CHAPTER-5

STRUCTURE OF SHATAVARIN-VII

Isolation of shatavarin-VII is described in chapter-2. It was isolated in the form of its acetate only. In present chapter tentative structure of shatavarin-VII is discussed.

Since structure of other glycosides isolated from shatavari were arrived at mainly with the help of spectral data, it was decided to use the same technique for structure determination of shatavarin-VII.

Peracetate of shatavarin-VII was also found to be +ve to Ehrlich's reagent[1]. It indicated that shatavarin-VII is also a furostanol saponin and not spirostanol saponin. On TLC, it is below shatavarin-I, and also it was eluted with more polar solvent system in column chromatography, so it was concluded that shatavarin-VII contains more number of sugars than shatavarin-I.

HYDROLYSIS OF PERACETATE OF SHATAVARIN-VII

Acetate of shatavarin-VII was refluxed with 2N sulfuric acid in dioxan for 20 hours. The aglycone and the sugars were separated. However, the aglycone part showed a number of spots on TLC, when it was passed through a column, the aglycone could not be isolated.

The aqueous part was neutralised by passing through an ion-exchange resin, concentrated and spotted on paper. On

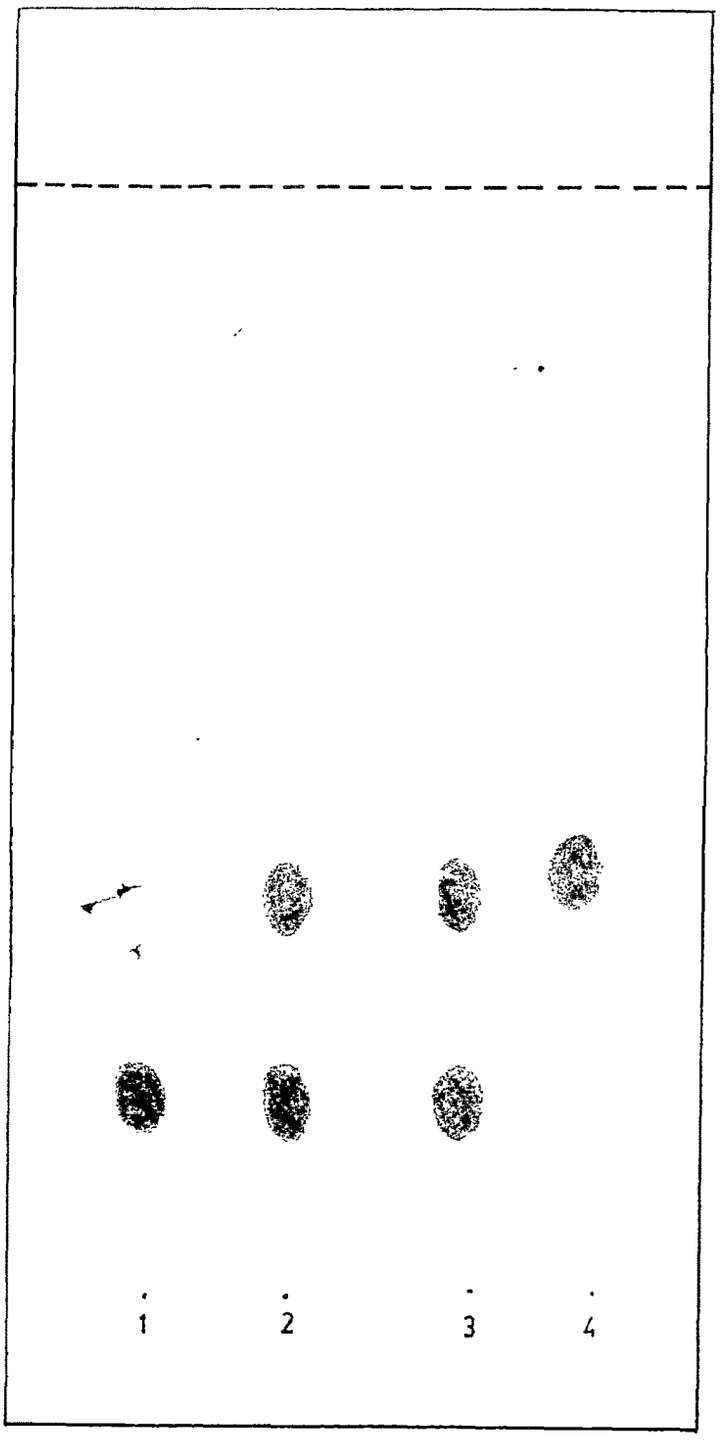

FIG. I : PAPER CHROMATOGRAM OF SUGARS OF SHATAVARIN-VII

SOLVENT SYSTEM : n-BuOH-HOAc-H O (4:1:5)(UPPER PHASE)
SPRAY REAGENT : ANILINE HYDROGEN PHTHALATE
TIME : 4 HOURS
SPOTS : 1) GLUCOSE 2) SUGARS OF SHATAVARIN-VII AFTER 4 HOURS 3) SUGARS OF SHATAVARIN-VII AFTER 20 HOURS 4) RHAMNOSE

paper chromatogram (Fig.1), the sugar part of acid hydrolysate of peracetate of shatavarin-VII showed only two spots. When spotted alongwith standard samples, the two corresponded to D-glucose* and L-rhamnose* (R_f values 0.26 and 0.44). The intensity of the two spots was almost equal.

When acetate of shatavarin-VII was treated with alkali to get shatavarin-VII, it was found that shatavarin-VII was water soluble, hence it was not tried to isolate as tentative structure could be assigned from its acetate derivative also.

IR spectrum of peracetate of shatavarin-VII was compared with the IR spectrum of peracetate of shatavarin-I. The two spectra are superimposable on each other. The four band absorption pattern of spirostanol saponins (bands at 850, 900, 920 and 987 cm^{-1}) is missing in both the spectra.

FABMS of peracetate of shatavarin-VII could not be recorded due to relative non polarity of the compound.

