CHEMICAL AND PHARMACOLOGICAL STUDIES OF THE POLAR CONSTITUENTS OF *LUFFA ECHINATA* AND *LUFFA CYLINDRICA*.

A DISSERTATION

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHILOSOPHY IN CHEMISTRY.

PRESENTED BY

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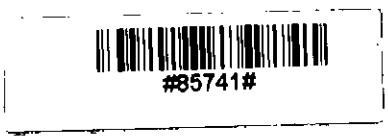

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The Author.

Contents

List of Figures

List of Table

CHAPTER 1

INTRODUCTION

1.1 General

Men and other animals have to rely on nature for existence in one way or another to a great degree and plant kingdom is of vital importance for them. It supplies us not only with food and shelter but also with medicine and raw materials for clothings. The civilization we are living in owes a good deal to nature. Along with cereal we find a number of plants in nature which have been in vital use from time immemorial as remedy for various diseases and decay of mankind. In Indian sub-continent the use of medicinal plants has a long, rich past background. It dates back to the very outset of civilization which once flourished in this area . The traditional treatment systems like Kaviriji, Ayurvedi and Unani are based upon indigenous medicinal plants. Even today these systems of treatment are fairly common amongst the village people of the sub continent.

The plant bodies have attracted the attention of Chemists and Pharmacists for their paramount importance and significance in certain spheres of human life. In consequence in the early period of civilization Natural product Chemistry attained a revolutionary advancement leading to systematization of Chemotheraphy and this fruitful discovery has expedited the research work for comparatively newer medicines. In the recent decades antibiotics, vitamins and hormones are undoubtedly the results of researches in the field of natural product chemistry. It is expected that in the years to come natural product Chemistry will afford more sophisticated medicines which will mitigate the sufferings of the vast masses of humanity.

It is easy to find Bangladesh replete with a vast majority of medicinal plants and herbs which are distributed all over the country in the jungles, hillocks and gardens. Hakims, Vaidays and Kavirajs have been using many of these plants as medicines so as to treat different diseases since time unknown. A systematic study on these plants may lead to the discovery of newer drugs or drugs with minimum or little side effects.

In the following section an outline of the chemical investigation on some plants classified on the basis of their application is given.

Anti-Diarrhoeal Agents

There is a host of plant bodies which are reported to be effective against diarrhoeal diseases. The syrup made from the extract of the leaves of **Andrographis paniculate** (Acanthaceae) is a popular ayurved preparation and is marketed as "Kalmegh"[1]. The capsules made from the dried leaves of **Hydrocotile asiatica** (Umbelliferae), **Poederia foetida** (Rubiaceae) and whole dried fruit **Aegle marmelos** (Rutaceae) have been found to be clinically efficacious against diarrhoea[2].

Anti-fertility Agents

A traditional contraceptive pill called 'Shanti Bari' is comprised of a mixture of exudate of **Acacia catechu**, powdered stem barks of **A.arabica** and the powdered seeds of **Tragia involuecrta**. Pharmacological study shows that the pill inhibit fertility of female rats to the extent of 87.5% without effecting

the oestrous cycles of the rats[3]. The shautal tribe of the country use the perennial herbs **Marsdenia tinctoria** (Asclapiadacease) as abortifacients. The alcohol extract of the plant showed antifertility activity[4]. The antifertility activity of the roots of **Plumbago zeylanica** was due to the presence of plumbagin a crystalline naphthoquinone compound[5]. The seeds of **Datura fastuosa** (Solanaceae) contain 10-12% of a nontoxic oil. Pharmacological study has revealed that the oil has a temporary sterile effect on female rats only and no effect on the male rats[6].

A few more plants e.g. **Abrus precatorius, Calotropis gigantea** and **Calotropis procera** are reputed to have antifertility effects.

Anti-Hypertensives

The famous hypotensive and tranquillizer reserpine has been isolated from the roots of **Rauvoleia serpentina**. Recent clinical trials on the capsules made from the dried powdered leaves of **Moringa oleifera** have shown encouraging results as antihypertensive[2].

Anti-Diabetes

The leaves of **Coccinia indica** are reported to have sugar lowering activity and clinical tests on the capsules made of it have proved to be so[2]. Chemical investigation on the roots of **Scoparia dulcis** which are believe to have antidiabetes property[7] has reported in the literature. Preliminary pharmacological tests show that the green fruits of **Momordica charantia** hasblood sugar lowering property.

1.2 Review on plants of the genus Luffa.

Luffa is one of the most important genera of the family Cucurbitaceae[8]. The seven species of the genus Luffa are distributed mostly in America, Australia, Africa and Asia. They are mainly tropical or sub-tropical herbs. In Bangladesh five of them have so far been identified and these are:

i) **Luffa aegyptiaca**(Synonym **Luffa cylindrica, Luffa pentandra**)

ii) **Luffa acutangula**

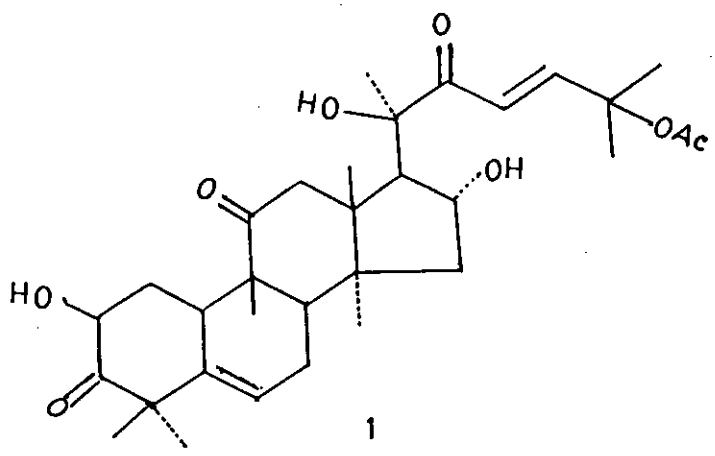
iii) **Luffa echinata** (Synonym **Luffa amara**)

