CHAPTER-4

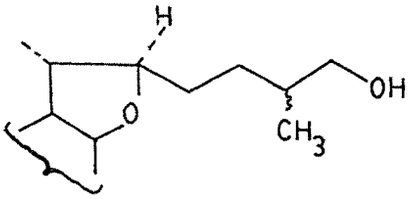
STRUCTURE OF SHATAVARIN-I

Isolation of shatavarin-I is described in chapter-2. The present chapter deals with the structure determination of it. The relevant evidence is described below.

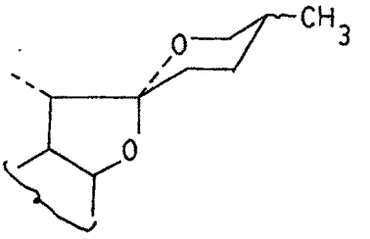
TLC STUDY OF SHATAVARIN-I

When the crude saponin mixture was sprayed with Ehrlich's reagent[1], four compounds gave red coloured spots (Fig.1). Shatavarin-I also gave red coloured spot with Ehrlich's reagent.

In 1968, Hutoh and Kiyosawa[2] reported that steroidal saponins with furostanol type[3] structure give positive test (red colour) to Ehrlich's reagent, whereas steroidal saponins with spirostanol type structure do not give red coloured spots, when sprayed with Ehrlich's reagent.



Furostane



Spirostane

Since shatavarin-I gives a positive Ehrlich's tests, it is a furostane type saponin and not a spirostanol type saponin.

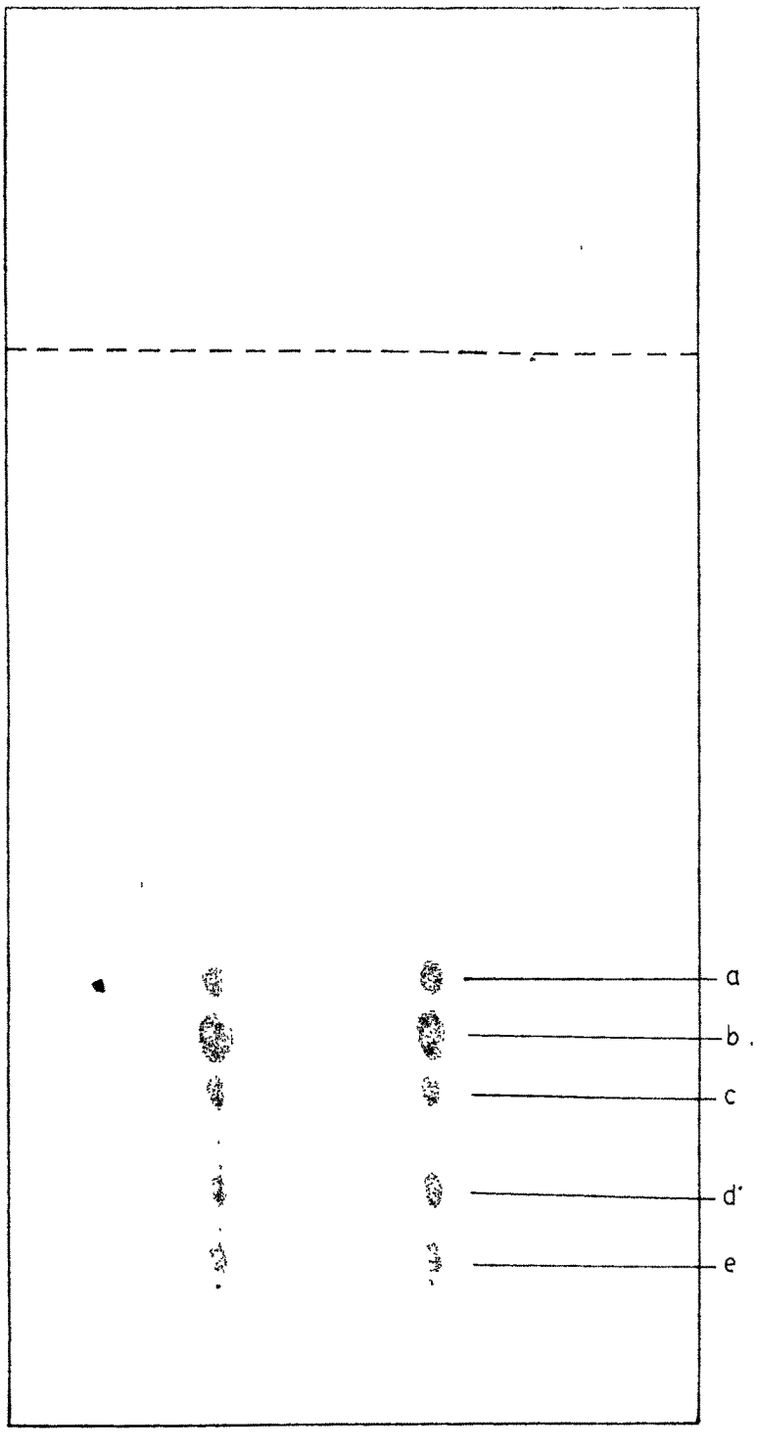


FIG.1 : TLC OF EHRLICH REAGENT +VE SAPONINS FROM SHATAVARI

SOLVENT SYSTEM : $CHCl_3$-CH_3OH-H_2O (65-35-10)
(LOWER PHASE)

SPRAY REAGENT : EHRLICH'S REAGENT

SPOTS : (a) SHATAVARIN-II (b) SHATAVARIN-I
(c) SHATAVARIN-V (d) SHATAVARIN-VII
(e) SHATAVARIN-VII

HYDROLYSIS OF SHATAVARIN-I

Shatavarin-I was hydrolysed by refluxing it with 2N sulfuric acid in dioxan[4]. The aglycone and the sugars were separated.

The aglycone was purified by chromatography over silica gel and crystallized several times to get a product (m.p. 197-200°) which analyzed for $C_{27}H_{44}O_3$, M^+, m/z 416. IR (KBr) showed -OH absorption at 3400 and C-O at 1060 cm^{-1}. Four absorption bands are observed at 850, 890, 912 and 987 cm^{-1} which are characteristic of a steroidal saponin[5]. The absorptivity of 912 band is stronger than that of 892 cm^{-1} band which indicates that it is a 'normal' sapogenin. The spectral data is comparable to the spectral data of sarsasapogenin[6-11]. The identity of the compound was confirmed by its comparison (m.m.p., CO-TLC) with an authentic sample*. The IR, ^{1}H-NMR and mass pectra are shown in Figs. 2, 3 and 4.

The comparable data is represented in Table-1.

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* Available in our Lab. collection.

