CHAPTER 2

SAPONINS OF SHATAVARI

SEPARATION

Roots from two different origins were studied for the glycoside content. Roots of commercial shatavari were purchased from the market (Baroda) and extracted. Five glycosides were obtained in pure form from the saponin fraction. Fresh roots of Asparagus racemosus (authentic*) were collected in Nov.-Dec. and extracted. Three glycosides were obtained in pure form from the saponin fraction. On comparison of the two extracts, it was found that the alcohol extract of A. racemosus was dark in colour and more hygroscopic (indicating presence of more sugars) and showed a different TLC pattern, whereas alcohol extract of commercial shatavari was more foamy, brown coloured and TLC pattern was the same as reported by Ravikumar[1].

Comparative study of shatavari and A.racemosus was done, the results are reported in Table 1.

It was found that both commercial shatavari and A. racemosus contained comp. B, and comp. A. Shatavarin-IV was isolated from shatavari (compared with authentic sample of shatavarin-IV, isolated earlier by Ravikumar). A new glycoside was isolated from A. racemosus, which was named

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(*) Identified in the Botany Dept., The M.S. University of Baroda.

as glycoside-AR_4 (AR - representing for A. racemosus) which has same R_f value as shatavarin-IV. IR spectrum is also similar to that of shatavarin-IV. Glycoside-AR_6 was obtained

TABLE - 1 : COMPARATIVE STUDY OF A. RACEMOSUS (AUTHENTIC) AND SHATAVARI (COMMERCIAL)

	A. RACEMOSUS		SHATAVARI
1)	Total acetone extract- 1.92% (% on dry roots).		Total acetone extract- 1.90% (% on dry roots).
2)	Total ethanol extract- 17.2%.		Total ethanol extract- 11.9%.
3)	Total n-BuOH extract- 21% of ethanol extract - Dark brown, foamy, highly hygroscopic powder. Showed 11 spots on TLC Compounds obtained -		Total n-BuOH extract- 26% of ethanol extract - Brown, foamy, hygroscopic powder. Showed 9 spots on TLC Compounds obtained -
i)	Comp. B (Crude), R_f 0.80.	i)	Comp. B - m.p. 271-4°, R_f 0.80.
ii)	Comp. A - m.p. 151-3° R_f 0.52.	ii)	Comp. A - m.p. 151-3°, R_f 0.52.
iii)	Glycoside-AR_4 - m.p. 212-15°, R_f 0.46.	iii)	Shatavarin-IV - m.p. 274-8°, R_f 0.46.
iv)	Glycoside-AR_6 - m.p. 142-9° (Crude), R_f 0.75.	iv)	Shatavarin-I - m.p. 179-86°, R_f 0.24.
		v)	Shatavarin-VII as acetate m.p. 142.5-144°.

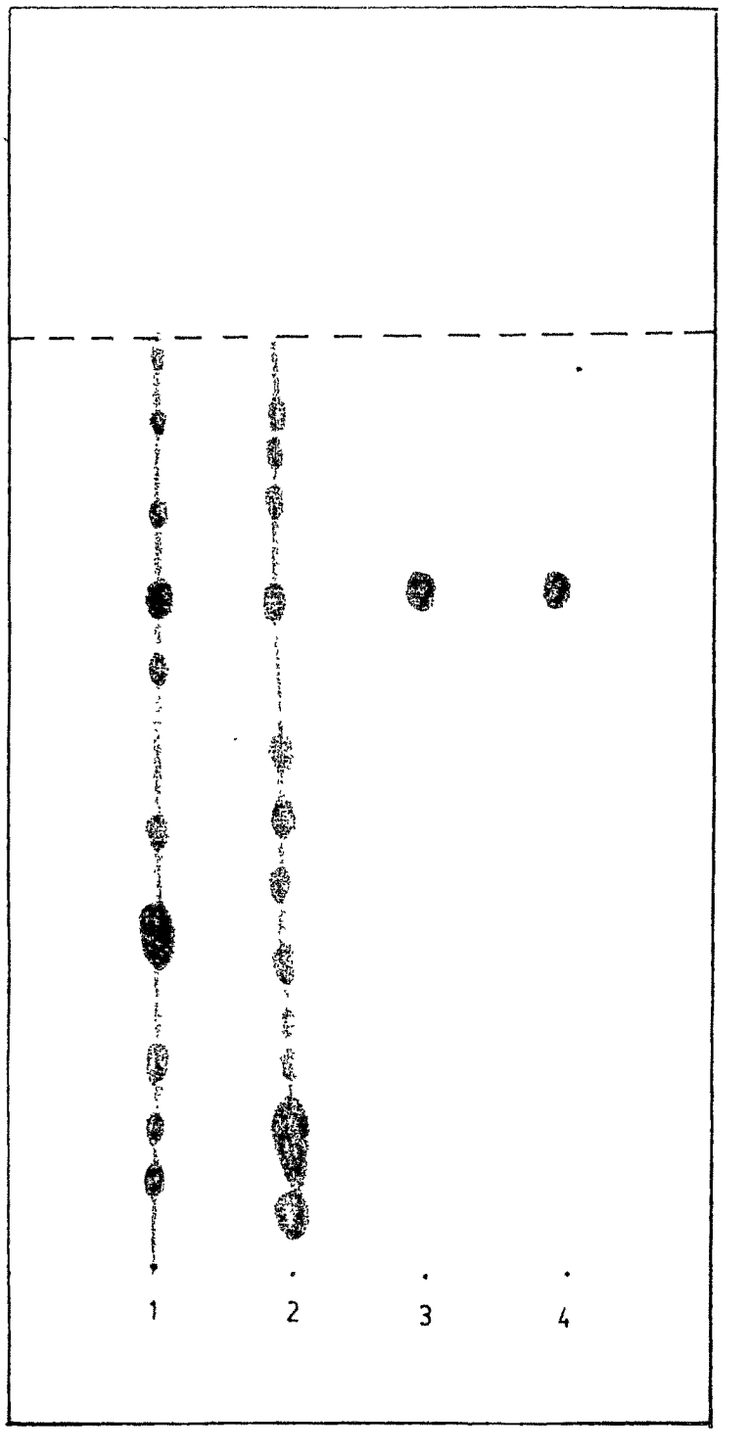


FIG.1 : COMPARATIVE TLC OF ALCOHOL EXTRACTS OF SHATAVARI AND A. RACEMOSUS

SILICA GEL G PLATE

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

SPRAY REAGENT : 1% VANILLINE IN 50% PHOSPHORIC ACID

SPOTS : 1) EtOH EXTRACT OF SHATAVARI
2) EtOH EXTRACT OF A. RACEMOSUS
3) SHATAVARIN-IV 4) GLYCOSIDE-AR_4

from A. racemosus, but was not found to be present in commercial variety. Shatavarin-I which is the major and active component was isolated from shatavari, but was found to be absent in A. racemosus.

Fresh roots of shatavari were obtained from Coimbatore. After drying and extraction, it was not found to contain any saponins (TLC). Only sugars were found to be present.

TABLE - 2 : COMPARISON OF SHATAVARI (COIMBATORE) AND A. RACEMOSUS

	SHATAVARI (COIMBATORE)	A. RACEMOSUS
1)	% of acetone extract 2.25% (on the basis of wt. of dry roots).	% of acetone extract 2.60% (on the basis of wt. of dry roots).
2)	% of alcohol extract 15% Dark brown, sticky mass.	% of alcohol extract 24% Dark brown, foamy, hygroscopic powder.
3)	n-BuOH extract -	n-BuOH extract - 21% of alcohol extract.
4)	TLC of n-BuOH extract No glycosides.	TLC of nBuOH extract 7 spots are there.

E X T R A C T I O N

Shatavari root powder (2 kg) was extracted in the soxhlet with hot acetone (6.5 Ltrs) for 48 hrs (twice).

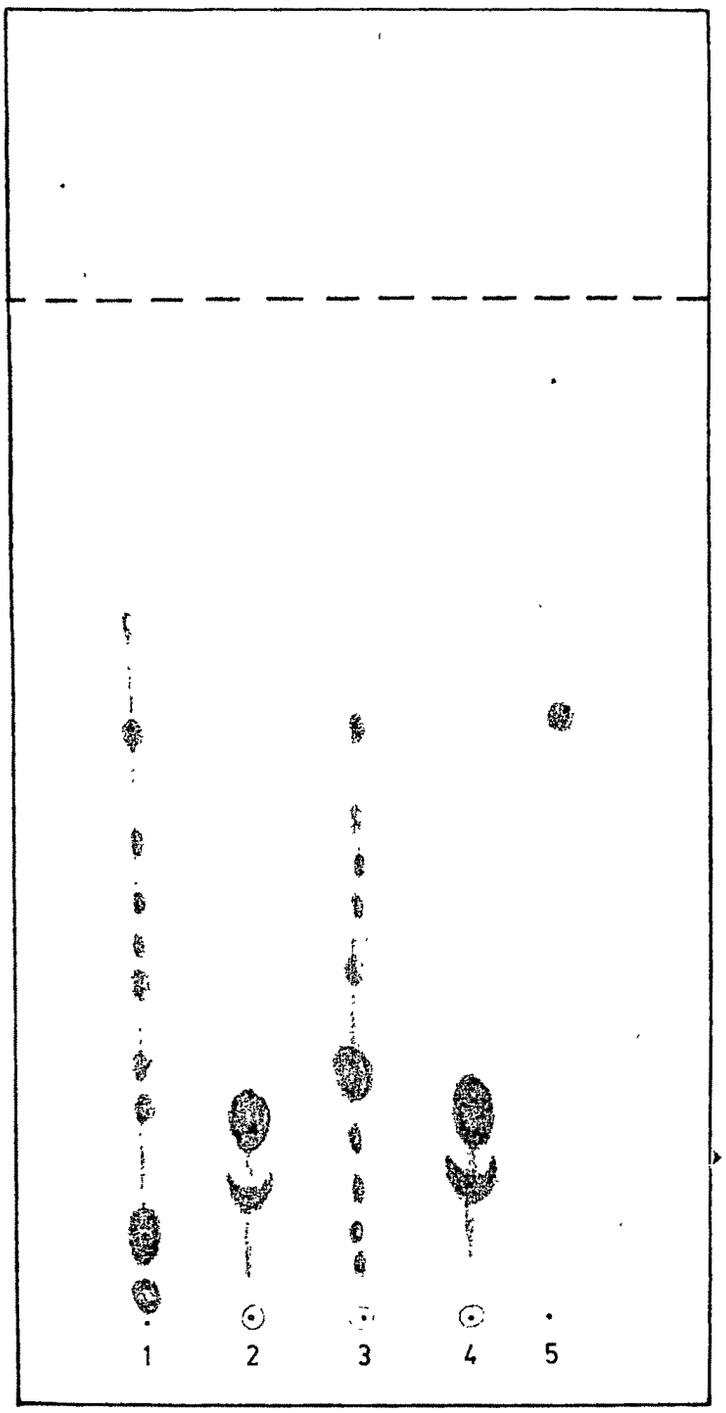


FIG.2 : COMPARATIVE TLC OF ALOCOHOL EXTRACTS OF SHATAVARI.

SILICA GEL G PLATES.

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

SPRAY REAGENT : 1% VANILINE IN 50% PHOSPHORIC ACID.

SPOTS : 1) EtOH EXTRACT OF A. RACEMOSUS. 2) EtOH EXTRACT OF SHATAVARI (KOIMBTUR). 3) EtOH EXTRACT OF A. RACEMOSUS. 4) EtOH EXTRACT OF SHATAVARI (KOIMBTUR). 5) SHATAVARIN-IV.

The solvent was removed from combined extracts to get 38.0 g. dark red oily material.

The root powder was further extracted with hot ethanol (95%, 6.5 Ltrs) for 48 hrs (twice). On removal of solvent, the combined extracts gave 238.0 g. brown, foamy, hygroscopic powder.

60.0 kg of wet roots of A. racemosus were collected in Nov., washed, cut and dried at room temp. for 15 days to get 5.4 kg of dry roots.

Water content : 91.0%.

The dried roots were powdered and 2 kg of root powder was extracted (twice) with hot acetone (6.5 Ltrs) in the Soxhlet for 48 hrs. each. The solvent was removed from the combined extracts to get 38.50 g. of dark brown, oily material.

The root powder was further extracted in the same unit with ethanol (95%, 6.5 Ltrs) for 48 hrs. (twice). Solvent was removed under vacuum from the combined extracts to get 345 g. dark brown, foamy, hygroscopic powder.

SEPARATION OF SAPONIN MIXTURE FROM NON-SAPONINS :

235 g. of alcohol extract of shatavari was dissolved in water (310 ml). The aqueous solution was extracted with

n-butyl alcohol saturated with water (165 ml x 10). The solvent was removed from combined butanol extracts to get brown, foamy, hygroscopic powder - Saponin mixture.

The aqueous part of the extract, on removal of water under vacuum gave 170 g. of black material.

315 g. of alcohol extract of A. racemosus was dissolved in water (422 ml). The aqueous solution was extracted with n-butyl alcohol saturated with water (222 ml x 8). Solvent was removed under vacuum from the combined organic extracts to get 69.0 g. of dark brown, foamy, hygroscopic powder - Saponin mixture.

From the aqueous part water was removed under vacuum to get 246 g. black sticky material.

DETAILED ANALYSIS OF SAPONIN MIXTURE :

As reported earlier, the saponin mixture from shatavari was found to be pharmacologically active[3-5], showing uterin blocking activity both spontaneous and induced by acetyl choline and oxytocin. The mixture also showed pronounced galactotropic activity. So a detailed analysis of saponin mixture was undertaken to identify the active compound.

Ravikumar had earlier isolated four compounds from the saponin mixture of shatavari. The compounds were Comp.A, Shatavarin-IV, Shatavarin-II and Shatavarin-I.

Similar work of separation of glycosides was also started with A. racemosus.

TLC OF SAPONIN MIXTURE

Number of solvent systems are described for the resolution of saponin mixture on silica gel plates[6-7]. However, the solvent system chloroform-methanol-water 65-35-10 (lower phase)[8] was found to be the best for saponin mixture.

TLC of ethanol extract of commercial shatavari showed at least 9 components. They are numbered 1-9 starting from the base upwards. 1-3 are possibly sugars, 4-7 are saponins and 8-9 are non saponins. Butanol extract showed 9 spots on TLC. Saponins 4-7 are named as Shatavarin-I, Shatavarin-II, Shatavarin-III and Shatavarin-IV. Saponins 1-3 are named as Shatavarin-VII, Shatavarin-VI and Shatavarin-V. Compounds 8 and 9 are named as Comp.A and Comp.B respectively. At least four compounds - Shatavarin-II, Shatavarin-I, Shatavarin-V and Shatavarin-VII were positive to the Ehrlich's reagent[9].

The alcohol extract of A. racemosus was resolved into at least 11 components on TLC. From the work described below it was clear that 1-3 are possibly sugars, 4-8 are saponins and the remaining compounds are non-saponins. The saponins 4-8 are named as glycoside-AR_1 to glycoside-AR_5 respectively, 9 is named as Comp.A and 10 and 11 are named as glycoside-AR_6 and Comp.B respectively.

ISOLATION OF INDIVIDUAL SAPONINS

Isolation of individual saponins was important to find out the active compound from the saponin mixture.

Saponin mixture from shatavari :

The saponin mixture from commercial shatavari was subjected to a broad cut chromatography over alumina using n-butyl alcohol saturated with water as eluent. Seven fractions were collected. (Chart-I, Fig.3, Table-3). FR. I (Chart-I, Table-3) was dark red, viscous, non-polar material. FR. II (Chart-I, Table-3) contained comp.B and coloured material. FR. III (Chart-I, Table-3) contained major comp.A with little comp.B and coloured material, with impurity of shatavarin-IV. FR. IV (Chart-I, Table-3)

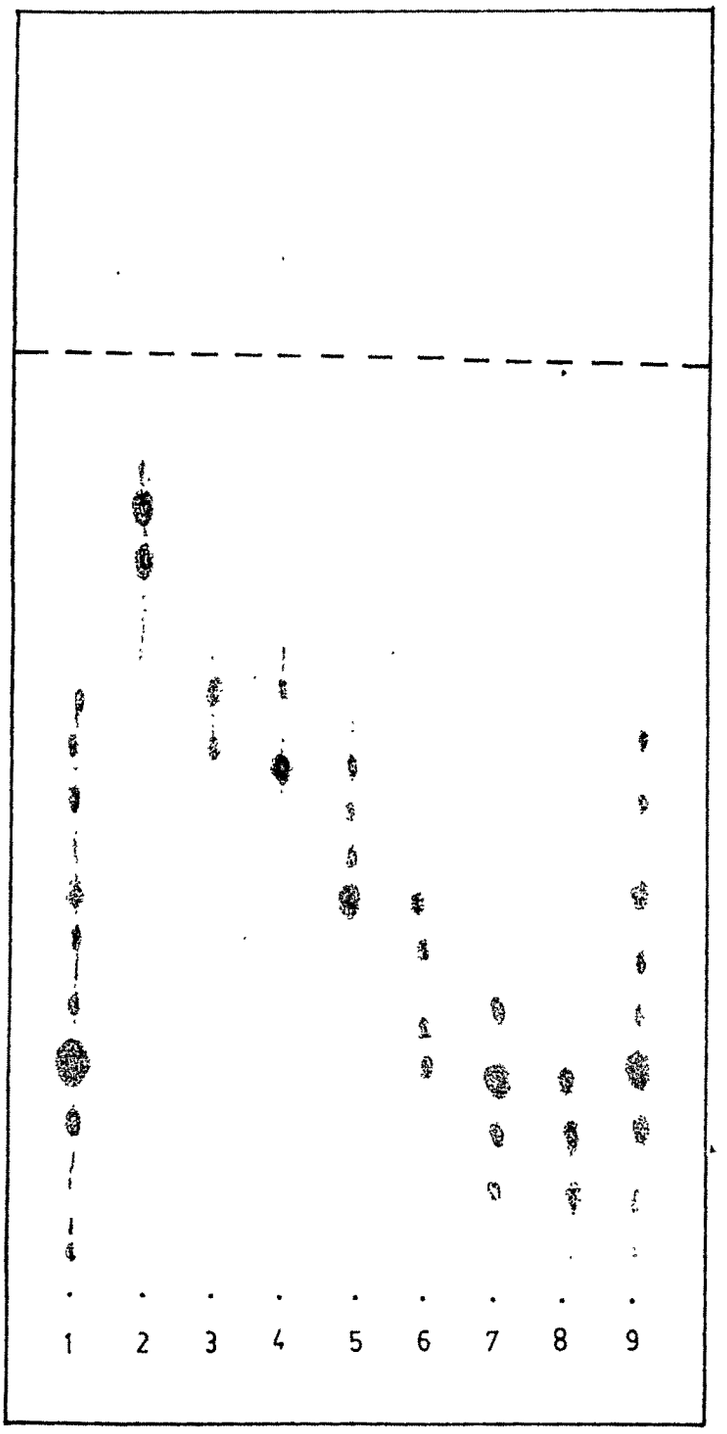


FIG.3 : TLC OF BROAD CUT CHROMATOGRAPHY OF TOTAL SAPONIN MIXTURE (SHATAVARI)

SILICA GEL G PLATE.

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

SPRAY REAGENT : 1% VANILINE IN 50% PHOSPHORIC ACID.

SPOTS : 1) TOTAL SAPONIN MIXTURE. 2) FR. I.
3) FR. II. 4) FR. III. 5) FR. IV.
6) FR. V. 7) FR. VI. 8) FR. VII.
9) TOTAL SAPONIN MIXTURE.

SOLVENT EXTRACTION OF SHATAVARI ROOTS

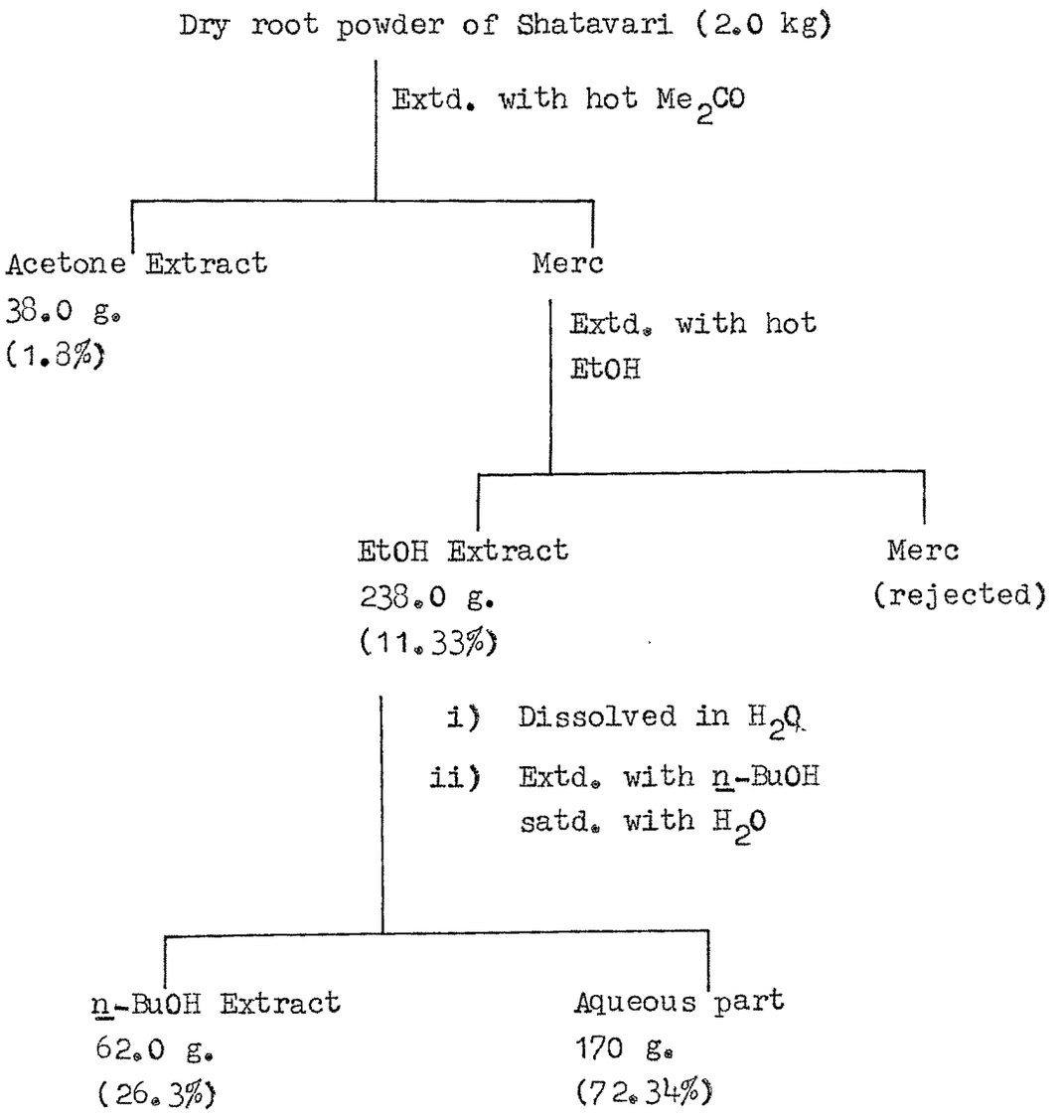
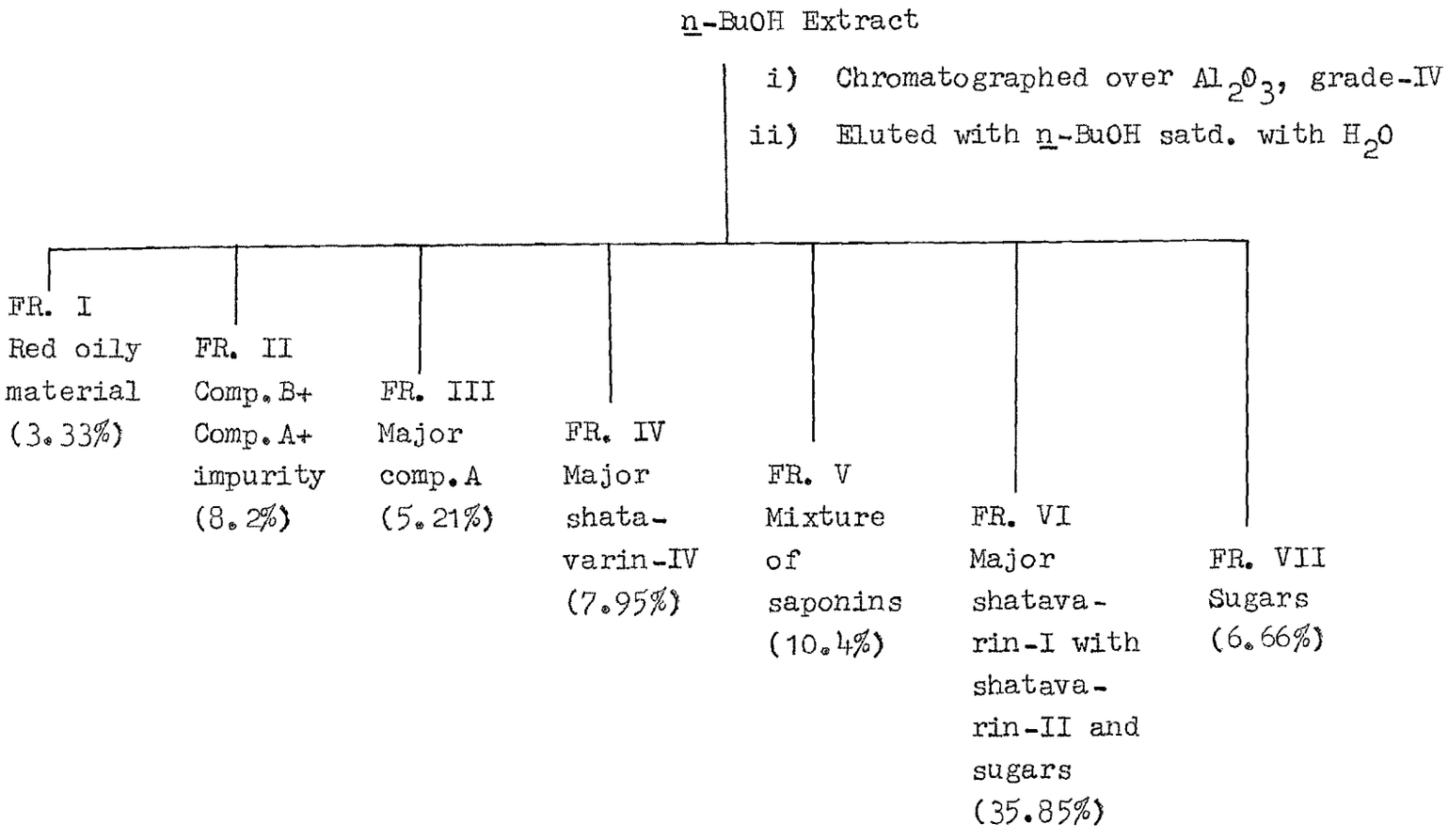
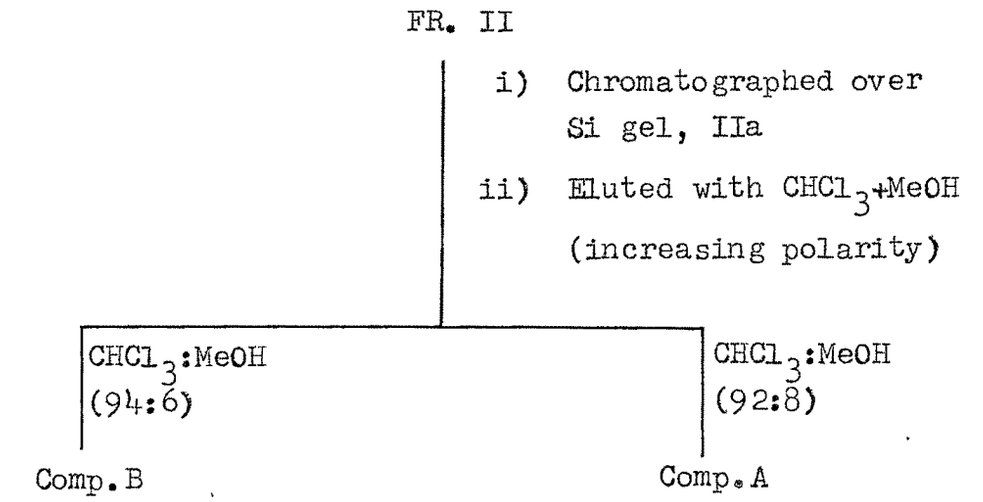