iv) **Luffa graveolens**

v) **Luffa operculata**

Of these **Luffa acutangula** and **Luffa cylindrica** are most common and they occur both as sweet and bitter. The sweet varieties are edible and widely consumed by the people. These plants are reported to have medicinal values and many parts of them have been subjected to chemical investigations.

Barua and his group[9] worked on the seeds of **Luffa acutangula** in India. They isolated a crystalline bitter principle cucurbitacin B(1), m.p 185°C and a saponin. Acid hydrolysis of the saponin yielded an acid genin which was identified as oleanolic acid (2), m.p 305°C.



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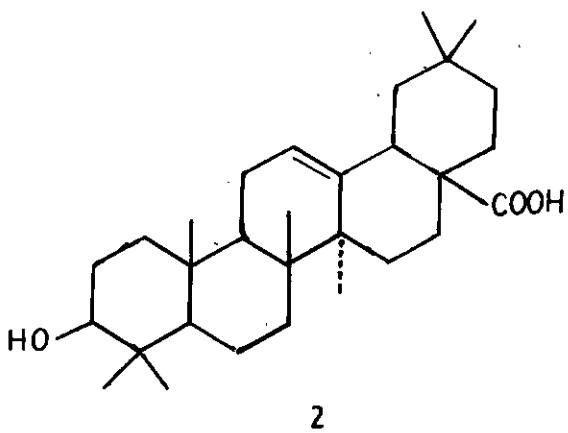

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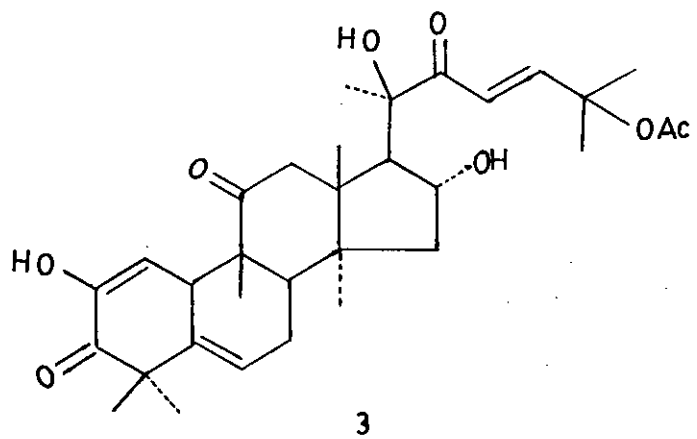
Chemical study on the fruits of **Luffa graveolens** was carried out by Bhakuni, Sarma and Kaul[10] and they were able to isolate two bitter principles namely cucurbitacin B(1). and cucurbitacin E (3), m.p 235°C.



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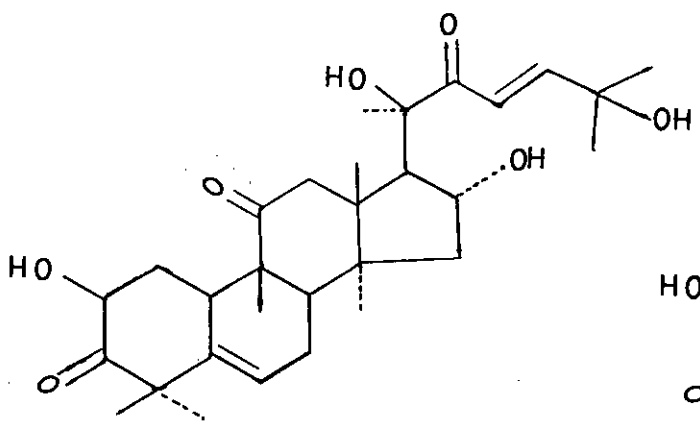
The plant **Luffa operculata** was shown to contain cucurbitacin B(1) and cucubitacin D(4), m.p 152°C by a group of German Chemists[11] in 1966. The plant **Luffa operculata** was also chemically investigated by Abreu Matos[12] and his group in Brazil. They reported the isolation of another bitter principle named isocucurbitacin B(5), m.p 228°C.



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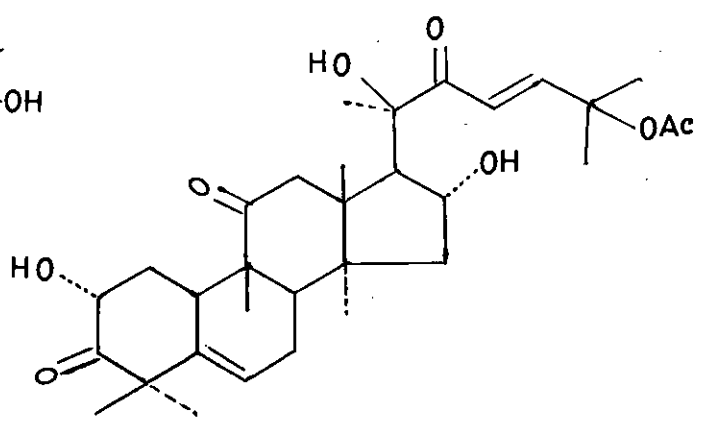