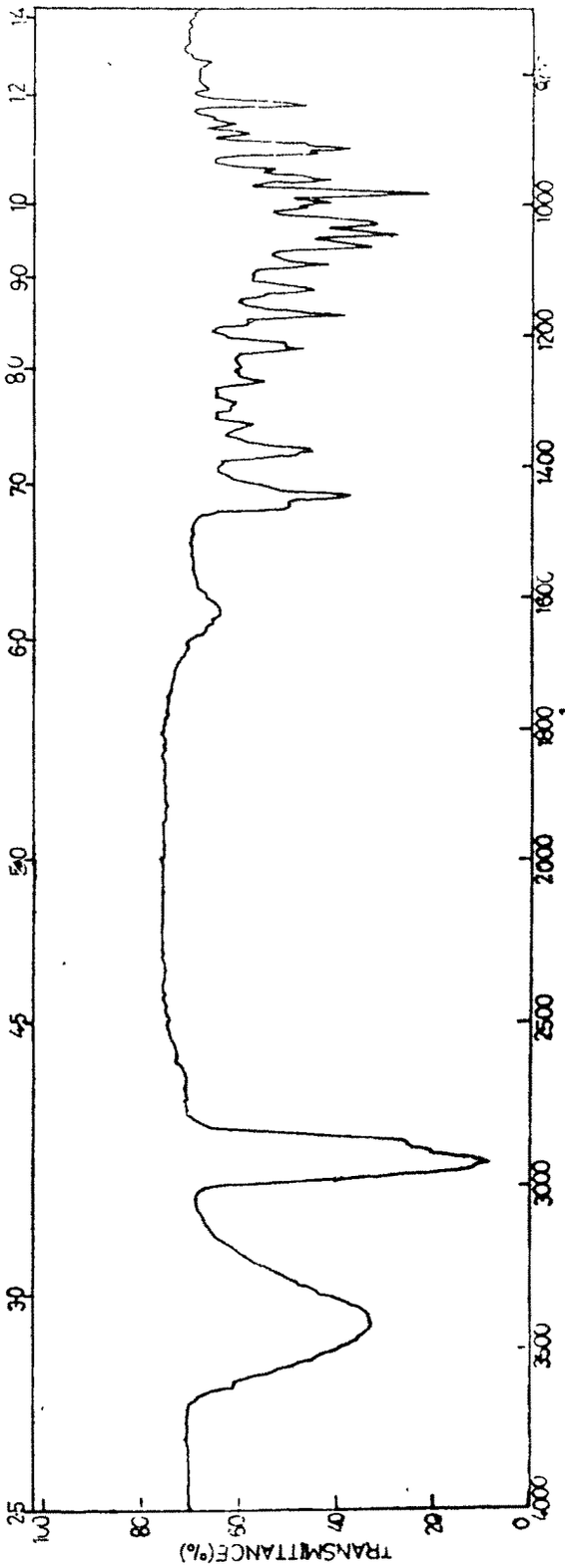


FIG.2 : IR SPECTRUM OF AGLYCONE OF SHATAVARIN-I

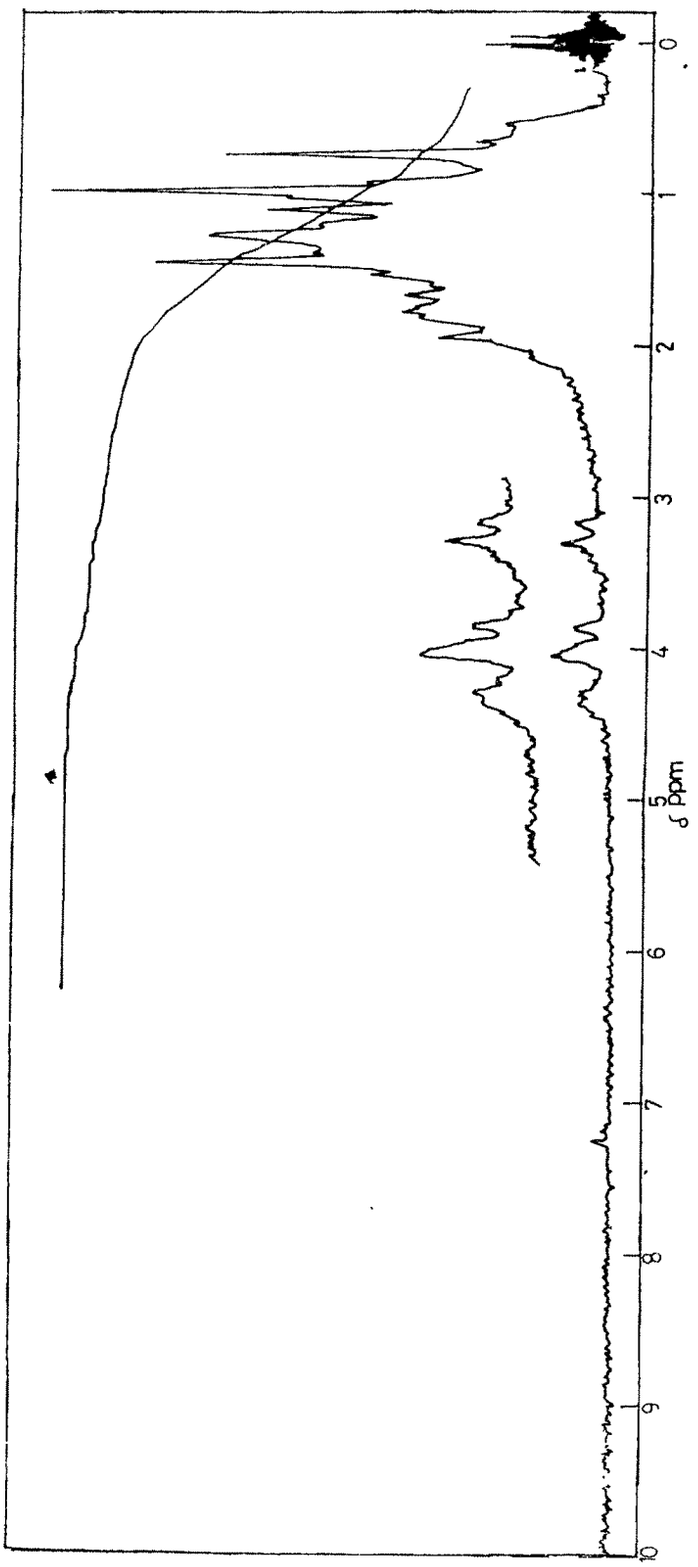


FIG.3 : ^{1}H-NMR SPECTRUM OF AGLYCONE OF SHATAVARIN-I

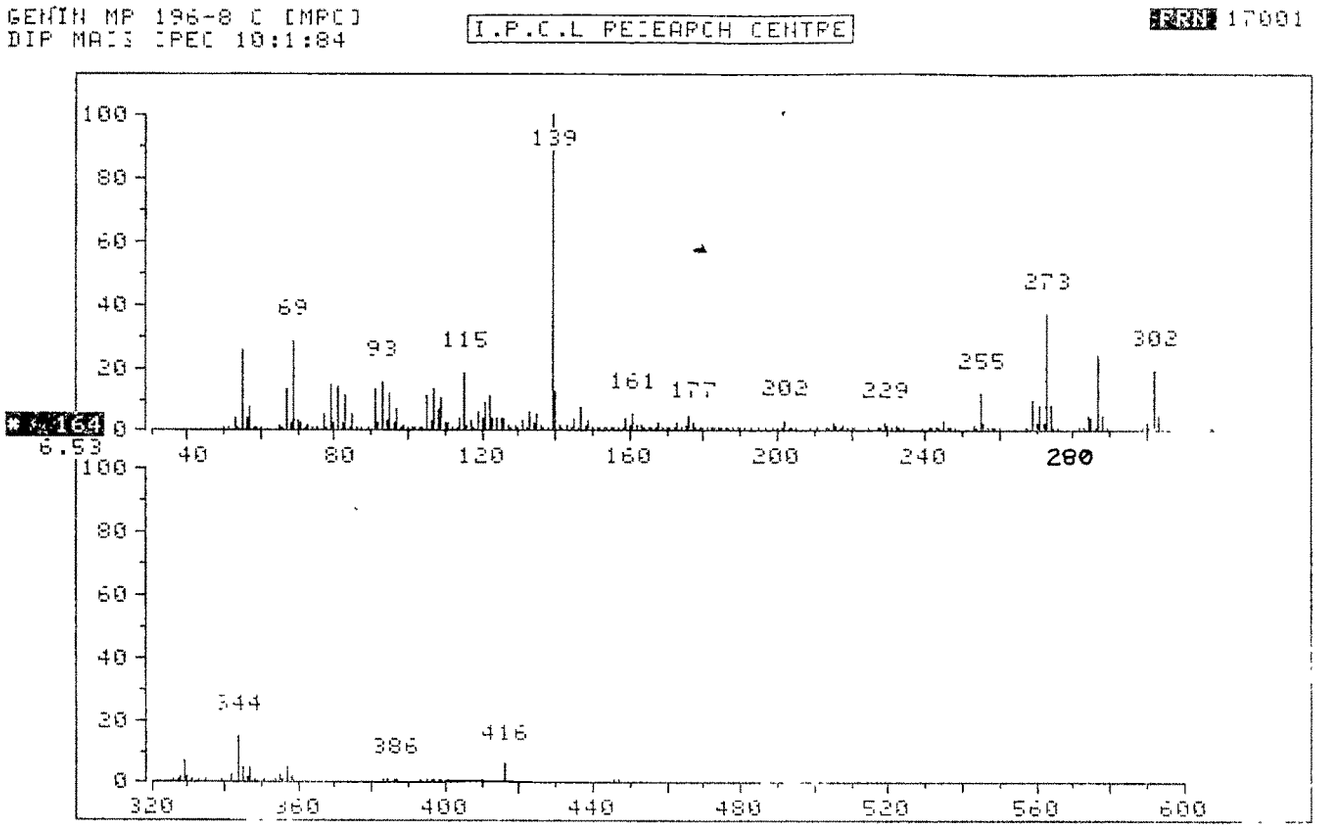


FIG.4 : MASS SPECTRUM OF AGLYCONE OF SHATAVARIN-I

TABLE-1 : COMPARISON OF THE PHYSICAL CHARACTERISTICS AND SPECTRAL PROPERTIES OF THE AGLYCONE WITH THOSE OF SARSASAPOGENIN

Sr. No.	Properties	From shatavarin-I	Literature[6-11]
1.	m.p.	197-200°	198-200°.
2.	$[\alpha]_D^{26}$	-76° ($CHCl_3$)	-78° ($CHCl_3$).
3.	TLC (R_f)+	0.5	0.5 (authentic sample).
4.	^{1}H-NMR ($CDCl_3$)	δ 4.38 3.85 (d) 1.28 1.1 0.99 0.77	δ 4.40 (16-H) 3.30/3.95 (26-H) 1.08 (27-H) 1.00 (21-H) 0.98 (19-H) 0.77 (18-H)
5.	IR	ν_{max}^{KBr} cm^{-1} 850, 890, 912 and 987, with 912 > 890 cm^{-1}.	ν_{max}^{KBr} cm^{-1} 850, 900, 920 and 986, with 920 > 900 cm^{-1}.

+ On silica gel G plate; solvent system: Benzene-ethyl acetate (1:1).

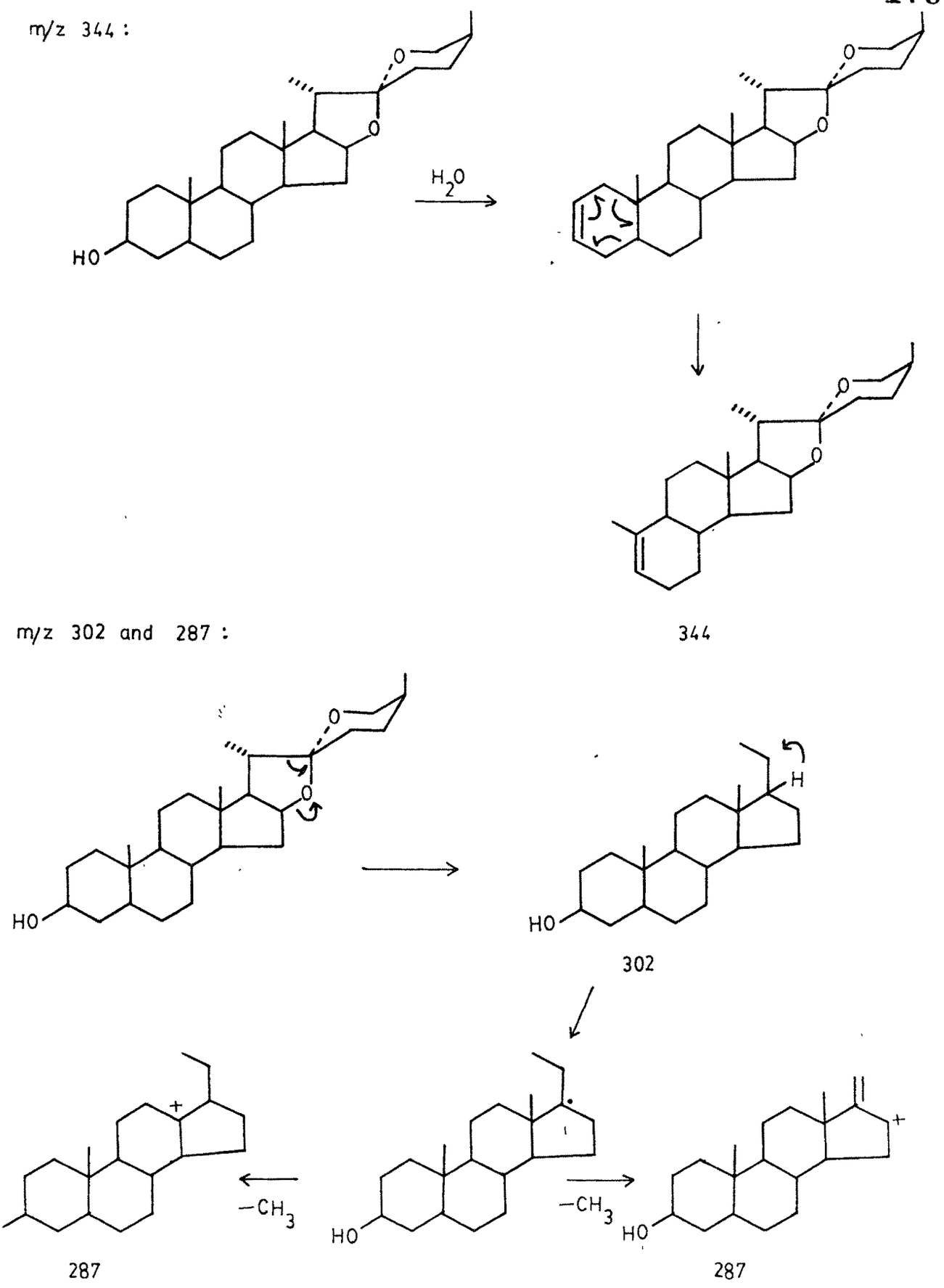
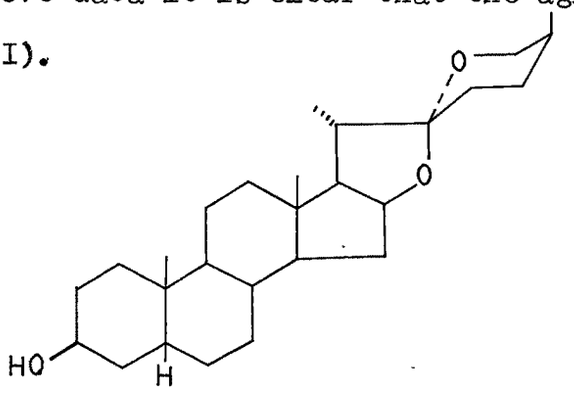


FIG.5 : FORMATION OF IONS IN THE MASS SPECTRUM OF SARSASAPOGENIN FROM SHATAVARIN-I.

SARSASAPOGENIN FROM SHATAVARIN-I.

From the above data it is clear that the aglycone is sarsasapogenin (I).



I

The electron impact fragmentation of steroidal sapogenins have been reported[12,13]. The mass spectrum of the aglycone is shown in Fig. 4 and genesis of important fragment ions is shown in Figs. 5-6.

Identification and estimation of sugars

The identification and quantitative estimation of the monosaccharide units of carbohydrate part of shatavarin-I was carried out by paper chromatography, TLC and GLC of the sugars obtained by hydrolysis of shatavarin-I. The aqueous part of acid hydrolysate of shatavarin-I was neutralised by passing through an ion exchange resin[14]. The sugars on paper chromatogram showed two spots with R_f values of 0.26 and 0.44. When samples of standard sugars were spotted, the two corresponded to D-glucose*(II) and L-rhamnose*(III) respectively (Fig.7). The spot corresponding to glucose

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* Naturally occuring glucose and rhamnose occur in D and L form respectively.

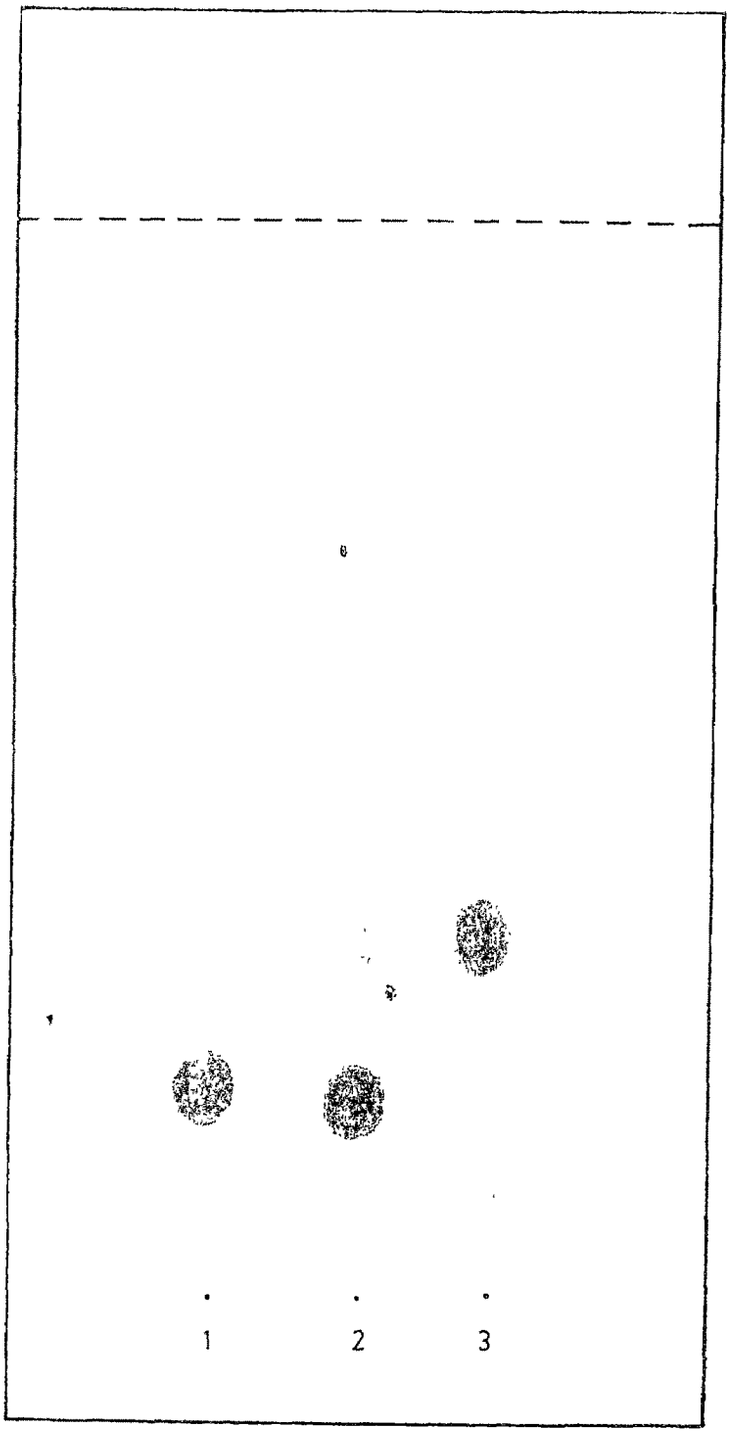


FIG.7 : PAPER CHROMATOGRAM OF SUGARS OF SHATAVARIN-I

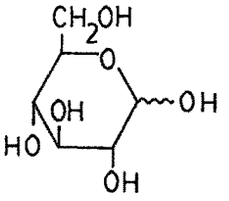
SOLVENT SYSTEM : n-BuOH-HOAc-H_2O (4:1:5)
(UPPER PHASE)

SPRAY REAGENT : ANILINE HYDROGEN PHTHALATE

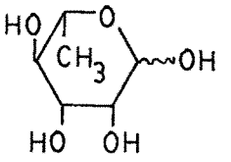
TIME : 4 HOURS

SPOTS : 1) D-GLUCOSE. 2) SUGARS OF SHATAVARIN-I
3) L-RHAMNOSE.

was more intense than the spot corresponding to rhamnose.



