3S). In the "C NMR spectrum, the signals due to the aglycone moiety were indicative of a 3,26-di-0-glycosylated 5α-furostane-3β,22,26-triol structure *Yahara et al. (1994).* The PROESY (phasesensitivc rotating frame nuclear Overhause effect spectra) spectrum of 3 confirmed the C-22 configuration to be a in which the cross peak was observed between the H-20 proton *(δ2.19m)* and the H-23 protons *(δ*1.97 *m).* The C-25 configuration of 3 was deduced to be a RS.-mixture based on the ^{1}H **NMR** signals at δ3.47 (^{1}H, *dd, J* = 7.8,8.5 Hz, Ha-26,*25S)* and 3.60 (1H, *dd,J* = 6.4, 9.3 Hz, Ha-26,*25R),* which was also determined by analysis of the ^{13}C NMR spectrum of prosapogcnin 3S. Therefore, the structure of 3 was established to be 26-0-β-D-glucopyranosyl (25R,S)-5α-furostane-3β,22α,26-triol-3-0-β-D-galactopyranosyl(l-2)-β-D-glucopyranosyl(l-4)-P-D-galactopyranoside, and was named tcrrestrosin H.

Compound 1 gave a red colour with Ehriich's reagent. Its molecular formula, $C_{45}H_{76}O_{20}$, was established by high-resolution negative FAB-mass spectrometry. The 'H NMR spectrum of I displayed three doublet signals of

anomeric protons at δ4.78, 4.90 and 5.24 with 7.9, 7.6 and 7.9 Hz coupling constants, respectively, diagnostic of a β-D-configurauon for allthree sugars (Table:1.1). In the ^{13}C NMR signals due to the aglycone moiety were indicative of a 3,26-di-O-glycosylated 5α-furostane-2α,3β,22α,26-tetrol structure *Yahara et al. (1994)*. Comparison of the ^{13}C NMR chemical shifts thus assigned with those of the reference methyl glycoside *Agrawal, Gupta and Thakur (1985)* and taking into account the known effect of the 0-glycosylation and the result of acid hydrolysis indicated that 1 has two terminal P-D-glucopyranosyI units and a 4-substituted β-D-gal-actopyranosyi unit.

On enzymatic hydrolysis with β-glucosidase, 1 yielded prosapogenin 1S and D-glucose. Compound 1S was identical with the reported prosapogenin, gito-genin 3-0-β3-D-glucopyranosyl(l-4)β-D galactopyr-Comparison of the ^{1}H and ^{13}C NMR spectra of 6 with those of 4 indicated that they have the same partial structures A, B, C and D-rings.



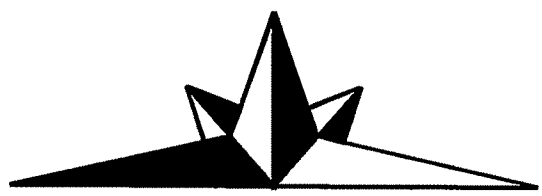
The 'H NMR spectrum of 4 showed the presence of two singlet and two doublet methyl signals while the ^{1}H NMR spectrum of 6 showed the presence of three singlet and only one doublet methyl signals. The difference between the two compounds is that 6 possesses a double bond between C-20 and C-22, which was suggested by the NMR signals at δH 1.72 (3H, *s*, 21-CH,) and 3.39 (1H, *d*, *J*= 10.3 Hz, 17-H) and two quaternary carbon signals at 8C 153.2 (C-22) and 103.2 (C-20) Agrwal, Jain and Pathak (1995) Dong and Han (1991). On acid hydrolysis. 6 gave hecogenin which was probably derived from its original aglycone by cyclization of the side chain. The assignments of the aglycone moiety were determined by DEPT, HSQC, HMBC and comparison with the aglycone moiety of 4. In the HMBC spectrum, the methyl protons at *6* 0.92 (18-CHJ) showed long-range correlation with the carbons at *S* 57.6 (C-13), 54.2 (C-14), 212.9 (C-12) and 56.2 (C-17), as shown in Fig. 1, indicating the attachment of the keto group at C-12. The methyl protons at *6* 0.68 (19-CH3) showed long-range correlation with the carbons at *6* 36.3 (C-10), 36.7 (C-l), 44.6 (C-5) and 55.6 (C-9). The methyl protons at δ 72 (21-CHi) showed long-range correlation with the carbons at δ 56.2 (C-17), 103,2 (C-20) and 153.2 (C-22). The methyl protons at δ 1.01 (27-CH,) showed long-range correlation with carbons at δ 31.4 (C-24), 33.8 (C-25) and 74.9 (C-26). Thus, its aglycone moiety was deduced to be a 5α-furost-20(22)-en-12-one-3β,26-diol structure. The C-25 configuration of 6 was δ, which was confirmed by the 'H NMR signals at δ 3.61 (1H, *dd*, *J* = 5.7, 9.3 Hz, Ha-26, *25R)*. The comparison of ^{1}H and ^{13}C NMR spectra of 6 with those of 4 indicated that they have the