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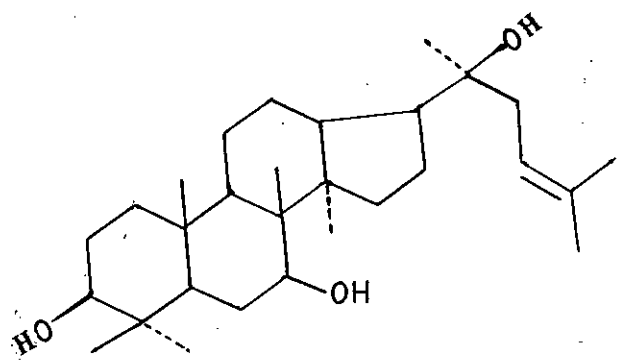
A group of Japanese Chemists[13] reported the isolation of eight damarane type triterpene glycosides named lupeosides A to H(6) from the dried herb of Luffa operculata. Their structures were determined on the basis of chemical and spectral evidences.



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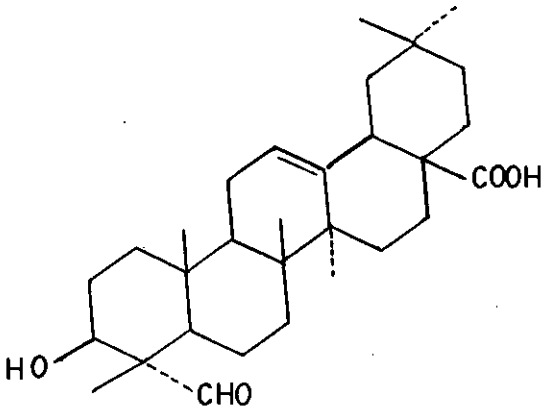
A: a 3.20-bis-O-β-D-glucopyranoside of (20s)-dammar-24-ene-3β,7β, 20-triol(Ag).
B: a 20-O-β-gentiobioside of Ag.
C: a 3-O-β-neohesperidoside-20-O-β-D-glucopyranoside of Ag.
D: a 3-O-β-D-glucopyranoside-20-O-β-gentiobioside of Ag.
E: a-3-O-β-neohesperidoside-20-O-β-D-xylopyranosyl-(1>6)-β-D-glucopyranoside of Ag.
F: a-3-O-β-neohesperidoside-20-O-β- gentiobioside of Ag.
G: a-3-O-β-neohesperidoside-20-O-β-gentiobioside of(20 s)-dammar-23-ene-3β, 7β,20,25-tetraol.
H: a-3-O-β-neohesperidoside-20-O-β-gentiobioside of (20s, 24s and R)-dammar-25-ene-3β,7β,20,24-tetraol.

A systematic study on the seeds of **Luffa aegyptica** (Luffa cylindrica) has been done by Varshney and his group[14,15]. They extracted the seeds with alcohol and obtained three saponins which were shown to be saponins of oleanolic acid (2), gypsogenin (7) and gypsogenin lactone (8) respectively by hydrolytic experiments. Further work on the seeds led these authors [16] to the isolation of two more saponins named aegyptinin A(9) and aegyptinin B(10), the partial structure of which was given on the basis of enzymic and alkaline hydrolysis.



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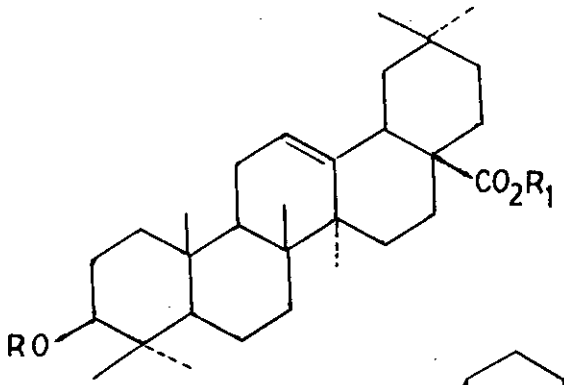

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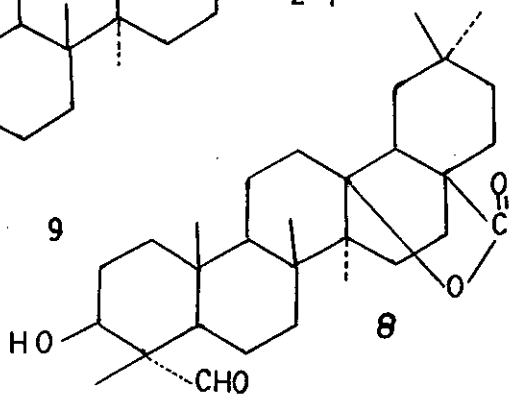