II



III

Thin layer chromatography : TLC of carbohydrates has been described on microcrystalline cellulose using the same solvent system as for PC. However, sugars can be separated on plain silica gel using n-butanol-acetic acid-ether-water (9:6:3:1).[15] The aqueous part containing sugars of shatavarin-I showed two spots on TLC with R_f values of 0.31 and 0.44. Fig.8 clearly shows that they correspond to standard D-glucose and L-rhamnose respectively.

The carbohydrate part of shatavarin-I hydrolysate was converted into the acetate derivatives by treatment with acetic anhydride and pyridine, GLC of which showed three peaks (Fig.9). Peak 1 was identified as due to L-rhamnose, peaks 2 and 3 with longer retention time arise from the acetate derivative of D-glucose. Calculation of areas under the curves established proportion of glucose and rhamnose to be 3:1. For comparison purpose, authentice D-glucose[+] and L-rhamnose[o] in proportion of 3:1 were acetylated and

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[+] BDH, GR; [o] SD fine chemicals.

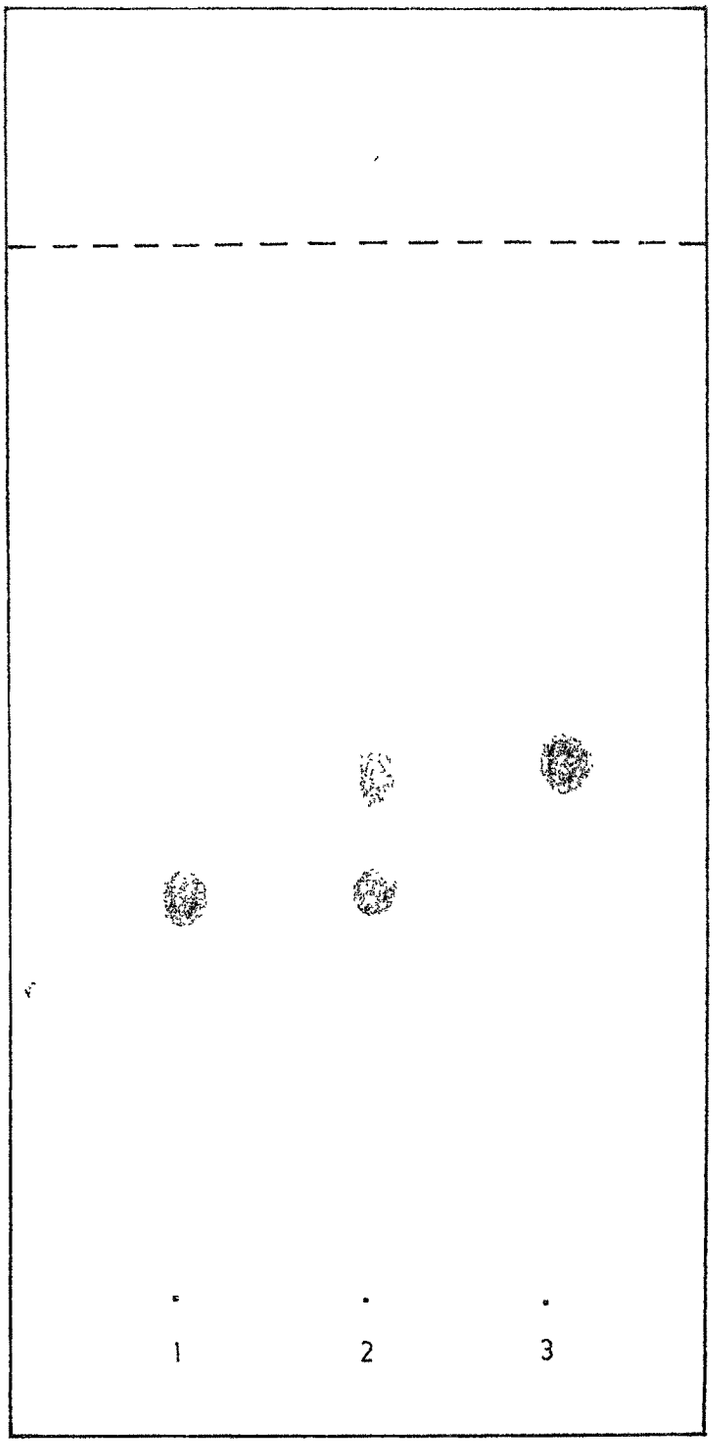


FIG.8 : TLC OF SUGARS OF SHATAVARIN-I

SOLVENT SYSTEM : n-BuOH-AcOH-Et_2O-H_2O (9:6:3:1)
(UPPER PHASE)

SPRAY REAGENT : THYMOL-SULPHURIC ACID.

SPOTS : 1) D-GLUCOSE. 2) SUGARS OF SHATAVARIN-I. 3) L-RHAMNOSE.

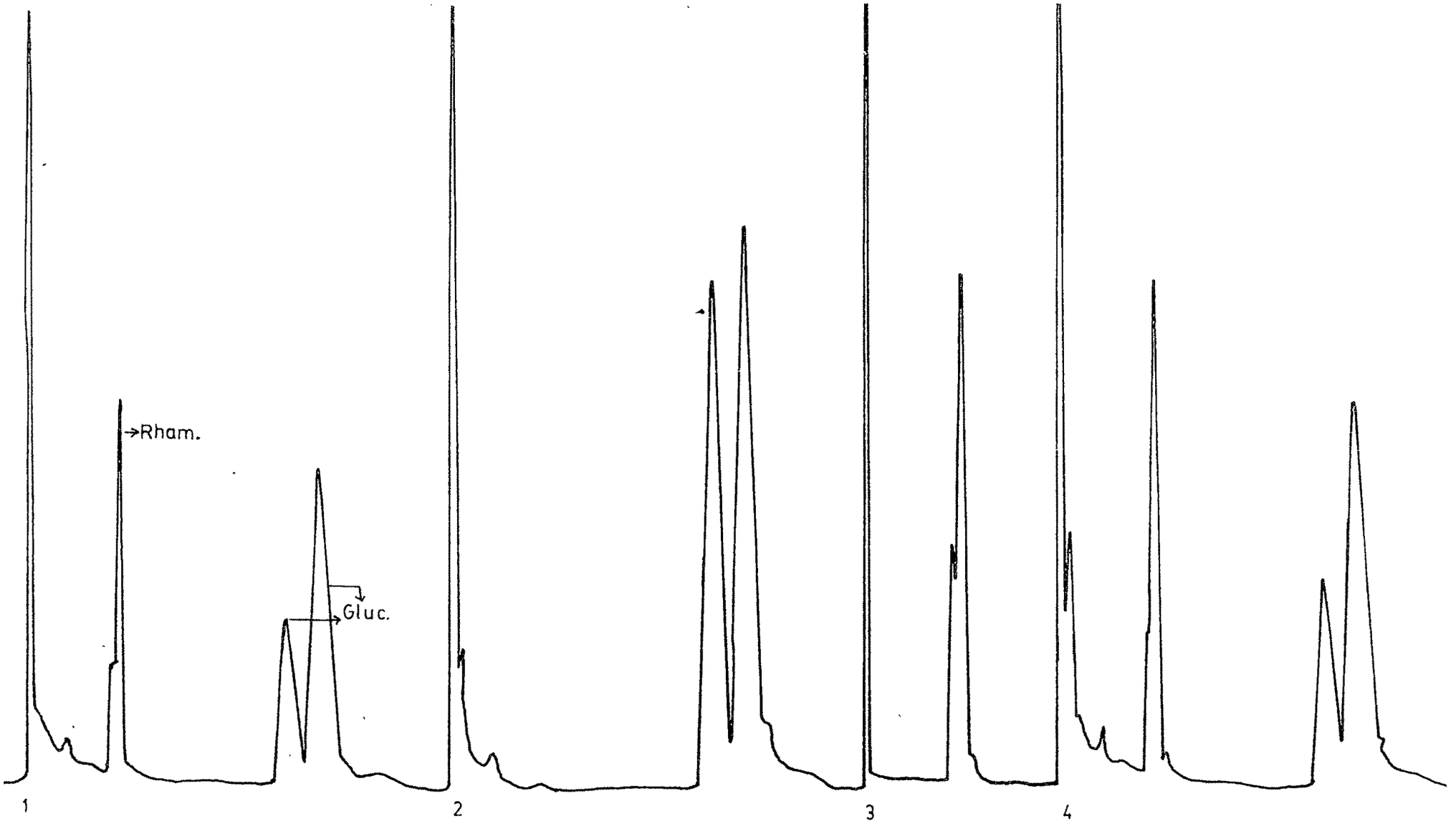


FIG.9 : GLC OF SUGARS OF SHATAVARIN-I.

COLUMN : 10% DCQF-1 ON CHROMOSORB W HP 60/80
NITROGEN FLOW : 60 ML/MIN. TEMP. : 210°.

1) MIXTURE OF GLUCOSE+RHAMNOSE (3:1). 2) GLUCOSE.
3) RHAMNOSE. 4) SUGARS OF SHATAVARIN-I.

analyzed by GLC (Fig.9).

Since shatavarin-I is a furostanol saponin and on hydrolysis it gives sarsasapogenin, the aglycon of shatavarin-I must be a precursor of sarsasapogenin. To get further information, shatavarin-I was hydrolysed enzymically.

ENZYMIC HYDROLYSIS OF SHATAVARIN-I

Shatavarin-I was hydrolysed enzymically at 50° using crude β-glucosidase at pH 5.0. After 24 hours, the mixture was separated into aqueous part and organic part. On removal of solvent from organic part, a product was obtained which was negative to Ehrlich's reagent. It was identified as shatavarin-IV* by CO-TLC, mixed TLC and m.p. Glucose could be identified in the aqueous part by paper chromatography (Fig.10). Shatavarin-IV under the same condtions did not undergo enzymic hydrolysis.

The fact that on enzymic hydrolysis shatavarin-I gives shatavarin-IV and glucose and that shatavarin-IV does not undergo enzymic hydrolysis proved that shatavarin-I has only one glucose more than shatavarin-IV. Following three conclusions were drawn from the above results.

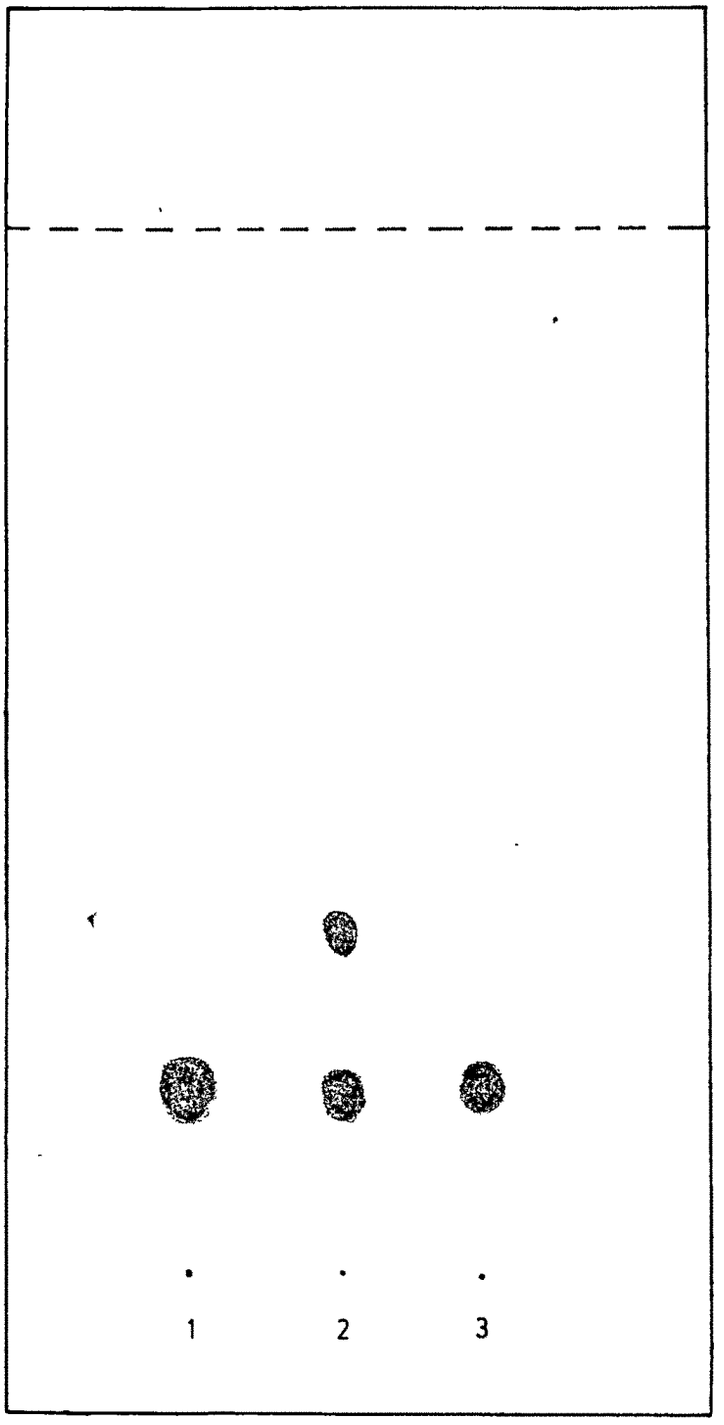


FIG.10 : PAPER CHROMATOGRAM OF SUGARS OF SHATAVARIN-I.