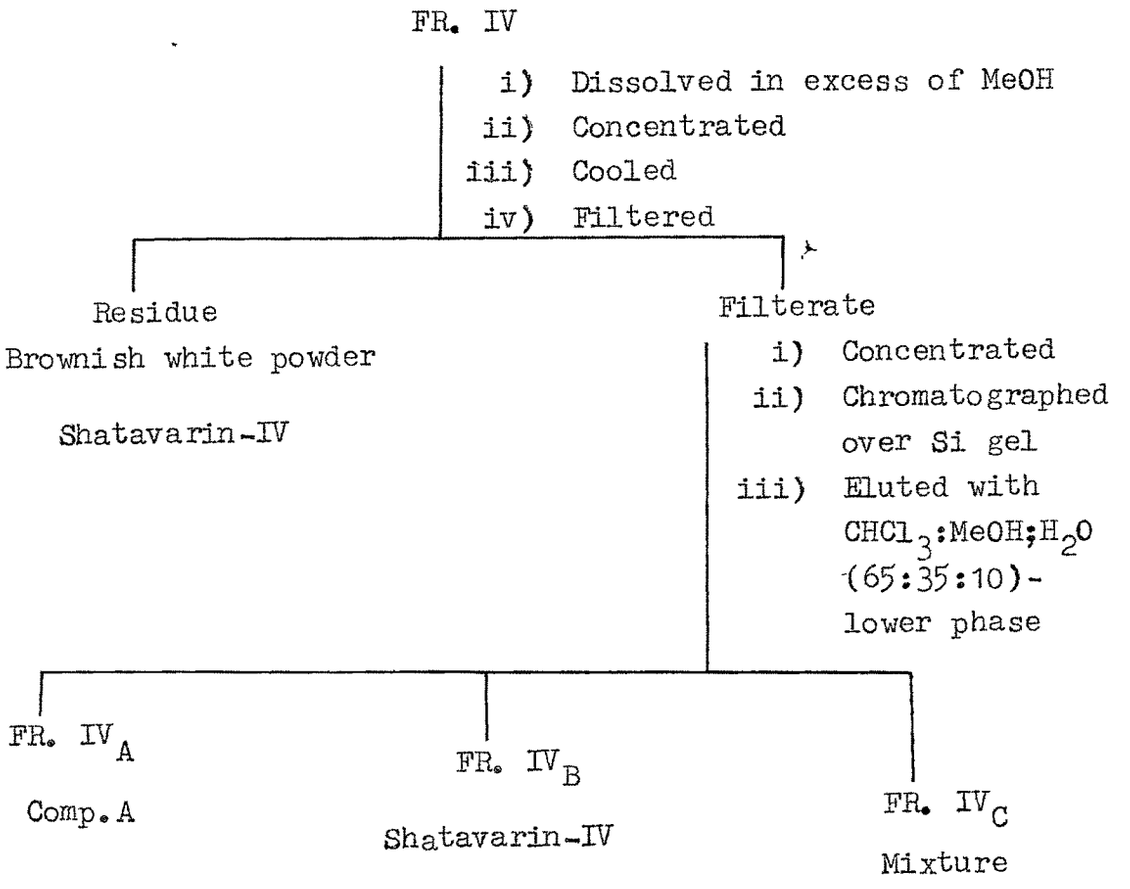
CHART-I : BROAD CUT CHROMATOGRAPHY OF n-BuOH EXTRACT



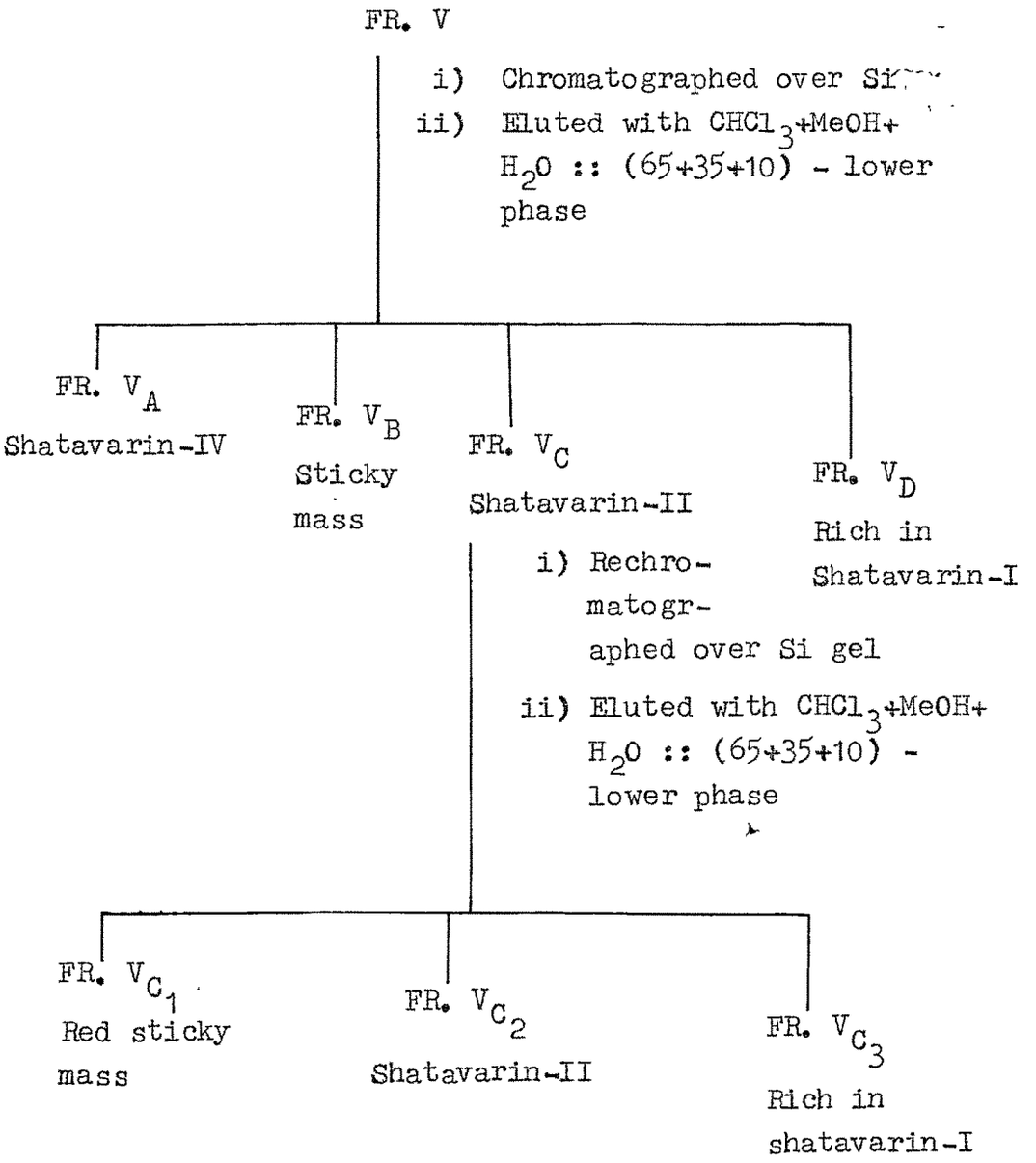
RECHROMATOGRAPHY OF FR. II (CHART-I)



RECHROMATOGRAPHY OF FR. IV (CHART-I)



RECHROMATOGRAPHY OF FR. V (CHART-I)



RECHROMATOGRAPHY OF FR. VI (CHART-I)

FR. VI + FR. V_{C_3} + FR. V_D

i) Dissolved in dry MeOH, precipitated with dry Me_2CO

ii) Filtered.

Precipitate (I)

Major shatavarin-I

Filtrate

i) Solvent removed

ii) Dissolved in dry MeOH, precipitated with dry Me_2CO

iii) Filtered.

Precipitate (II)

Filtrate (I)

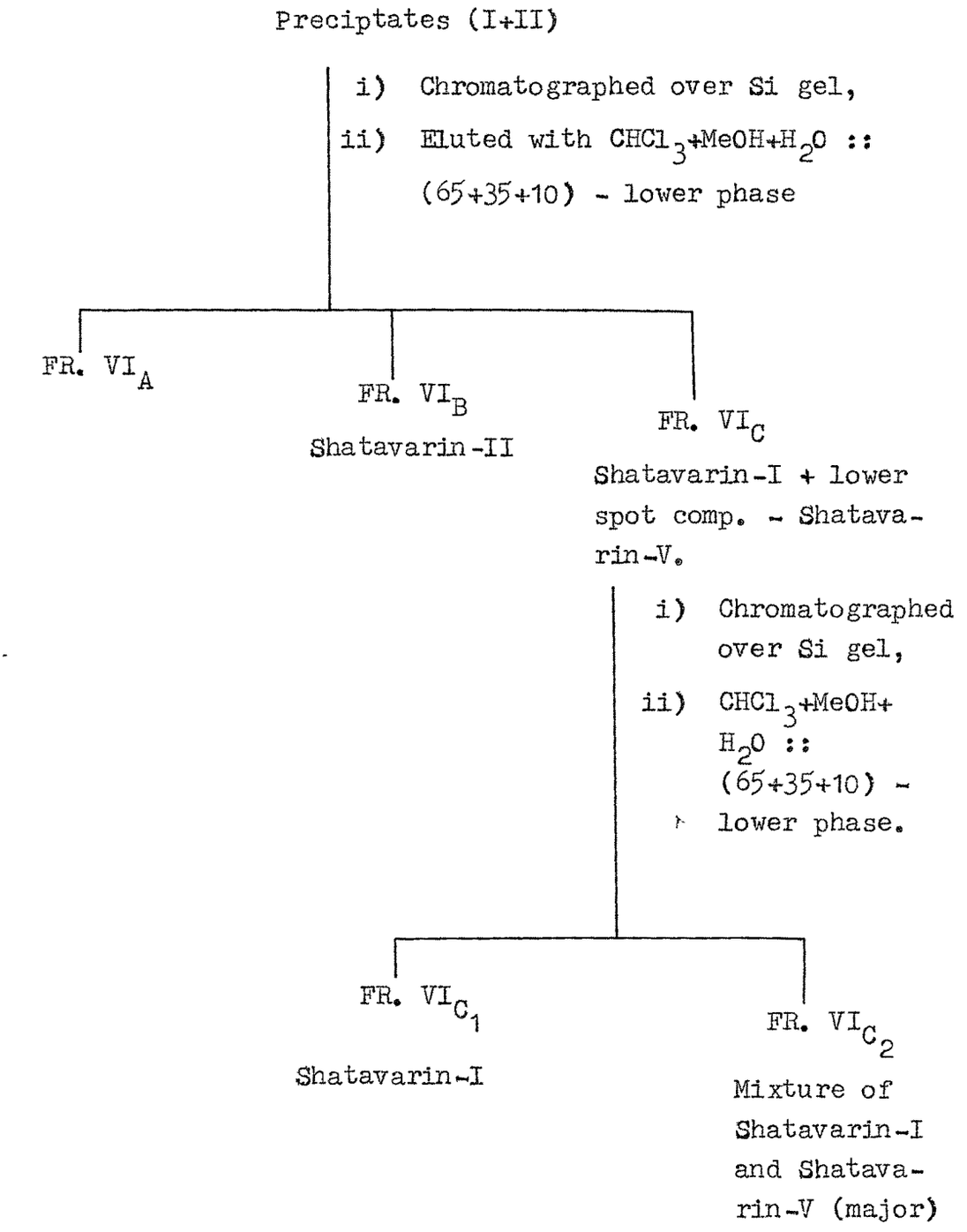

Preciptates (I+II)
i) Chromatographed over Si gel,
ii) Eluted with $CHCl_3$+MeOH+H_2O :: (65+35+10) - lower phase
FR. VI_A
FR. VI_B
Shatavarin-II
FR. VI_C
Shatavarin-I + lower spot comp. - Shatavarin-V.
i) Chromatographed over Si gel,
ii) $CHCl_3$+MeOH+ H_2O :: (65+35+10) - lower phase.
FR. VI_{C_1}
Shatavarin-I
FR. VI_{C_2}
Mixture of Shatavarin-I and Shatavarin-V (major)

CHART-II : RECHROMATOGRAPHY OF FILTRATE

i) Solvent removed

ii) Acetylated with Ac_2O/Pyridine at r.t.

iii) Chromatographed over Si gel, IIa

iv) Eluted with mixture of C_6H_6: EtOAc

C_6H_6:EtOAc (7:3)	C_6H_6:EtOAc (7:3)	C_6H_6:EtOAc (6:4)	C_6H_6:EtOAc (1:1)
Acetate of Shatavarin-IV	Acetate of Shatavarin-I	Mixture	Acetate of Shatavarin-VII

TABLE - 11 : PHYSICAL CHARACTERISTICS OF THE COMPOUNDS ISOLATED FROM THE SAPONIN MIXTURE OF SHATAVARI

Sr. No.	Properties	Comp.B	Comp.A	Shatavarin-IV	Shatavarin-I	Acetate of shatavarin-VII
1.	m.p.	271-4°	151-3°	274-6°	179-86°	142.5-44°
2.	$[\alpha]_D^{27}$		- 140°	- 68.6° (C_5H_5N)	- 35° (CH_3OH)	- 192° ($CHCl_3$)
3.	TLC (R_f)	0.85	0.52	0.46	0.24	
4.	Solubility in MeOH	Less soluble	Soluble	Less soluble	Soluble	Soluble
5.	Water of crystallization	-	-	-	Present (IR 1650 cm^{-1})	-
6.	Molecular formula	-	$C_{10}H_{20}O_6$	$C_{45}H_{74}O_{17}$	$C_{51}H_{84}O_{22}\cdot 5H_2O$	

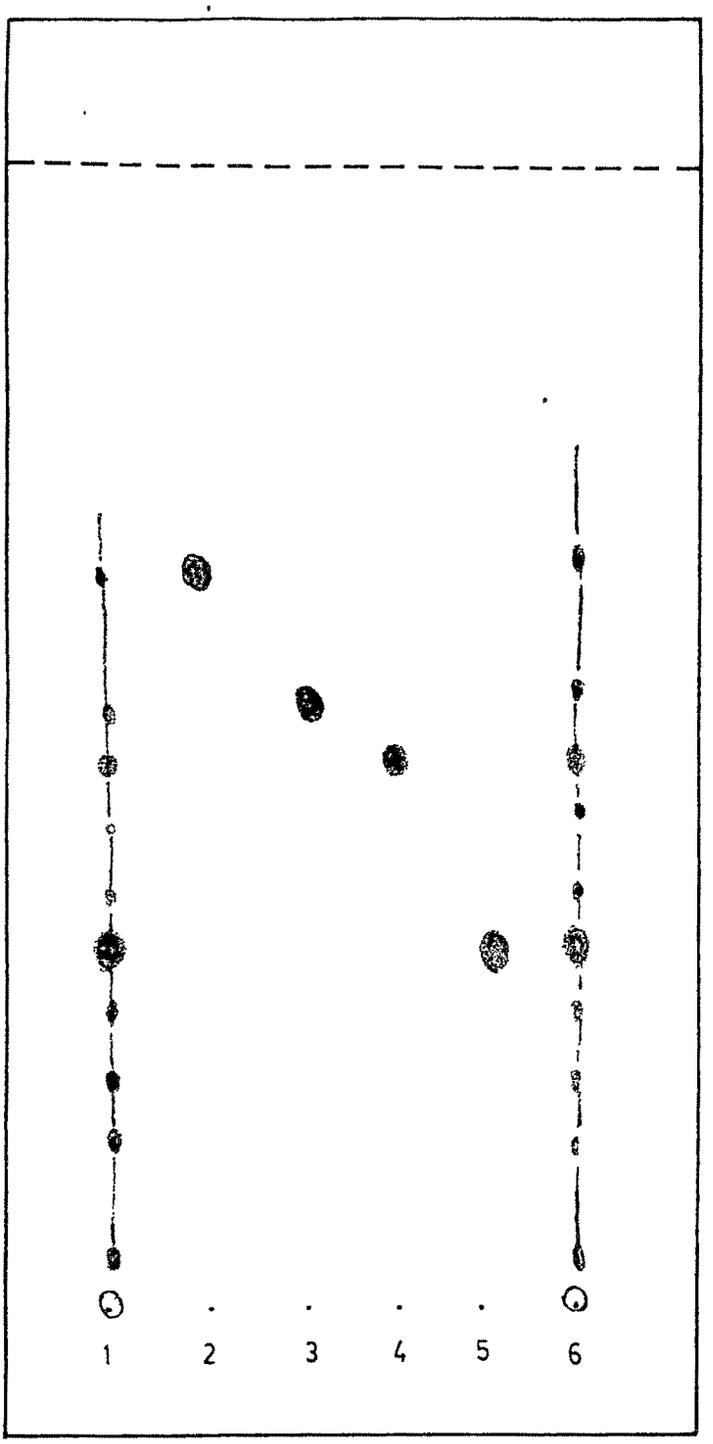


FIG.5 : TLC OF PURE GLYCOSIDES ISOLATED FROM SHATAVARI

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

SPRAY REAGENT : 1% VANILLIN IN 50% PHOSPHORIC ACID

SPOTS : 1) TOTAL SAPONIN MIXTURE 2) COMP.B.
3) COMP.A. 4) SHATAVARIN-IV.
5) SHATAVARIN-I. 6) TOTAL SAPONIN MIXTURE.

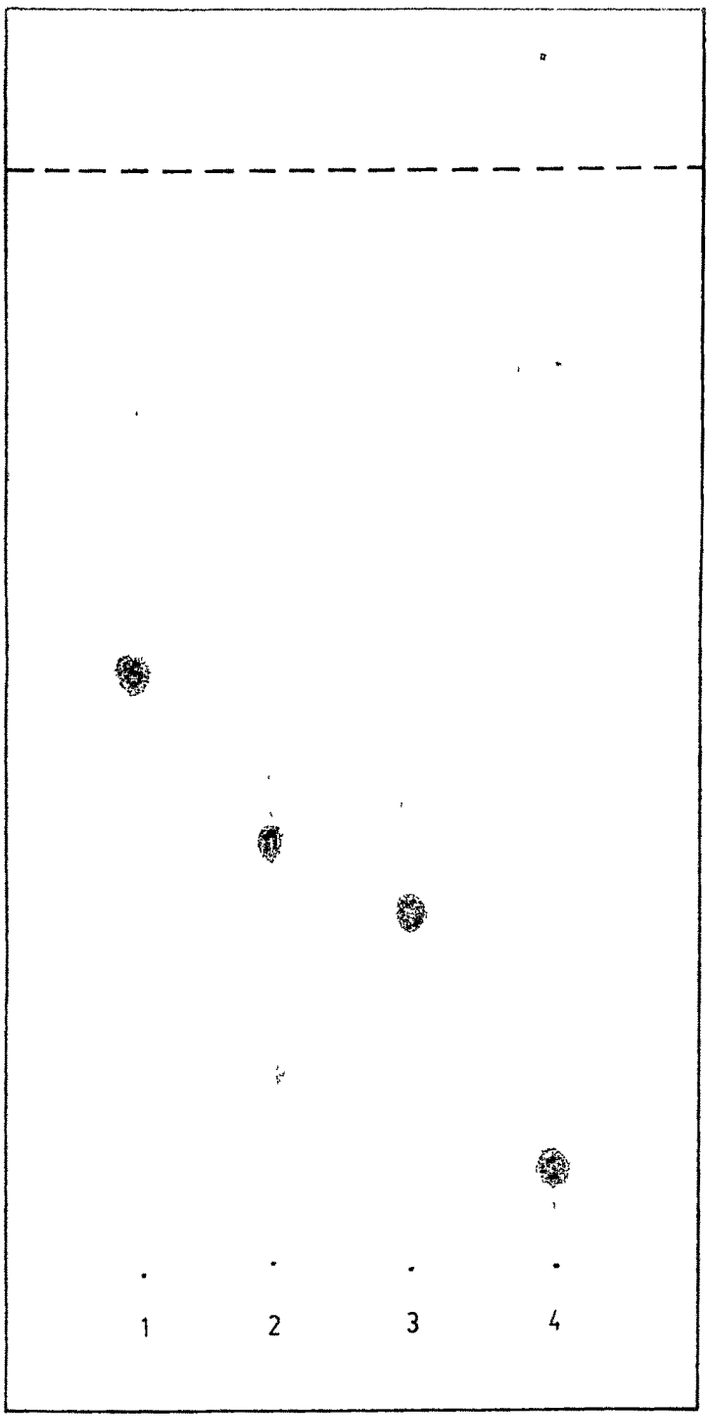


FIG.6 : TLC OF ACETATES OF SAPONINS FROM SHATAVARI.