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

As reported earlier for shatavarin-IV and shatavarin-I, ^{13}C-NMR spectroscopy was used chiefly for the determination of points of attachment of sugars. ^{13}C-NMR spectrum of peracetate of shatavarin-VII was recorded in deuterochloroform. The ^{13}C chemical shifts of the aglycone was assigned by comparison with the values in the ^{13}C-NMR spectrum of peracetate of shatavarin-I and according to known chemical shift rules[2]. Those of sugars were by comparison of the values in peracetate of shatavarin-IV and peracetate of shatavarin-I.

The ^{13}C-NMR spectrum of peracetate of shatavarin-VII was slightly different from that of peracetate of shatavarin-IV and peracetate of shatavarin-I. In the ^{13}C-NMR spectrum five signals appear in the range 99-101 ppm, which are assignable to the anomeric carbon centre of monosaccharides, indicating that there are five monosaccharide residues. There are two signals at 19.6 and 19.9 which are assigned to rhamnose C-6, as glucose and rhamnose are the only two sugars obtained by acid hydrolysis. Since there are five anomeric signals, there are five sugars, two of which are rhamnose, hence the remaining three are due to glucose.

The spectrum differs from that of peracetate of shatavarin-I. Signals for C-20, C-21, C-22, C-23, C-16

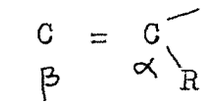
and C-17 carbon atoms appear at unusual position in the spectrum of peracetate of shatavarin-VII.

A signal appears at 154.8 ppm. In olefinic compounds, signals are observed in the range 120-155 ppm.[2] A signal appears at 99.394 ppm considering these two carbon atoms as double bonded carbon atoms, the two correspond to C-22 and C-20 carbon atoms respectively. In case of Δ^5 sapogenins (e.g. diosgenin), the C-5 and C-6 carbon atoms appear at 120-130 ppm only.[3] The C-21 and C-23 carbon atoms are found at 23.895 and 42.388 ppm which are downfield by + 9.5 and + 15.2 ppm respectively. The C-16 and C-17 carbons appear at 77.6 and 68.8 ppm respectively. The C-26 signal is observed at 70.746 ppm.

Thus, from ^{13}C-NMR spectrum it is concluded that there is a double bond between C-20 and C-22. ^{13}C Chemical shift value for each carbon atom can be calculated using additive parameters and substituent effects.[4] The shift values for C-20 and C-22 carbon atoms can be calculated as follows.

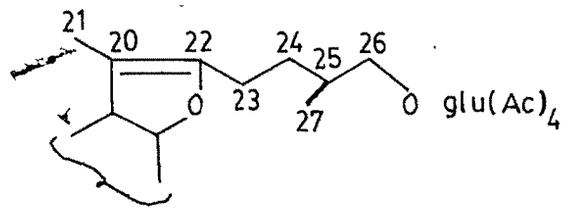
A series of alkylcyclohexenes and 1-alkylcyclopentenes were studied and the results reported.[5] As expected from acyclic olefins, pronounced changes are caused by 1-alkyl substituents. A list of parameters were developed by Savitsky and Namikawa.[6] For α-substituents β-nucleus

absorbs at higher field. Carbon atom bearing alkyl group moves to lower field by + 10 ppm while the second olefinic nucleus (i.e. β-carbon atom) is shielded by 8 ppm.[5]



R = alkyl group.

In case of peracetate of shatavarin-VII, the structure can be infered as (I).



I

Considering the parent value 123.3 ppm for olefin[7], the values are calculated as 123.3 + Zi.

For C-22 carbon,

123.3 + 29.4 = 152.7 (-O-CH-CH< at C-22).

152.7 + 14.6 = 167.3 (-CH_2-CH_2-CH(-CH_3)-O-gluc Ac)$_4$ at C-22).

167.3 - 16.7 = 151.2 (-CH< and -CH_3 on C-20).

Observed value for C-22 is 154.8, which is well in accord with the calculated value. The difference in the two values may be due to strain in ring.

The value for C-20 can be accounted by examining vinylic derivative shifts. The shieldings of vinylic derivatives have been examined in detail. The β carbon atoms observe a shift of over 55 ppm due to substitution at α- carbon atom. This can be rationalised depending upon the substituent. If a free electron pair is adjacent to the double bond, one more cannonical form of the type $\overset{+}{X}=CH-\bar{C}H_2$ will contribute to the overall electron distribution, thereby increasing the electron density at the carbon atom, causing a more shielding effect. When oxygen and nitrogen are present as substituents, such an effect is more predominating e.g. -OMe and $-OCH_2CH_3$ make the β-carbon atom resonate at 85.5 and 84.6 ppm respectively[8]. Thus -OR substituent causes an upfield shift of -33-40 ppm for the β carbon atom. Calculation for C-20 signal shift,:

123.7	-	39.8	= 83.9	(-OR on C-22)
83.9	-	8.9	= 75.0	($-CH_2-CH_2-\underset{CH_3}{CH}-O$-glu(Ac)$_4$ on C-22.
75.0	+	26.1	= 101.1	(two alkyl substituents on C-20)

The signal is observed at 99.319 ppm, only 2 ppm upfield of calculated value.

Due to double bond between C-20 and C-22, C-17 carbon atom, being adjacent carbon observes a downfield shift of 6 ppm and is observed at 68.808 ppm. The C-23 carbon atom being α- to the double bonded carbon atom bearing an -OR functional group is more deshielded and appears at 42.38 ppm. The C-21 carbon atom again being allylic shifts downfield to 23.8 ppm. C-26 carbon

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

Carbon No.	Aglycone in peracetate of shatavarin-I+	Aglycone in peracetate of shatavarin-VII
1.	30.177	30.2
2.	26.478	26.595
3.	75.035	75.325
4.	30.177	30.292
5.	34.991	34.9
6.	26.419	26.478
7.	26.419	26.478
8.	34.991	34.932
9.		40.334
10.	34.932	34.932
11.	21.253	21.253
12.		40.040
13.		40.334
14.		
15.	30.177	33.17
16.	77.615	77.615
17.	62.115	68.808

(contd.)