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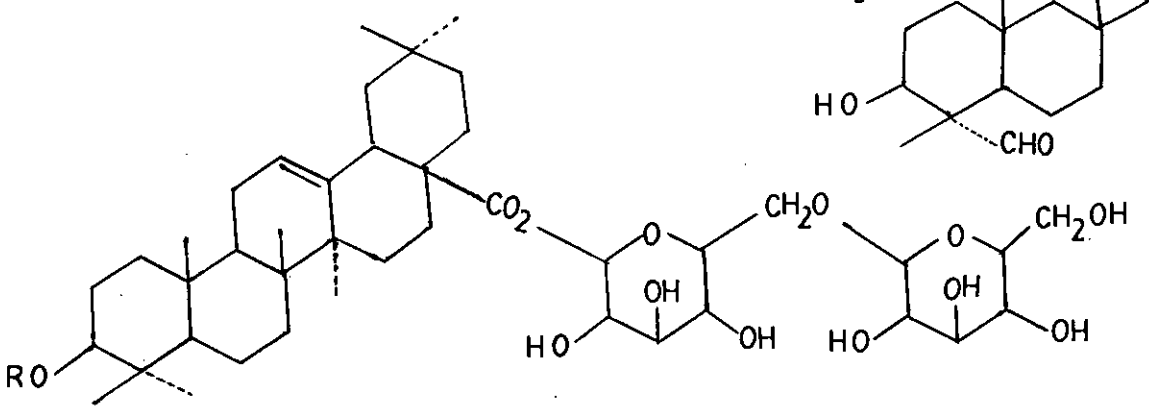
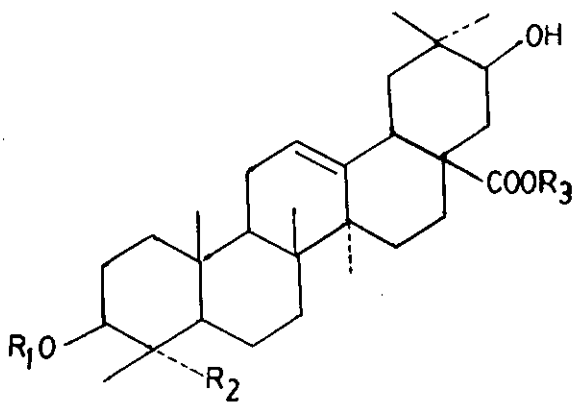


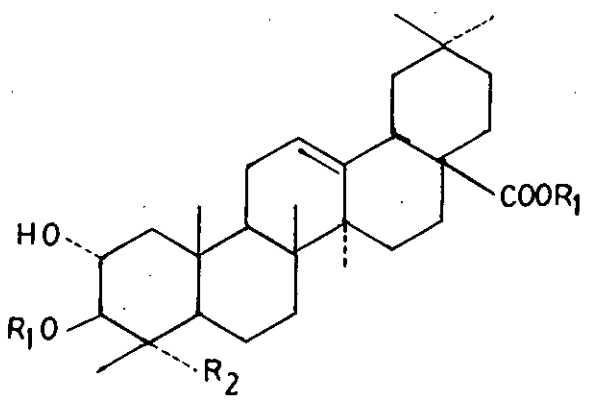
10

	R	R_1	RR_1
9.	glucoronic acid glucose,arabinose xylose,rhamnose	arabinose xylose rhamnose	glucuronic acid glucose,arabinose xylose,rhamnose
10.	glucoronic acid galactose,glucose arabinose, xylose rhamnose.		

In the recent years Arihara[17] and his co-workers have reported a more thorough chemical investigation on Luffa cylindrica. They reported the isolation of eight new saponins named lucyosides A to H (11-18), besides two known saponins named ginsenosides-Re (19) and ginsenosides-Rg_1 (20) from the herb of Luffa cylindrica. Extensive spectral studies enabled them to determine the structures of the compounds and in some cases these

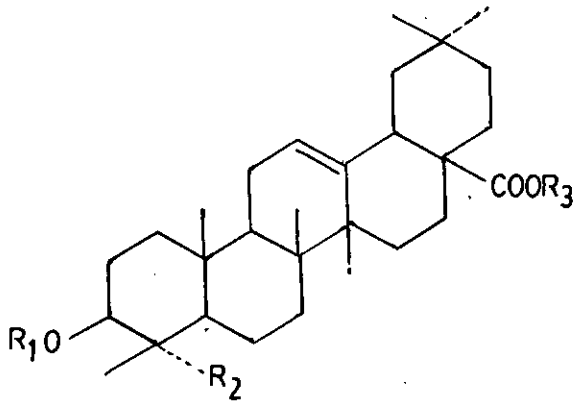
were compared by chemical correlations. Later Arihara[18] and his group reported the separation of another saponin named lucyoside I(21) from the herb of Luffa cylindrica, the structure of which was established to be 3-O-ß-D-glucopyranosyl arjunolic acid. Their investigation on the fruits of Luffa cylindrica led them to the isolation of four new saponins named lucyosides J to M(22-25) along with five known saponins mamed lucyosides A(11), E (15), F(16), 3-O-ß-D-glucopyranosyl hederagenin (26) and 3-O-ß-D-glucopyranosyl oleanolic acid (27). The structures of lucyosides (J-M) were established as 3,28-O-bis-ß-D-gluco-pyranosyl-21ß-hydroxy gypsogenin, 3-O-ß-D-glucopyranosyl gypsogenin, 3-O-ß-sophorosyl-28-O-ß-D-glucopyranosyl gypsogenin and 3-O-(6-acetuxy -ß-O-D-glucopyranosyl)-28-O-ß-D-glucopyranosyl gypsogenin respectively.