SOLVENT SYSTEM : BENZENE-ACETONE (30:20)

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

SPOTS : 1) ACETATE OF SHATAVARIN-IV. 2) ACETATE OF SHATAVARIN-II. 3) ACETATE OF SHATAVARIN-I. 4) ACETATE OF SHATAVARIN-VII.

mainly shatavarin-IV with little shatavarin-III and upper impurity. FR. V (Chart-I, Table-3) contained mixture of all saponins. FR. VI (Chart-I, Table-3) consisted of shatavarin-II (little), shatavarin-I (major) and more polar saponins shatavarin-VII and shatavarin-V. FR. VII (Chart-I, Table-3) was the mixture of sugars (major) and polar saponins (in a small amount).

From extensive chromatographies, six compounds were obtained in the state of purity. (Fig. 5 and 6, Table-10). The compounds are comp.B, comp.A, shatavarin-IV, shatavarin-II (as acetate), also shatavarin-I and shatavarin-VII (as acetate). Shatavarin-II, shatavarin-I, shatavarin-V and shatavarin-VII were positive to Ehrlich's reagent. (Fig.4). Table-10 summarises the physico-chemical characteristics of these compounds.

ISOLATION OF COMP.B :

Rechromatography of FR. III (Table-3, Fig.3) over silica gel using chloroform + methanol (with increasing polarity) for elution (Table-4) gave comp.B. Comp.B was also obtained from methanol solution of FR. II (Table-3), in which it was separated as a white powder. It was

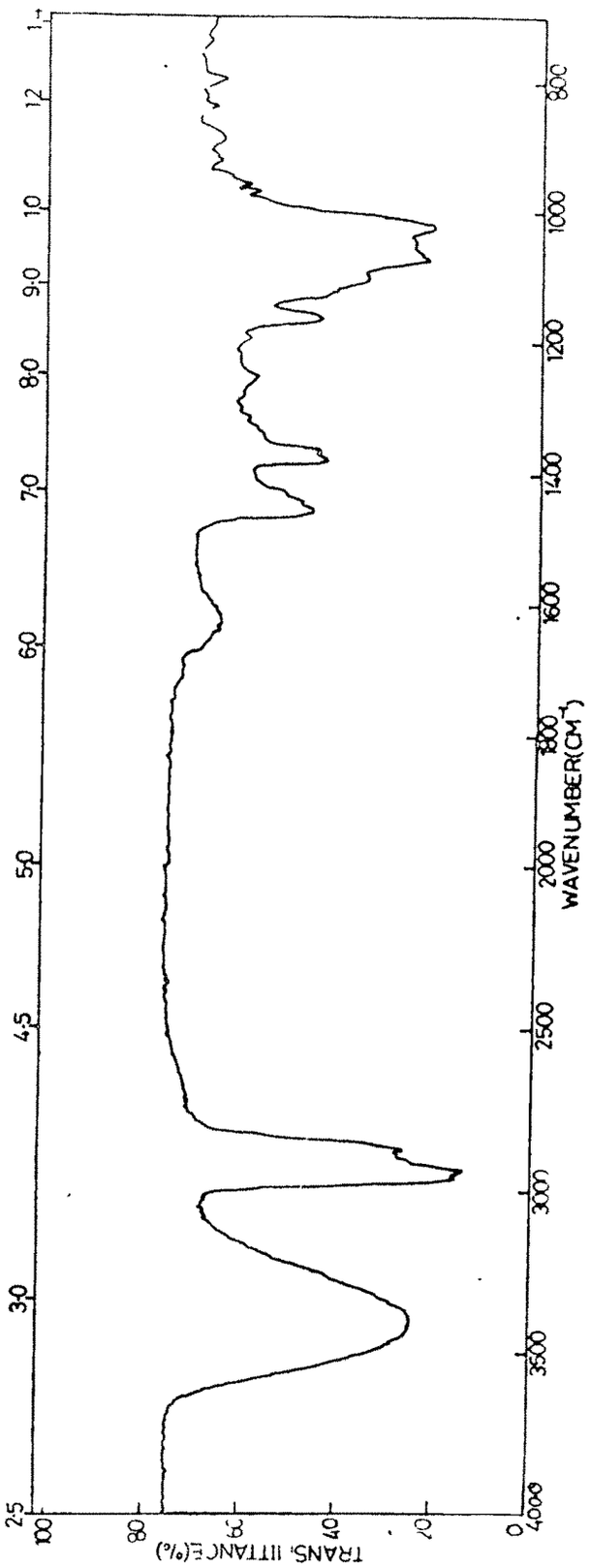


FIG. 7 : IR SPECTRUM OF COMP. B

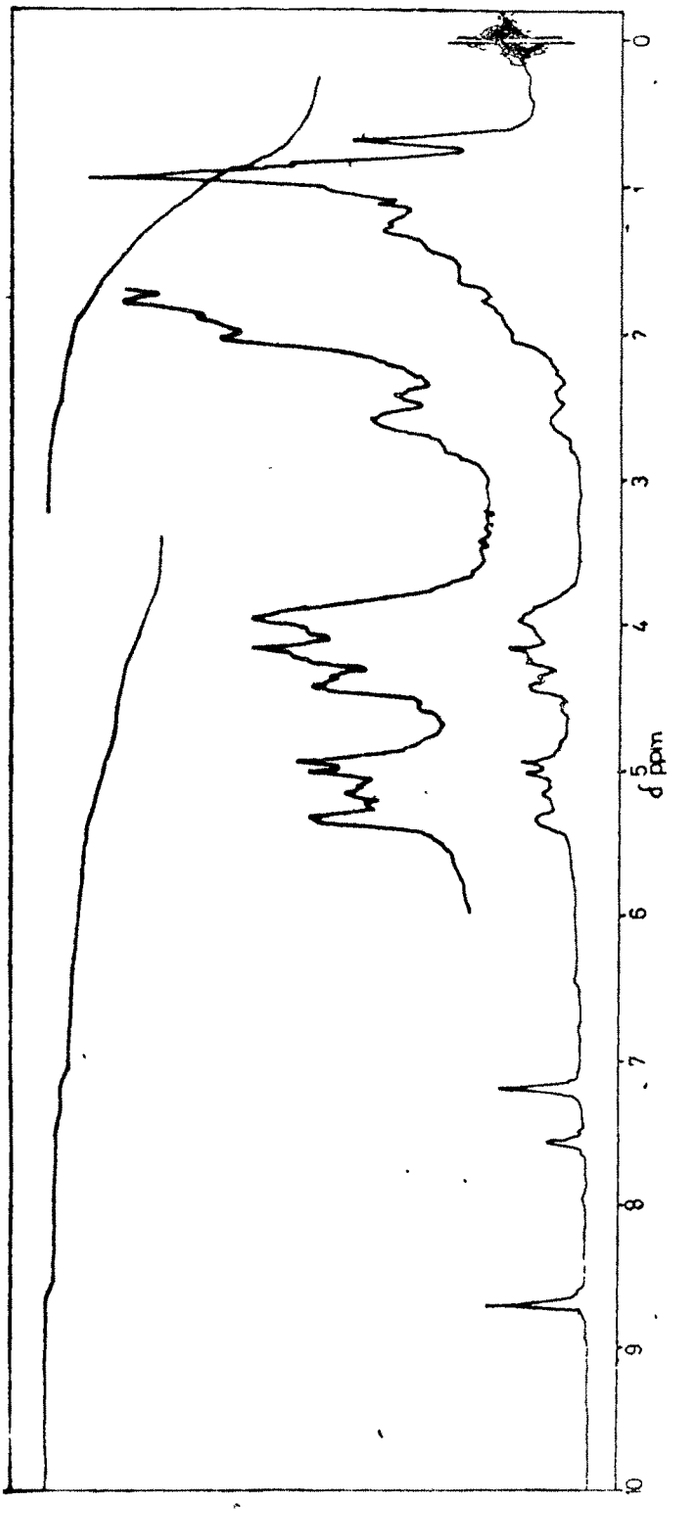


FIG.8 : ^{1}H-NMR SPECTRUM OF COMP.B. (C_5D_5N)

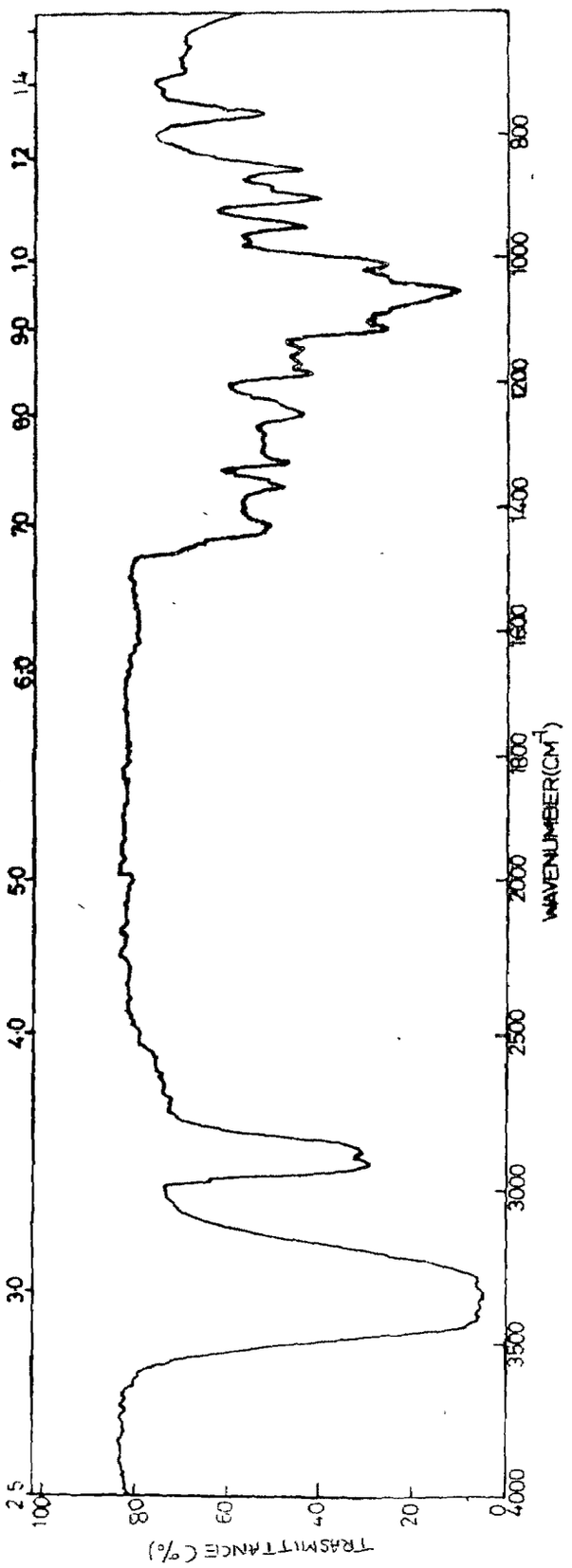


FIG.9 : IR SPECTRUM OF COMP.A

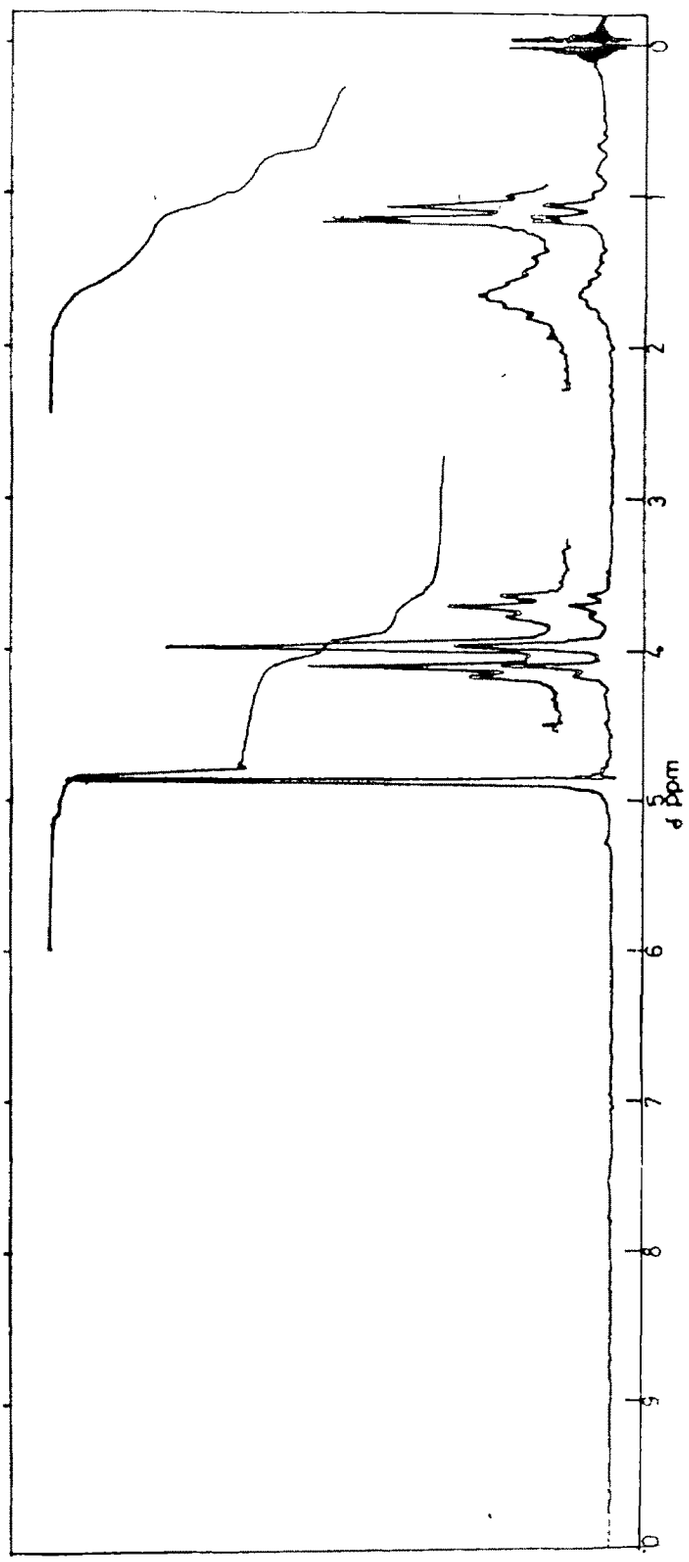


FIG.10 : ^{1}H-NMR SPECTRUM OF COMP.A (D_2O)

scale ?

FIG.11 : ^{13}C-NMR SPECTRUM OF COMP.A (D2O)

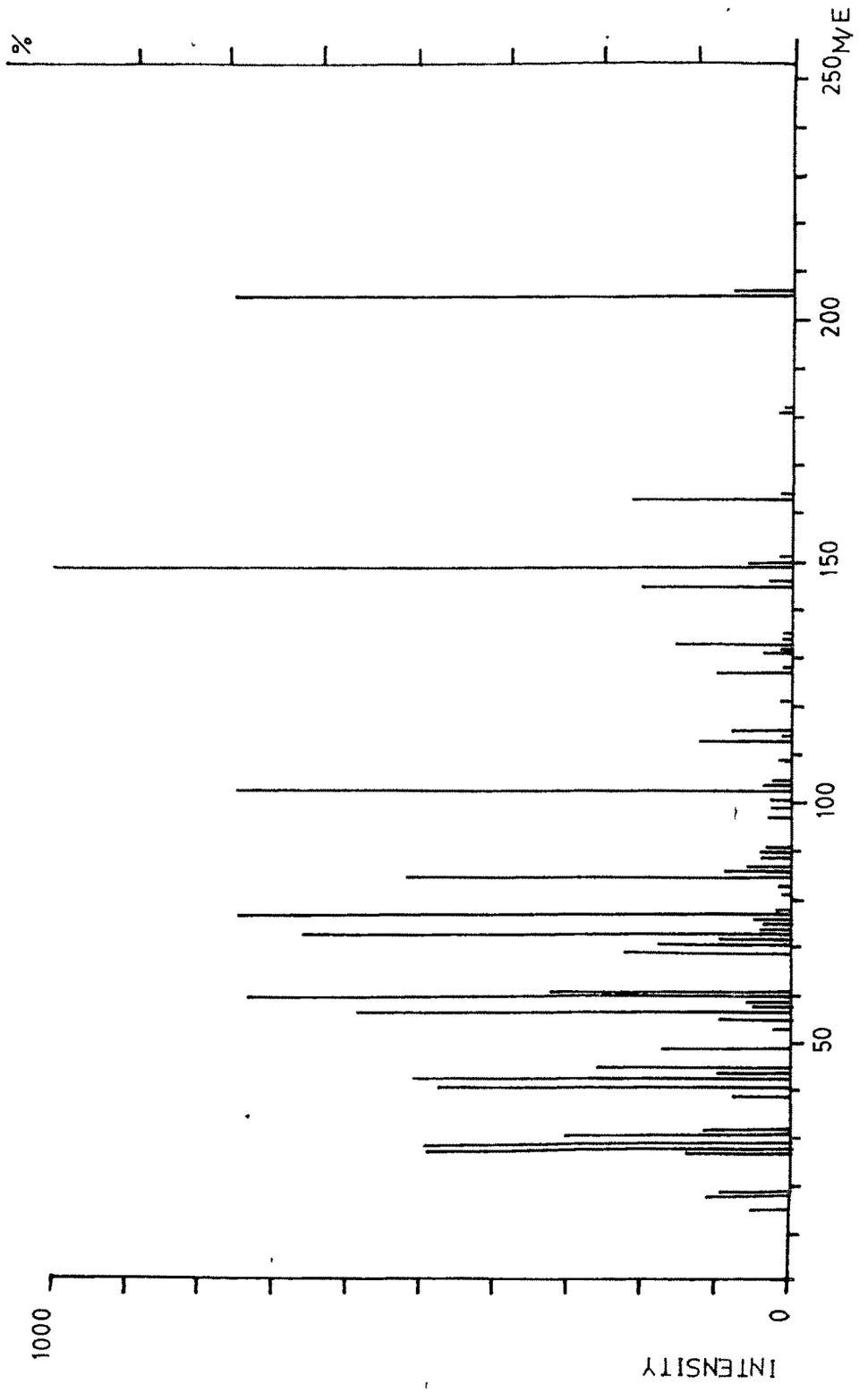


FIG.12 : MASS SPECTRUM OF COMP.A.

crystallized from hot methanol + ethanol (1:1), (Fig.5). m.p. 271-4°, IR (KBr) is shown in Fig.7. ^{1}H-NMR is shown in Fig.8.

ISOLATION OF COMP.A :

FR. III (Table-3) was subjected to rechromatography over silica gel using chloroform + methanol (increasing polarity) for elution (Table-4). FR. III_C gave comp.A - single spot compound which was crystallized from methanol to get pure comp.A as needles (Fig.5). m.p. 151-3°, IR (KBr), PMR (D_2O) and ^{13}C NMR (D_2O) are shown in Figs 9, 10 and 11 respectively. Mass spectrum is shown in Fig. 12.

Structure of comp.A is discussed later in this chapter.

ISOLATION OF SHATAVARIN-IV :

FR. III_G (Table-4) and FR. IV (Table-3, Fig.3) were combined. The brown powder was dissolved in excess of methanol and concentrated to 50%. On cooling, shatavarin-IV separated out as a white powder which was filtered and washed several times with cold methanol to remove the traces of impurities. Shatavarin-IV remained as insoluble white powder. Solvent was removed from the mother liquor and the residue was dried under vacuum to get brown, foamy powder which was subjected to chromatography over silica

gel using chloroform + methanol + water :: 65 + 35 + 10 (lower phase) for elution. (Table-5). FR. IV_F gave pure shatavarin-IV. Shatavarin-IV was repeatedly recrystallized from hot methanol to get pure shatavarin-IV as a white crystalline powder. (Fig.5). m.p. 274-8°. The compound analyzed for $C_{45}H_{74}O_{17}$. IR (KBr) and ^{1}HNMR are shown in Figs 13 and 14 respectively.

ISOLATION OF SHATAVARIN-I :

FR. VI (Table-3) was dissolved in dry methanol and precipitated with dry acetone. The precipitates were filtered and washed with dry acetone. Solvent was removed from the mother liquor and the residue redissolved in dry methanol and precipitated with dry acetone. The precipitates were filtered and washed with dry acetone. The two precipitates were combined and subjected to chromatography over silica gel using chloroform + methanol + water :: 65 + 35 + 10 (lower phase) as eluent. (Table-6). FR. VI_C which was rich in statavarin-I, was further rechromatographed over silica gel (Table-7) to get pure shatavarin-I (Fig.5). m.p. 179-86°. The compound analyzed for $C_{51}H_{84}O_{22}$ $5H_2O$. IR (KBr) and ^{1}HNMR are shown in Figs 15 and 16 respectively.

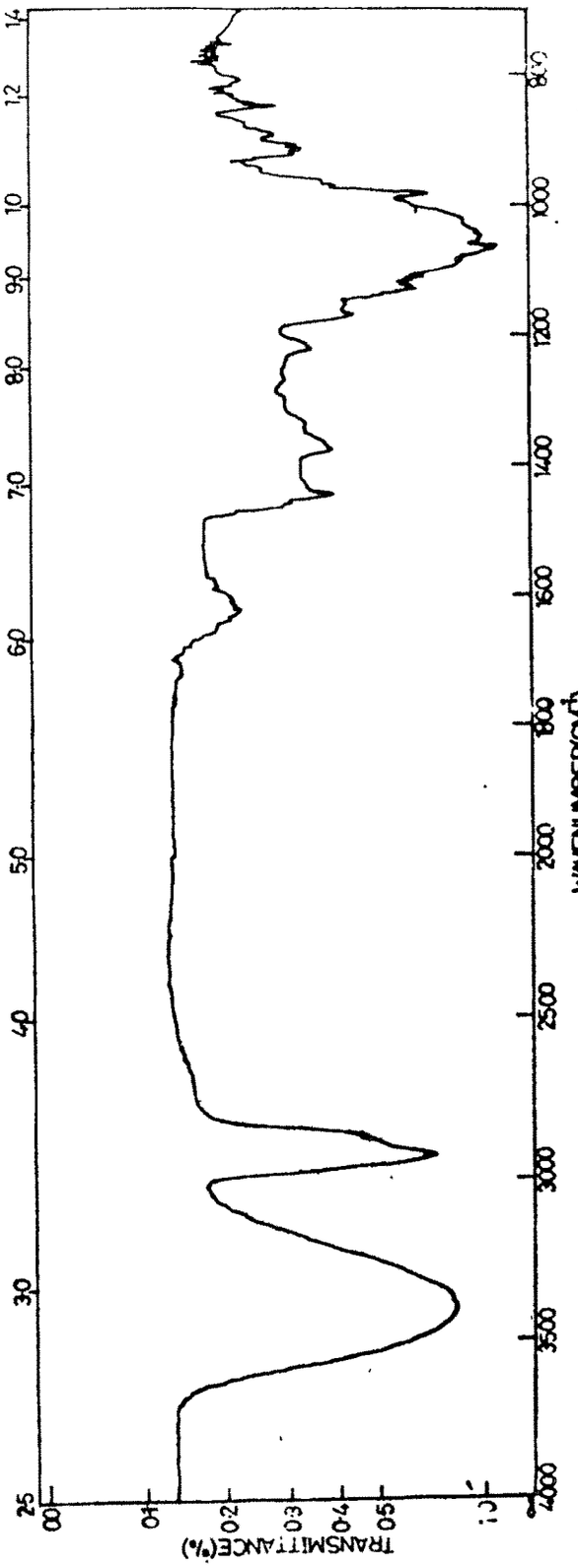


FIG.13 : IR SPECTRUM OF SHATAVARIN-IV.