TABLE-1 : (contd.)

Carbon No.	Aglycon in peracetate of shatavarin-I+	Aglycone in peracetate of shatavarin-VII
18.	17.201	17.260
19.	26.392	23.895
20.		99.394
21.	17.2	23.895
22.	99.4	154.873
23.	26.478	42.388
24.	26.392	26.008
25.	26.392	26.243
26.	70.159	70.746
27.	17.20	17.260

* δ ppm from TMS

\+ Chapter-4.

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

Carbon No.	Methyl -β-D-glucopyranoside tetraacetate	∝-L-rhamnopyranoside tetraacetate	Sugars of peracetate of shatavarin-VII	
C-1	101.4		100.216	Gluc.1
C-2	70.358		77.084 (+6.7)	
C-3	72.853		72.096 (-0.8)	
C-4	67.923		75.325 (+7.4)	
C-5	72.853		72.977 (+0.1)	
C-6	61.593		61.763 (+0.2)	
C-1'			101.038	Gluc.2
C-2'			70.100 (-0.2)	
C-3'			72.096 (-0.8)	
C-4'			67.809 (-0.10)	
C-5'			71.509 (-1.3)	
C-6'			62.409 (+0.9)	
C-1''			100.747	Gluc.3
C-2''			70.100 (-0.2)	
C-3''			71.802 (-1.0)	
C-4''			67.869 (-0.1)	
C-5''			71.509 (-1.3)	
C-6''			62.115 (-0.6)	

(contd.)

TABLE-2 (contd.)

Carbon No.	Methyl -β- D-glucopyranoside tetraacetate	α-L-rhamnopyranoside tetratetraacetate	Sugars of peracetate of shatavarin-VII	
C-1'''		91.25	100.864	Rham.1
C-2'''		69.24	67.869 (-1.38)	
C-3'''		69.03	67.869 (-1.17)	
C-4'''		71.20	70.746 (-0.46)	
C-5'''		69.31	68.808 (-0.51)	
C-6'''		17.57	19.609 (+2.03)	
C-1''''			99.394	Rham.2
C-2''''			67.81 (-1.43)	
C-3''''			67.81 (-1.22)	
C-4''''			76.675 (+5.47)	
C-5''''			68.808 (-0.51)	
C-6''''			19.902 (+2.34)	

* δ ppm from TMS.

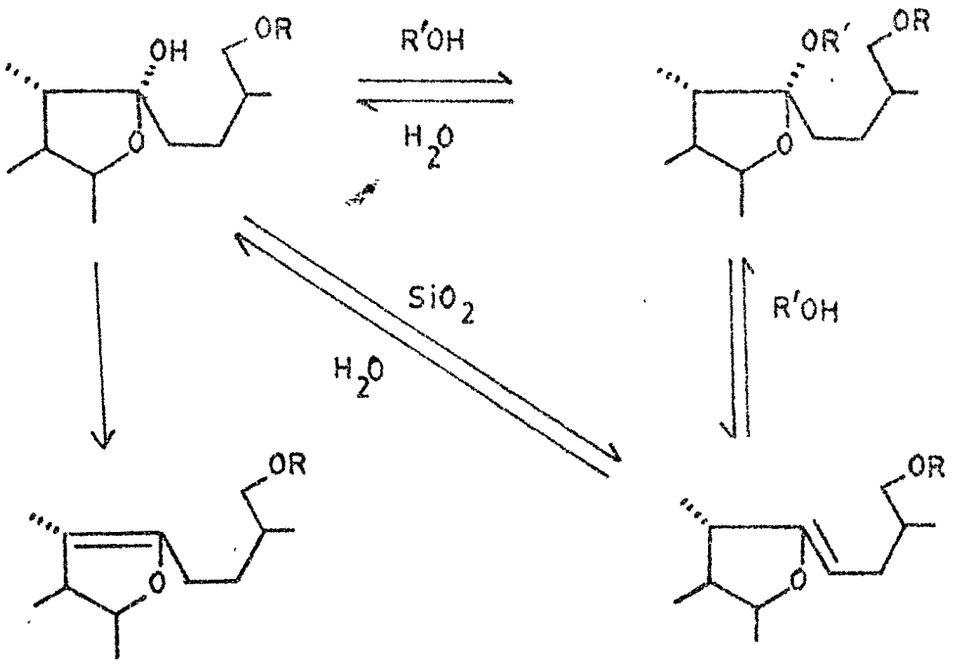
+ Data collected by the author.

atom is glucosidated and hence observes a downfield shift of + 6-7 ppm and is found at 70.74 ppm.