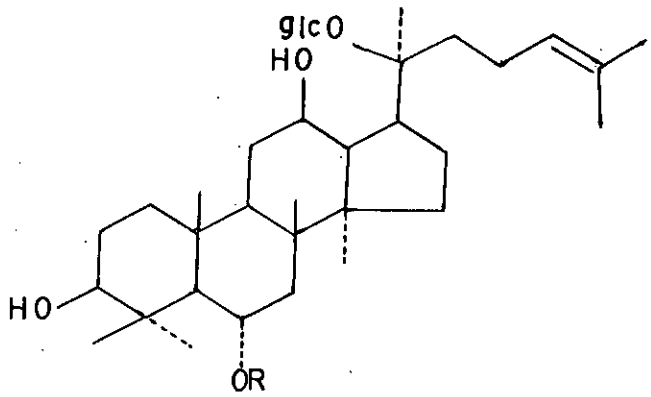


11. R_1, R_3 =glc, $R_2=CH_2OH$
13. R_1, R_3 =glc, $R_3=CH_3$

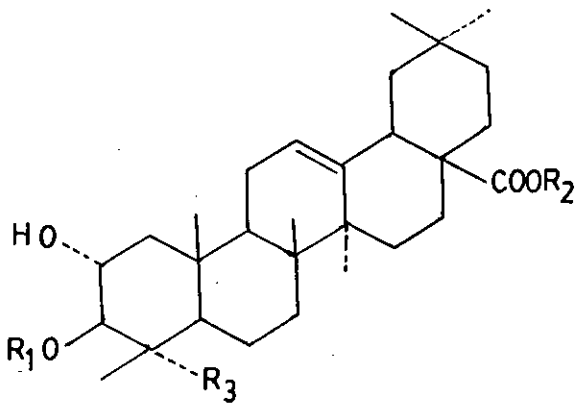
12. R=glc, $R_2=CH_2OH$
14. R_1=glc, $R_2=CHO$
17. R_1=glc, $R_2=CH_3$



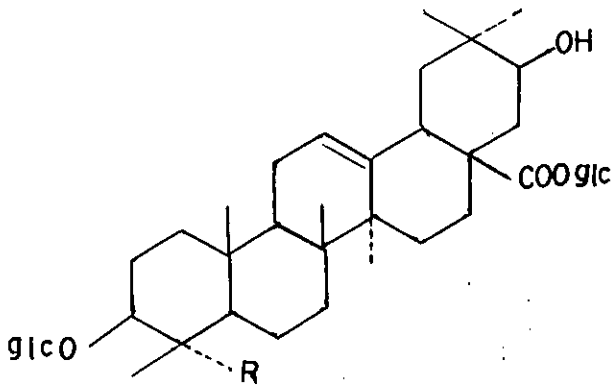
15. R_1, R_3=glc, R_2=CH_2OH
16. R_1, R_3=glc, R_2=CHO
18. R_1, R_3=glc, R_2=CH_3



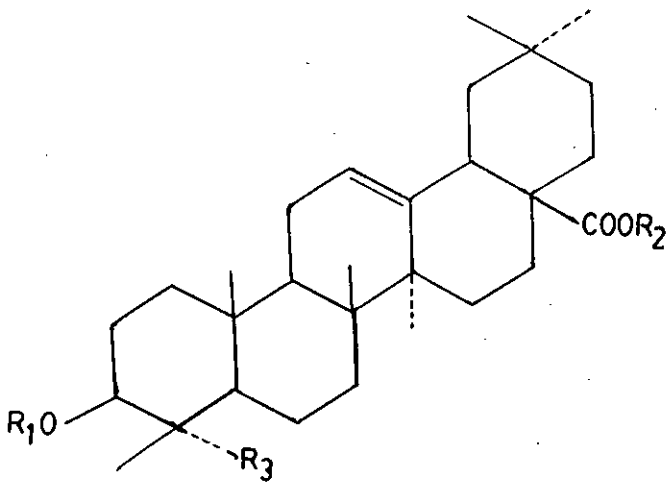
19. R=glc $\frac{2}{}$ rham
20. R=glc



21. R=glc, R_2=H, R_3=CH_2OH



22. R=CHO



23. R_1=glc, R_2=H, R_3=CHO

24. R_1=glc^{2}-glc R_2=glc, R_3=CHO

25. R_1=glc^{6}Ac, R_2=glc, R_3=CHO

26. R_1=glc, R_2=H, R_3=CH_2OH

27. R_1=glc, R_2=H, R_3=CH_3

1.3 Review on Luffa echinata.

Luffa echinata(Luffa amara) is one of the important members of vast group of medicinal plants of Bangladesh and locally known as Ghoshalata. It is a climbing plant, which is met in different parts of the Indian sub-continent. The fruit, for which the plant is grown, matures during the rainy season. Sowing should be made from March to the beginning of June. When the young plants are about 4 inches high, supports should be given for them to climb on.

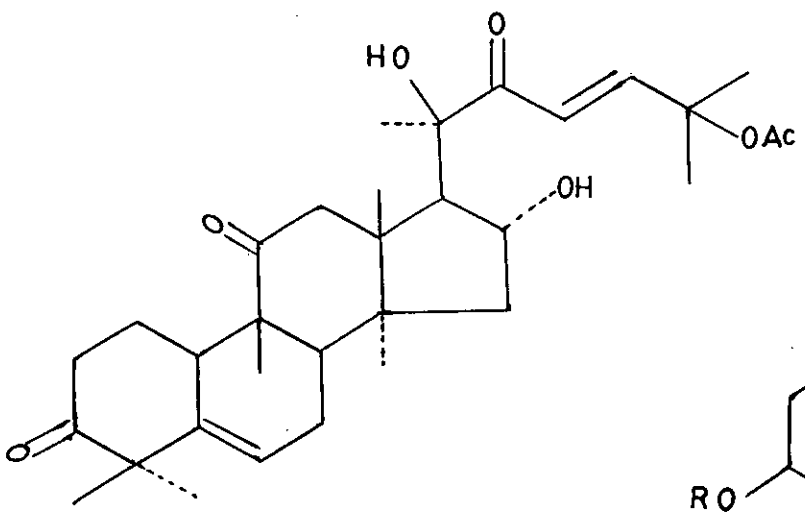
Every part of the plant is bitter and the fruit is mentioned in indigenous medicine as cathertic and emetic [8]. It has a bad taste; cures chronic bronchitis and lung complaints. In the Konkan, a few grains of the bitter fibrous contents of the fruit are given in infusion in cholera after each stool; in putrid fevers, the infusion is applied to the whole body, and in jaundice it is applied to the head and also given internally; the infusion has also a reputation as a remedy for colic. The fruit is considered in North India as a powerful remedy for dropsy. It has also purgative properties.