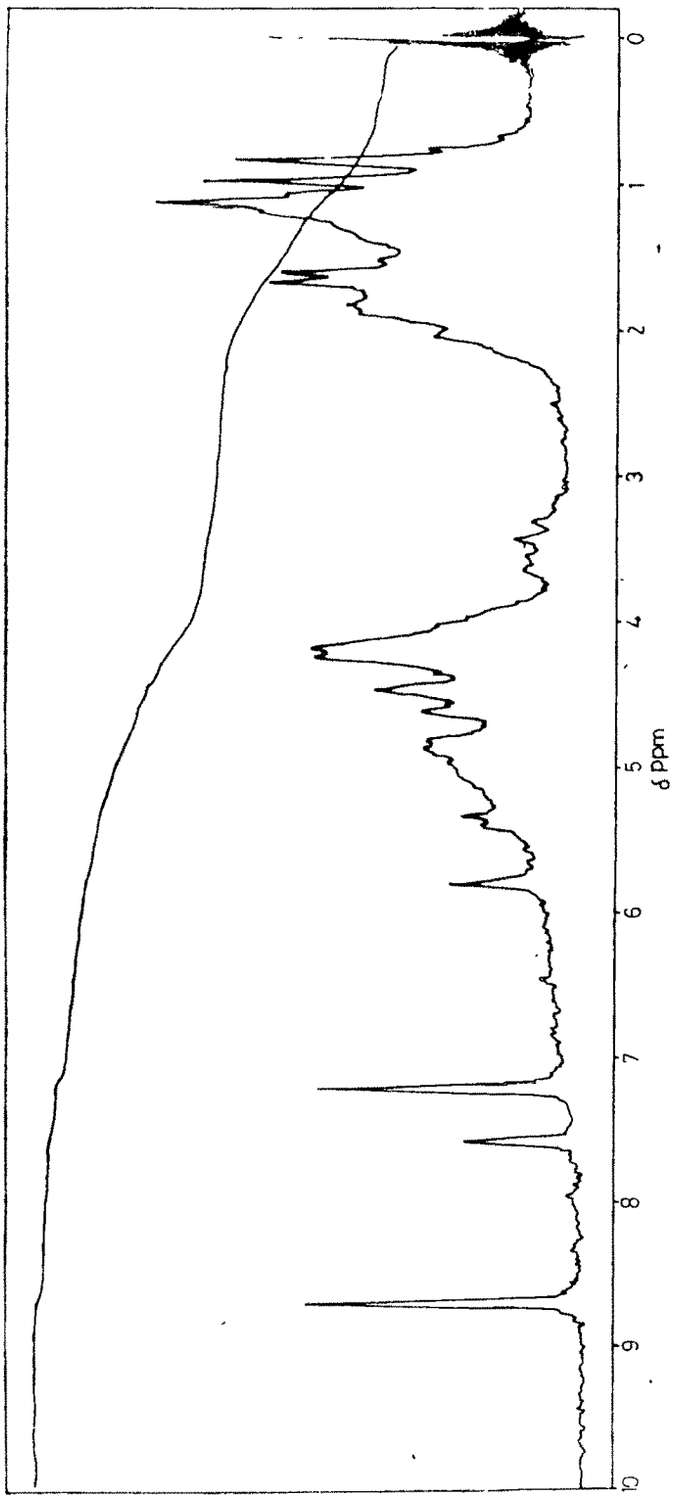


FIG.14 : ^{1}H-NMR SPECTRUM OF SHATAVARIN-IV (C_5D_5N)

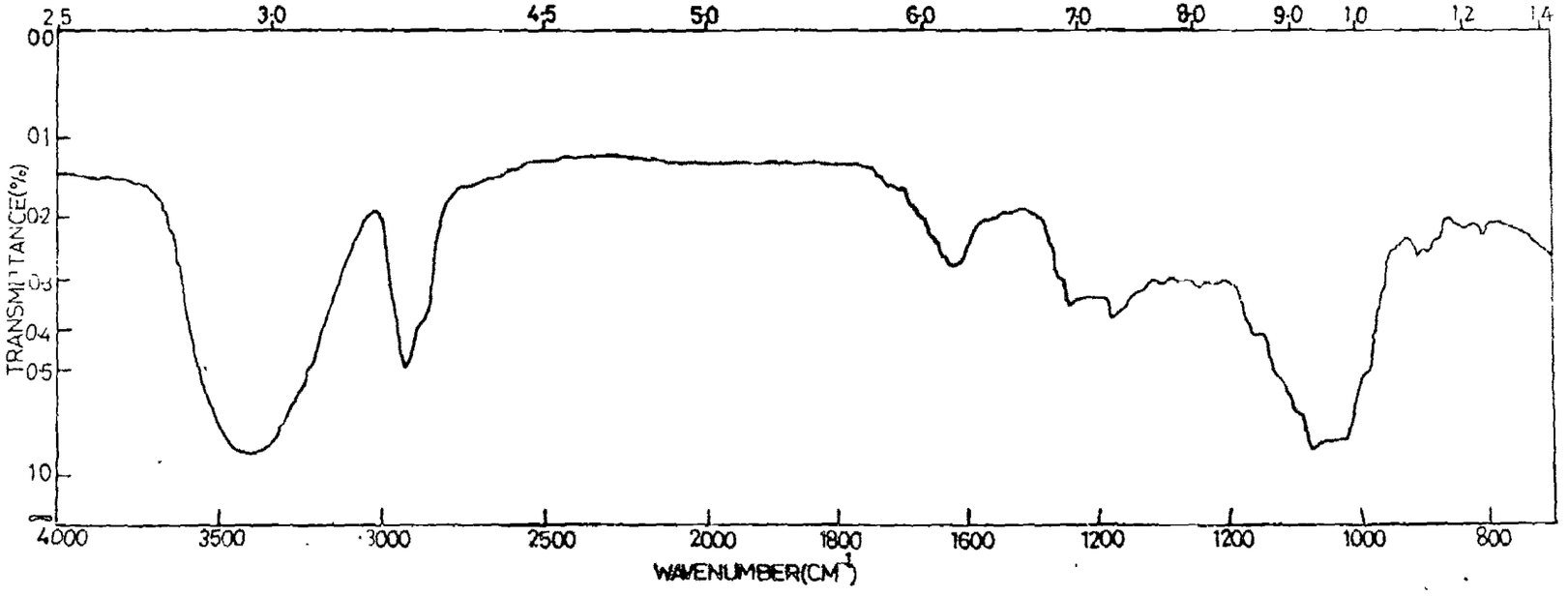


FIG.15 : IR SPECTRUM OF SHATAVARIN-I.

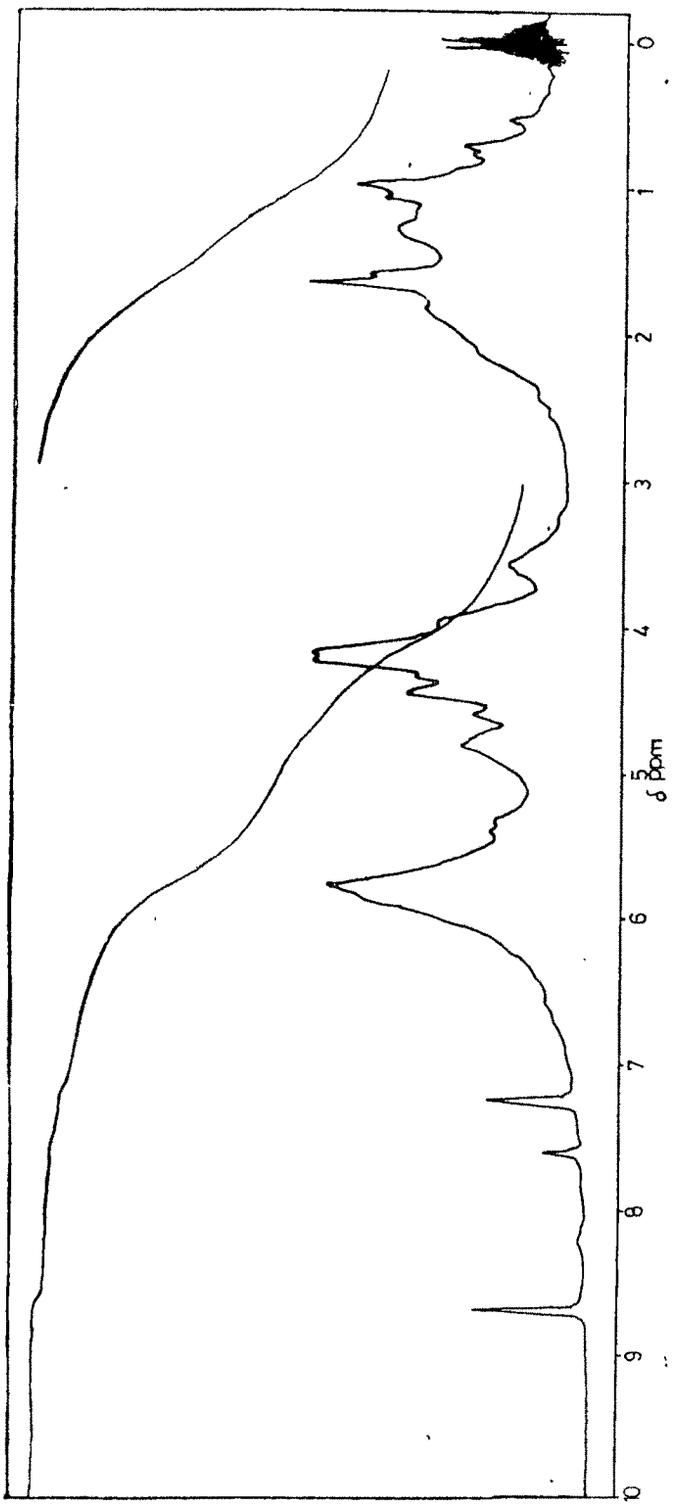


FIG.16 : ^{1}H-NMR SPECTRUM OF SHATAVARIN-I (C_5D_5N)

ISOLATION OF SHATAVARIN-II :

FR. V (Table-3) which contained mixture of saponins was subjected to rechromatography over silica gel (Table-7) using chloroform + methanol + water :: 65 + 35 + 10 (lower phase). A fraction was obtained which was a sticky solid showing a single spot between shatavarin-IV and shatavarin-II. Rechromatography of FR. V_5 (Table-8,9) gave shatavarin-II with slight impurity m.p. 192-8°.

ISOLATION OF SHATAVARIN-VII :

Shatavarin-VII was isolated in the form of its acetate (Chart-III). The mother liquor from FR. VI (Table-3) after precipitation of shatavarin-I, contained shatavarin-II, shatavarin-I, shatavarin-V, shatavarin-VI and shatavarin-VII. Solvent was removed completely and the residue was dried under vacuum. It was acetylated with acetic anhydride in pyridine at room temp. Acetic anhydride and pyridine were removed under vacuum. The residue was taken up in chloroform, washed with aqueous sodium carbonate solution and $\frac{N}{2}$ hydrochloric acid to remove traces of acetic acid and pyridine. Light brown, foamy powder was obtained as residue after the removal of chloroform, which was subjected to chromatography over silica gel using benzene + ethyl acetate (increasing

polarity) for elution (Table-10).

FR. 4 (Table-10) showed acetate of shatavarin-II with slight impurity (TLC). It was obtained as white foamy powder, m.p. 65.5-69°. (Fig.6). FR. 6 (Table-10) contained acetate of shatavarin-I, which was obtained as whitish yellow foamy powder, m.p. 112-15°. It was repeatedly recrystallized from dry ethanol to get pure acetate of shatavarin-I (Fig.6), m.p. 119.5-121°.

FR. 10 (Table-10) was a light yellow foamy powder which showed a single spot on TLC. It was named as acetate of shatavarin-VII, m.p. 136-39°. It was repeatedly recrystallized from hot ethanol (dry) to get white, heavy crystals, m.p. 142.5-144°. IR (KBr), PMR ($CDCl_3$) are reported in Figs 17 and 18 respectively.

Structure of comp.A is discussed in the present chapter, structure of shatavarin-IV is discussed in chapter-3, that of shatavarin-I in chapter-4 and tentative structure of acetate of shatavarin-VII is discussed in chapter-5.

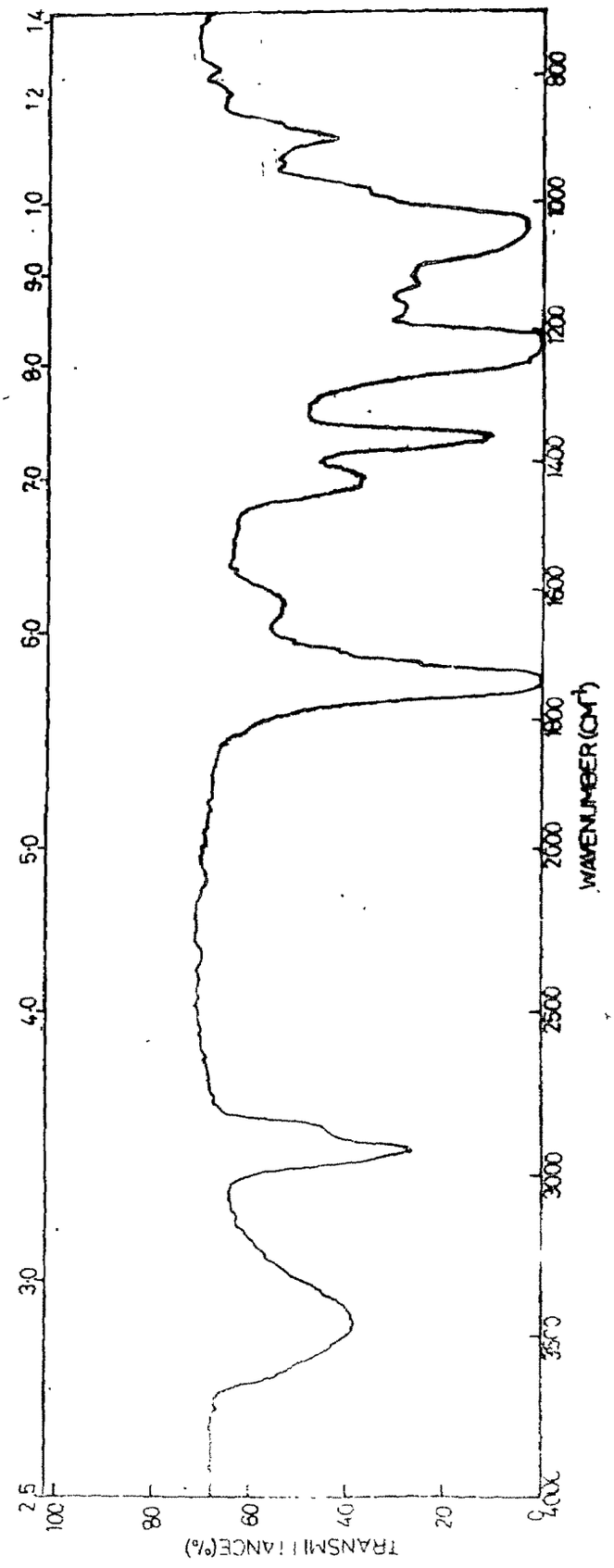


FIG.17 : IR SPECTRUM OF PERACETATE OF SHATAVARIN-VII

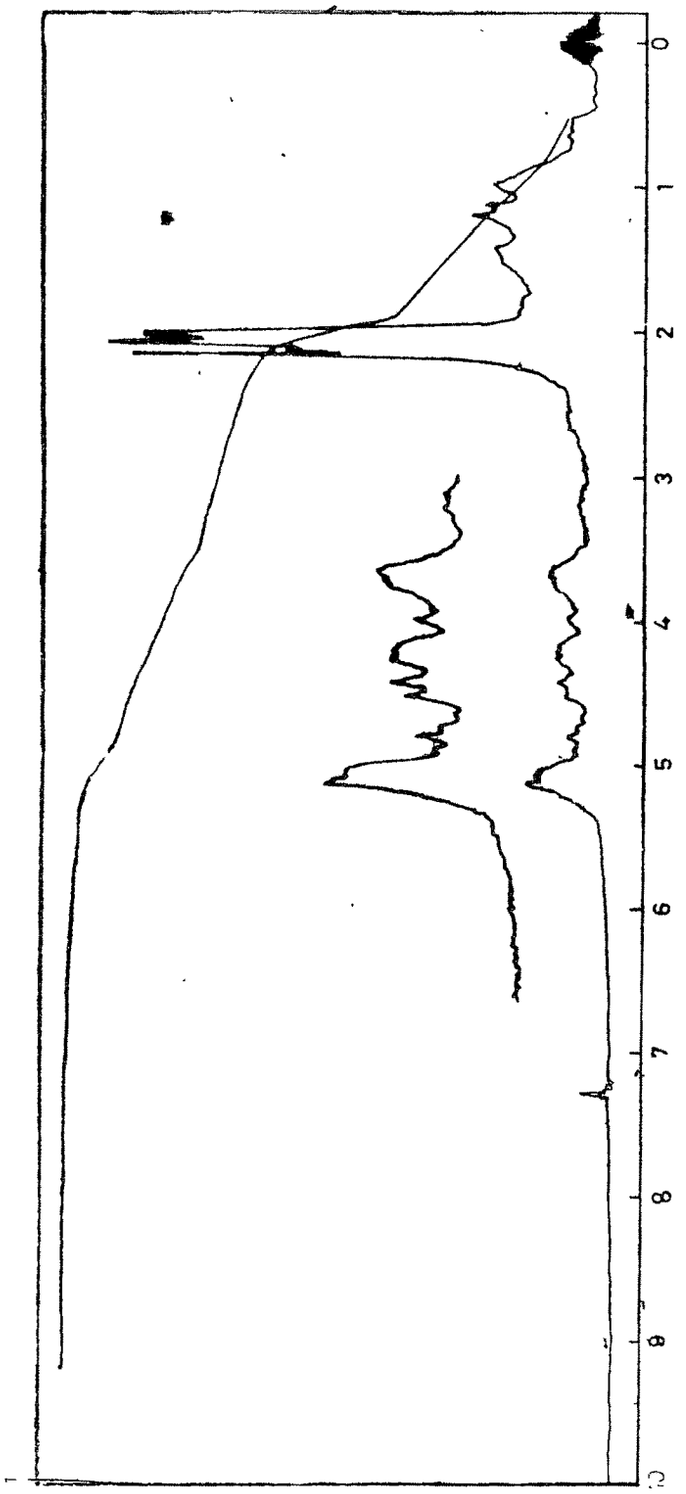


FIG. 18 : ^{1}H-NMR SPECTRUM OF ACETATE OF SHATAVARIN-VII ($CDCl_3$)

Saponin mixture from A. racemosus

The saponin mixture was first subjected to a broad cut chromatography over alumina using n-butyl alcohol saturated with water for elution. Seven fractions were collected. (Chart-II, Fig.19, Table-12). FR. I (Chart-II, Table-12) was dark red, oily, least polar material. FR. II (Chart-II, Table-12) contained comp.B with coloured material. FR. III (Chart-II, Table-12) mainly contained comp.A with a small amount of comp.B and glycoside-AR_4. FR. IV (Chart-II, Table-12) contained glycoside-AR_4 as major compound with glycoside-AR_6 and glycoside-AR_3. FR. V (Chart-II, Table-12) was the mixture of saponins. FR. VI (Chart-II, Table-12) contained major glycoside-AR_1 with glycoside-AR_2 and sugars. FR. VII (Chart-II, Table-12) contained mainly sugars.

From these broad cuts it had been possible to isolate three compounds in a state of purity. (Chart-2, Fig.20, Table-18). These compounds are comp.B, comp.A and glycoside-AR_4. Table-18 summarises the physico chemical characteristics of these compounds.

ISOLATION OF COMP.B :

FR. II (Chart-II, Table-12) was subjected to rechromatography over silica gel using chloroform + methanol

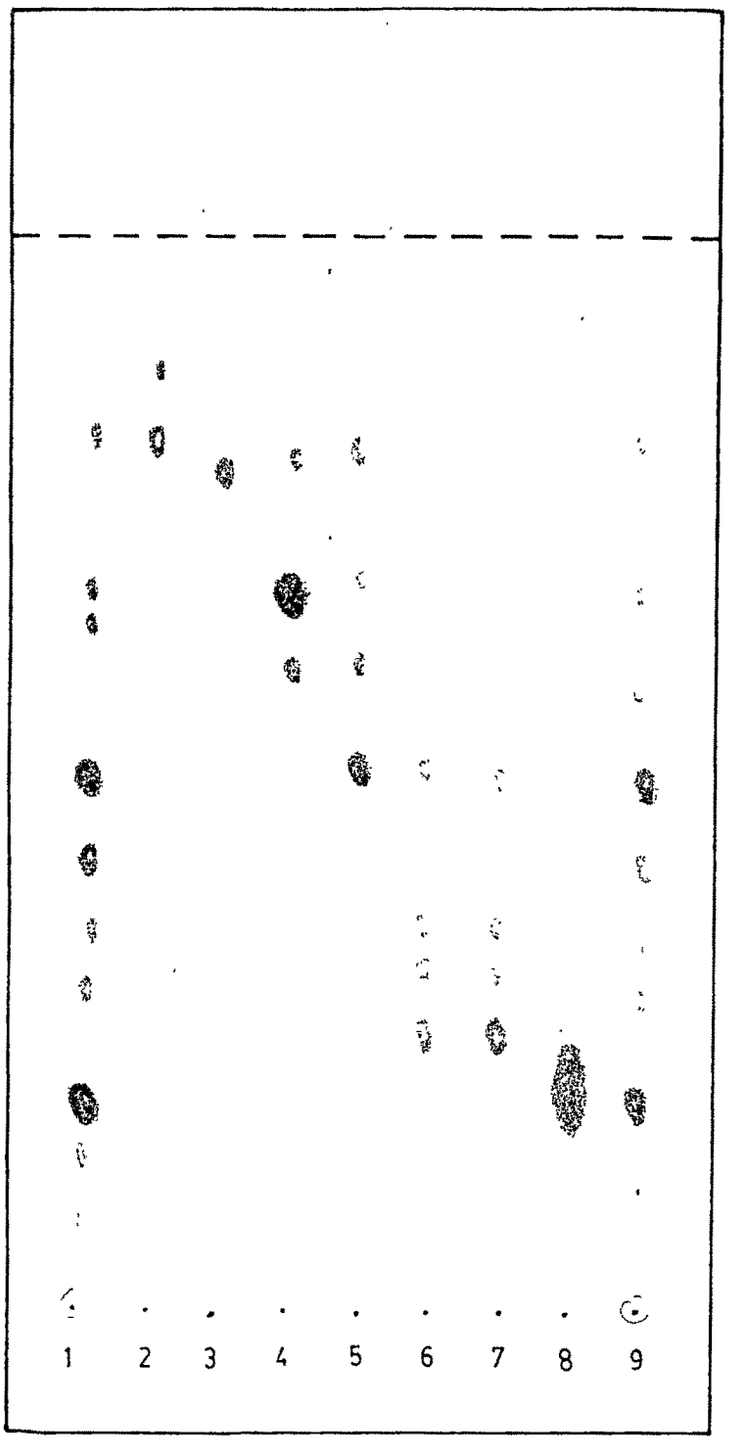


FIG.19 : TLC OF BROAD CUT CHROMATOGRAPHY OF A. RACEMOSUS
SILICA GEL G PLATE
SOLVENT SYSTEM : $CHCl_3$-MeOH-H_2O (65:35:10)(LOWER PHASE)
SPRAY REAGENT : 1% VANILINE IN 50% PHOSPHORIC ACID
SPOTS : 1) TOTAL SAPONIN MIXTURE. 2) FR. I
3) FR. II. 4) FR.III 5) FR. IV 6) FR. V.
7) FR. VI. 3) FR. VII. 9) TOTAL SAPONIN
MIXTURE.