SOLVENT SYSTEM : n-BuOH-HOAc-H_2O (4:1:5) (UPPER PHASE).

SPRAY REAGENT : ANILINE HYDROGEN PHTHALATE.

TIME : 4 HOURS.

SPOTS : 1) D-GLUCOSE. 2) SUGARS OF SHATAVARIN-I (ACID HYDROLYSIS). 3) SUGAR OF SHATAVARIN-I (ENZYME HYDROLYSIS).

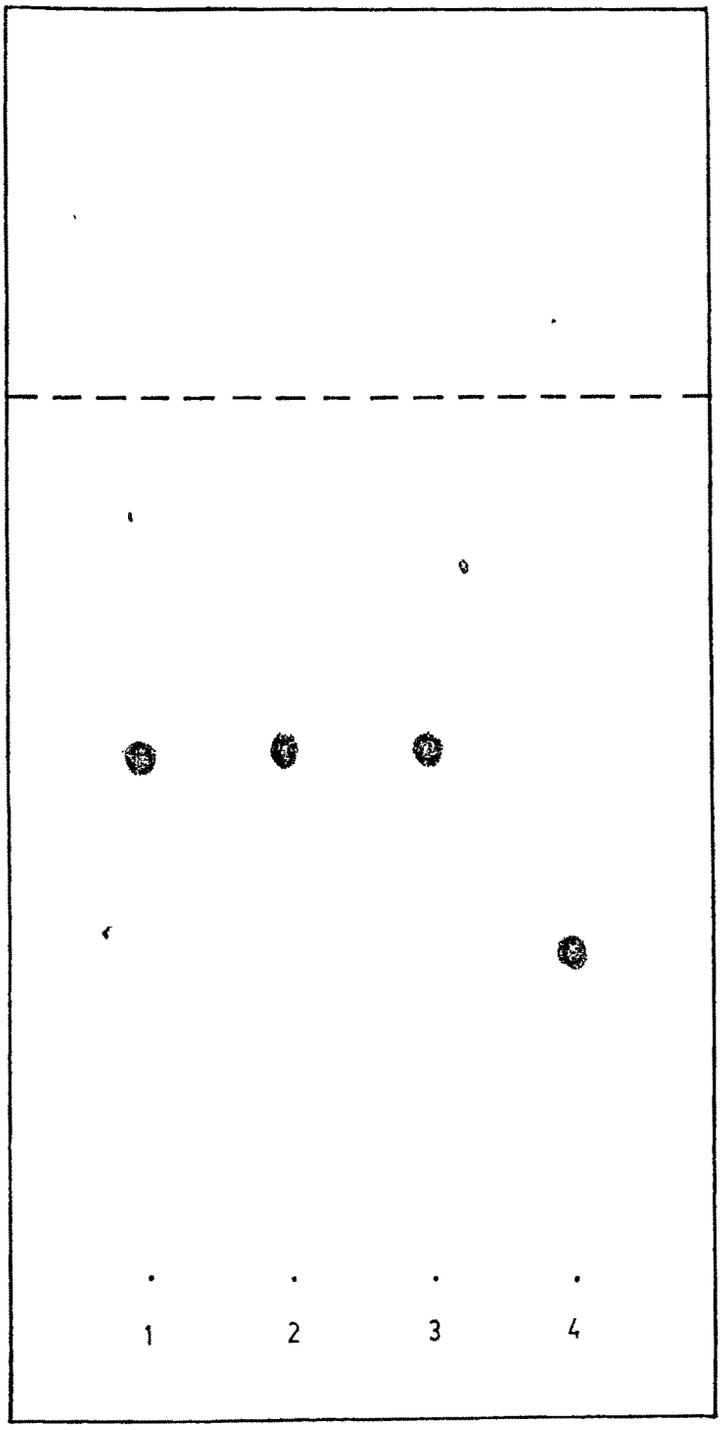


FIG.10.a : TLC OF ENZYME HYDROLYSIS PRODUCT OF SHATAVARIN-I.

SOLVENT SYSTEM : $CHCl_3$-CH_3OH-H_2O (65:35:10)
(LOWER PHASE)

SPRAY REAGENT : 1% VANILLINE IN 50% PHOSPHORIC AICD.

SPOTS : 1) SHATAVARIN-IV. 2) ENZYME HYDROLYSIS PRODUCT OF SHATAVARIN-IV. 3) ENZYME HYDROLYSIS PRODUCT OF SHATAVARIN-I.
4) SHATAVARIN-I.

(i) Shatavarin-I is a glycoside with two sugar chain attachments. The two glycosylation sites are at C-3 and C-26. As shatavarin-IV does not undergo enzymic hydrolysis, the glucose from C-3 sugar chain is not cleaved by the enzyme, indicating that it must be glucose from C-26 position which undergoes cleavage, simultaneous cyclization gives rise to spirostanol saponin shatavarin-IV.

(ii) The sugar sequence and the position of the linkages of monosaccharides at C-3 are the same as that in case of shatavarin-IV.

(iii) Only β-glucose is connected at C-26.

It was in 1967, when Tschesche et al.[16,17] isolated first furostanol saponin from Smilax aristolochiaefolia MILL. This gave a supporting evidence to Marker's[18] suggestion that spirostanols might be artefacts produced by acid hydrolysis of glycosides having ring E or F opened aglycone. This was in contrast to Wall's observation.[19]

In furostanol saponins the glucose at C-26 position protects the furostanol saponin from ring closure, but rupture of the C-26 glycosidic bond then causes the formation of spirostanol glycosides.

It was now assumed that shatavarin-I has two sugar chain attachments, one at C-3 and the other at C-26. To confirm the C-26 sugar chain attachment, shatavarin-I was oxidized by CrO_3 by the method of Tschesche *at al.*[16,20] (Fig.11). The first step was Marker's oxidation[21] by Wall's[22] procedure. Acetate of shatavarin-I (1) was treated with acetic anhydride to get the product (2) which was exposed to CrO_3 in acetic acid at 15°. The resulting ester-ketone (3) was hydrolysed with potassium hydroxide in t-butanol, to get the Δ^{16}-pregnenolon-glycoside (4) with the sugar linkages at C-3; and the acid glycoside (with six carbon atoms aglycone) as δ-Hydroxy-γ-methyl valeric acid-glucoside (6). Product (4) was hydrolysed by treatment with hydrochloric acid, the resulting aglycone was acetylated to get 3β-acetoxy-5β-preg-16-ene-20-one (5). λ_{max} 240 nm. IR: ν^{KBr}_{max} cm^{-1} 1730, 1668 cm^{-1} (for Δ^{16}-20-keto-steroids). Mass: M^+ m/z 358. Compound (6) was treated with acetic anhydride followed by diazomethane to get δ-hydroxy-γ-methyl valeric acid methyl ester-glucoside acetate (7), which was identified by its mass spectrum: m/z 331, 243, 242, 200, 169, 157, 145, 141, 129, 115, 109, 97, 89 and 81.

FABMS OF SHATAVARIN-I

FABMS of shatavarin-I (Fig.16) was recorded and it was found to be different from that of spirostanol saponins.