The plant is reported to be useful in the treatment of leprosy, vaginal tumours, leucoderma, uterine complaints and in conjuction with Hisbiscus rosanencis for gonorrhoea[19].

Every part of the plant, either alone or in combination, has been recommended for the treatment of snake-bite[8]. The root of this plant is laxative, anthelmintic, analgesic; cures tumours, bronchitis, piles, vaginal discharges, jaundice.

Because of its medicinal value different parts of **Luffa echinata** have drawn the attention of Chemists.

Chemical examination of the seeds of **Luffa echinata** by some Indian Chemists resulted in the isolation of cucurbitacin B (1),[20,21,22], 2-deoxy cucurbitacin B(28)[23], β-sitosterol (29) and a saponin.[24]. Acid hydrolysis of the saponin furnished glucose, rhamnose and a sapogenin, which was identified as oleanolic acid (2). They also reported the isolation of two triterpene alcohols which were provisionally named as echinatol A, ($C_{30}H_{52}O_2$)m.p 144°C and echinatol B, ($C_{30}H_{50}O_2$) m.p 166°C respectively. No structure for these two alcohols could be furnished.



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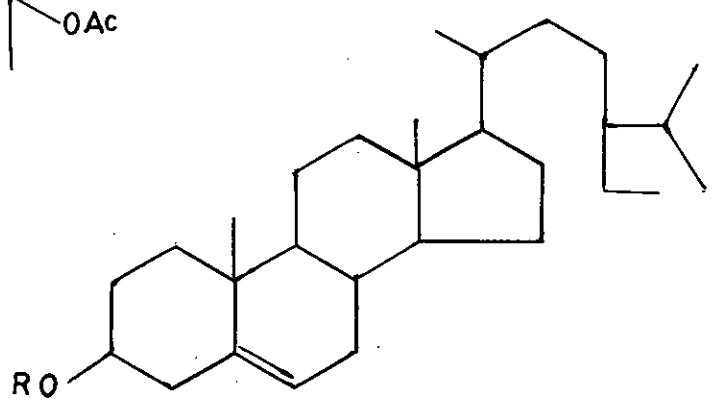
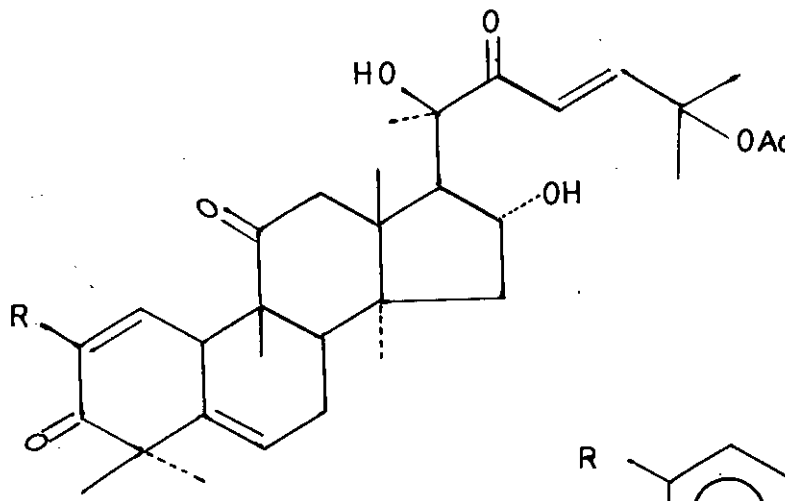


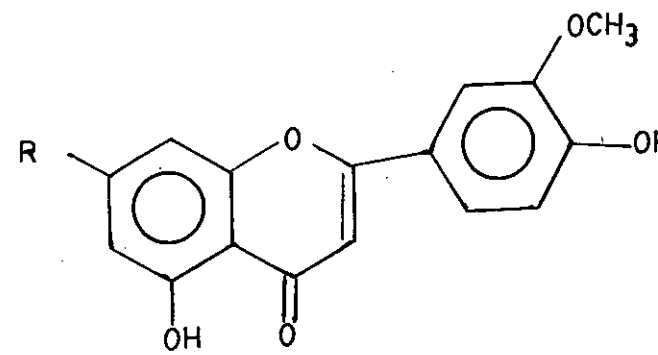
29· R = H
30· R = glc

Khorana [25] et al.carried out chemical investigation on the fruits of Luffa echinata. They obtained an alkane hentriacontane and a saponin from the ethanol extract of the fruits. Acidic hydrolysis of the saponin yielded a sapogenin identified as gypsogenin (7). Glucose, xylose and rhamnose were identified as sugar in the molar ratio of 3:2:1.