SOLVENT EXTRACTION OF *ASPARAGUS RACEMOSUS* ROOTS

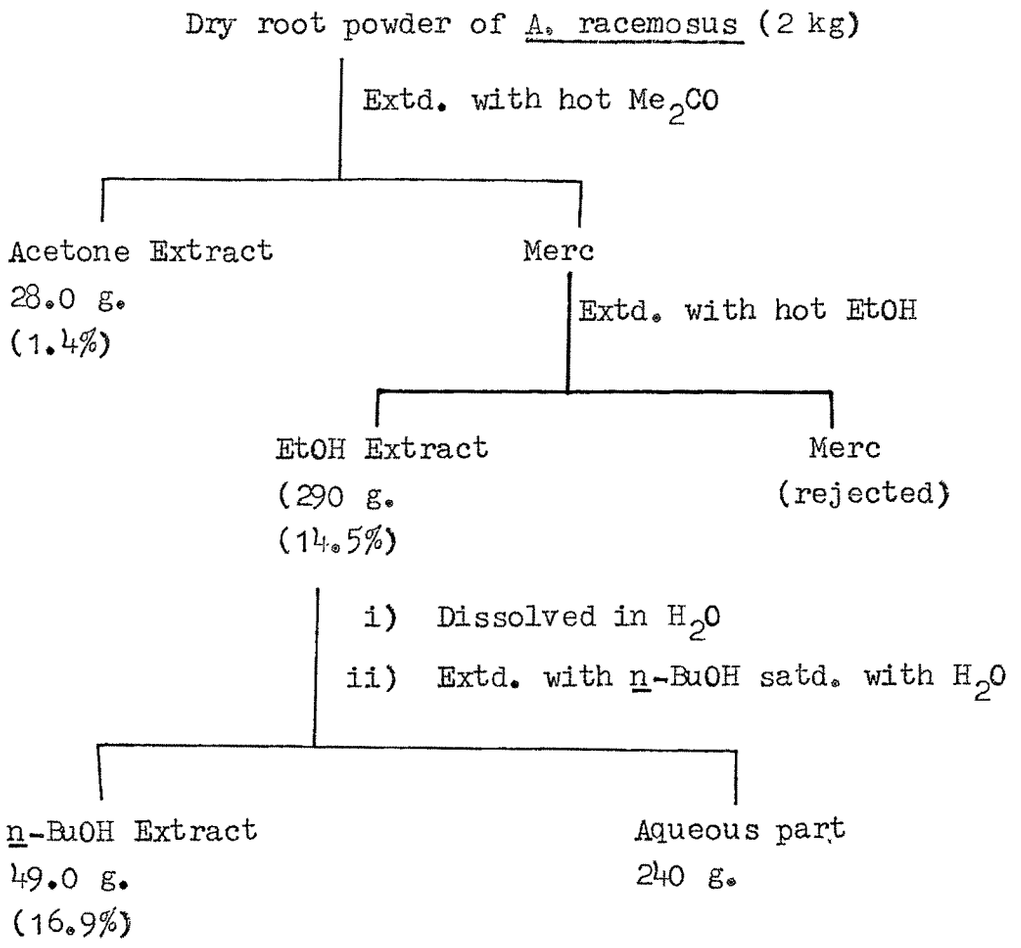
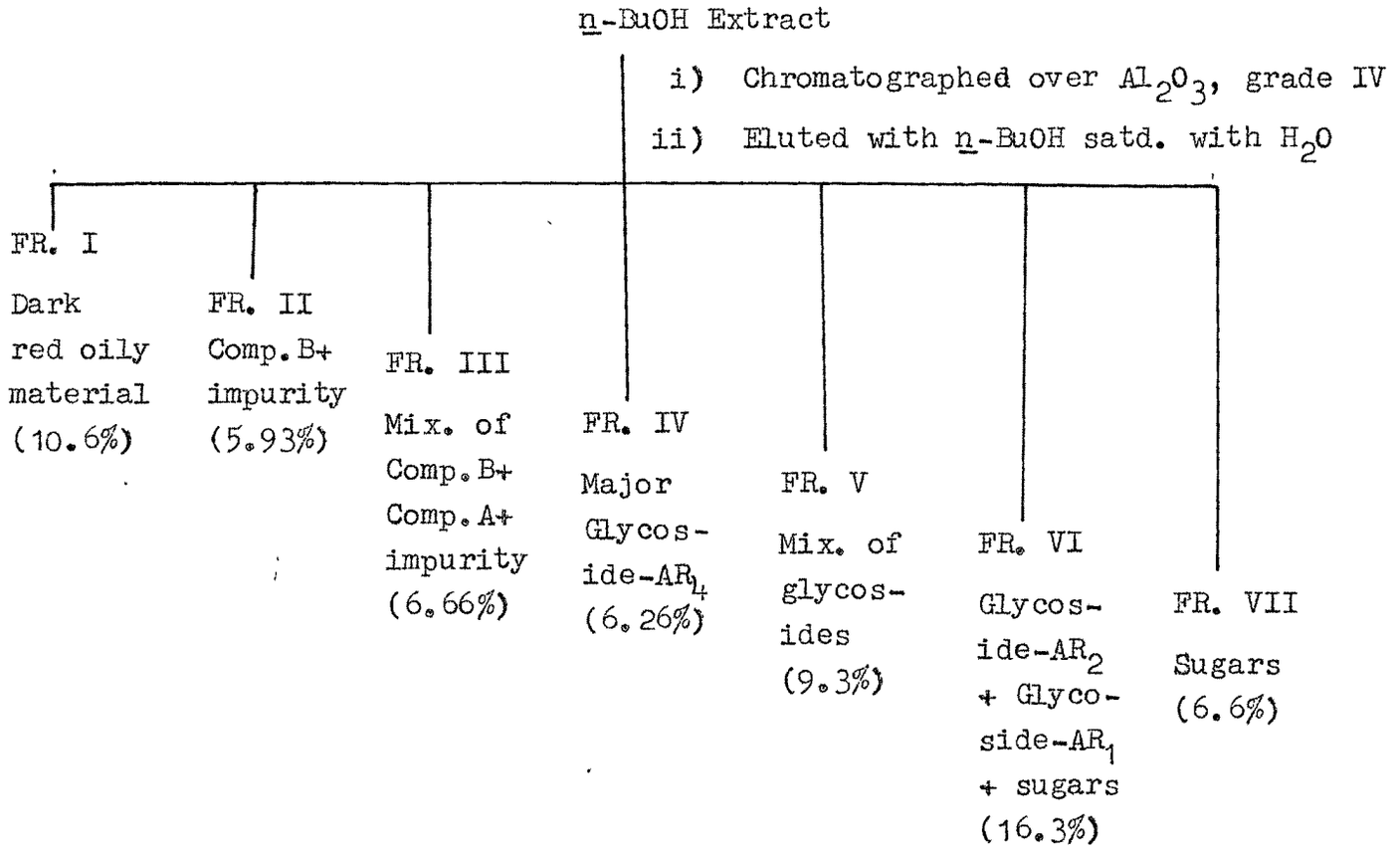
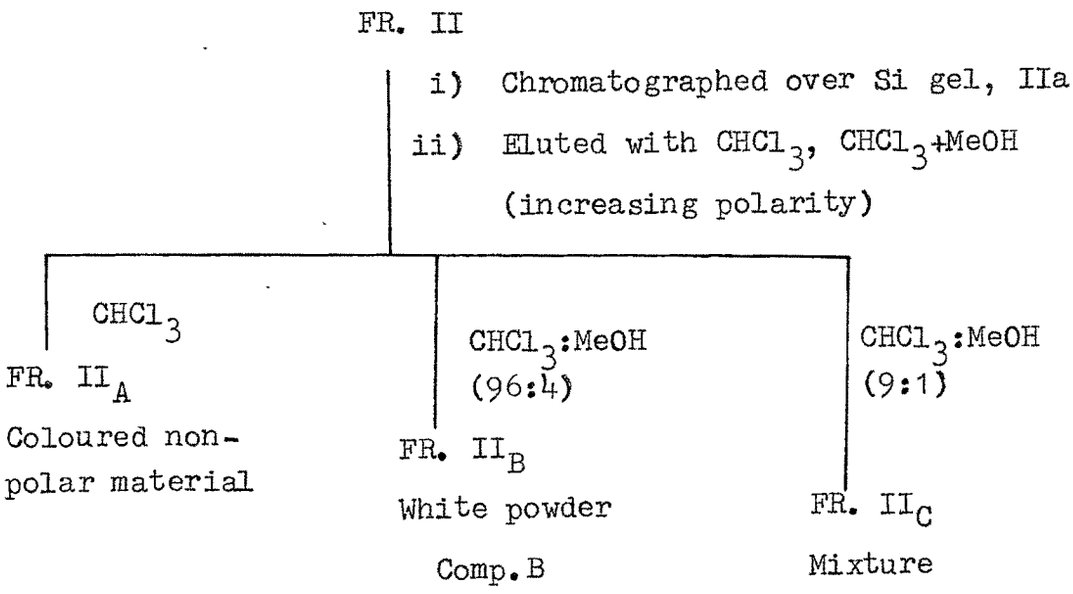


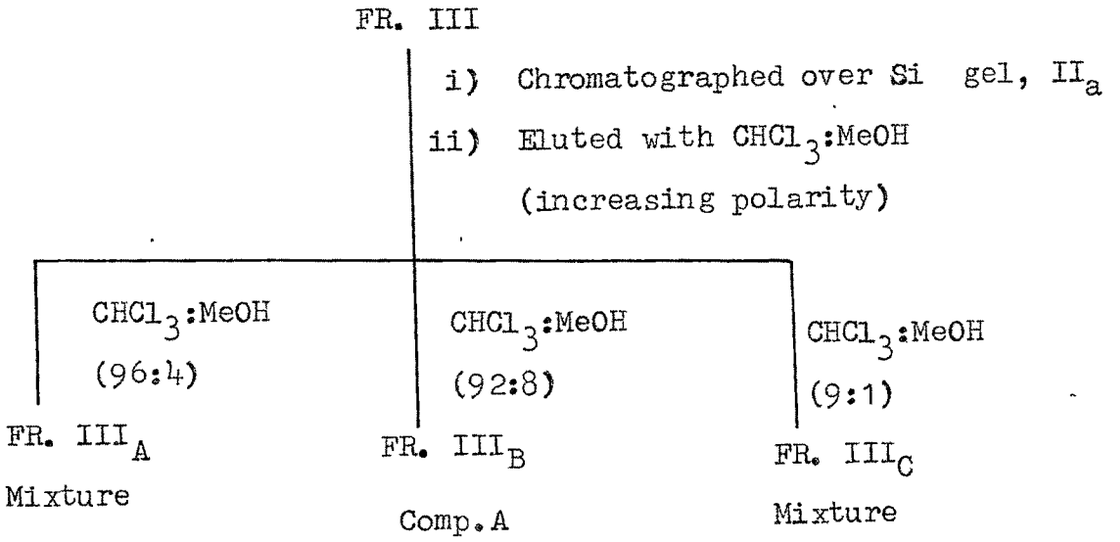
CHART-III : BROAD CUT CHROMATOGRAPHY OF n-BuOH EXTRACT



RECHROMATOGRAPHY OF FR. II (CHART-III)



RECHROMATOGRAPHY OF FR. III (CHART-III)



RECHROMATOGRAPHY OF FR. IV (CHART-III)

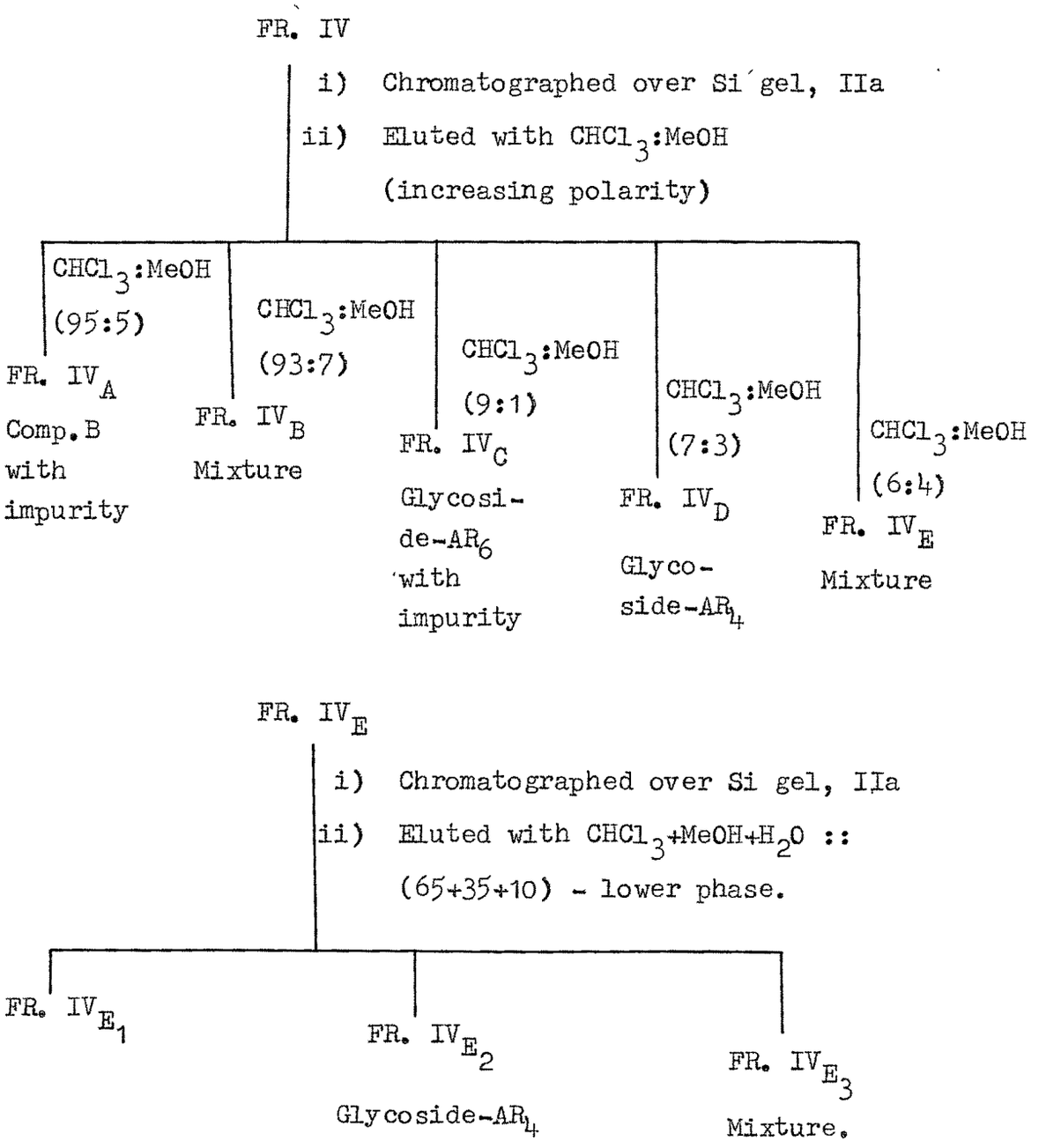


TABLE - 18 : PHYSICAL CHARACTERISTICS OF THE COMPOUNDS ISOLATED FROM THE SAPONIN MIXTURE OF A. RACEMOSUS

Sr. No.	Properties	Comp. B	Comp. A	Glycoside-AR_4
1.	m.p.	246-49°	151-3°	212-15°
2.	$[\alpha]_D^{27}$	-	- 140°	-
3.	TLC (R_f)	0.80	0.52	0.46
4.	Solubility in CH_3OH	Less soluble	Soluble	Less soluble
5.	Water of crystallization	-	-	-
6.	Molecular formula	-	$C_{10}H_{20}O_6$	-

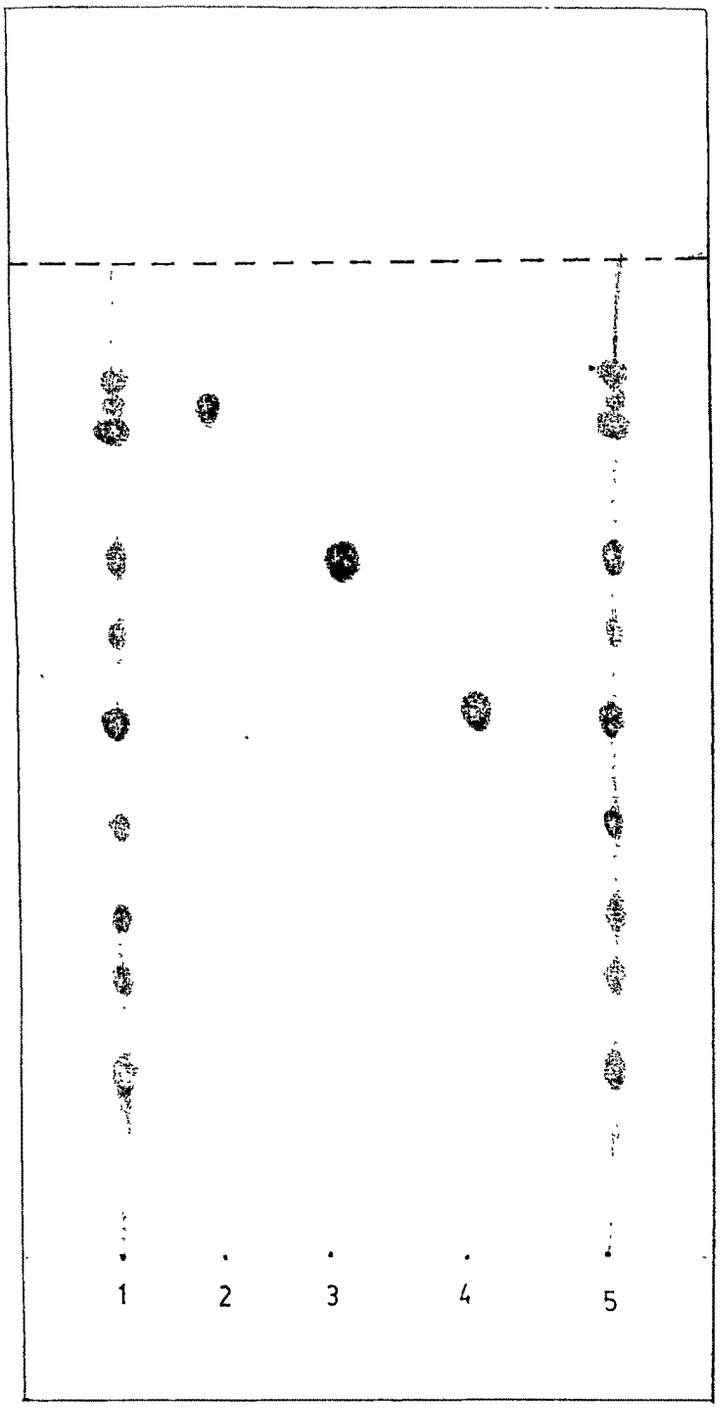


FIG. 20 : TLC OF PURE GLYCOSIDES ISOLATED FROM A. RACEMOSUS

SILICA GEL G PLATE

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

SPRAY REAGENT : 1% VANILLIN IN 50% PHOSPHORIC ACID

SPOTS : 1) TOTAL SAPONIN MIXTURE 2) COMP. B
3) COMP. A 4) GLYCOSIDE-AR_4
5) TOTAL SAPONIN MIXTURE

(with increasing polarity) as eluent. (Table-13 and 14). Pure comp.B. was crystallized from hot methanol as a white powder. (Fig.20). It was found to be identical with comp.B isolated from shatavari.

ISOLATION OF COMP.A :

FR. III (Chart-II, Table-12) which mainly contained comp.A and coloured material was subjected to rechromatography over silica gel using chloroform + methanol (with increasing polarity) for elution. (Table-15). It was then crystallized from methanol to get pure comp.A as needles. (Fig.20). It was found to be identical with comp.A isolated from shatavari.

ISOLATION OF GLYCOSIDE-AR_4:

FR. IV (Chart-II, Table-12) consisting mainly of glycoside-AR_4 and glycoside-AR_6 was subjected to rechromatography over silica gel using chloroform + methanol (with increasing polarity) as eluent. (Table-16). Glycoside-AR_6 was obtained in an impure form, as brown, foamy solid, m.p. 142-49° (decomposes). Glycoside-AR_4 which separated as a white powder in methanol solution was subjected to rechromatography over silica gel using chloroform + methanol + water :: 65 + 35 + 10 (lower phase) for elution. (Table-17). FR IV_{7B} contained pure

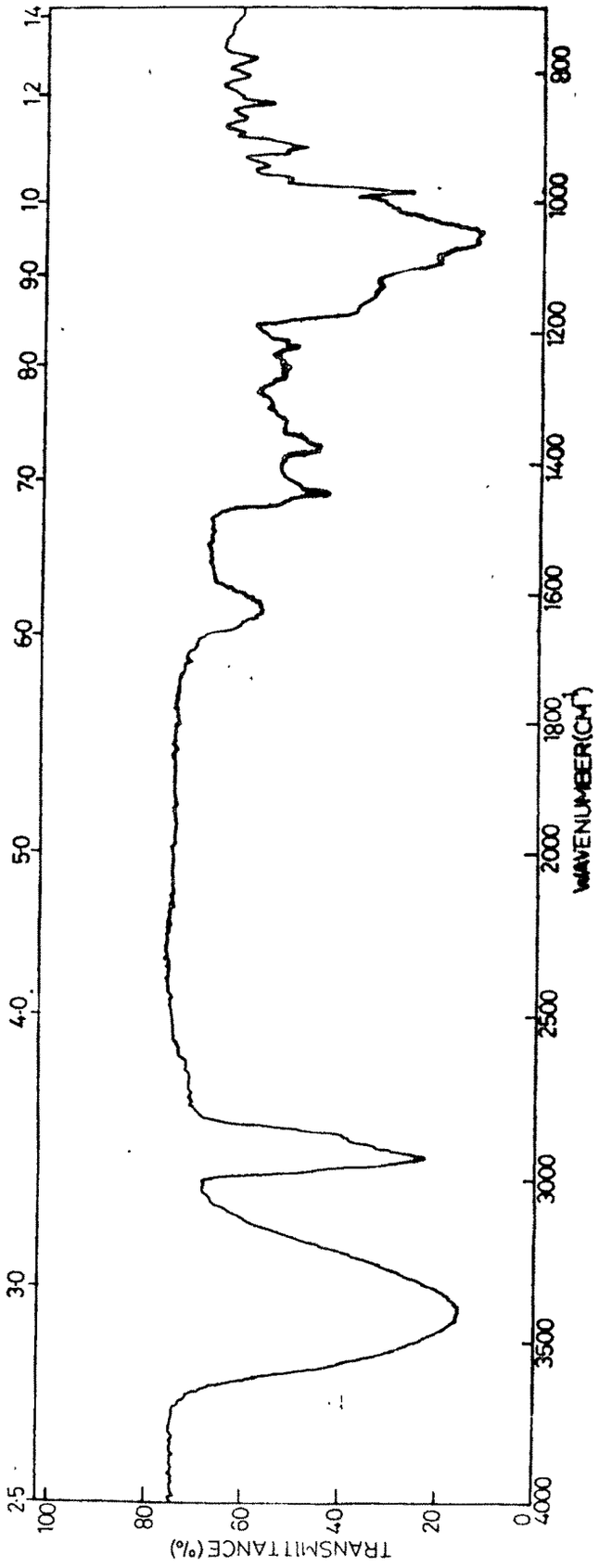


FIG. 21 : IR SPECTRUM OF GLYCOSIDE-AR_4

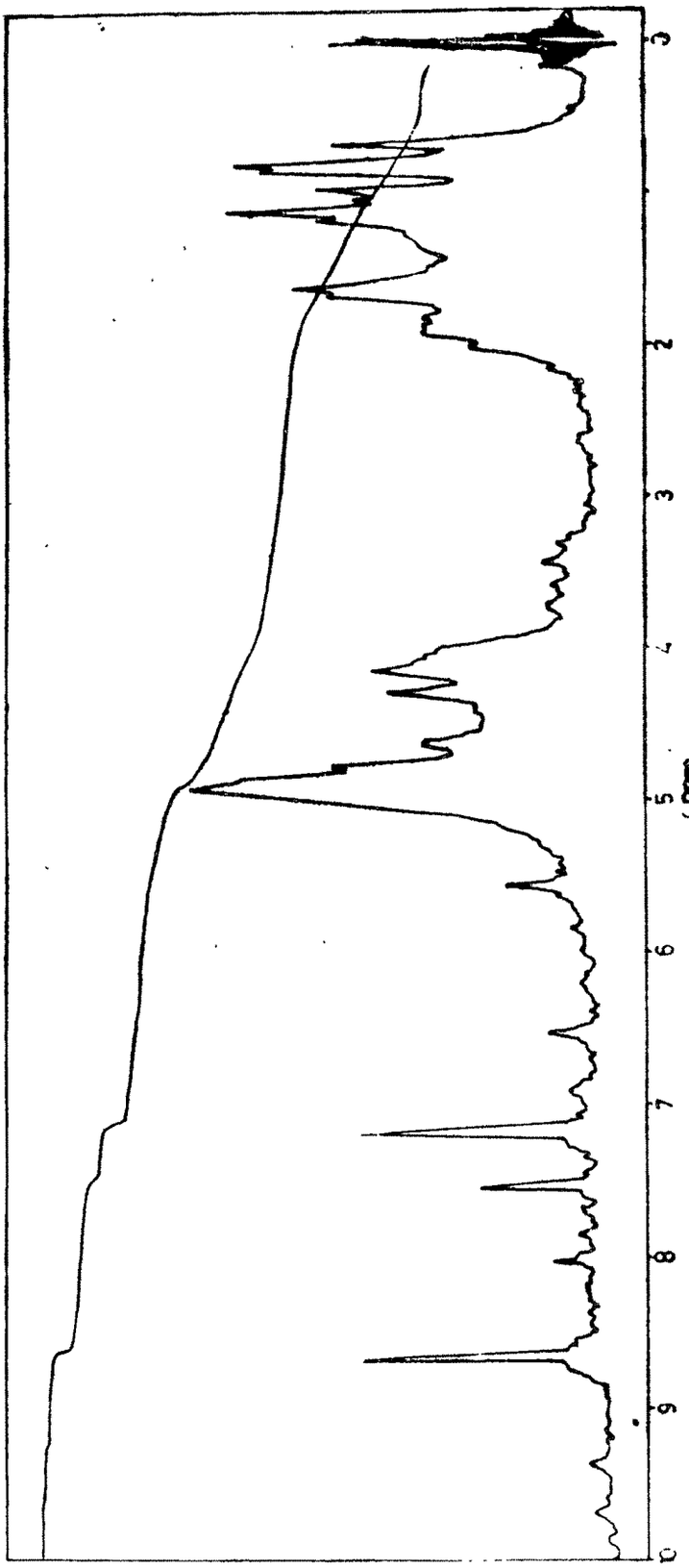


FIG. 22 : ^{1}H-NMR SPECTRUM OF GLYCOSIDE-AR_4

glycoside-AR_4, which showed a single spot on TLC. It was repeatedly recrystallized from hot methanol to get pure glycoside-AR_4 (Fig. 20), m.p. 212-15°, IR (KBr) and FABMS are shown in Figs 21 and 22 respectively. Structure of comp.A is discussed below. Structures of comp.B and glycoside-AR_4 are discussed in chapter-6.