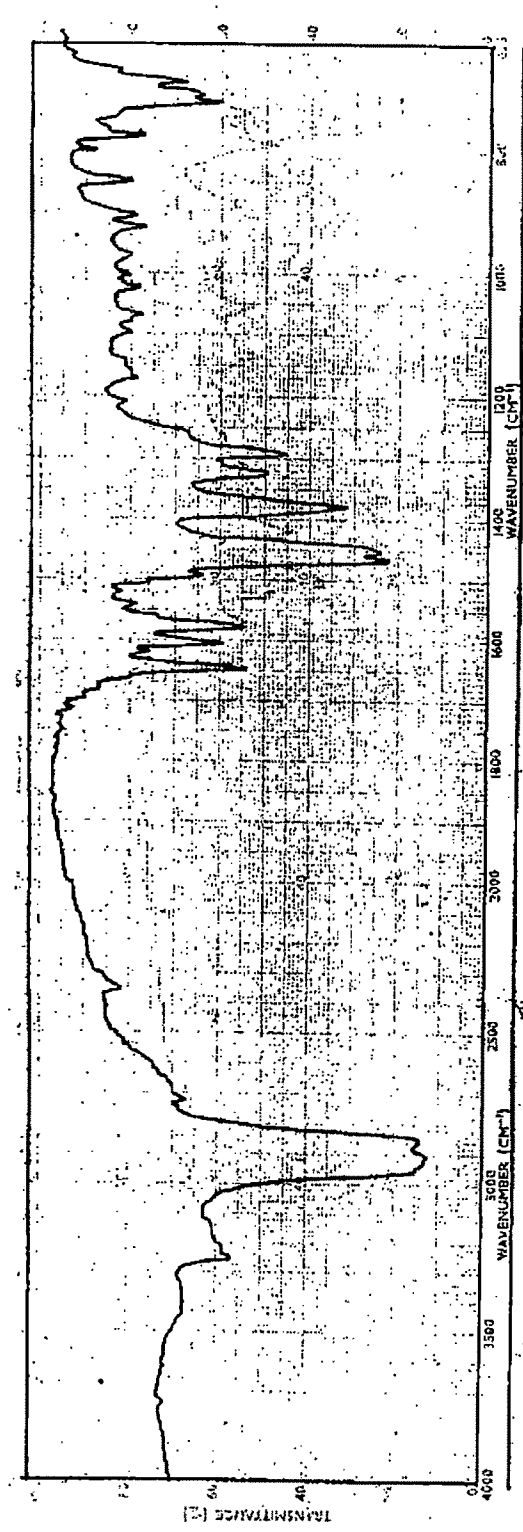
same sugar sequence. Thus, the structure of 6 was established to be 26-0-β-D-glucopyranosyl (25β)-5α-furost-20(22)-en-12-one-3β,26-diol-3-0-β-D-galactopyranosyl(1 -2)-β-D-glucopyranosyl(1-4)-β-D-galactopyranoside and was named terrestrosin K.