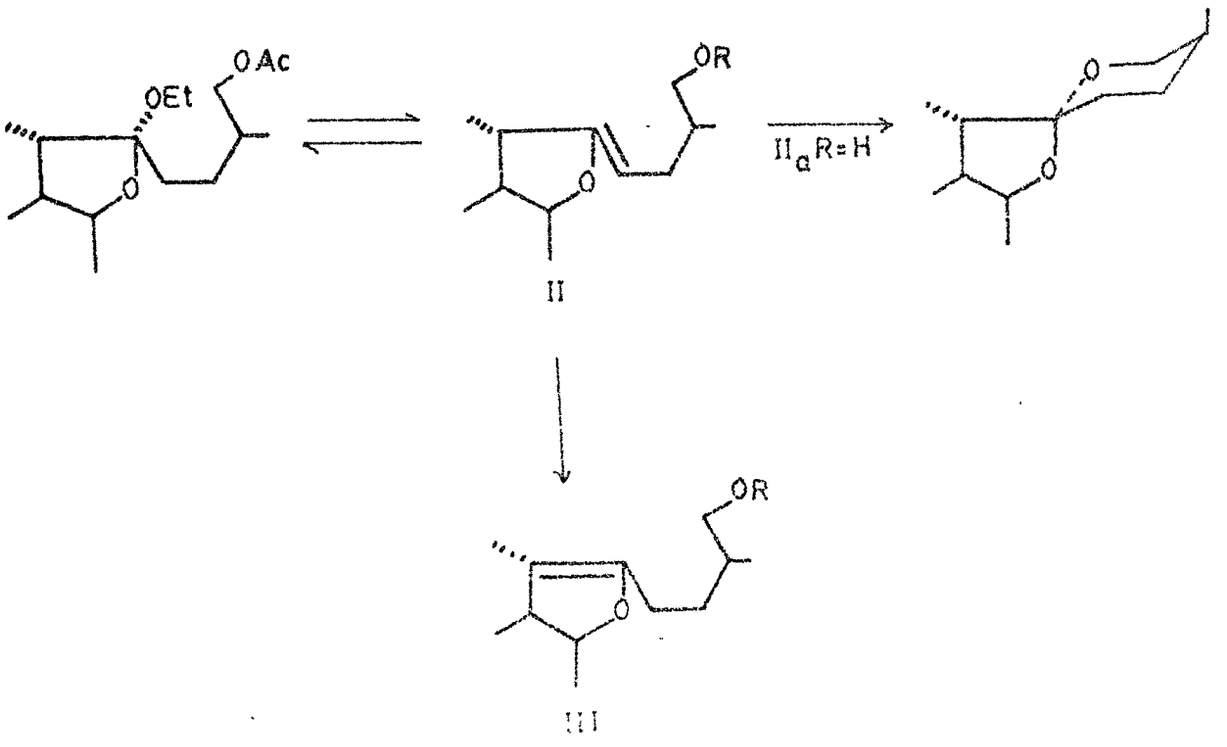
In case of sugar linkages, ^{13}C-NMR helps in determining the interglycosidic linkages. The signals of C-2' and C-4' of glucose-1 have been deshielded by glycosylation and appear at + 6.7 and + 7.4 ppm downfield respectively, indicating that it is glycosylated at C-2' and C-4' and that C-2' is rhamnosylated and C-4' is glucosylated. In case of rhamnose also C-4' signal is shifted downfield by + 5.9 ppm, indicating that it is rhamnosylated at C-4'.

From the biogenesis point of view, it may be that shatavarin-VII has a glucose at C-26 and at C-3 the linkages of the sugars are the same as in case of shatavarin-I, with one more rhamnose connected at rhamnose 4 position. The double bond between C-20 and C-22 must have been generated during isolation. It is possible that on silica gel column, the double bond is generated by the loss of a water molecule in the original compound or by elimination of a molecule of acetic acid. In furostanol saponins, the following reactions are observed[9] (scheme-1).

Hirschmann and Hirschmann[10] observed destruction of ketal (I) on silica gel - celite column, yielding a mixture



SCHEME-1



SCHEME-2

containing the hemiacetal (II) and (IIa) which is converted to more stable isomer (III) at room temp. in presence of acetic acid or phosphoric acid (Scheme-2).

To confirm the C-26 sugar chain attachment, shatavarin-VII was oxidized by CrO_3 by the method of Tschesche et al.[9,11] (Fig.2). The first was Marker's oxidation[12] by Wall's[13] procedure. Acetate of shatavarin-VII (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-pregn-16-ene-20-one (5). λ_{max} 240 nm. IR: ν_{max}^{KBr} cm^{-1} 1740, 1670 (For Δ^{16}-20-keto-steroids). Mass m/z 358 M^+. 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.