STRUCTURE OF COMP.A

Isolation of comp.A is described above, structure elucidation is given below. The relevant evidence, as summarised under several headings is given below.

NATURE OF THE COMPOUND :

Comp.A was obtained as colourless needles from methanol. It is soluble in water, alcohol and hot ethyl acetate, but insoluble in other organic solvents such benzene, petroleum ether, ether and chloroform. It gave positive tests for sugars. It gave green colour in the anthrone test[10], it was +ve to Molisch's test[11], +ve to Keller-Killiani reaction[12], +ve to resorcinol test[13]. It developed red colour with Seliwanoff's reagent in 2 minutes, indicating that it is a ketohexose[14].

All the physical data, spetral data and chemical data were collected and the compound was analyzed as below.

HYDROLYSIS OF COMP.A :

Comp.A was hydrolysed by refluxing with sulfuric acid. After neutralisation and concentration, the aqueous part was spotted on paper and on silica gel plate. It showed only a single spot. When spotted alongwith standard samples, it corresponded to fructose. (Figs 23, 24). Formation of fructose on hydrolysis indicated that camp.A is a fructoside.

From the elemental analysis and ^{13}C NMR spectrum, the molecular formula was derived as $C_{10}H_{20}O_6$.

ANALYSIS OF IR SPECTRUM :

In the IR spectrum the region 900-1500 cm^{-1} is very crowded. It exhibits absorptions due to tetraphydropyran ring at 865 and 775 cm^{-1} [15,16] besides absorptions for OH, CH_3 and CH_2 suggesting that fructose is a pyranose and not a furanose.

According to Hudson's rule[17] for equioptical rotations, molecular optical rotation of glycosides is

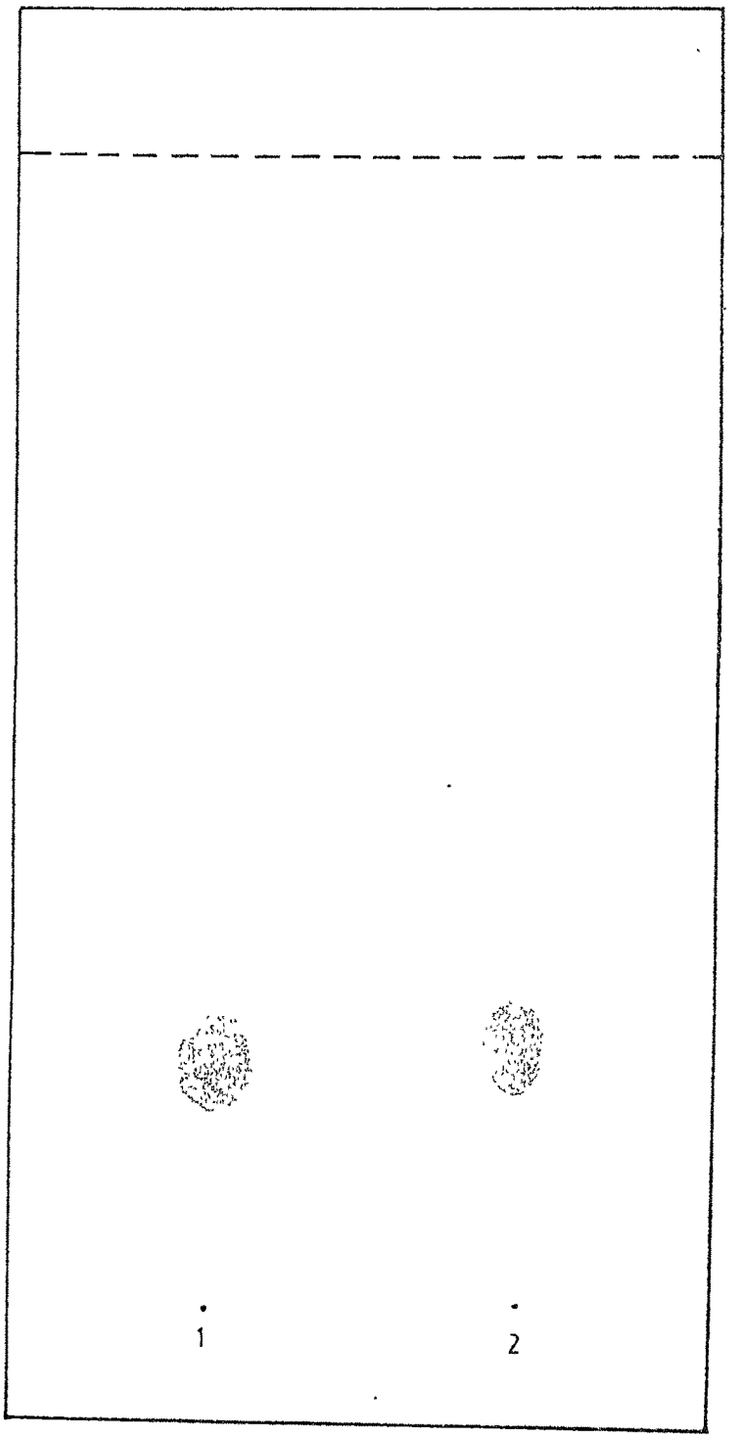


FIG. 23 : PAPER CHROMATOGRAM OF SUGAR OF COMP. A.

SOLVENT SYSTEM : n-BuOH-HOAc-H_2O (4:1:5) (UPPER PHASE)
SPRAY REAGENT : (i) SATURATED SOLUTION OF $AgNO_3$.
(ii) 5% ETHANOLIC NaOH SOLUTION.
(iii) 20% $Na_2S_2O_3$ SOLUTION.
TIME : 4 HOURS.
SPOTS : 1) FRUCTOSE. 2) SUGAR OF COMP. A.

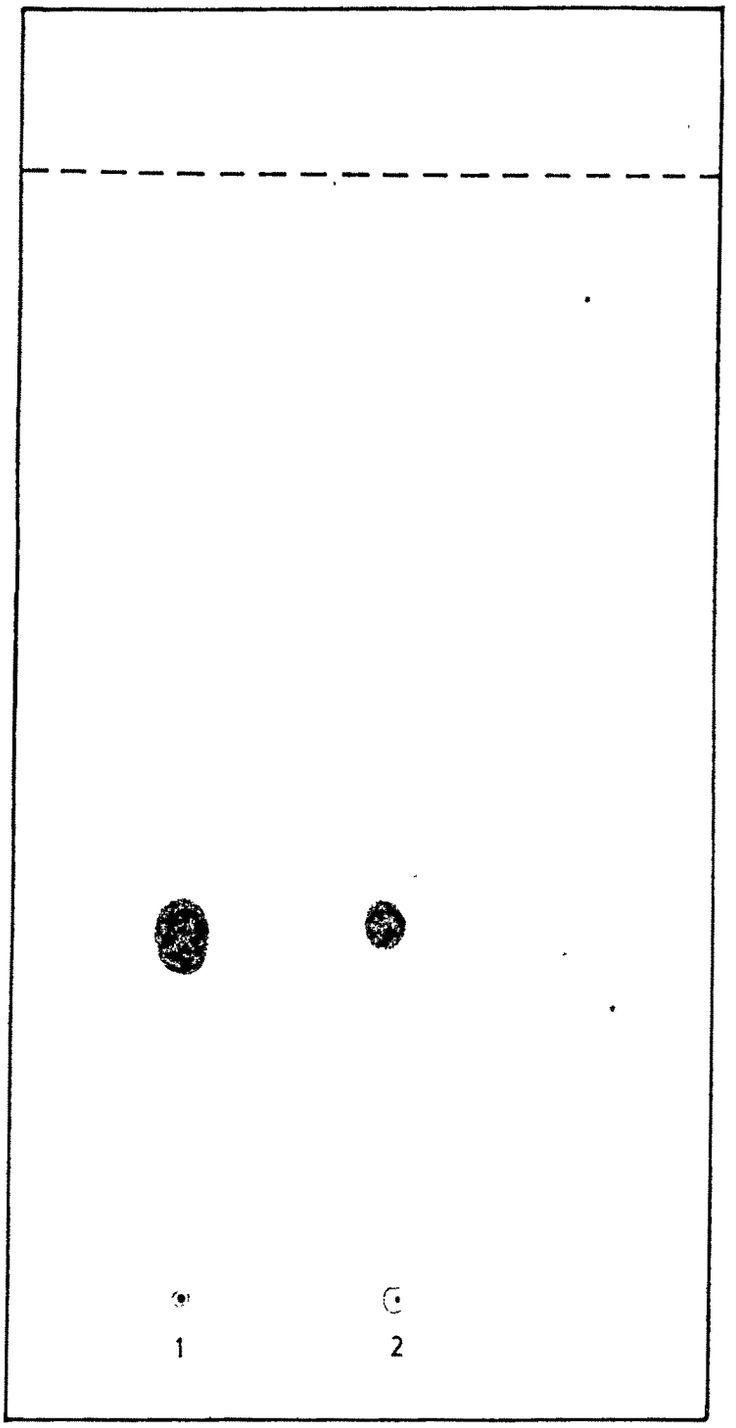


FIG. 24 : TLC OF SUGAR OF COMP. A.

SOLVENT SYSTEM : n-BuOH-HOAc-Et_2O-H_2O (9:6:3:1)
SPRAY REAGENT : THYMOL-SULFURIC ACID.
SPOTS : 1) FRUCTOSE.
2) SUGAR OF COMP. A.

less affected by the substituents, compared to their anomers. The optical rotation of comp.A, $[\alpha]_D^{27}$ - 140° is close to that of methyl-β-D-fructopyranoside[18] of -173° (α-type, +80°).

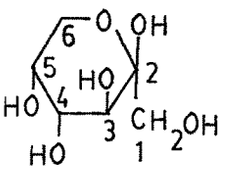
ANALYSIS OF ^{13}C NMR :

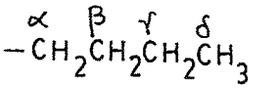
In the ^{13}C NMR spectrum, 10 singlets are obtained, indicating the presence of 10 carbon atoms in the compound. 6 carbon atoms account for fructose and the remaining 4 carbon atoms form the aglycone. From the data given below, it was clear that the aglycone must be from $CH_3CH_2CH_2CH_2OH$. i.e. The compound may be n-Butyl-β-D-fructopyranoside.

No.	δ ppm	No.	δ ppm
1.	101.17	6.	62.12
2.	70.41	7.	61.53
3.	69.89	8.	32.01
4.	69.14	9.	19.47
5.	64.53	10.	13.88

From the comparison of the spectral data with ^{13}C NMR data of β-D-fructopyranoside[19-21], the signals 1, 2, 3, 4, 5 and 7 correspond to C-2, C-4, C-5, C-3,

C-6 and C-1 of fructose respectively. The signals 6, 8, 9, 10 correspond to α, β, γ and δ carbon atoms of n-butyl group[22].





The values for the fructose residue are well in accord with the values for β-pyranose forms and do not agree with the furanose form.

ANALYSIS OF MASS SPECTRUM :

In the mass spectra it is known that, when fructose takes up a furanose type conformation, a stable fragment ion of furan structure is formed by liberation of substituent at C-2, followed by deavage between C-5 and C-6[23], whereas if it is a pyranose type only the liberation of substituent at C-2 occures and a stable fragment ion pyran appears[24]. In the case of pentacetate of β-D-fructopyranose, m/e 331 for $M^+ - OCOCH_3$ is observed .

Formation of important ions in the mass spectrum of comp.A are explained in Figs. 25-28. No molecular ion peak is obtained.

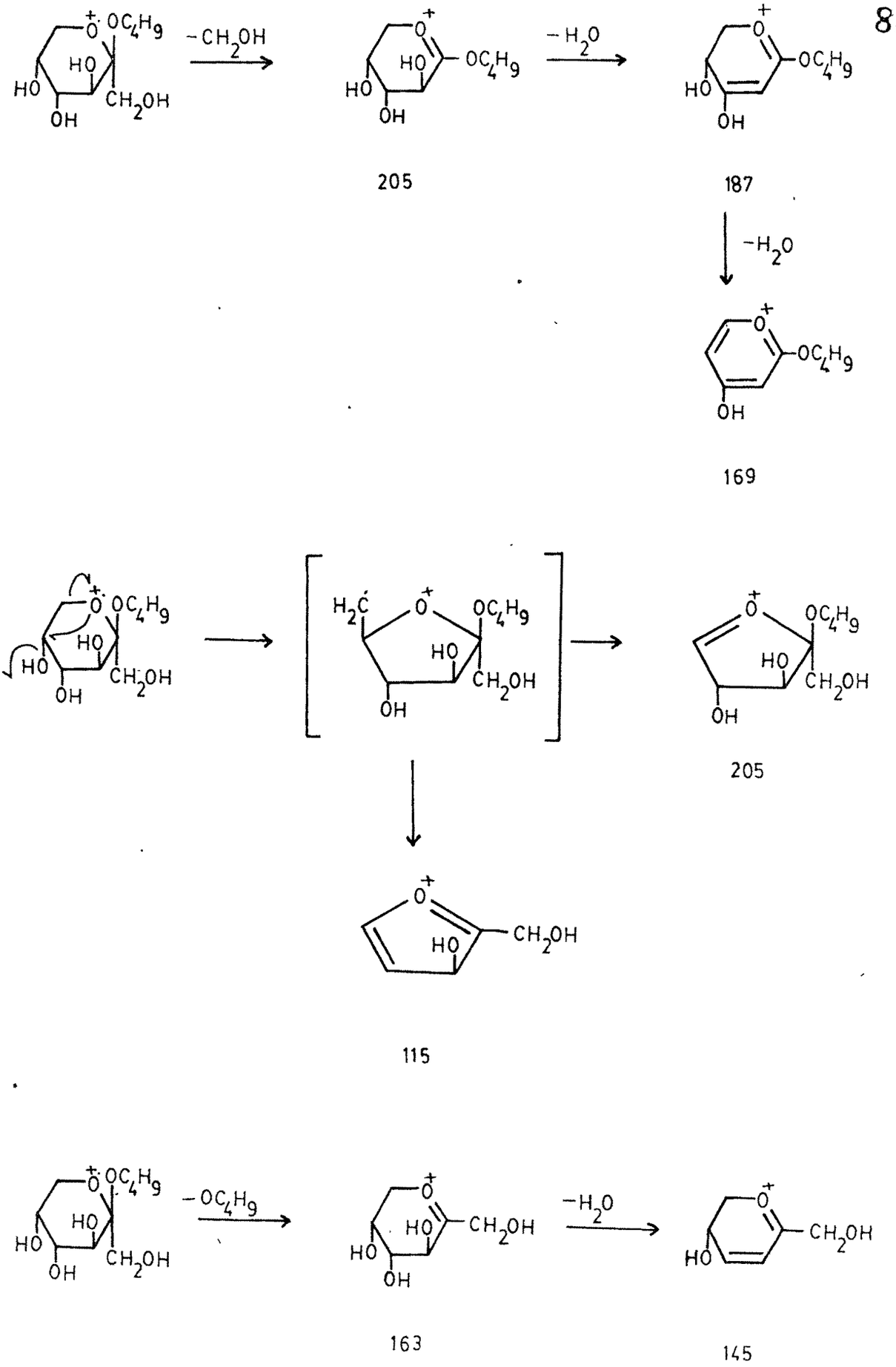


FIG. 25 : FORMATION OF IONS IN THE MASS SPECTRUM OF COMP. A.

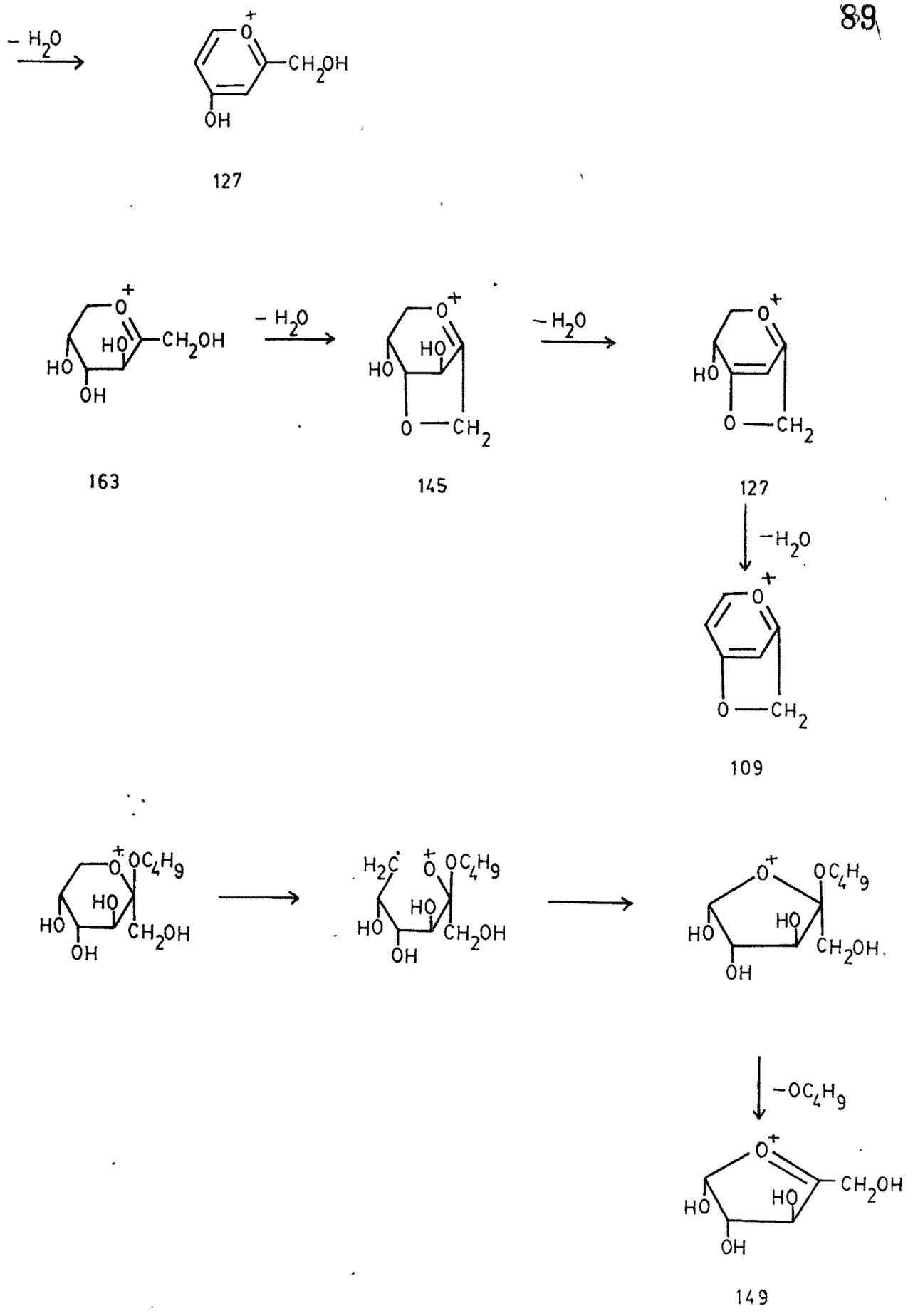
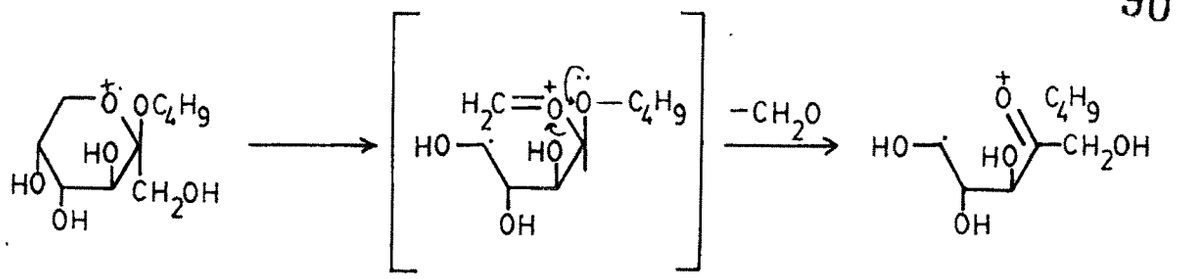


FIG. 26 : FORMATION OF IONS IN THE MASS SPECTRUM OF COMP. A.