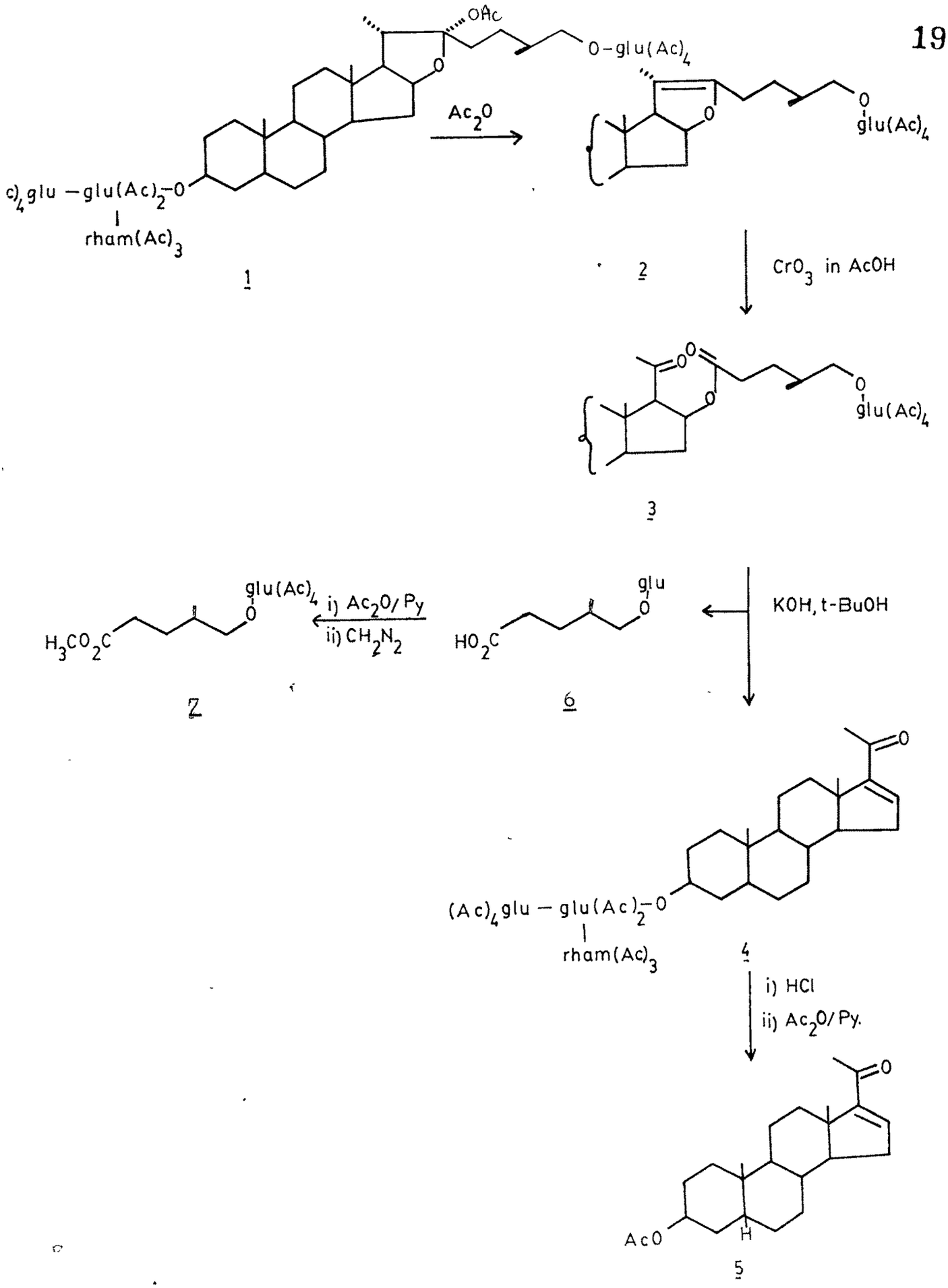


FIG.11 : CrO_3 OXIDATION OF SHATAVARIN-I

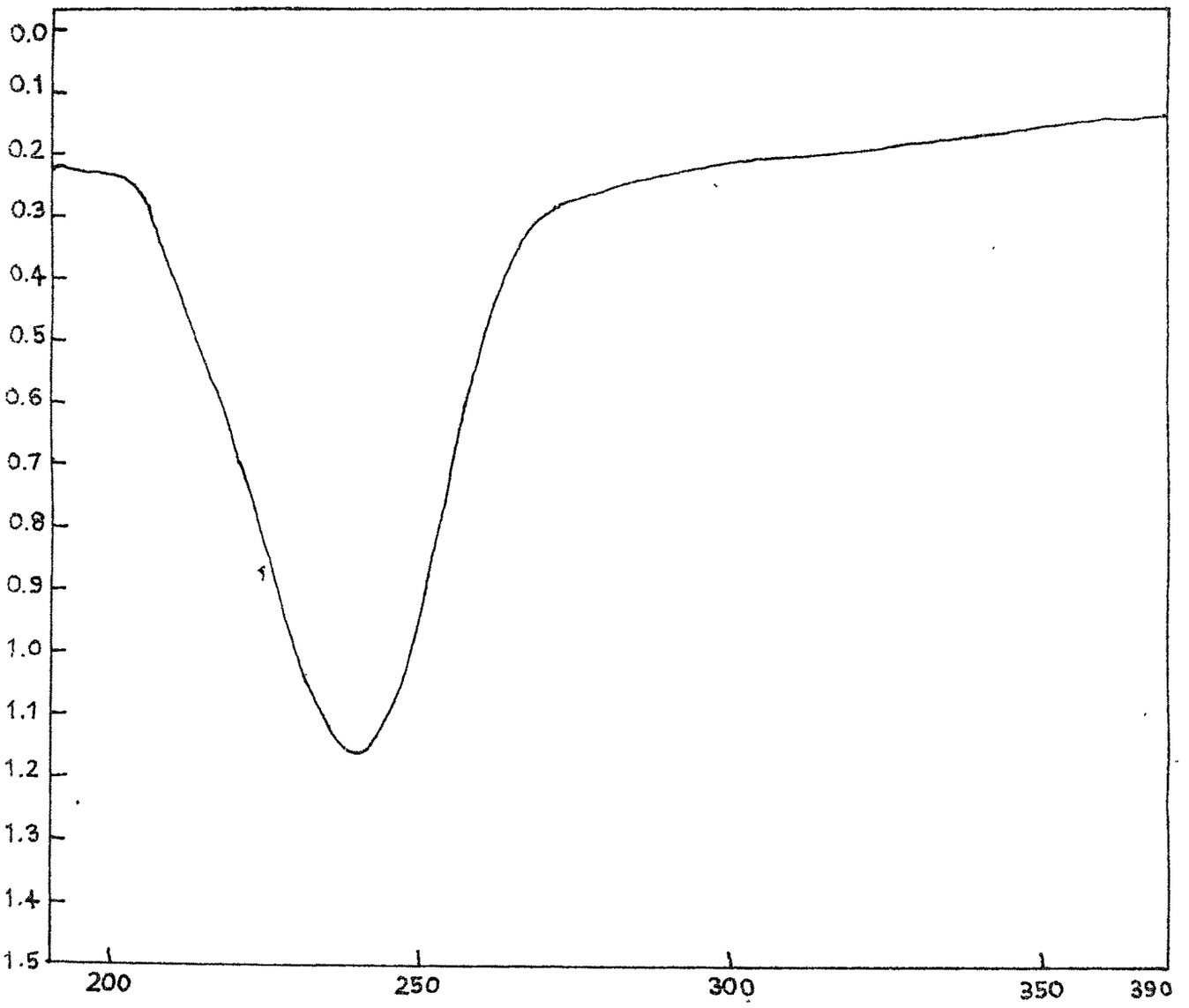


FIG.12 : UV SPECTRUM OF (5)

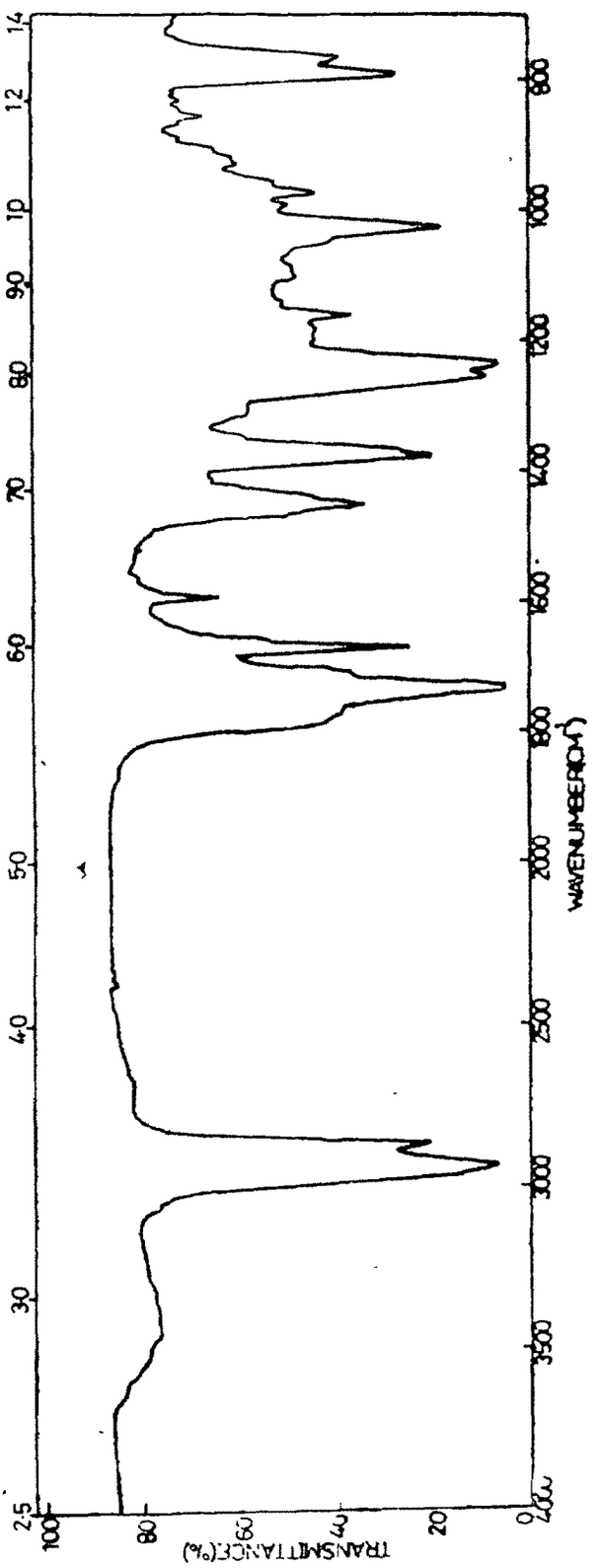


FIG.13 : IR SPECTRUM OF (5)

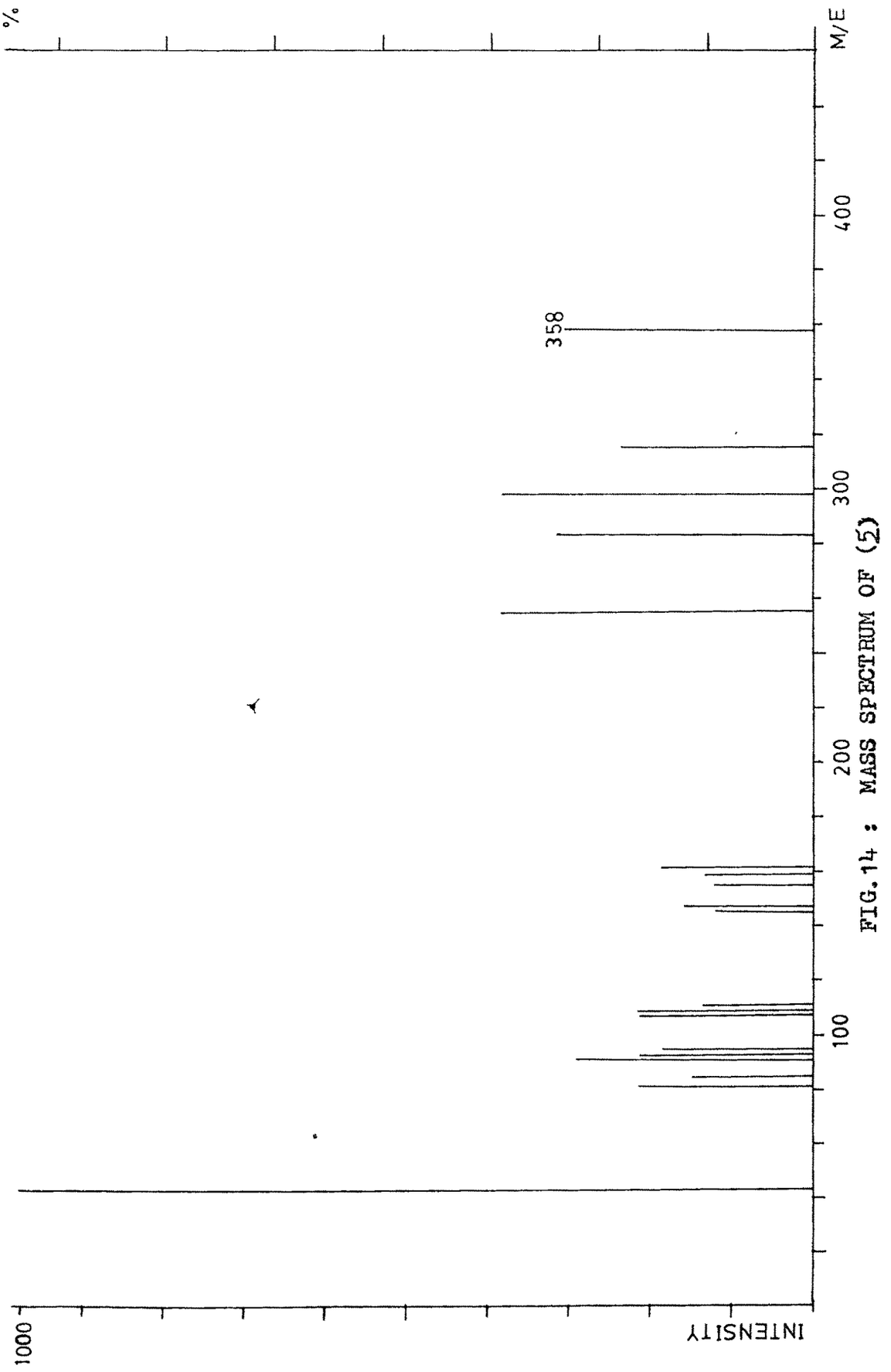


FIG.14 : MASS SPECTRUM OF (5)

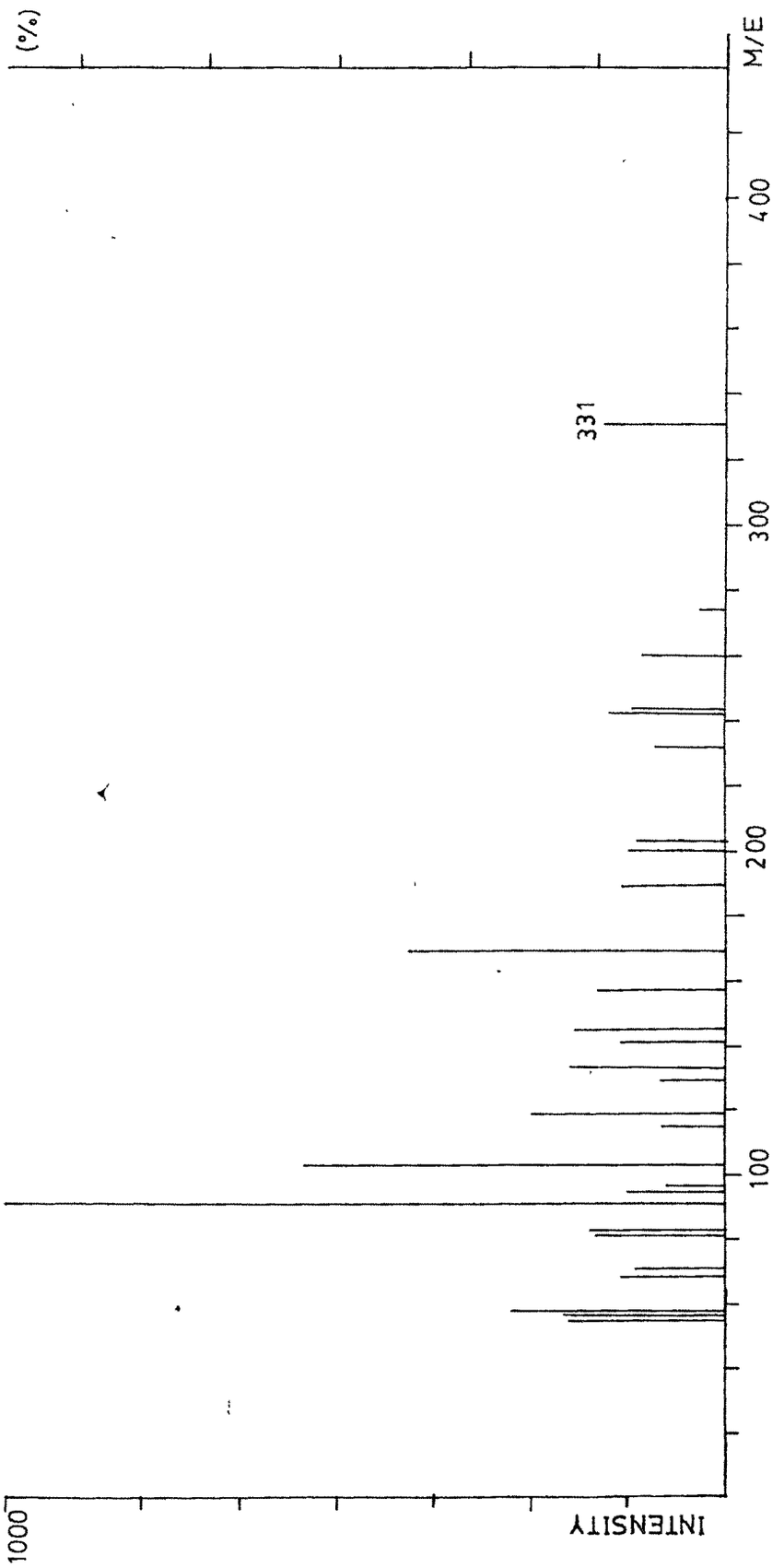


FIG.15 : MASS SPECTRUM OF (2)