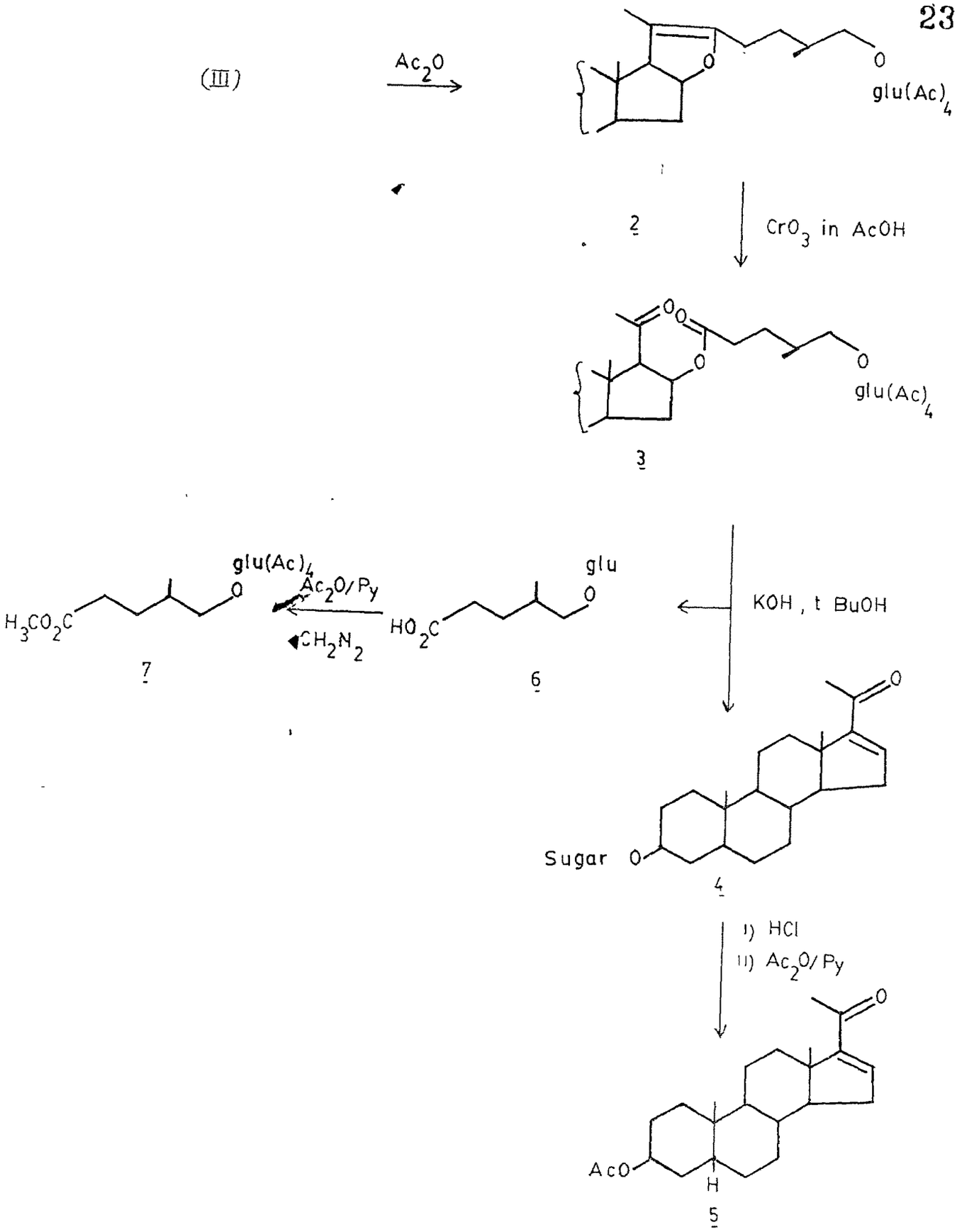


FIG. 2 : CrO_3 OXIDATION OF SHATAVARIN-VII

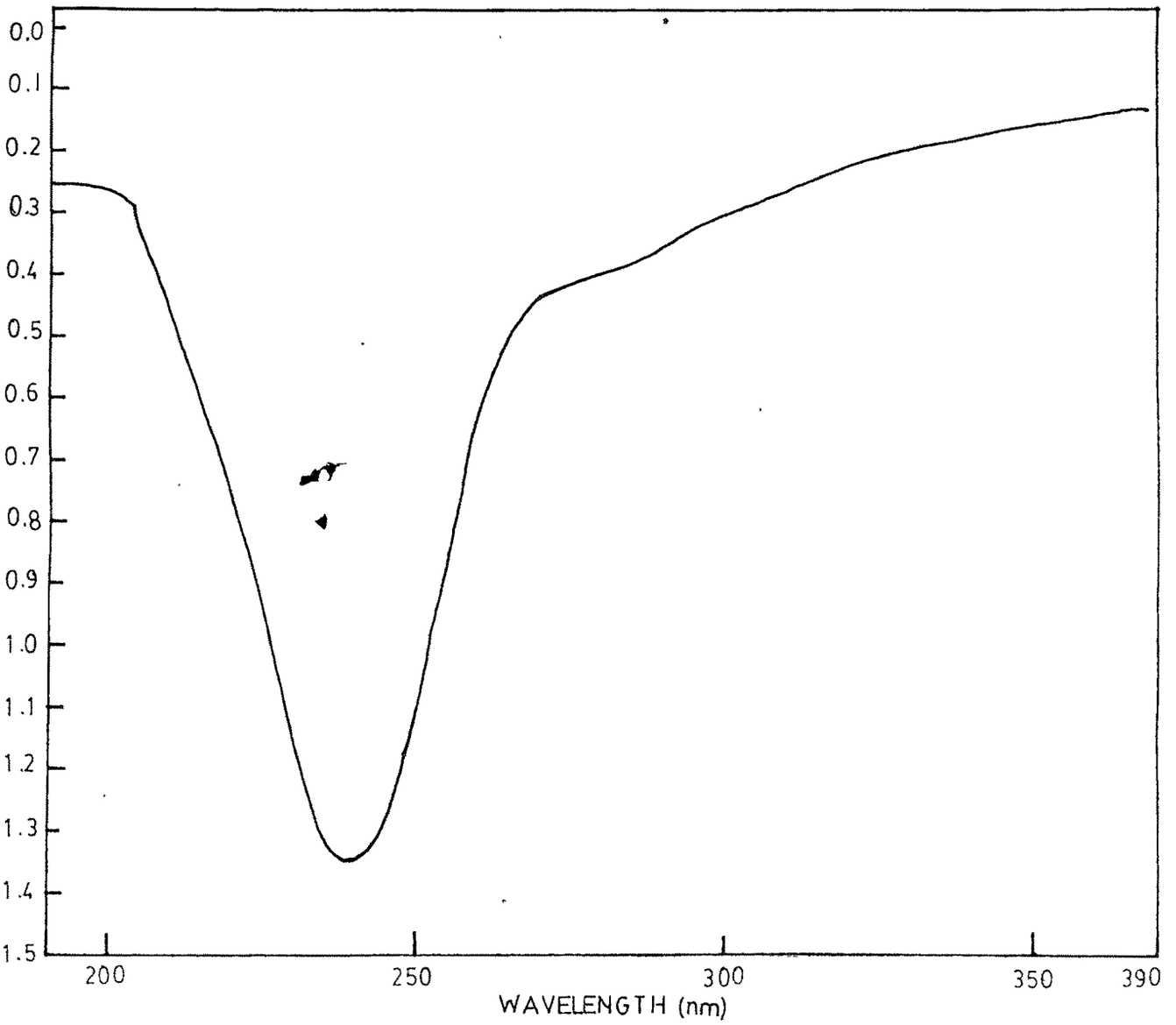


FIG. 3 : UV SPECTRUM OF (5)