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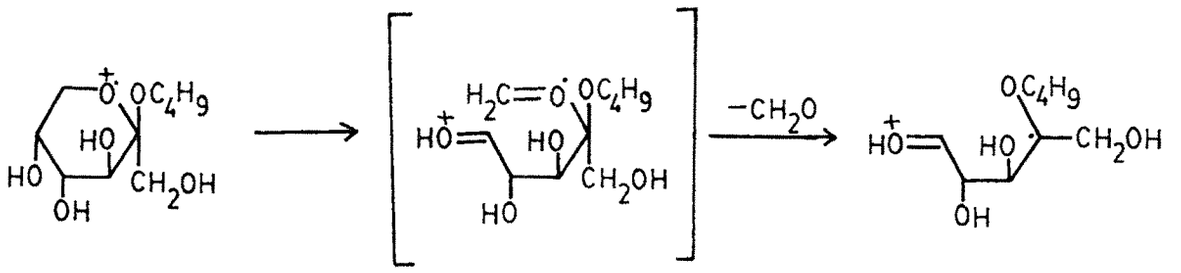

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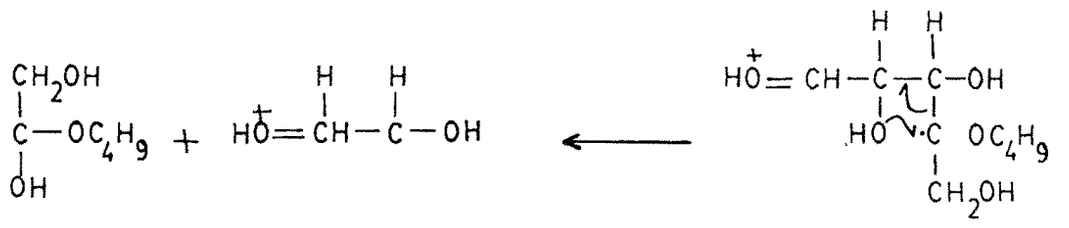

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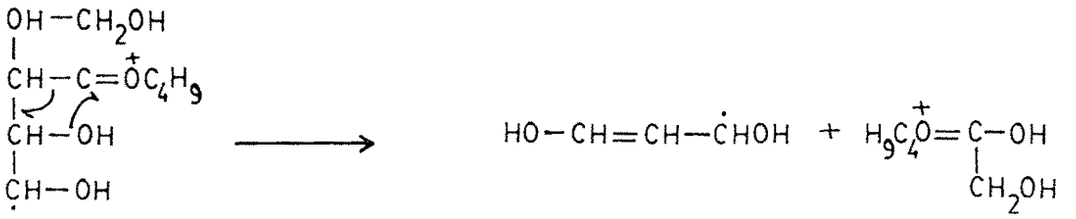

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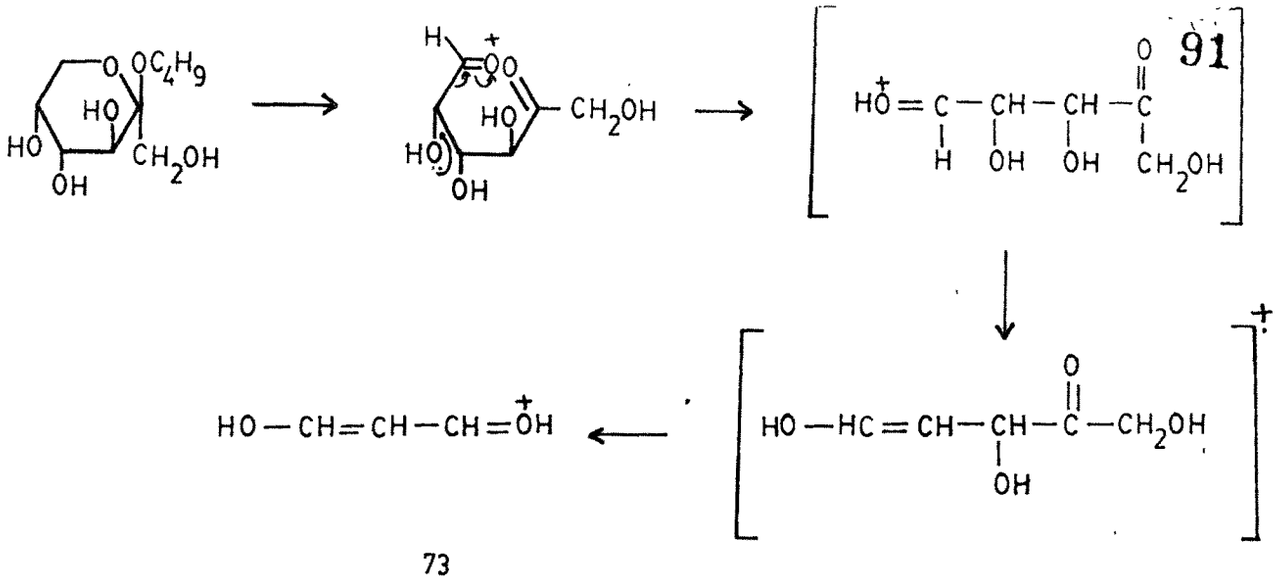
FIG. 27 : FORMATION OF IONS IN THE MASS SPECTRUM OF COMP. A.



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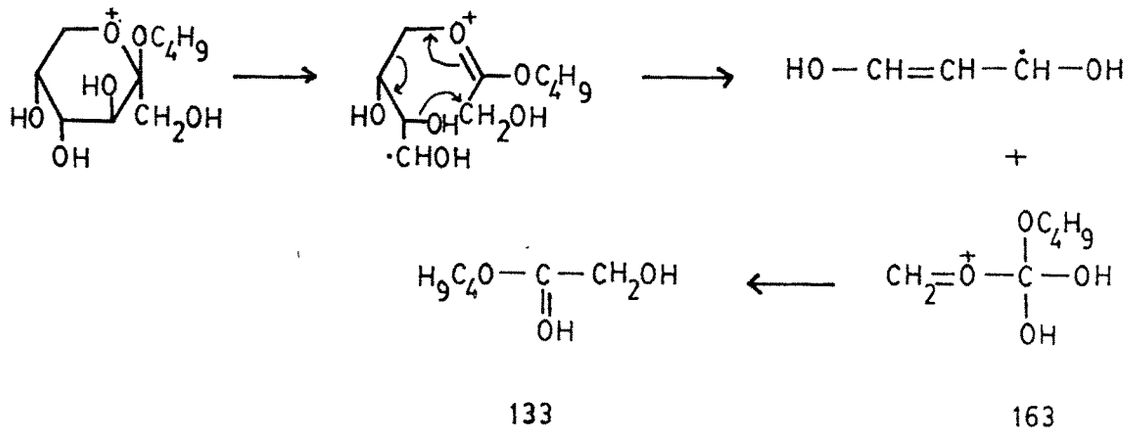

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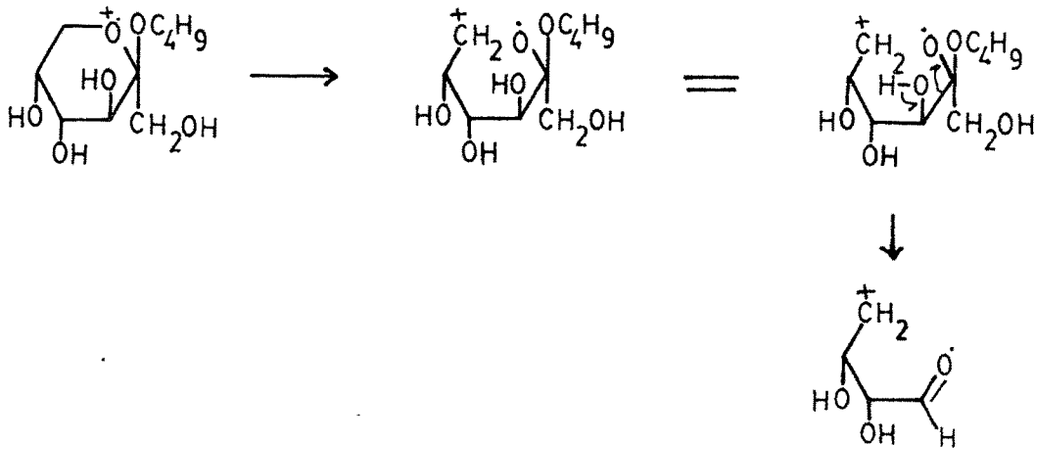

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FIG.28 : FORMATION OF IONS IN THE MASS SPECTRUM OF COMP. A.

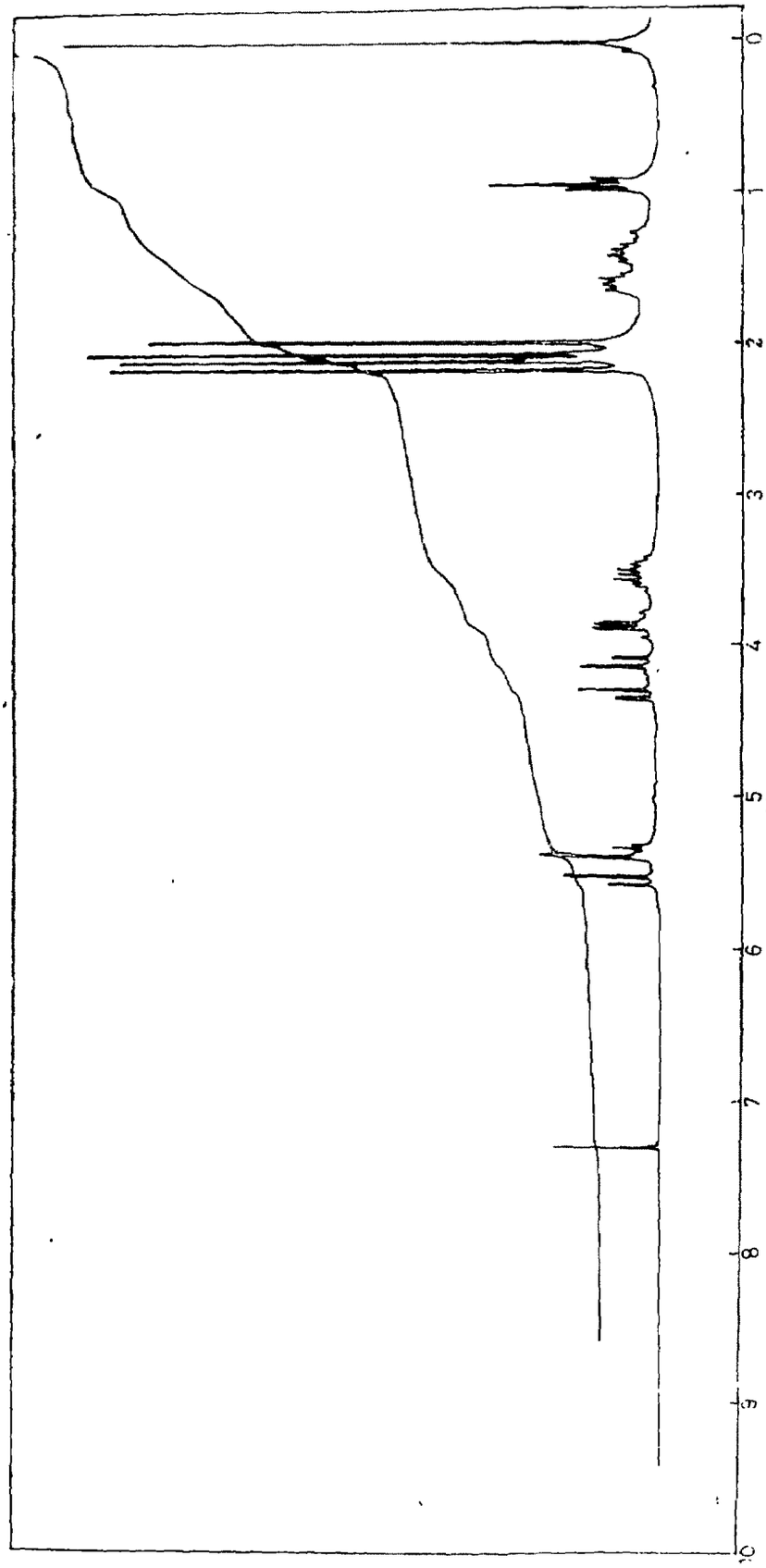


FIG. 29 : ^{1}H-NMR SPECTRUM OF TETRAACETATE OF COMP. A

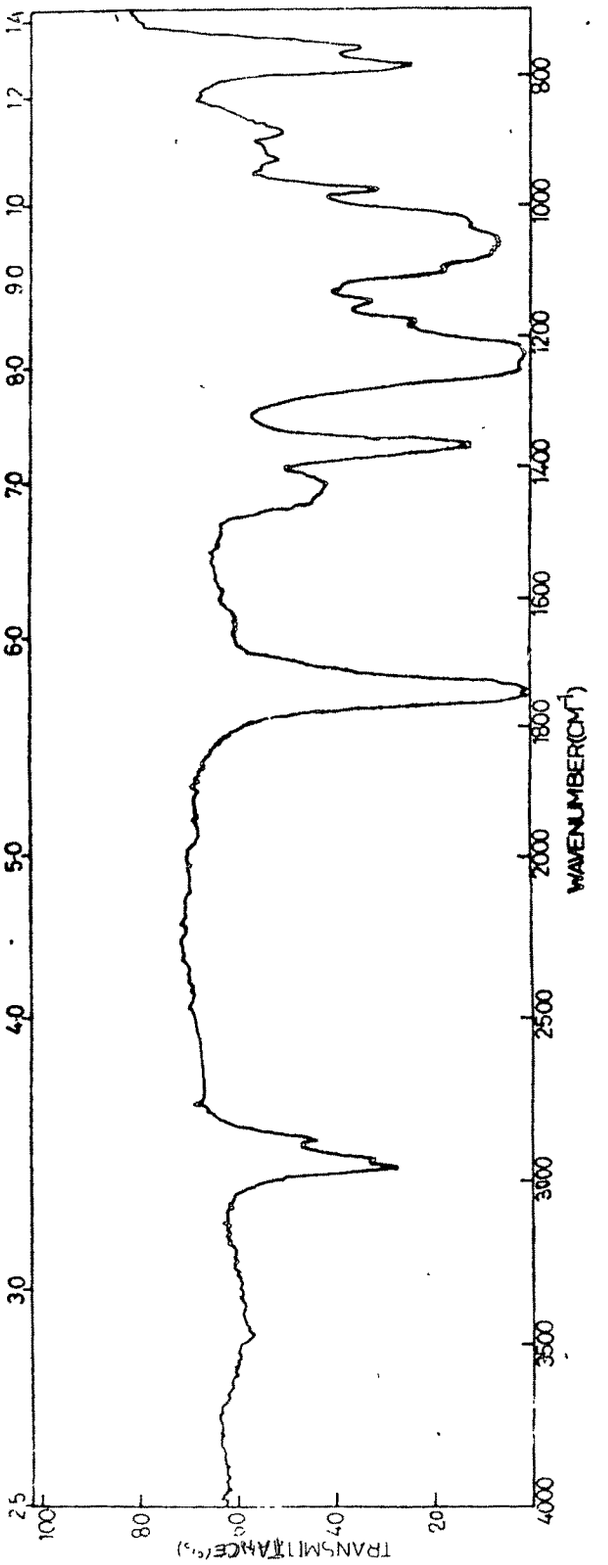


FIG. 30 : IR SPECTRUM OF TETRAACETATE OF COMP. A

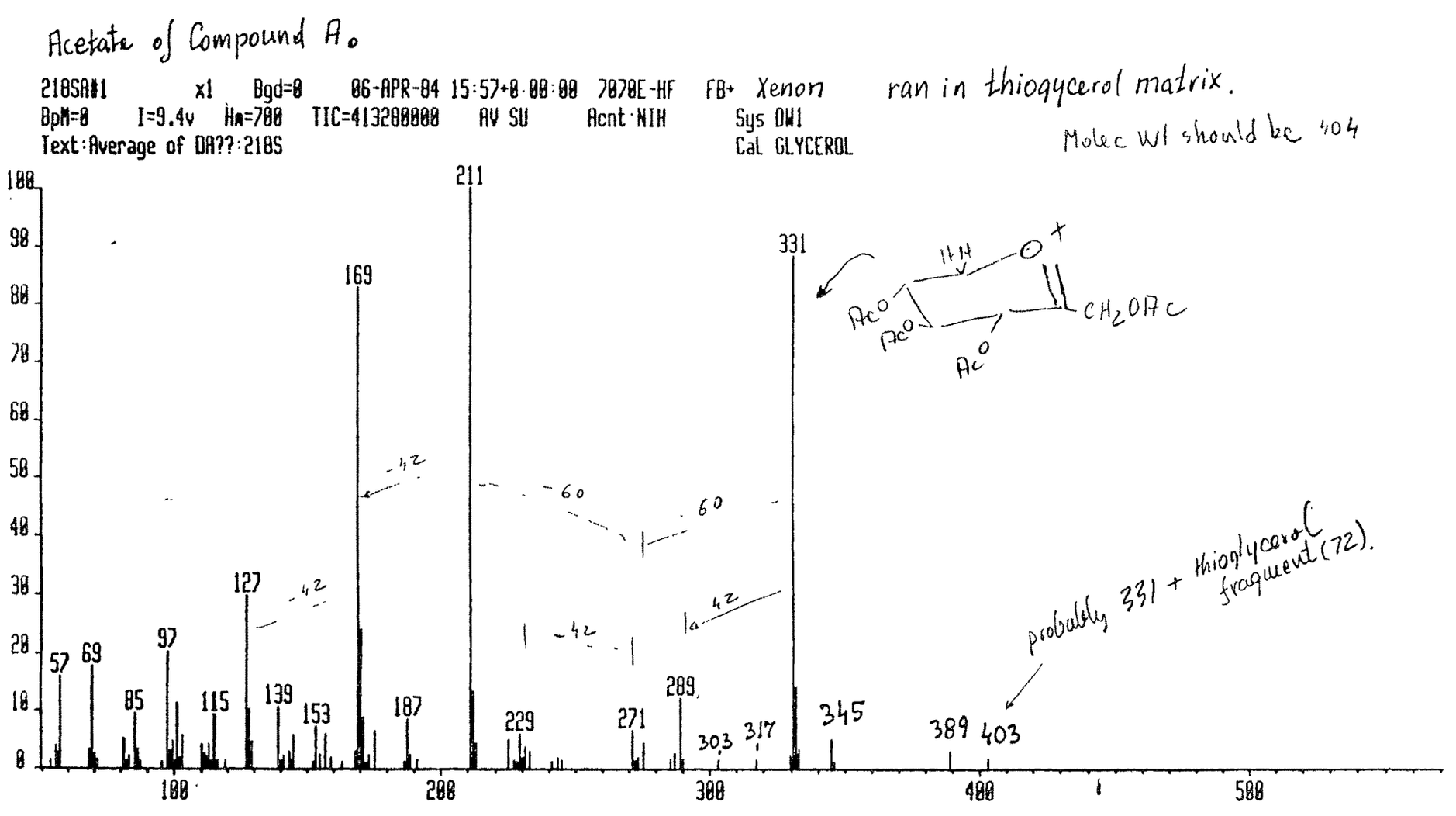


FIG.31 : FAB^{+}_{MS} OF TETRAACETATE OF COMP. A.

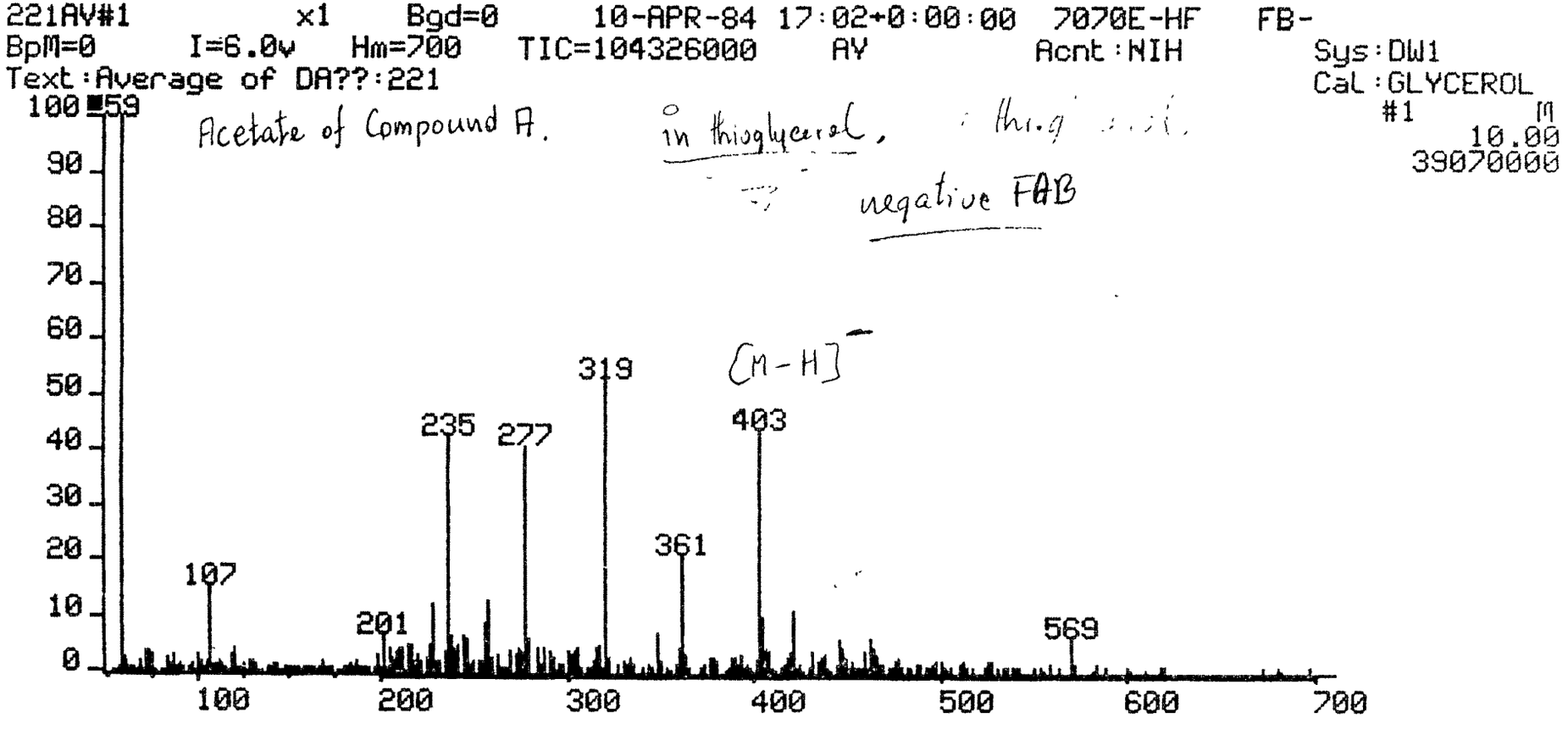


FIG.32 : FAB⁻MS OF TETRAACETATE OF COMP.A.

Fast atom bombardment mass spectra (FABMS)[25] of peracetate of comp.A were recorded in thioglycerol. In +ve FAB, no pseudomolecular ion at $[M + H]^+$ or molecular ion at $[M]^+$ was observed. However a strong peak was observed at m/e 331, formed by the loss of butyl group from the molecule. Formation of important ions is explained in Fig.23. In the -ve FABMS however, a strong $[M-H]^-$ peak is observed at m/e 403. Thus confirming the molecular weight of peracetate of comp.A to be 404.

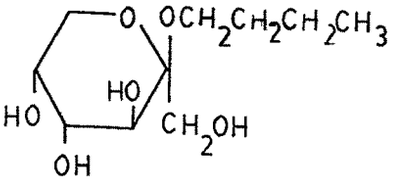
Comp.A is similar to n-Butyl-β-D-fructopyranoside, which was earlier isolated by Shin Matsuura[26] and Munekazu Inuma, from the Calix of Diospyrose kaki.

COMPARISON OF COMP.A AND n-BUTYL-β-D-FRUCTOPYRANOSIDE

	COMP.A	n-BUTYL- -D-FRUCTOPYRANOSIDE
1)	m.p. 151-3°	m.p. 149-51°
2)	$[\alpha]_D^{27}$ -140° (CH_3OH; C, 0.9).	$[\alpha]_D^{25}$ -138° (CH_3OH).
3)	Found: C, 50.68%; H, 8.34%.	Found: C, 50.83%; H, 8.53%.
4)	Mass: m/e 206, 205, 163, 149, 145, 133, 127, 115, 103, 77.	Mass: m/e 236 M^+, 206, 205, 149, 103, 77.
5)	IR: ν_{max}^{KBr} cm^{-1} 3280-3400, 2910, 2880, 1430, 1110, 950, 900.	IR: ν_{max}^{KBr} cm^{-1} 3400, 2950, 1400, 1120, 1060, 912, 890, 865, 780, 665.
6)	NMR (D_2O): δ1.15 (3H, $-CH_2-C\underline{H}_3$), 1.65 (4H, br, $-C\underline{H}_2-C\underline{H}_2$), 3.7 (2H, t, $-O.C\underline{H}_2$), 3.95-4.1 (7H) ppm.	NMR (CD_3OD): δ0.95 (3H, t, $-CH_2-C\underline{H}_3$), 1.51 (4H, br, $-C\underline{H}_2-C\underline{H}_2$), 3.55 (2H, t, $-O-C\underline{H}_2$), 3.35-3.92 (7H) ppm.
7)	^{13}C-NMR (D_2O): δ 13.88, 19.47, 32.07, 61.53, 62.12, 64.53, 69.14, 69.89, 70.41, 101.17 ppm.	^{13}C-NMR ($(CD_3)_2SO$): δ 13.4, 18.9, 31.8, 59.4, 62.1, 63.7, 68.9, 69.3, 100 ppm.

TETRAACETATE OF COMP.A	TETRAACETATE
8) Mass: m/e 331, 289, 271, 229, 211, 187, 169, 127 - (FAB^+). m/e - 403 - $[M-H]^{(-)}$ - (FAB^-).	8) Mass: m/e 404 M^+, 331, 275, 233, 211, 170, 149, 126, 109.
9) NMR ($CDCl_3$): δ 0.96 (3H, t, $-CH_2\underline{CH_3}$), 1.26 and 1.54 (4H, br, $-\underline{CH_2}-\underline{CH_2}$), 1.95-2.15 (12H, each S, 4 x $COC\underline{H_3}$), 3.5 (2H, $-O-\underline{CH_2}-CH_2$), 3.8 (2H, (C-1)-$\underline{CH_2}$), 4.05-4.18 (2H, (C-6)-$\underline{H}$) and 5.28 (3H, (C-3,4,5)-$\underline{H}$).	9) NMR ($CDCl_3$): δ 0.96 (3H, t, $-CH_2-\underline{CH_3}$), 1.38 (4H, br, $-\underline{CH_2}-\underline{CH_2}$), 1.99-2.18 (12H, each S, 4 x $COC\underline{H_3}$), 3.57 (2H, $-O-\underline{CH_2}-CH_2$), 3.89 (2H, (C-1)-$\underline{CH_2}$), 4.22 (2H, (C-6)-$\underline{H}$), 5.40 (3H, (C-3,4,5)-$\underline{H}$).