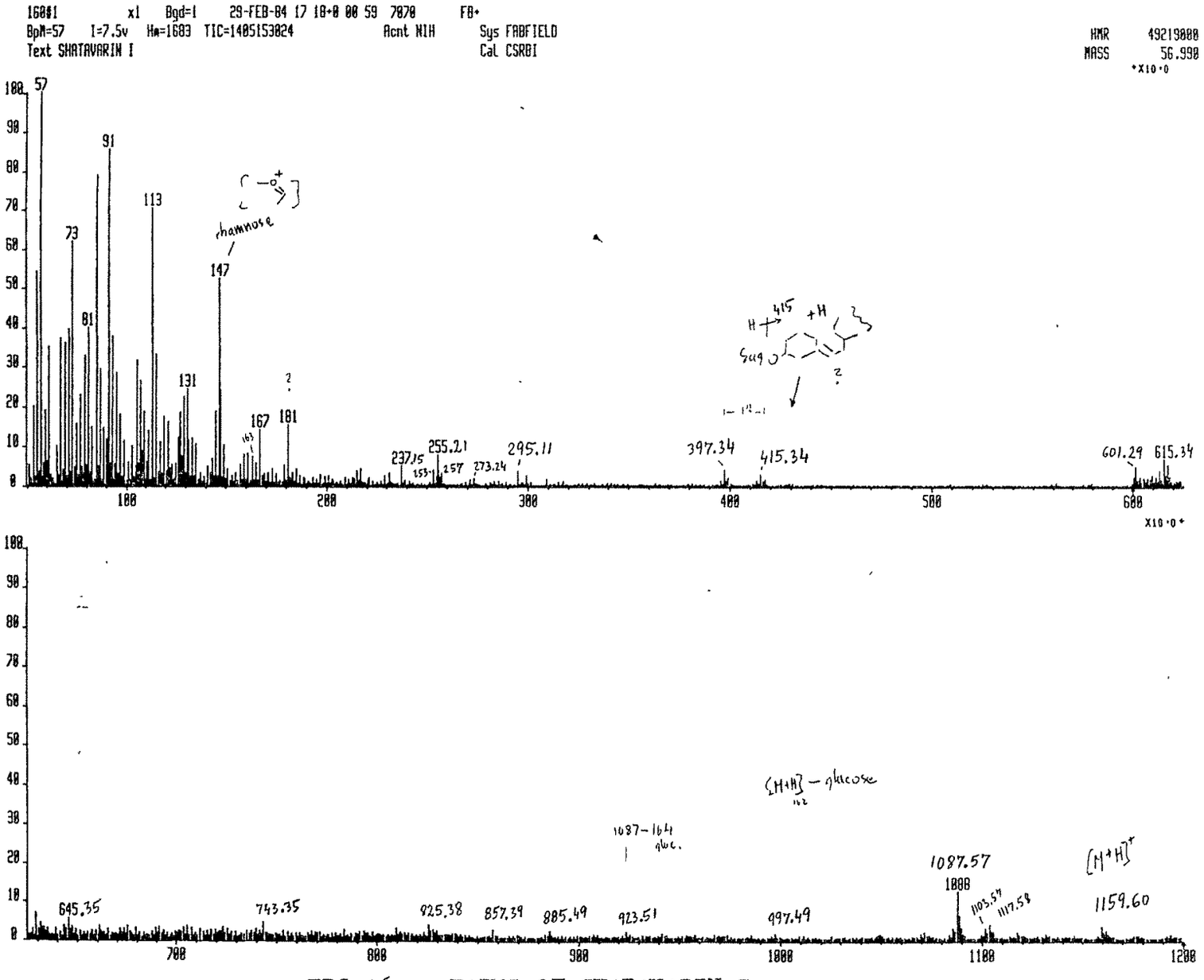


FIG.16 : FABMS OF SHATAVARIN-I

No molecular ion M^+ or pseudomolecular ion $[M^+ + H]$ are observed. However in different solvents and using different matricies, a strong peak appears at m/z 1088 which can be attributed to $[M^+ + Na - H]$. It has been reported that in plasma-desorption-mass-spectra[23] and FD-MS[24] of steroidal saponins peaks are observed corresponding to $[M^+ + Na]$, $[M^+ + 2Na]$ and $[M^+ + 2Na - H]$. Calculated molecular weight of shatavarin-I is 1066. No M^+, $[M^+ + H]$ or $[M^+ - H_2O]$ at m/z 1066, 1067 or 1048 are observed. The peak at m/z 1159, which is observed in the spectra can be assigned to $[M^+ + 93]$. When glycerol is used as a solvent[25] it gives peaks at mass numbers corresponding to $(92n + 1)^+$ and $(92n-1)^-$, with values of n upto 15 being detectable. The ion at $M^+ + 93$ may arise by the formation of $[M^+ + (92n+1)]$ cluster ion, where n = 1, or by the formation of cluster ion $(M^+ + Na-H + 72)$, 72 may be the fragment of the solvent glycerol.

The fragmentation pattern can be explained as follows. The ions are mainly derived from 1088. The peak at m/z 925 is observed by the loss of 163 mass units from 1088. This loss of 163 mu corresponds to loss of glucose. The peak at m/z 615 corresponds to the ion formed by the loss of 310 mu from 925. This loss corresponds to loss of glucose+rhamnose. The ion at m/z 744 arises by the loss of glucose and a water molecule from the ion at 925. The ion at m/z 415 corresponding to dehydrosarsasapogenin is formed by the cleavage of

all the sugars, followed by dehydration at C-22.

Apart from the above mentioned ions, others ions are also observed which do not originate from 1088. The formation of these ions can be explained as follows. The ion at m/z 997 arises from 1159 by the loss of glucose. The ion at m/z 885 is derived from the molecular ion at m/z 1066 by the loss of glucose with simultaneous loss of water molecule.

The ions at m/z 397, 285, 273 and 255 are characteristic of steroid nucleus. The ion at 139 which is characteristic of spirostanol saponins is not observed here; which further confirms that shatavarin-I is not a spirostanol saponin. The formation of the fragment ions is shown in Fig. 17. Figs 18 and 19 show the important ions formed in the FABMS.

Ions	m/z
$[M^+ + Na - H]$	: 1088
$[M^+ + 93]$	: 1159
$[(M^+ + Na-H)-gluc] = 1088-163$	: 925
$[(M^+ + Na-H-gluc)-(gluc+H_2O)] = 925-163-18$	: 744
$[(M^+ + Na-H-gluc)-(gluc+rham)] = 925-310$	: 615
$[(M^+ + Na-H-gluc-glu-rham)-gluc-H_2O] - Na$ $= 615-163-18-23$	: 415

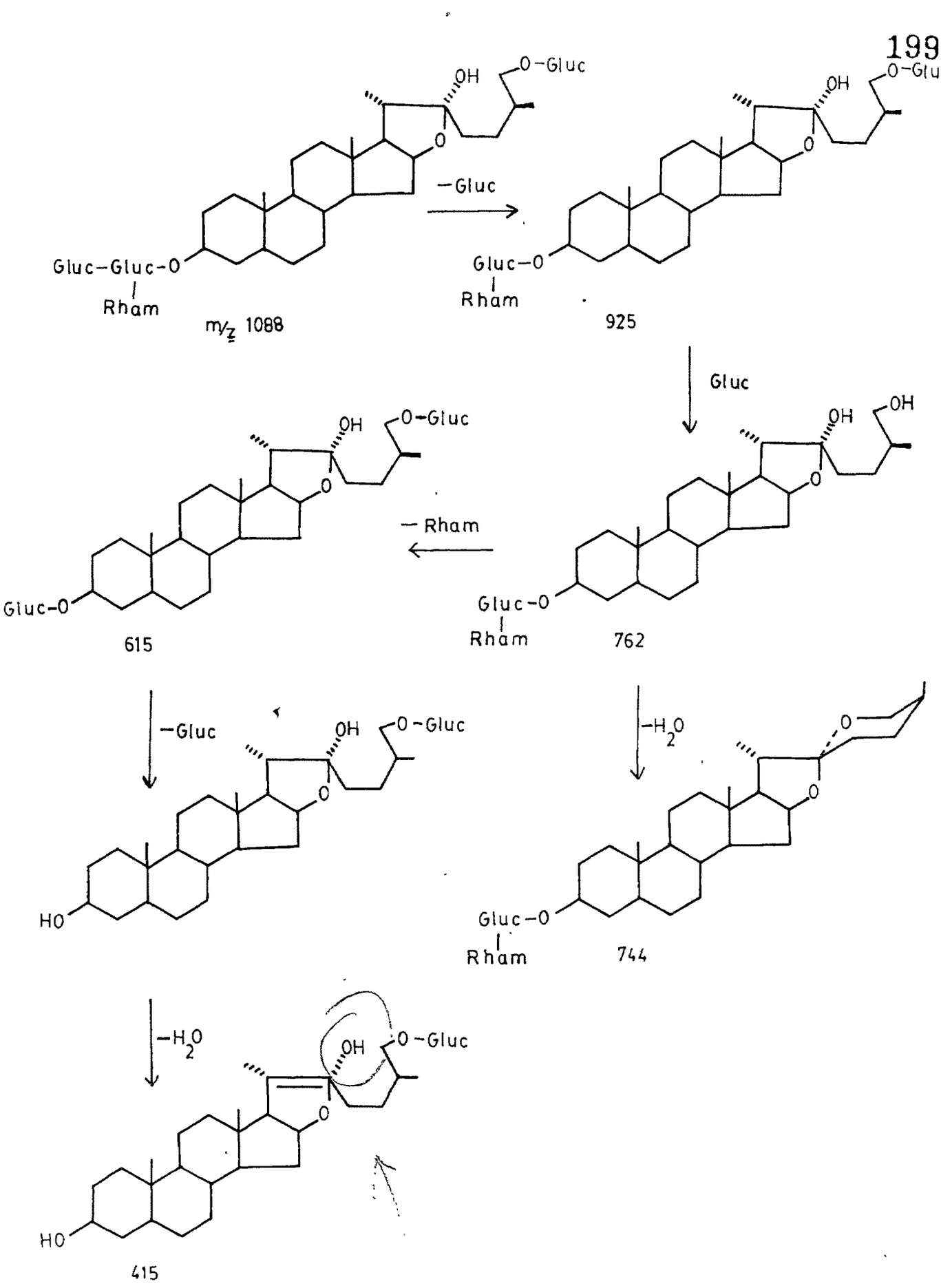


FIG.18 : FORMATION OF IONS IN THE FAB$^+$MS OF SHATAVARIN-I.

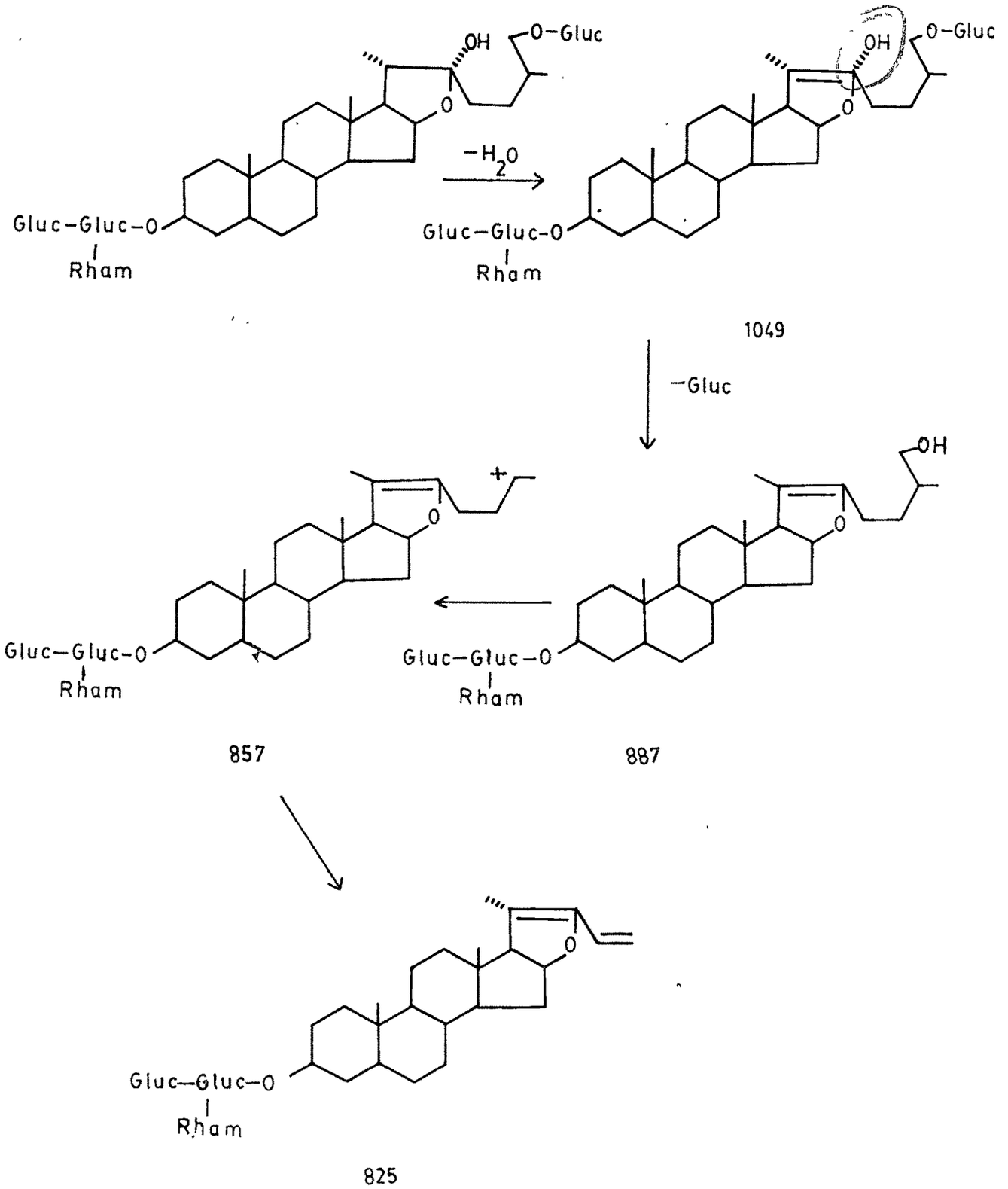


FIG. 19 : FORMATION OF IONS IN THE FAB$^+$MS OF SHATAVARIN-I.