In addition to 6, the 25R,S-mixture, $[\delta]o^{25}$ + 5.3° (pyridinc) was also isolated. Compound 6a was established to be the 25R, S-mixture based on the 'H NMR signals at δ 3.47 (1H, dd, j= 7.0,8.8 Hz. Ha-26,255) and 3.60 (1H, *dd, J* = 6.0,9.0 Hz, Ha-26, 25.R).

The absolution configurations of the sugars were determined in the course of our studies of the spiro-stanol saponins from the fruits of *T. terrestris Wang et al. (1996)*. We assumed the same configurations for the sugar moieties of the furostanol saponins found in the same extract.

The amount of the isolated saponins indicated that the furostanol saponins were the major constituents of the fruit extract:

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1400
1200

Fig. No. 1

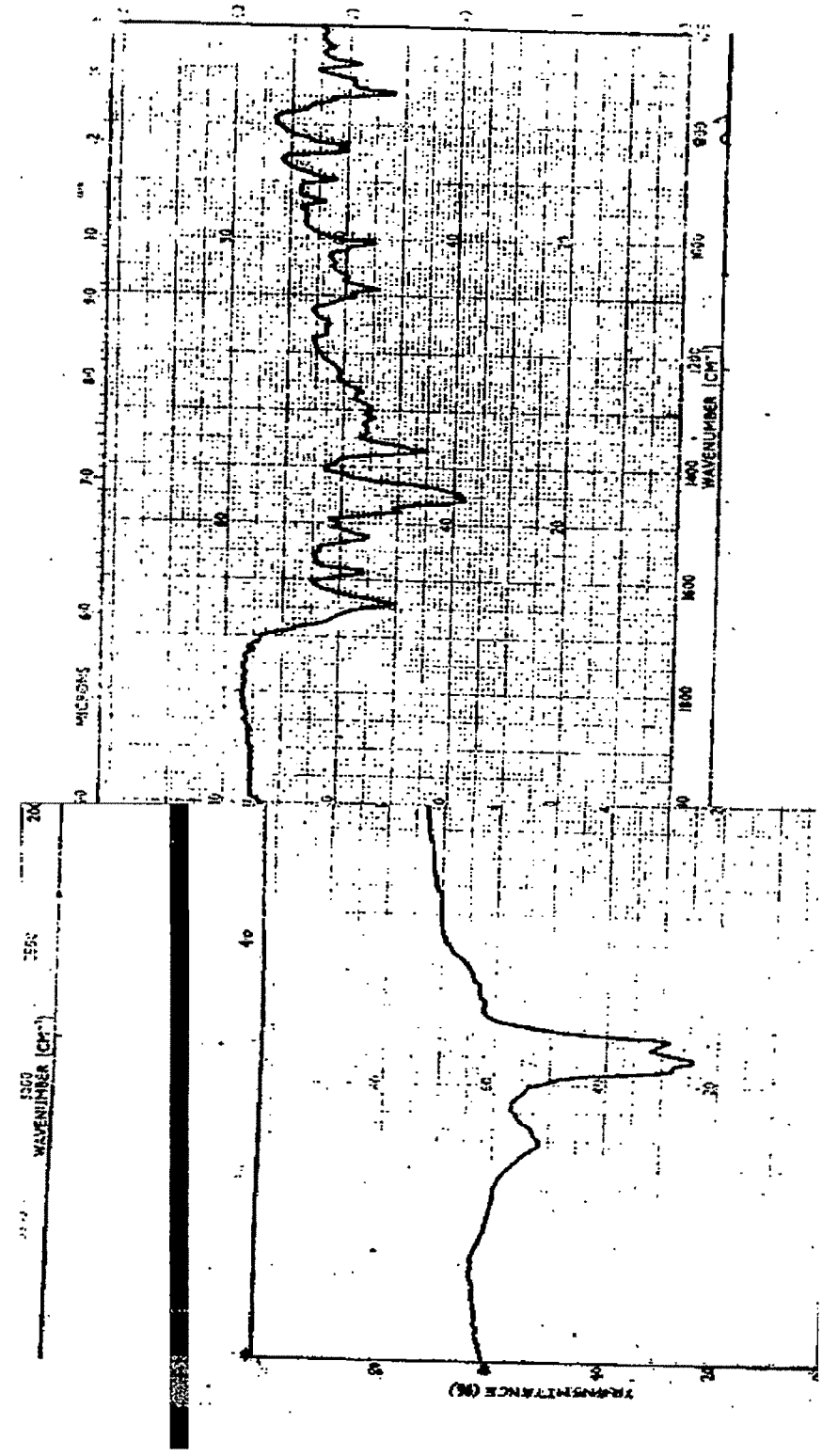
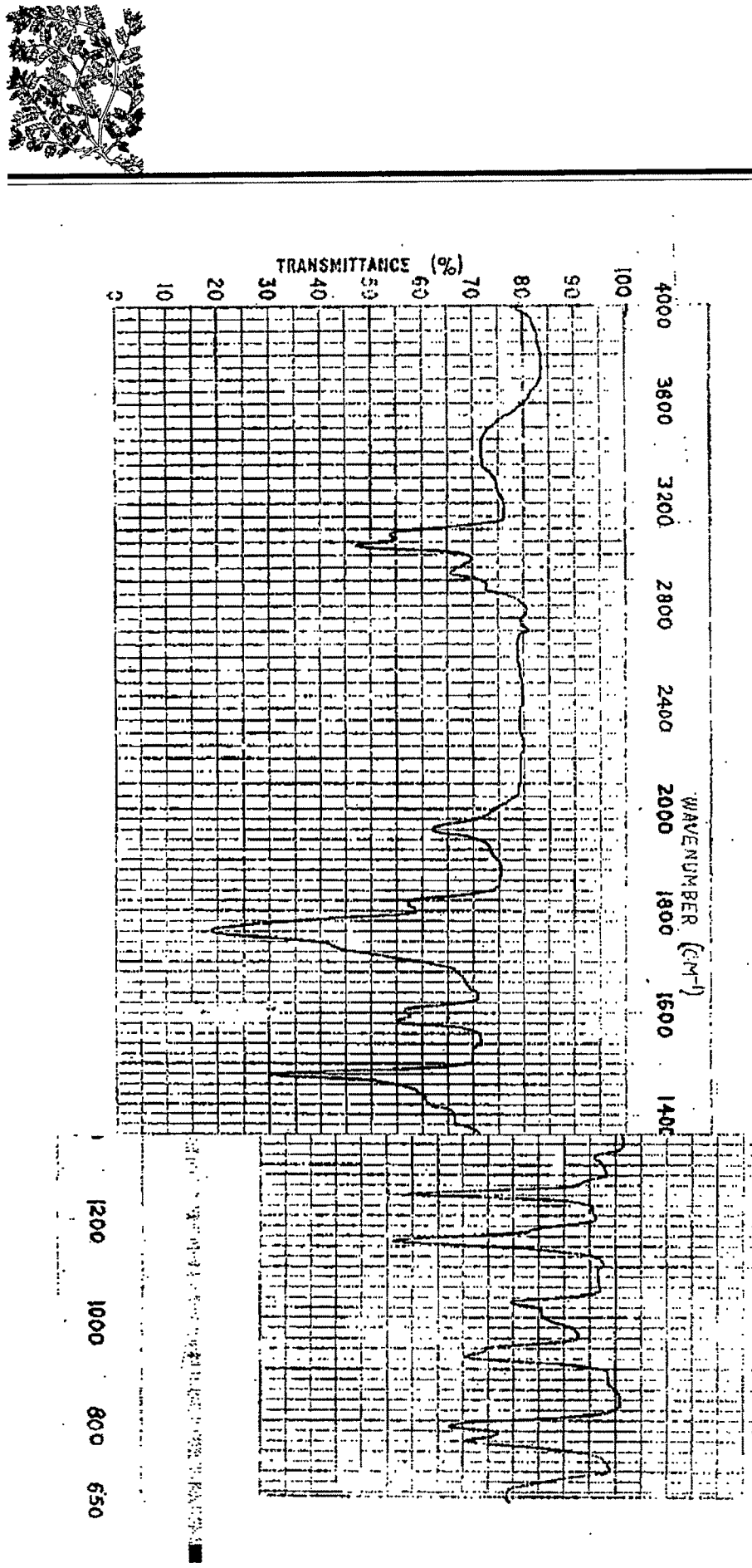