Thus, structure of comp.A is assigned as n-Butyl-β-D-fructopyranoside.



Since this type of compounds do not occur naturally, it was assumed that this compound may be an artefact of the extraction with n-butyl alcohol.

For confirmation, the total ethanol extract was subjected to broad cut chromatography over silica gel using chloroform + methanol + water :: 65 + 35 + 10 (lower phase) for elution. No fraction containing comp. A was obtained form it. This made it clear that Comp. A is an artefact of extraction with butanol, and is not present in the original saponin mixture.

E X P E R I M E N T A L

All melting points are uncorrected. All the melting points were recorded in melting point apparatus. Optical rotations were recorded in Schmidt-Haenshc polarimeter. The IR spectra were recorded on a Perkin Elmer Infra cord model-55. The ^{1}H-NMR spectra were recorded on Perkin Elmer R-32 model NMR spectrometer. The positions were determined with tetramethylsilane (TMS) as internal standard. The signal positions were recorded in parts per million (ppm) units starting from TMS as zero. The FABMS were recorded using VG Micromass 7070E-HF mass spectrometer. Xenon (8 KeV) was used to bombard the sample dissolved in glycerol.

Alumina used for chromatography was the commercial basic alumina which was washed with 10% nitric acid at 90°, followed by washing with water till neutral. It was activated at 450° and various grades were prepared and standardized according to Brockmann procedure[27]. Thin layer chromatography was carried out using silica gel G (230 mesh) with 13% $CaSO_4$. Silica gel used for column chromatography was standardized according to the procedure of Hernandez et al.[28] Visualisation of the spots was done

by spraying either with vanillin/phosphoric acid reagent[29] followed by heating the plate at 120° for 10 mins., or with Ehrlich's reagent[9]. All the chromatographies were monitored by TLC.

TLC solvent system[30] used for sugars was n-butanol-acetic acid-ether-water :: 9-6-3-1 - time period 4 hrs. Visualization of the spots was done by spraying with thymol-sulfuric acid reagent[31], followed by heating the plate at 120° for 4 hrs. Paper chromatography was carried out by ascending technique on Whatman No. 1 filter paper using n-butanol-acetic acid-water :: 4-1-5 (Upper phase)[32] - time period - 5 hrs. Visualization of the spots was done by spraying either with saturated solution of silver nitrate[33], followed by 5% ethanolic NaOH, followed by washing with 20% $Na_2S_2O_3$ solution, or by spraying with aniline hydrogen phthalate[34].

FROM COMMERCIAL SHATAVARI

The butanol extract was subjected to broad cut chromatography over alumina grade-IV.

TABLE-3 : CHROMATOGRAM

Material : 60 g. adsorbed on 120 g. alumina.

Adsorbent : 1.2 kg. Al_2O_3-IV.

Column dimension : 6.25 cm x 46 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr (gms)	Remarks
I	n-BuOH saturated with water	150ml x 3	2.00	Coloured, non-polar material.
II	- ,, -	150ml x 7	4.9286	Red sticky material, contains comp.B and comp.A.
III	- " -	150ml x 8	3.1376	Containing mixture of comp.A, and shatavarin-IV.
IV	- " -	200ml x 3	4.7700	Major shatavarin-IV.
V	- " -	200ml x 6	6.2400	Mixture of shatavarin-IV (little), shatavarin-III, shatavarin-II and shatavarin-I (little).

...conted.

TABLE-3 (Contd.)

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
VI	n-BuOH saturated with water	200ml x 20	21.5100	Contains major shatavarin-I with shatavarin-II (little) and shatavarin-V and shatavarin-VII.
VII	- " -	200ml x 6	4.0000	Sugars.
		Total...	46.5862 (77.6%)	

Comp.B :

Comp.B was deposited in the methanol solution of fractions containing comp.B. On filteration, it was obtained as white powder which was insoluble in all organic solvents and water. It was crystallized from hot methanol+ethanol (1:1). (Fig.5). m.p.271-4°. IR, FAB^+ recorded.

Comp.A :

FR. III (Table-3) was subjected to chromatography over silica gel.

TABLE-4 : CHROMATOGRAM

Material : 9 g. adsorbed on 20 g. silica gel

Adsorbent : 200 g. silica gel, IIa.

Column dimension : 4 cm x 38 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$	100ml x 5	-	-
2.	$CHCl_3$+MeOH :: 95+5	100ml x 5	0.2000	Contains comp.B
3.	$CHCl_3$+MeOH :: 90+10	100ml x 6	1.2850	Comp.A with coloured impurity and a small amount of upper impurity.
4.	$CHCl_3$+MeOH :: 85+15	100ml x 12	2.8647	White powder single spot, comp.A.
5.	$CHCl_3$+MeOH :: 85+15	100ml x 9	0.4350	Mixture.
6.	$CHCl_3$+MeOH :: 80+20	100ml x 5	0.8046	Mixture.
7.	$CHCl_3$+MeOH :: 80+20	100ml x 11	2.0552	Slightly impure shatavarin-IV.
8.	$CHCl_3$+MeOH :: 75+25	100ml x 4	0.5000	Mixture.
9.	MeOH	100ml x 4	0.5000	Mixture.
		Total...	8.6395 (95%)	

FR. 4 was crystallized from methanol to get pure comp.A (needles). (Fig.5), m.p. 151-3°, $[\alpha]_D^{27}$ -140° (CH_3OH; C, 0.9), Found: C, 50.68%; H, 8.32%). The compound analyzed for $C_{10}H_{20}O_6$. Calculated: C, 50.8%; H, 8.4% . IR (KBr), ^{1}H-NMR (D_2O), ^{13}C-NMR (D_2O) and mass spectra are recorded.

Shatavarin-IV :

FR. 7 (Table-4) was combined with FR. IV (Table-3). The combined fractions were tried to dissolve in minimum quantity of methanol (hot). Insoluble shatavarin-IV was filtered out and dissolved in excess of methanol. The solution was concentrated to 60%. It was cooled to room temp. and then to 0°. Shatavarin-IV deposited as brownish white powder (single spot) which was filtered out and washed with cold methanol. The filterate was mixed with the mother liquor which was concentrated and cooled to get more shatavarin-IV. After filteration, the solvent was removed from the filterate, the brown foamy solid which was obtained as the residue subjected to chromatography.

TABLE-5 : CHROMATOGRAM

Material : 4.5 g. adsorbed on 13 g. silica gel.

Adsorbent : 135 g. silica gel, activated at 120° for 8 hrs.

Column dimension : 2.3 cm x 77.5 cm.

Fr. No.	Eluent	Vol. of Fr.	Wt. of Fr. (gms)	Remarks
1.	$CHCl_3$+MeOH+H_2O :: 65+35+10 - lower phase	25 ml x 10	0.0152	-
2.	- " -	25 ml x 5	0.3144	Coloured material.
3.	- " -	25 ml x 2	0.4550	Major comp.A + impurity (lower).
4.	- "-	25 ml x 2	0.4363	Mixture of comp.A + shatavarin-IV.
5.	- " -	25 ml x 6	1.6596	Shatavarin-IV.
6.	- " -	25 ml x 4	0.2000	Mixture.
		Total...	3.0805 (69%)	

Shatavarin-IV was dissolved in minimum quantity of hot methanol, boiled, filtered and cooled to room temp. and then to 0°. The crystals were filtered and washed with cold

methanol. After four crystallization (constant melting point), shatavarin-IV was obtained as white crystalline powder. (Fig.5). m.p. 274-8°, $[\alpha]_D^{27}$ -67° (C_5H_5N; C, 1), Found: C, 60.09%; H, 8.4%. Analyzed for $C_{45}H_{74}O_{17}$ (Calculated : C, 60.95%, H, 8.35%).

Shatavarin-I :

11.84 g. of FR. VI (Table-3) was dissolved in 20 ml dry methanol (refluxed and distilled over magnesium methoxide) and 100 ml dry acetone (distilled and dried over anhydrous K_2CO_3) was added to it. The precipitates obtained were filtered and washed with dry acetone. The two filterates were combined and solvent was removed. The residue was dried under suction. It was redissolved in dry methanol (20 ml) and dry acetone (100 ml) was added to it. The precipitates were filtered and washed with dry acetone. The combined precipitates were subjected to chromatography over silica gel. Solvent was removed from the combined filterates to get brown foamy powder.

TABLE- 6 : CHROMATOGRAM

Material : 10 g. adsorbed on 25 g. silica gel.

Adsorbent : 500 g. silica gel.

Column dimension : 4.5 cm 75 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$+MeOH+H_2O :: 65+35+10 - lower phase.	100ml x 8	-	-
2.	- " -	100ml x 4	0.1287	Shatavarin-II with upper impurity.
3.	- " -	100 ml x 2	0.3384	Shatavarin-II almost pure(90%)
4.	- " -	100ml x 2	0.5000	Mixture of shatavarin-II and shatavarin-I.
5.	- " -	100ml x 8	6.7370	Major shatavarin-I with little impurity of shatavarin-V.
6.	- " -	100ml x 4	0.6600	Mixture of shatavarin-I and shatavarin-V.
7.	- " -	100ml x 5	0.4861	Major shatavarin-V.
8.	- " -	100ml x 5	0.5000	Mixture.
		Total...	9.3502 (93%)	

FR. 5 (Table-6) was subjected to rechromatography over silica gel.

TABLE-7 : CHROMATOGRAM

Material : 3.5 g. adsorbed on 7 g. silica gel.

Adsorbent : 700 g. silica gel.

Column dimension : 4.5 cm x 95 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$+MeOH+H_2O :: 65+35+10 - lower phase.	100ml x 18	0.1017	-
2.	- " -	100ml x 2	0.3187	Major shatavarin-II.
3.	- " -	100ml x 8	1.6662	Shatavarin-I.
4.	- " -	100ml x 6	0.6000	Mixture of shatavarin-I and shatavarin-V.
5.	- " -	100ml x 2	0.1561	Major shatavarin-V.
6.	- " -	100ml x 8	0.3500	Mixture.
		Total...	3.1981 (91%)	

FR. 3 (Table-7) contained pure shatavarin-I (yellow foamy powder). (Fig.5) m.p. 179-86°, $[\alpha]_D^{27}$ - 35°,

Found: C, 53.74%; H, 8.604%. Analyzed for $C_{51}H_{85}O_{22}.5H_2O$ (Calculated: C, 53.73%; H, 8.34%). IR (KBr), ^{1}H-NMR, FAB^+ are recorded.

Shatavarin-II :

Shatavarin-II could be obtained with 90% purity only. FR. VI (Table-3) was subjected to rechromatography over silica gel.

TABLE-8 : CHROMATOGRAM

Material : 7.4 g. adsorbed on 15.g. silica gel

Adsorbent : 450 g. silica gel. IIa.

Column dimension : 4.5 cm x 72 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$+MeOH+H_2O :: 65+35+10 - lower phase.	50 ml x 20	-	-
2.	- " -	50 ml x 5	0.1200	Shatavarin-IV.
3.	- " -	50 ml x 5	1.3500	Sticky mass-a yellow-green spot below shatavarin-IV with slight impurity.

(contd.)

TABLE-8 (contd.)

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
4.	$CHCl_3$+MeOH+H_2O :: 65+35+10 - lower phase.	50 ml x 4	1.4900	Mixture of comp. above shatavarin-II and shatavarin-II.
5.	- " -	50 ml x 9	2.2700	Major shatavarin-II.
6.	- " -	50 ml x 15	1.500	Contains shatavarin-I and shatavarin-V.
		Total...	6.8300 (92%)	

FR. 5 (Table-8) was subjected to rechromatography.

TABLE-9 : CHROMATOGRAM

Material : 2.27 g. adsorbed on 5 g. silica gel.

Adsorbent : 135 g. silica gel.

Column dimension : 2.3 cm x 80 cm

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (grms)	Remarks
1.	$CHCl_3$+MeOH+H_2O :: 65+35+10 - lower phase.	25 ml x 18	0.2000	-

(conted)

TABLE-9 (contd)

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (grms)	Remarks
2.	$CHCl_3$+MeOH+H_2O :: 65+35+10 - lower phase.	25 ml x 3	0.5000	Almost pure comp. above shatavarin-II.
3.	- " -	25 ml x 3	0.2500	Mixture of shatavarin-II and compound above it.
4.	- " -	25 ml x 10	1.0400	Shatavarin-II (90%).
5.	- " -	25 ml x 6	0.0900	Shatavarin-I with shatavarin-V.
		Total...	2.0500 (91%).	

FR. 4 (Table-9) contained shatavarin-II with some polar impurities. It was obtained as a foamy solid. m.p. 192-98°.

Shatavarin-VII :

7.24 g. of the residue from the mother liquor of FR. VI (Table-3) after precipitation for shatavarin-I was acetylated with 120 ml acetic anhydride (freshly distilled) in 100 ml pyridine (refluxed and distilled over KOH) at room temp. for

48 hours. Acetic anhydride and pyridine were removed at 65-75° under high vacuum (30-25 mm). The residue was taken up in chloroform (500 ml), washed with water (250 ml), 5% aqueous sodium carbonate (250 ml x 3), water (200 ml), $\frac{N}{2}$ hydrochloric acid (250 ml x 2) and finally with water (200 ml x 4). After drying over anhydrous sodium sulfate, chloroform was distilled off and the residue was dried under vacuum to get 11 g. of the acetylated mixture (foamy powder) which was subjected to chrmatography over silica gel.

TABLE-10 : CHROMATOGRAM

Material : 4.75 g. adsorbed on 10 g. silica gel.

Adsorbent : 200 g. silica gel, activated at 120° for 8 hours.

Column dimension : 4 cm x 40 cm.

Fr. No.	Eluent	Vol. of Fr.	Wt. of Fr. (gms)	Remarks
1.	Benzene+ethyl acetate :: 80+20	100ml x10	-	-
2.	Benzene+ethyl acetate :: 75+25	100ml x 5	-	-
3.	Benzene+ethyl acetate :: 70+30	100ml x 3	0.2818	Sticky mass, single spot.

(contd)

TABLE-10 (contd)

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
4.	Benzene+ethyl acetate :: 70+30	100ml x 1	0.1182	Acetate of shatavarin-II.
5.	- " -	100ml x 2	0.3682	Mixture of acetate of shatavarin-II and acetate of shatavarin-I.
6.	- " -	100ml x 12	1.2533	Acetate of shatavarin-I.
7.	- " -	100ml x 5	0.2650	Acetate of shatavarin-I with slight impurity.
8.	Benzene+ethyl acetate :: 60+40	100ml x 10	1.0000	Mixture.
9.	Benzene+ethyl acetate :: 50+50	100ml x 7	0.2746	Mixture.
10.	- " -	100ml x 20	0.6684	Acetate of shatavarin-VII.
11.	- " -	100ml x 8	0.1400	Acetate of shatavarin-VII with slight impurity.

(contd)

TABLE-10 (contd)

Fr. No.	Eluent	Vol. of Fr.	Wt. of Fr. (gms)	Remarks
12.	Ethyl acetate	100ml x 5	0.0736	Mixture.
13.	MeOH wash	100ml x 5	0.3000	Mixture.
		Total...	4.4331 (93%)	

FR. 4 is a white foamy solid which contained acetate of shatavarin-II (95%). m.p. 65.5-69°.

Acetate of shatavarin-I :

FR. 6 is a yellowish white foamy solid, containing acetate of shatavarin-I (m.p. 112-15°). It was repeatedly recrysallised from hot ethanol (dry, refluxed and distilled over magnesium ethoxide) to get white, heavy crystals. m.p. 119.5-121°. IR (KBr), ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) recorded. $[\alpha]_D^{27}$ - 17.5°

Acetate of shatavarin-VII :

FR. 10 - light yellow foamy powder showed a single spot and was named as acetate of shatavarin-VII. It was repeatedly recrystallized from hot ethanol (dry) to get white, heavy crystals. m.p. 142.5-144°. IR (KBr), ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$) recorded. $[\alpha]_D^{27}$ - 19°.

Similarly, butanol extract of A. racemosus was subjected to broad cut chromatography over alumina.

TABLE-12 : CHROMATOGRAM

Material : 30 g. adsorbed on 60 g. alumina.

Adsorbent : Al_2O_3- IV, 1 kg.

Column dimension : 4.5 cm x 70 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
I	n-BuOH saturated with water.	150ml x 3	3.2036	Dark red, oily, non polar material.
II	- " -	150ml x 3	1.7800	Coloured material contains comp.B.
III	- " -	150ml x 8	2.0091	Comp.A (major) with little comp.B and glycoside-AR_4.
IV	- " -	150ml x 8	1.8800	Glycoside-AR_4 (major) with glycoside-AR_3 and glycoside-AR_2.
V	- " -	150ml x 10	2.7931	Mixture of all saponins.
VI	- " -	150ml x 10	4.900	Mixture of glycoside-AR_1 and sugars.
VII	- " -	150ml x 10	2.000	Sugars.
		Total...	18.5658 (61.8%)	

Comp.B :

FR. II (Table-12) was subjected to flash chromatography.[35]

TABLE-13 : CHROMATOGRAM

Material : 0.530 g. adsorbed on 1 g. silica gel.

Adsorbent : 20 g. silica gel, 200-300 mesh.

Column dimension : 5" x 3.2 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$	15 ml x 10	-	-
2.	$CHCl_3$+MeOH :: 98+2	15 ml x 10	0.0587	Coloured material.
3.	$CHCl_3$+MeOH :: 95+5	15 ml x 7	0.1170	Major comp.B.
4.	$CHCl_3$+MeOH :: 93+7	15 ml x 10	0.1028	Comp.B with slight impurity.
5.	$CHCl_3$+MeOH :: 90+10	15 ml x 10	0.0713	Mixture.
6.	$CHCl_3$+MeOH :: 80+20	15 ml x 10	0.0706	Mixture.
		Total...	0.4024 (75%)	

FR. 3 and FR. 4 were combined together and subjected to rechromatography.

TABLE-14 : CHROMATOGRAM

Material : 0.200 g. adsorbed on 1.0 g. silica gel.

Adsorbent : 15 g. silica gel, grade II.

Column dimension : 23.5 cm x 1.6 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$	40 ml x 3	0.0033	-
2.	$CHCl_3$+MeOH :: 97+3	40 ml x 6	0.0310	-
3.	$CHCl_3$+MeOH :: 96+4	40 ml x 6	0.1377	Yellowish white powder - single spot - comp.B.
4.	$CHCl_3$+MeOH :: 90+10	40 ml x 4	0.0113	Mixture.
		Total...	0.1833 (91%)	

FR. 3 gave comp.B.

Comp.A :

Comp.A was obtained from the rechromatography of FR. III (Table-12).

TABLE-15 : CHROMATOGRAM

Material : 1.5 g. adsorbed on 3 g. silica gel.

Adsorbent : 60 g. silica gel, activated at 120°.

Column dimension : 2.0 cm x 37.5 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$	50 ml x 4	0.0149	Red oily material
2.	$CHCl_3$+MeOH :: 96+4	50 ml x 6	0.0952	Red oily material
3.	$CHCl_3$+MeOH :: 95+5	50 ml x 4	0.0313	Comp.A with slight impurity.
4.	$CHCl_3$+MeOH :: 93+7	50 ml x 7	0.5877	Comp.A.
5.	$CHCl_3$+MeOH :: 90+10	50 ml x 4	0.1700	Comp.A with lower impurity.
6.	$CHCl_3$+MeOH :: 85+15	50 ml x 5	0.3500	Mixture.
		Total...	1.0491 (69%)	

FR. 4 contained comp.A which was crystallized from methanol as needles. m.p. 151-3°.

Glycoside-AR_4 :

FR. IV (Table-12) was subjected to rechromatography over silica gel.

TABLE-16 : CHROMATOGRAM

Material : 3.94 g. adsorbed on 7 g. silica gel.

Adsorbent : 100 g. silica gel, grade IIa.

Column dimension : 56 cm x 2.3 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$	100ml x 2	0.0148	-
2.	$CHCl_3$+MeOH :: 96+4	100ml x 4	0.2835	Red oily material
3.	$CHCl_3$+MeOH :: 95+5	100ml x 3	0.0652	Comp.B with coloured impurity.
4.	$CHCl_3$+MeOH :: 93+7	100ml x 2	0.2800	Mixture.
5.	$CHCl_3$+MeOH :: 90+10	100ml x 18	1.2058	Brown foamy powder, glycoside-AR_6 with some impurity.
6.	$CHCl_3$+MeOH :: 80+20	100ml x 2	0.1226	Mixture of glycoside-AR_5 and glycoside-AR_4.
7.	$CHCl_3$+MeOH :: 70+30	100ml x 10	1.1702	Almost pure glycoside-AR_4.
8.	$CHCl_3$+MeOH :: 60+40	100ml x 2	0.2160	Mixture.

(contd)

TABLE-16 : CHROMATOGRAM (contd)

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
9.	MeOH wash	100ml x 2	0.1386	Mixture.
		Total...	3.4867 (88%)	

FR. 7 was subjected to rechromatography over silica gel to get pure glycoside-AR_4.

TABLE-17 : CHROMATOGRAM

Material : 0.4950 g. adsorbed on 1 g. silica gel.

Adsorbent : 40 g. silica gel, IIa

Column dimension : 1.2 cm x 78 cm.

Fr. No.	Eluent	Vol.of Fr.	Wt.of Fr. (gms)	Remarks
1.	$CHCl_3$+MeOH+H_2O :: 65+35+10 - lower phase.	10ml x 10	0.0028	-
2.	- " -	10ml x 7	0.3583	Glycoside-AR_4.
3.	- " -	100ml x 5	0.0529	Glycoside-AR_4 with lower impurity.
		Total...	0.4140 (83%)	

FR. 2 was repeatedly recrystallized from hot methanol to get white crystalline powder (Fig.16). m.p. 212-15°, Found: C, 59.82%; H, 8.57%. Analyzed for $C_{45}H_{74}O_{16}\cdot H_2O$ (Calculated: C, 60.41%;H, 8.46%).

Hydrolysis of comp.A :

50 mg of comp.A was hydrolysed by refluxing with 10 ml 2N sulfuric acid for 2 hours. It was cooled to room temp. and diluted to 25 ml, neutralised with ion exchange resin (Amberlite IRA-400, pretreated with 10% aqueous NaOH followed by water till neutral). The resin was filtered and filterate concentrated under vacuum. The residue was spotted on paper with a standard sample of D-fructose. It showed a spot corresponding to fructose. (R_G 0.27). On TLC also it showed a spot corresponding to fructose. (R_f 0.38).

Acetate of comp.A :

0.1280 g of comp.A was acetylated with 4 ml acetic anhydride (freshly distilled) in 4 ml dry pyridine (refluxed and distilled over KOH) for 48 hours. Pyridine and acetic anhydride were removed under vacuum (25 mm, bath temp.65-75°). The residue was taken up in chloroform and washed with water (25 ml), 5% aquous Na_2CO_3 (25 ml x 3), water (25 ml), $\frac{N}{2}$ HCl (25 ml x 2) and finally with water (25 ml x 4), dried over

anhydrous sodium sulfate and chloroform was distilled off to get 0.1866 g. of sticky, viscous material. IR, ^{1}H-NMR and FAB^{+} mass spectra recorded.

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SUMMARY

A comparative study of A. racemosus Willd (from M.S. University of Baroda) and commercial shatavari (Baroda market) was carried out. Five compounds namely comp.B, comp.A, shatavarin-IV, shatavarin-I and shatavarin-VII (as acetate) were obtained in pure form. Shatavarin-II and acetate of shatavarin-II were obtained in slightly impure form, from commercial shatavari. Three compounds were obtained from A. racemosus in pure form. The compounds were comp.B, comp.A and glycoside-AR_4.

Structure of comp.A is discussed. On hydrolysis, it gave fructose. From its physical characteristics and spectral data, compound is found to be identical with n-Butyl- -D-fructopyranoside. It was formed as an artefact of extraction with butanol.