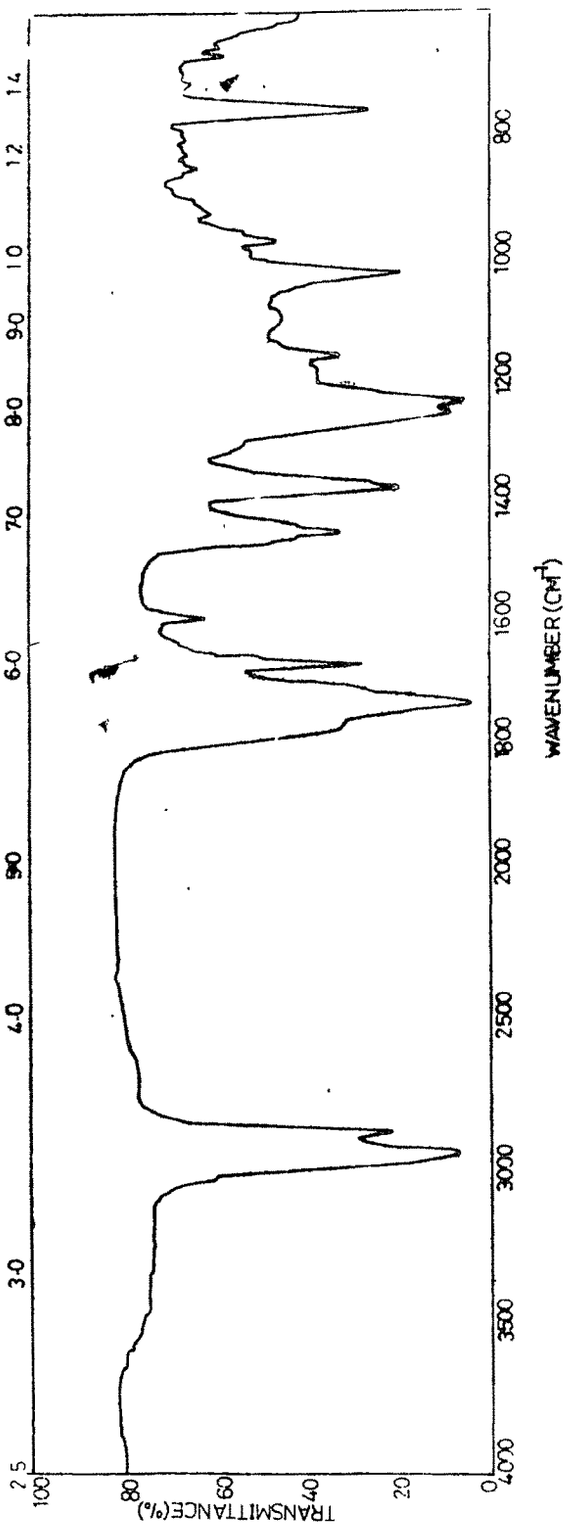


FIG. 4 : IR SPECTRUM OF (5)

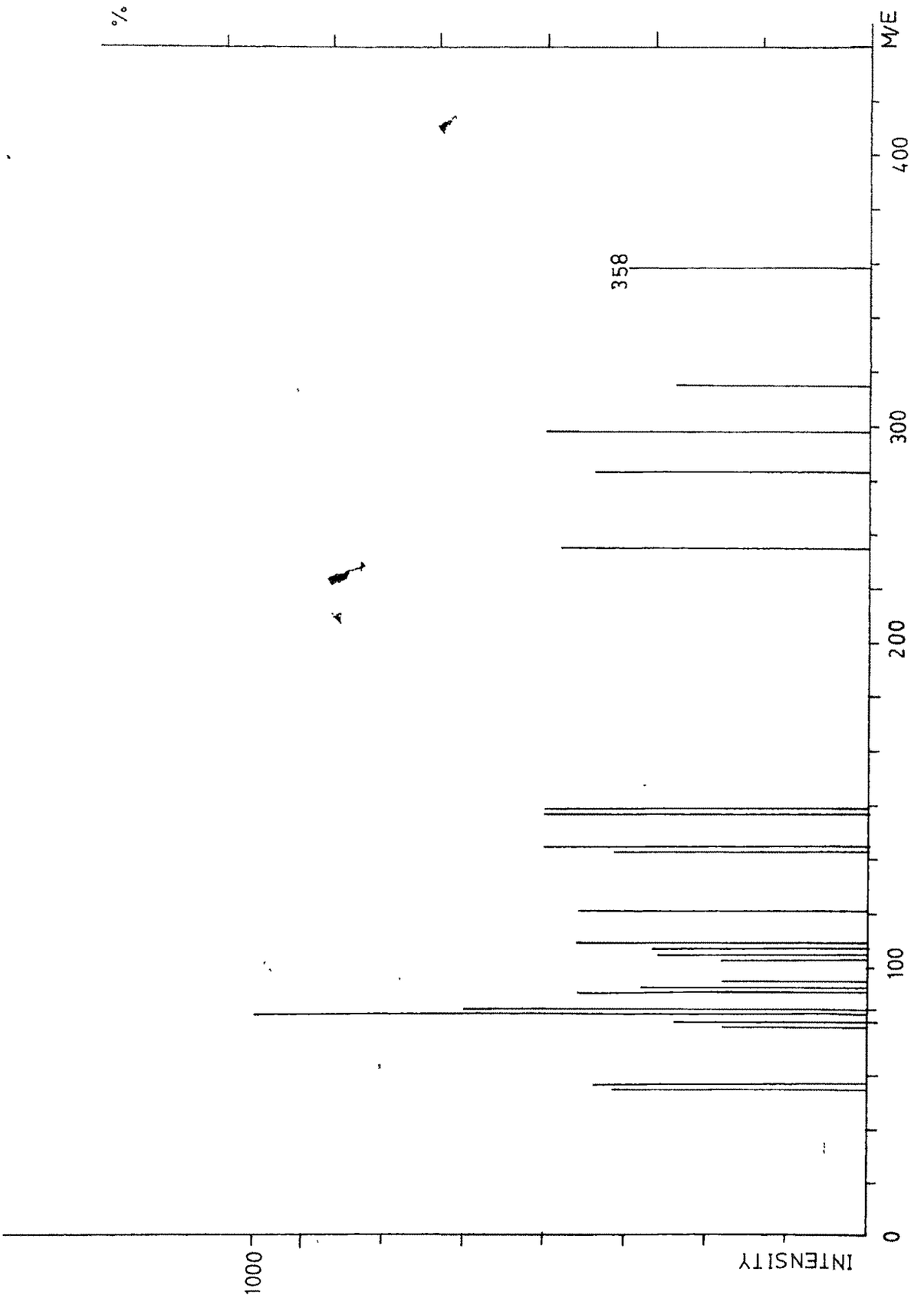


FIG: 5 : MASS SPECTRUM OF (5)