Fig. No. 2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800
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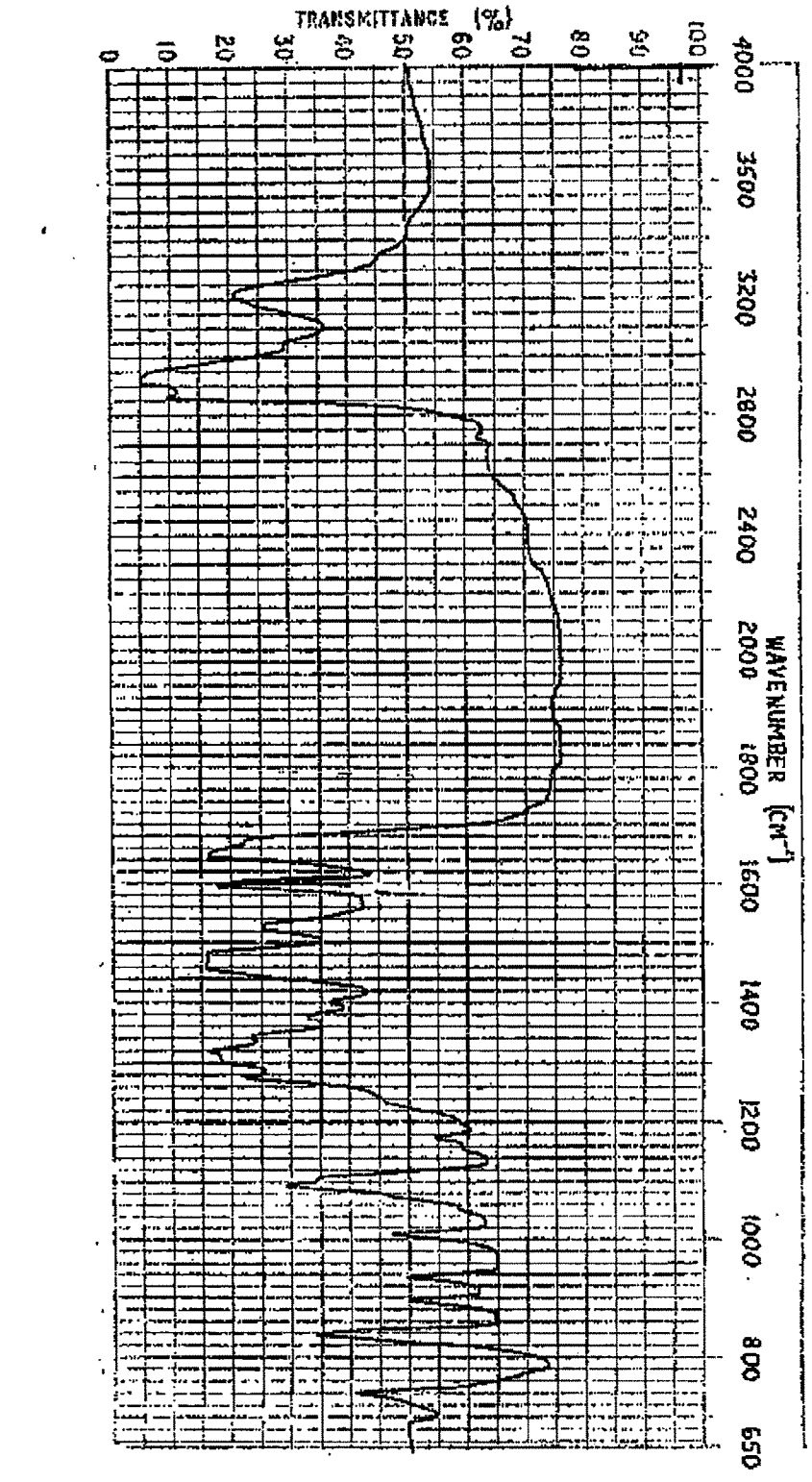
Fig. No. 3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650
WAVENUMBER (cm⁻¹)

Fig. No. 4