$[M^+ + 93\text{-gluc}]$ = 1159-163 : 996

$[M^+\text{-gluc-}H_2O]$ = 1066-163-18 : 885

Fig.17 : Fragments formed in the FAB$^+$MS of shatavarin-I.

^{13}C-NMR OF SHATAVARIN-I

The ^{13}C-NMR of peracetyl shatavarin-I (IV) was recorded in deuterochloroform. The signal assignment of glycoside was by comparison with those in its corresponding spirostanl aglycone sarsasapogenin[26,27] and known chemical shift rules.[28] Chemical shifts and glycosylation shifts i.e. chemical shift changes from aglycone and methyl glycoside to sarsasapogenin are shown in Table-1. The spectrum is not well-resolved, but most values can be assigned. Table-2 shows the chmical shifts of the sugars of peracetyl shatavarin-I.

In the ^{13}C chemical shifts of the aglycone, there is a change in the shift values of C-22 and C-26 carbon atoms. In case of spirostanol saponins, the C-22 carbon atom is connected to two oxygen atoms, which form two different rings, one forms a five membered ring and the other forms a six membered ring, the two rings are non-planar. Due to the two spiro rings, C-22 carbon atom resonates at 109.5.

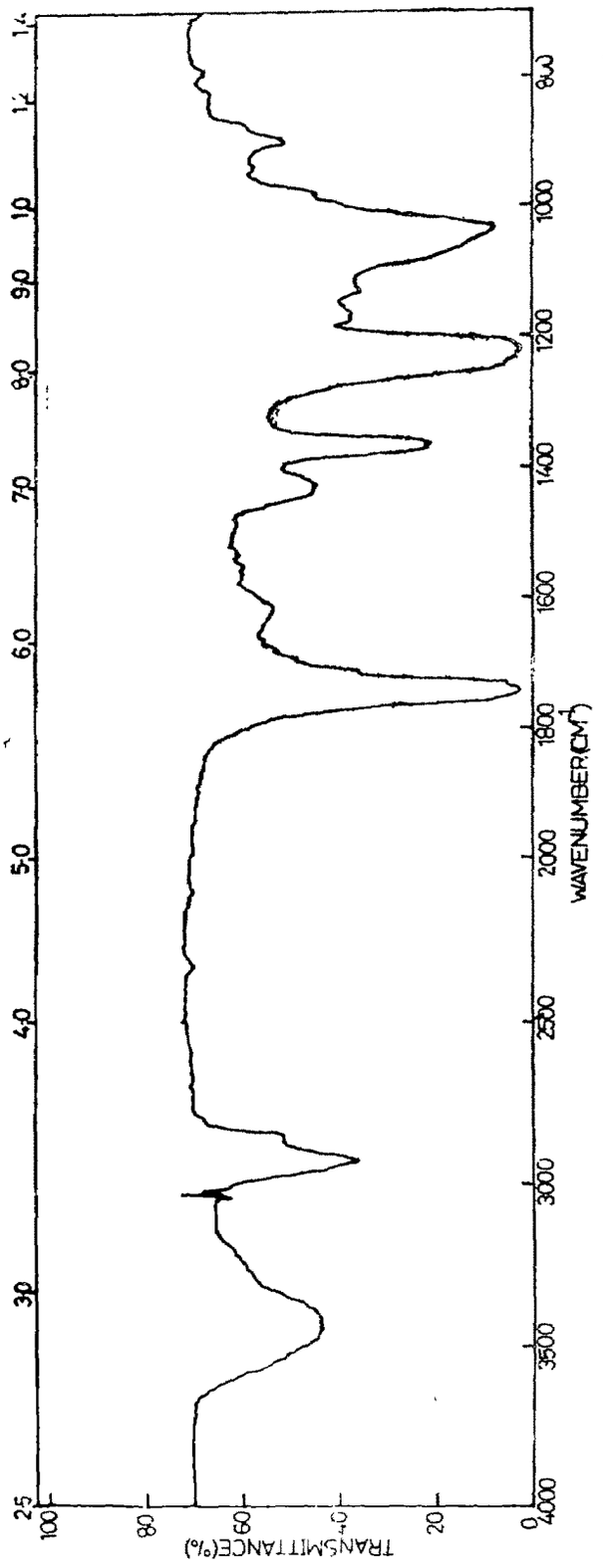


FIG. 20 : IR SPECTRUM OF PERACETATE OF SHATAVARIN-I

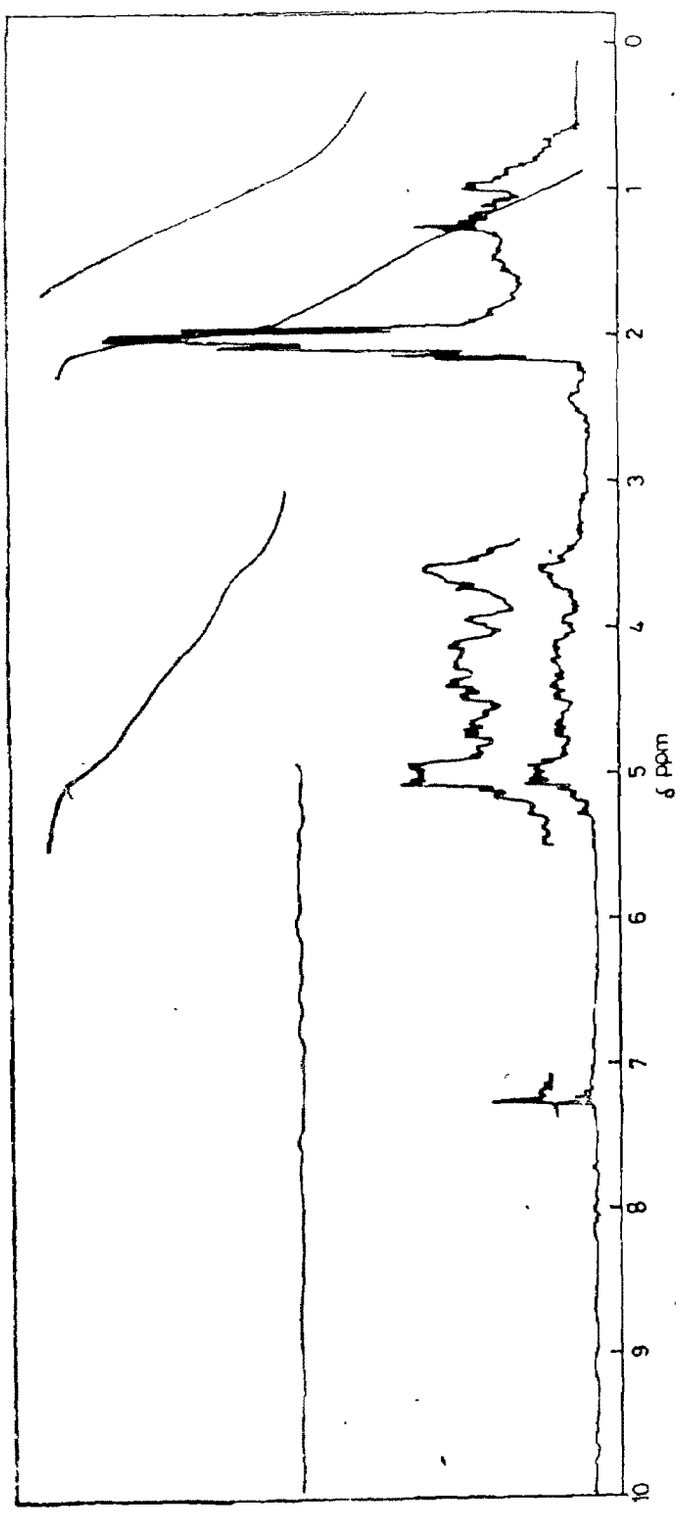


FIG.21 : ^{1}H-NMR SPECTRUM OF PERACETATE OF SHATAVARIN-I

TABLE-1 : ^{13}C-NMR ASSIGNMENTS* OF AGLYCONE IN PERACETATE OF SHATAVARIN-I.

Carbon No.	Sarsasapogenin[26,27]	Aglycone in peracetate of shatavarin-I
1.	29.9	30.177 (+0.2)
2.	27.8	26.478 (-1.4)
3.	67.0	75.035 (+8.0)
4.	33.6	30.177 (-3.5)
5.	36.5	34.991 (-1.6)
6.	26.6	26.419 (-0.2)
7.	26.6	26.419 (-0.2)
8.	35.3	34.991 (-0.4)
9.	40.3	
10.	35.3	34.991 (-0.4)
11.	20.9	21.253 (+0.3)
12.	39.9	
13.	40.6	
14.	56.4	
15.	31.7	30.177 (-1.6)
16.	80.9	77.615 (-3.3)
17.	62.1	62.115 (0.0)

(contd.)

TABLE-1 : (contd.)

Carbon No.	Sarsasapogenin[26,27]	Aglycone in peracetate of shatavarin-I
18.	16.5	17.201 (+0.7)
19.	23.9	26.392 (+2.4)
20.	42.1	
21.	14.3	17.2 (+2.9)
22.	109.5	99.4 (-10.1)
23.	27.1	26.478 (-0.7)
24.	25.8	26.392 (-0.5)
25.	26.0	26.392 (+0.3)
26.	65.0	70.159 (+5.1)
27.	16.1	17.20 (+1.2)

* δ ppm from TMS.

TABLE-2 : ^{13}C-CHEMICAL SHIELDINGS* OF SUGARS OF PERACETATE OF SHATAVARIN-I.

Carbon No.	Methyl-β-D-glucopyranoside tetraacetate	α-L-rhamnopyranoside tetraacetate	Sugars of peracetate of shatavarin-I	
C-1	101.4		99.394	Gluc.1
C-2	70.358		77.084 (+6.7)	
C-3	72.853		72.096 (-0.8)	
C-4	67.923		75.149 (+7.2)	
C-5	72.853		72.977 (+0.1)	
C-6	61.593		61.998 (+0.4)	
C-1'			100.27	Gluc.2
C-2'			70.159 (-0.2)	
C-3'			72.096 (-0.8)	
C-4'			67.810 (-0.1)	
C-5'			71.333 (-1.5)	
C-6'			62.409 (+0.9)	
C-1''			100.98	Gluc.3
C-2''			70.159 (-0.2)	
C-3''			71.920 (-0.9)	
C-4''			67.810 (-0.1)	
C-5''			71.744 (-1.1)	
C-6''			62.155 (+0.6)	

(contd.)

TABLE-2 (contd.)