A systematic chemical examination was undertaken on the fruits of Luffa echinata by Seshadri and Vydeeswaran [26] in India. They extracted the air dried fruits successively with light petroleum ether, ether, acetone, alcohol and alcohol-water mixture (60:40) respectively and reported the isolation of cucurbitacin E (elaterin) (3), m.p 234°C, isocucurbitacin B,(5) and elaterin glucoside from petroleum ether and ether extract. The structure of elaterin glucoside was established as elaterin 2-O-ß-D-glucopyranoside (32). A flavone glycoside along with elaterin glucoside was identified from acetone extract. Acidic hydrolysis of flavone glucoside yielded chrysoeriol and glucose and its structure was identified as chrysoeriol 7- glucoside (33). Another flavone glycoside, a steroidal glucoside and a flavone were isolated by them from the alcohol extract and their structures were established as chrysoeriol 7-O-ß-D-glucopyranosidyl (2-1) D-apiofuranoside (34), ß-sitosterol glucoside (30) and graveobioside B(35), m.p 212°C respectively. In the aqueous-alcohol extract they identified the presence of apigenin, m.p >310 and luteolin (36).

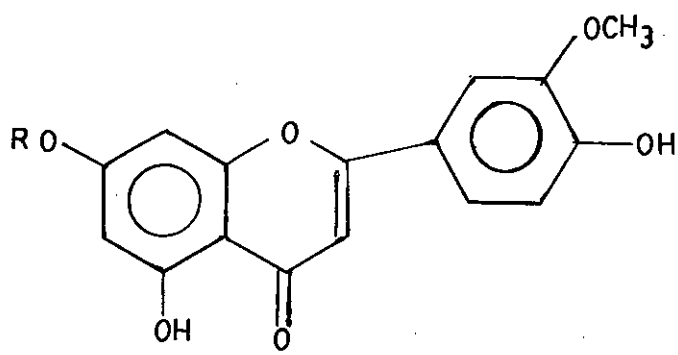


32. R = glc

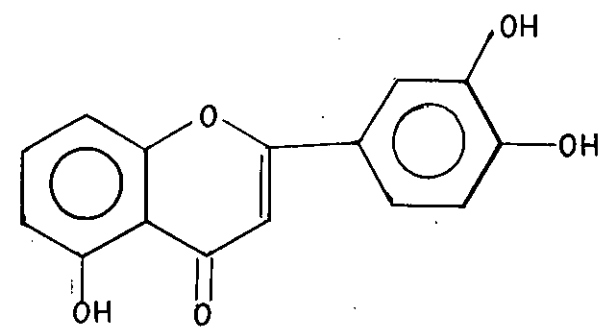


33. R = glc

34. R = $C_{11}H_{19}O_9$



35. R = $C_6H_{11}O_5$



36

The chemical constituents of various Luffa plants are summarized in Table 1; the medicinal uses of the plants are shown in Table 2.

Table 1

Chemical constituents of the plant of the Luffa genus

Species	Part of the plant	Constituents
Luffa echinata	seeds	cucurbitacin B, amarinin
	fruits	cucurbitacin B,cucurbitacin E, isocucurbitacin B,elaterin 2-O-ß-D-gluco pyranoside, two flavonoid glycoside, ß-sitosterol glucoside.
Luffa graveolens	fruits	cucurbitacin B,cucurbitacin E
Luffa operculata	plant	cucurbitacin B, cucurbitacin D,gypsogenin, gypsogenin lactone.
Luffa acutangula	fruits	saponin of oleanolic acid,cucurbitacin B
Luffa cylindrica	seeds	saponin of oleanolic acid,
	fruits & herbs	2-ginsenoside and nine saponins, saponins of hederagenin, gypsogenin, oleanolic acid,arjunolic acid gypsogenin lactone.

Table 2

Medicinal uses of the plant of the Luffa genus

Species	Part of the plant	Medicinal uses
Luffa cylindrica	fruits	laxative, removes biliousness, useful in leprosy, expectorant, piles, fever, haematuria, syphilis, bronchitis, lactagogue (Indo-china), diuretic (Combodia), tumours, and poultice
Luffa cylindrica (bitter variety	seeds	emetic, cathartic
Luffa acutangula	fruits	anthelmintic, stomachic, antipyretic, cures biliousness, asthma, bronchitis, antidote for snake venom.
	leaves	stomachic, antibilious, antipyretic, cure bronchitis, leprosy, ringworm (Combodia).
Luffa echinata	plant	laxative, carminative, cures biliousness, anaemia, liver complaints, leucoderma, bronchitis, ascites, jaundice, tumours, tuberculous glands, vaginal tumours, gonorrhoea, leprosy, emetic, anthelmintic, asthma and piles.
	fruits	bitter tonic, diuretic, skin disease, asthma, purgative, jaundice.
	roots	laxative, anthelmintic, analgesic, cures tumours, bronchitis, piles, vaginal discharges and jaundice.

1.4 Objective of the Research

The plants Luffa echinata and Luffa cylindrica (bitter variety) are two of the important medicinal plants of Bangladesh. They belong to the genus Luffa under the family of Cucurbitaceae. Every part of these plants are bitter and they are reported to possess a variety of medicinal properties[8]. The fruits and seeds are mentioned in indigenous medicine as cathartic and emetic. Luffa echinata is reported to be useful in the treatment of leprosy, vaginal tumours, leucoderma, uterine complaints and in conjunction with Hisbiscus rosanencisfor gonorrhoea[19]. The fruits of Luffa echinata are widely used as remedy for jaundice by the Hakimi dispensary School in Bangladesh.