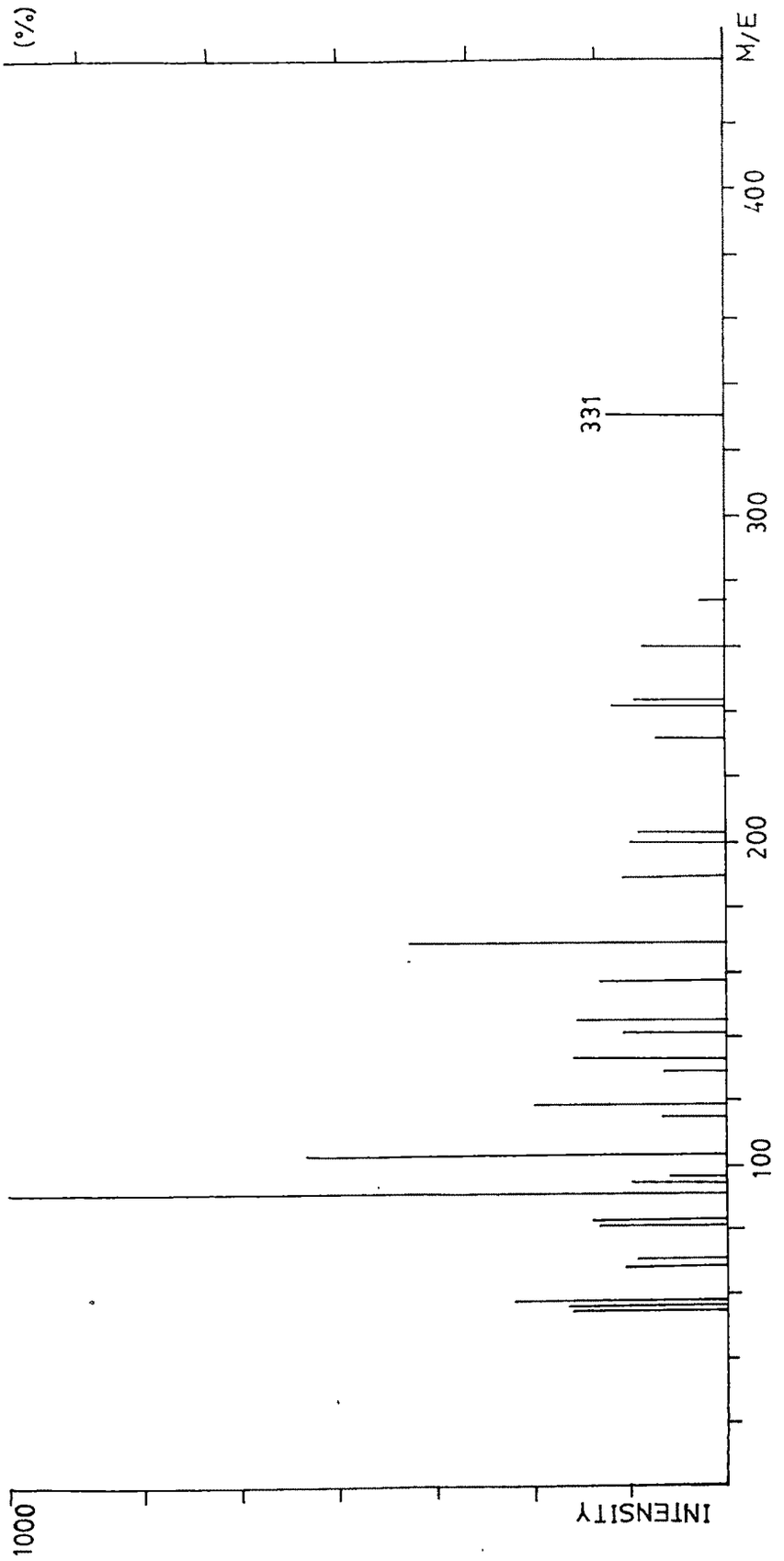
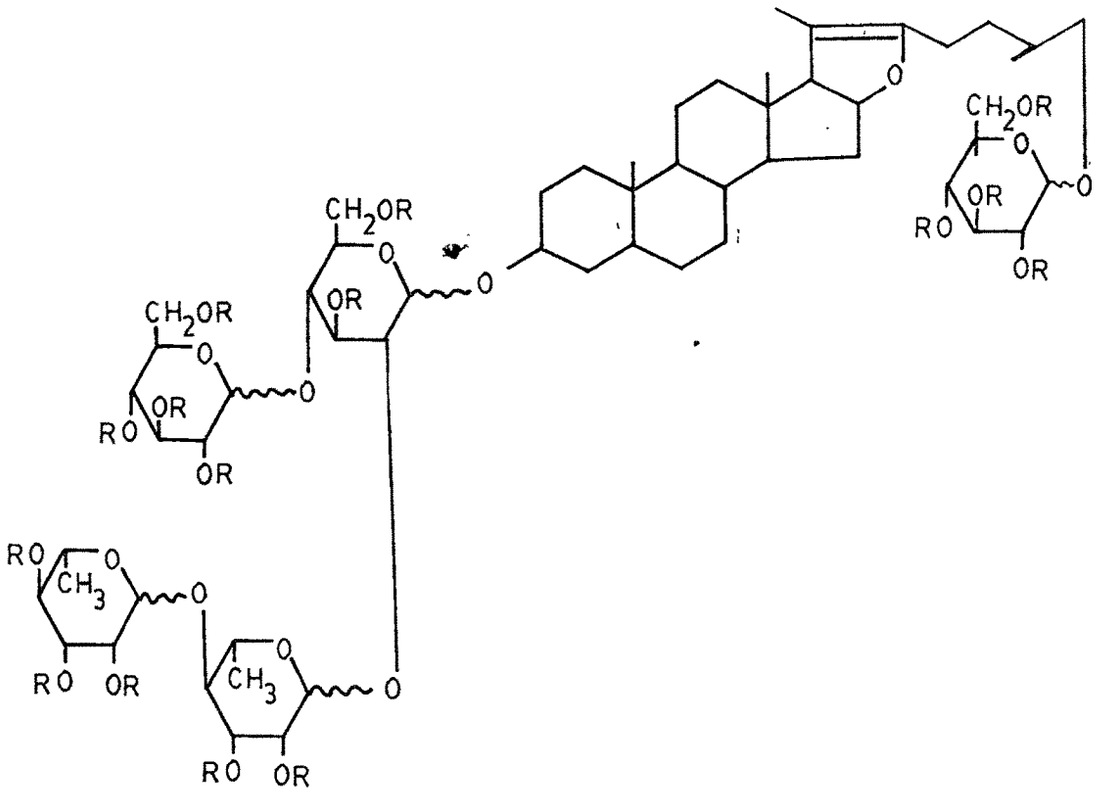