Carbon No.	Methyl-β-D-glucopyranoside tetraacetate	α-L-rhamnopyranoside tetraacetate	Sugars of peracetate of shatavarin-I	
C-1'''		91.25	99.394	)
C-2'''		69.24	67.81 (-1.43)	)
C-3'''		69.03	67.81 (-1.22)	) Rham.
C-4'''		71.20	70.746 (-0.5)	)
C-5'''		69.31	68.63 (-0.7)	)
C-6'''		17.57	17.20 (-0.3)	)

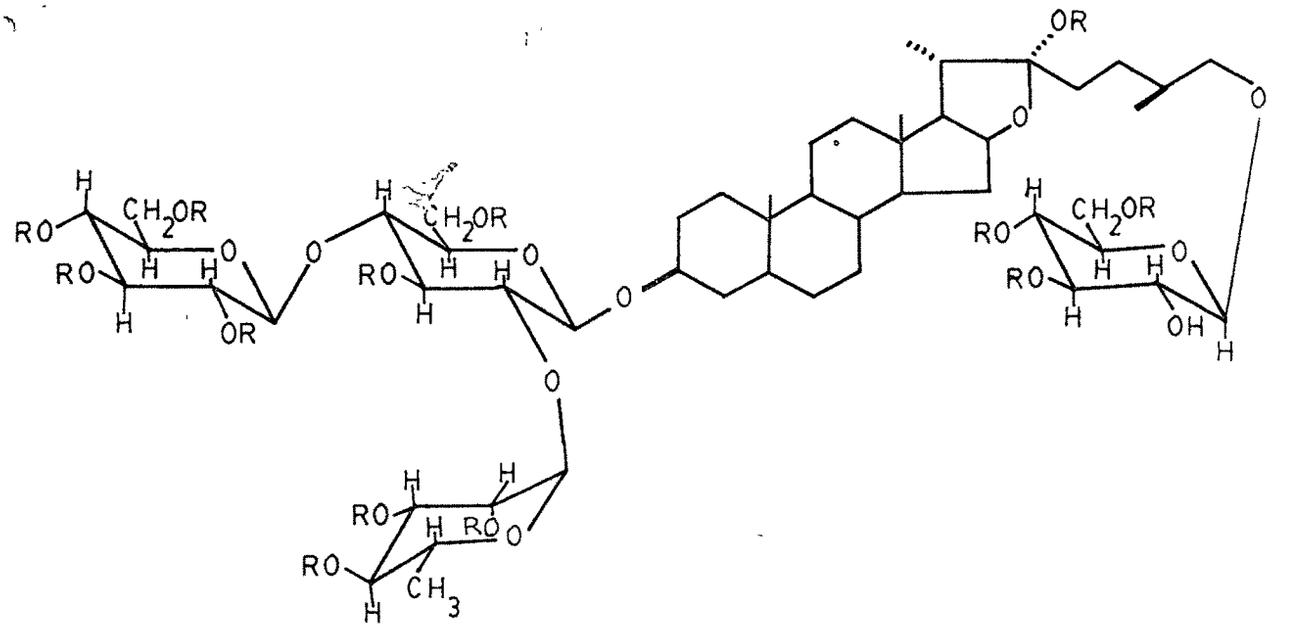
* δ ppm from TMS.

Shatavarin-I is a furostanol saponin and there is no spirosystem in it. C-22 carbon atom is connected to two oxygen atoms. One oxygen atom forms a five-membered ring and the other forms $-OCOCH_3$. C-22 carbon atom is thus comparable to the anomeric carbon atom of a furanose ring sugar. The anomeric carbon atom of a furanose ring sugar resonates between 98-100 ppm. The C-22 carbon atom in peracetyl shatavarin-I (IV) resonates at 99.4 ppm, which is well in accord with its structure.

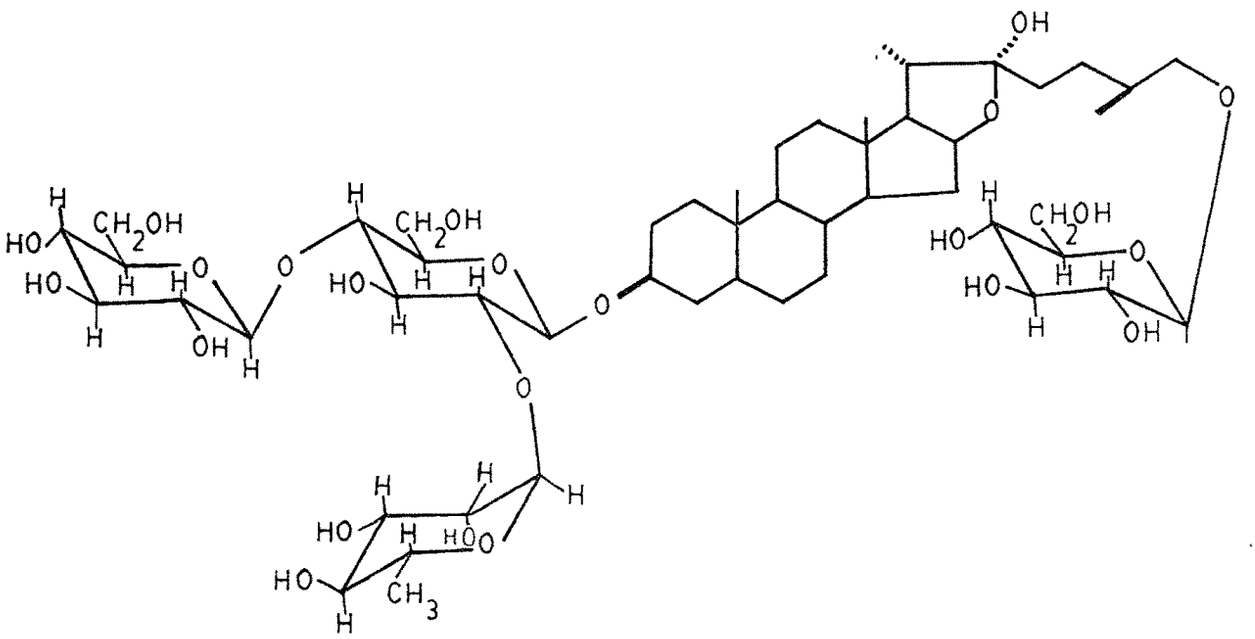
In case of spirostanol saponins C-26 carbon atom forms an ether ring, whereas in a furostanol saponin, it is glycosylated and hence is observed at a slightly lower field.

^{1}H-NMR study of peracetyl shatavarin-I revealed that glucose units are β-linked and rhamnose is α-linked.

From the above results, shatavarin-I is formulated as 3-o-{α-L-rhamnopyranosyl-(1$\rightarrow$2)-[β-D-glucopyranosyl-(1$\rightarrow$4)]-β-D-glucopyranosyl}-26-o-{β-D-glucopyranosyl}-5β(25 S)-furostane-3β,22α,26-triol (V).



IV R=-$COCH_3$



V

Column dimension : 0.9 cm x 18 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	Benzene	15 ml x 6	-	-
2.	Benzene+ethyl acetate :: 99+1	15 ml x 6	-	-
3.	Benzene+ethyl acetate :: 98+2	15 ml x 6	-	-
4.	Benzene+ethyl acetate :: 97+3	15 ml x 6	0.085 g.	White powder.
5.	Benzene+ethyl acetate :: 95+5	15 ml x 6	0.007 g.	rejected.
		Total...	0.092 g. (92%)	

White solid from FR. 4 was repeatedly recrystallized from methanol to get the product (55 mg), m.p. 197-200°, $[\alpha]_D^{26}$ - 75° ($CHCl_3$; C, 1); IR (KBr), ^{1}H-NMR ($CDCl_3$) and mass spectra are recorded. Found : C, 77.65%; H, 10.70%. Calculated for $C_{27}H_{44}O_3$: C, 77.87%; H, 10.58%.

Filtrate obtained after removal of the aglycone was neutralised with anion exchange resin** (300 ml) and

** Amberlite-IRA-400, pretreated with 10% NaOH and then washed with water till neutral.

filtered. The resin was washed with water (100 ml). Water was then removed from the combined extracts under suction to get 140 mg of sugars.

Paper chromatography : This was carried out on Whatman No.1 filterpaper by upward irrigation with the organic phase of n-butanol-acetic acid-water (4:1:5) for 4 hours and sprayed with aniline hydrogen phthalate. Two spots: One with R_f value 0.26 and the other with R_f value 0.44.

Thin layer chromatography : This was carried out on silica gel G (with 13% $CaSO_4$) plates using the solvent system n-butanol-acetic acid-ether-water (9:6:3:1) and sprayed with thymol/sulfuric acid. Two spots: One with R_f value 0.31 and the other with R_f value 0.44.

Acetylation : 50 mg of the sugar mixture from the hydrolysate in dry pyridine (2 ml) was acetylated with acetic anhydride (2 ml) by refluxing it at 145-150° for 4 hours. Acetic anhydride and pyridine were removed under vacuum (25 mm, 75-85°). The residue was taken up in chloroform (50 ml), washed with water (20 ml), $\frac{N}{2}$ hydrochloric acid (20 ml x 2), water (20 ml), 5% aqueous sodium carbonate solution (20 ml x 2) and finally with water (20 ml x 3), dried with anhydrous sodium sulfate and solvent was removed. The residue was taken up in dry chloroform and used for GLC.

under the conditions mentioned in the text. Glucose and rhamnose were found to be in the ratio of 3:1. Acetate of standard glucose and rhamnose and mixture of glucose-rhamnose :: 3-1 were prepared and compared by GLC.

ENZYMIC HYDROLYSIS OF SHATAVARIN-I

Shatavarin-I (30 mg) was dissolved in water (0.5 ml) and buffer (pH 5.0, 0.5 ml) and enzyme (crude β-glucosidase) were added to it. The mixture was incubated at 50° for 24 hours. After 24 hours, it was diluted with water (20 ml) and extracted with n-butanol saturated with water (25 ml x 3). Solvent was removed from the combined organic extracts to get the product. When spotted on TLC, it corresponds to shatavarin-IV, which was confirmed by mixed TLC and m.p.

The aqueous part when spotted on paper, on paper chromatogram, (run under the conditions mentioned earlier) showed only one spot corresponding to glucose.

PERACETYL SHATAVARIN-I

Shatavarin-I (152 mg) was dissolved in dry pyridine (15 ml) and acetic anhydride (15 ml) was added to it. The mixture was kept at room temp. for 48 hours. Acetic anhydride and pyridine were removed under vacuum (25 mm, 75-85°). The residue was taken up in chloroform (100 ml),

washed with water (25 ml), 0.5% aqueous sodium carbonate solution (25 ml x 3), water (25 ml), $\frac{N}{2}$ hydrochloric acid (25 ml x 2) and finally with water (25 ml x 4), dried with anhydrous sodium sulfate. Solvent was removed to get 0.200 g. foamy powder which was repeatedly recrystallized from dry ethanol to get white heavy crystals. m.p. 119-121.5°, $[\alpha]_D^{26}$ - 17.7° ($CHCl_3$; C, 1.13), IR ν_{max}^{KBr} cm^{-1} 1740 (-OAc), ^{1}H-NMR ($CDCl_3$), CH_3CO- signals are observed at δ 1.95, 1.99, 2.02, 2.04, 2.08, 2.14 and 2.16. ^{13}C-NMR ($CDCl_3$) recorded.

CrO_3 OXIDATION OF SHATAVARIN-I

Shatavarin-I acetate (1 g) was taken up in acetic anhydride (20 ml), refluxed (1 hr), cooled and water (10 ml) added. The mixture was dried under reduced pressure and to the residue was added acetic acid (15 ml) and sodium acetate (250 mg). To the mixture at 15° was added CrO_3 (800 mg) in 50% acetic acid (15 ml) over 15 mins. with continous stirring for 2 hours. The reaction mixture was diluted with water (50 ml) and extracted with ether (50 ml x 3). The ether extract was evaporated to dryness and the residue (1 g) taken up in t-butanol (25 ml) and KOH (1.5 g) in water (15 ml) added. The mixture was stirred at 30° for 4 hours under nitrogen. Water (20 ml) added, t-butanol removed and extracted with n-butanol (50 ml x 3). 3β-Hydroxy-5β-

pregn-16-ene-20-one acetate : The n-butanol extract was concentrated to dryness and the residue purified by column chromatography over silica gel using chloroform-methanol-water :: 65-35-10 (lower phase) as eluent. The purified glycoside was hydrolysed by refluxing with 5% HCl-toluene (20 ml) for 4 hours. The reaction mixture was cooled and the toluene phase separated, evaporated and acetylated as usual to get the product. IR: ν_{max}^{KBr} cm^{-1} 1730, 1668, 958, 930, 890, 820 (reported 1724, 1662, 958, 920, 895, 820). EI-MS: m/z 358 M^+ (reported EI-MS (probe) 70 eV: m/z 358 M^+ . UV λmax: 240 nm (reported max 239 nm).

δ-Hydroxy-γ-methyl valeric acid methyl ester-glucoside acetate : The above aqueous phase was adjusted to pH 3.0 with 2N hydrochloric acid and extracted alternately with n-butanol and chloroform. The aqueous phase was neutralised with 2N NaOH and evaporated. The residue was acetylated, worked up as usual and treated with diazomethane (30 ml) for 12 hours. The reaction mixture was evaporated and purified by chromatography on silica gel using benzene+ethyl acetate as eluent, to yield a syrup. EI-MS : m/z 331, 243, 242, 200, 169, 157, 145, 141, 115, 109, 129, 97, 89 and 81.

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SUMMARY

Structure of shatavarin-I is discussed. By TLC, it was found to be Ehrlich +ve furostanol saponin. By acid hydrolysis, the aglycone was identified as sarsasapogenin. The sugars found to be present were D-glucose and L-rhamnose in the proportion of 3:1. By enzymic hydrolysis, the products obtained were identified as shatavarin-IV and D-glucose. On chromic acid oxidation the two products obtained were identified as 3β-hydroxy-5β-pregn-16-ene-20-one acetate and δ-hydroxy-γ-methyl valeric acid-methyl ester glucoside acetate. The FAB mass spectral study of shatavarin-I and ^{13}C-NMR study of its peracetyl derivative confirmed the structure of shatavarin-I formulated as 3-o-{α-L-rhmnopyranosyl (1→2)-[β-D-glucopyranosyl (1→4)]-β-D-glucopyranosyl}-26-o-(β-D-glucopyranosyl)-5β(25 S)-furostane-3β,22α,26-triol. This was substantiated by chemical evidence.