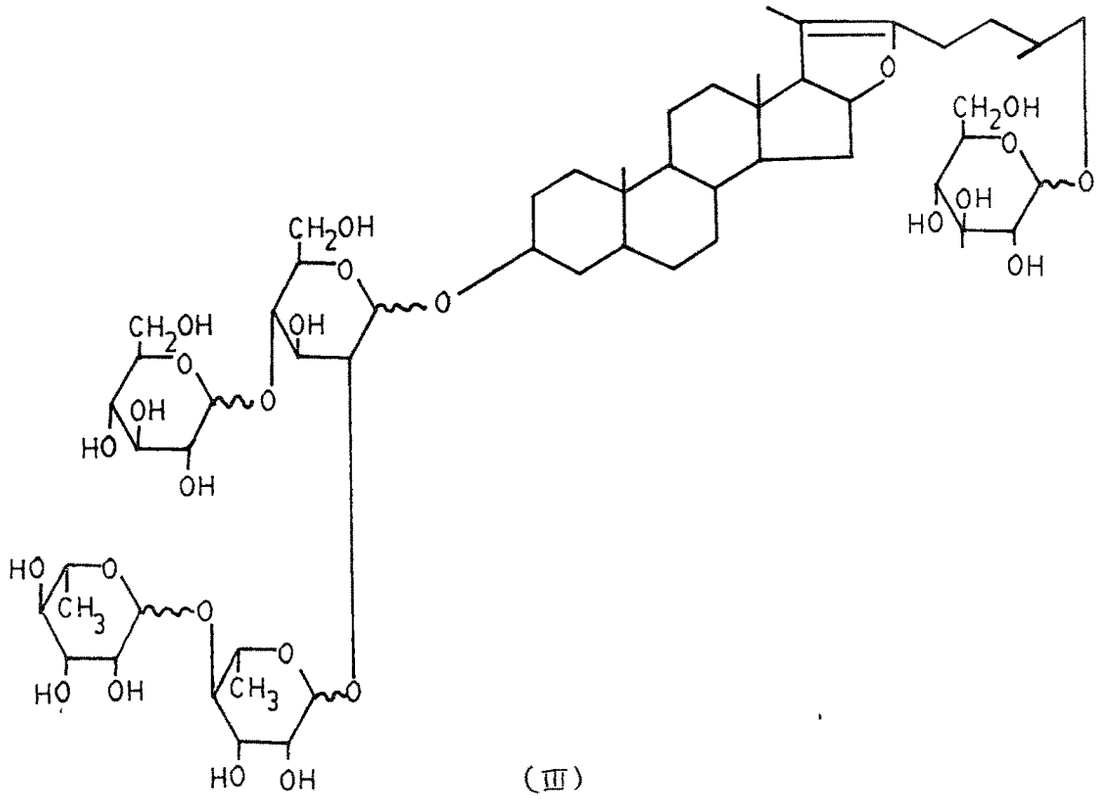
FIG. 6 : MASS SPECTRUM OF (Z)

Schulten et al.[14] prepared a compound with a double bond at $^{20-22}$ from a furostanol saponin by acid hydrolysis of a furostanol saponin.

From the above results, structure of acetate of shatavarin-VII was formulated as (II) and that of shatavarin-VII as (III).



(Ⅱ) R= CH_3CO-



(Ⅲ)

EXPERIMENTAL

For general remarks refer to page no. 100 and 160.

HYDROLYSIS OF ACETATE OF SHATAVARIN-VII

Acetate of shatavarin-VII (300mg) was heated on a boiling water bath with 2N sulfuric acid (70 ml) and dioxan (30 ml) for 20 hours. It was cooled to room temp. and diluted with water (150 ml), extracted with benzene (100 ml x 4). The combined benzene extracts were washed with water (100 ml x 4) and dried with anhydrous sodium sulfate. Solvent was evaporated to get 0.1200 g. of the aglycone part which on TLC showed a number of spots. When subjected to chromatography, only mixture was obtained.

The aqueous part was neutralised with anion exchange resin* (300 ml) and filtered. The resin was washed with water (100 ml). Water was then removed from the combined extracts under suction to get 150 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 of 0.44.

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

CrO_3 OXIDATION OF SHATAVARIN-VII

Shatavarin-VII 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 mi) and extracted with ether (50 ml x 3). The ether extracts 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} 1740, 1670, 970, 930, 830 (reported : 1724, 1662, 958, 920, 895, 820). EI-MS : m/z 358 M^+ . (reported EI-MS (probe) 70 eV : m/z 358 M^+ . λ_{max} : 240 nm. (reported λ_{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 with n-butanol and chloroform alternately. The aqueous phase was neutralised with 2N NaOH and evapor-

ated. 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, 97, 89, and 81.

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SUMMARY

The tentative structure of shatavarin-VII is discussed. By TLC it was found to be Ehrlich +ve furostanol saponin. By acid hydrolysis, the sugars obtained were D-glucose and L-rhamnose. On chromic acid oxidation the two products obtained were identified as 3β-acetoxy-pregn-16-ene-20-one and δ-hydroxy--√-methyl valeric acid methyl ester-glucoside acetate. The ^{13}C-NMR study revealed the presence of a double bond and 2 rhamnose and 3 glucose units. From the above results a tentative structure (III) was formulated for shatavarin-VII.