Different parts of Luffa echinata have been the subjects of chemical investigation for isolation of the active principles. The fruits and seeds have been reported to contain cucurbitacin B(1), cucurbitacin E(3),isocucurbitacin B(5), 2-deoxycucurbitacin B(28), β-sitosterol(29), graveobioside B(35), luteolin(36), and glycosides of oleanolic acid(2), gypsogenin(7), elaterin(32), chrysoeriol(33),(34) and β-sitosterol(30).

The plants of the genus Luffa have been shown to contain physiologically active glycosides. But the work on the glycosides for Luffa echinata appears to be incomplete and no such investigation on the glycoside for Luffa cylindrica (bitter variety) is reported. The present work was therefore undertaken to isolate and characterize the different components including the glycosides of the fruits of Luffa echinata and Luffa cylindrica (bitter variety). Study on pharmacological activity of different extracts of the plants was also planned. The results of the investigations are discussed in this dissertation.

CHAPTER 2

EXPERIMENTAL

2.1 General

Melting points of various compounds were determined by GallenKamp melting point apparatus.

Ultra-violet spectra were recorded in ethanol and chloroform solution on a Shimadzu UV-visible spectrophotometer, Model UV-160A at the Chemistry Department of Dhaka University, Dhaka.

IR spectra were recorded on a Shimadzu spectrophotometer, Model IR 470. Samples were run usually as solid emulsion in nujol mull. The spectra were run at the Chemistry Department of Dhaka University.

^{1}H NMR spectra were recorded by using a varin unit XL-500 NMR spectrometer system in the Department of Organic Chemistry, Chalmers University of Technology, Goteborg, Sweden. In each run, TMS was taken as internal standard during the recording of spectra.

^{13}C NMR spectra were recorded by using a varian unit-XL-500 ^{13}C NRM spectrometer system in the Department of Organic Chemistry, Chalmers University of Technology, Goteborg Sweden.

The mass spectra were recorded on MS 902 spectrometer and on a Vacuum General Micromass 7070 F spectrometer using the direct insertion technique at the BCSIR laboratory, Chittagong.

Thin layer chromatography (TLC)

The glass plates (7.6X2.7 cm) used for TLC were thoroughly cleaned and dried; the glass plates were coated with a layer (0.02 cm) of silica gel by spreading an emulsion of silica gel (8 gm in 16 ml water) with the help of a spreader. These were then allowed to stand for twenty four hours for drying and warmed to 70-85°C before use.

Preparative thin layer chromatography

The preparative glass plates (25 X 23 cm) were cleaned and dried. The plates were coated with a slurry (silica gel 70 gm. mixed with 140 ml of water) with the help of a spreader to yield a coating of 0.5 cm thickness. The plates were left to stand at room temperature until their surface became completely dry; the plates were then heated at 110°C in an electrical oven befor use to increase activation.

Column Chromatography

The chromatographic column was prepared by slurry method using silica gel (70 - 230 mesh. BDH, England) as the stationary phase. The column was half filled with the appropriate solvent (chromatographically pure) and the slurry in the same solvent poured into it so that the packing was compact and uniform. Air bubble was avoided by making the column as fast as possible and allowing the solvent to run drop by drop through the stopcock of the column during packing. The solvent was allowed to run through the column for some time and then the column was ready for use.

Paper Chromatography

Paper chromatography was carried out on Whatmann No.1 paper. For the separation of monosaccharides appropriate solvent mixture was prepared from n-butanol : pyridine : water (10:3:3).

Drops of solutions of substances to be chramatographed were individually applied to the paper by means of a capillary tube and the paper was dried. The paper was then placed in a suitable container so that it can be irrigated with the mobile phase (descending technique) without losses by evaporation. When the solvent travelled the required distance the paper was removed from the container and the paper was dried. Then the paper is soaked in 5% NaOH solution and finally washed by water until the spots became visible.

Drying of pyridine

Laboratory grade pyridine was taken in a quickfit round bottom flask fitted with a reflux condenser which inturn was connected to a calcium chloride guard tube. It was then refluxed for six hours in the presence of sufficient amount of potassium hydroxide pellets and then distilled (b.p.114-115°C) with careful exclusion of moisture. The distilled pyridine was subsequently allowed to stand on solid potassium hydroxide pellets.

Purification of acetic anhydride

Acetic anhydride was distilled before use and the fraction collecting at 138-140°C was used for reactions.

Luffa echinata

2.2 Extraction of the fruits of Luffa echinata.

The dry fruits of Luffa echinata were collected from the local market of Dhaka and were used directly for extraction. The brown dry fruits (800 gm) were soaked in chloroform in a 5 litre container at room temperature. The extract was collected after 48 hours and two more such extractions were carried out at intervals of 48 hours. The combined chloroform extracts (7.5 litres) was evaporated to dryness under reduced pressure at 45°C. This gave a deep green solid mass (40 gm). The crude extract was partially soluble in petroleum ether, n-hexane, methanol; soluble in chloroform, ethyl acetate, diethyl ether but insoluble in water.

2.3 Fractionation of chloroform extract with n-hexane.

The deep green crude chloroform extract was stirred with a large volume of n-hexane at room temperature for 1.5 hours. The n-hexane soluble fraction was collected by filtration and the residue was treated twice more with n-hexane in a similar fashion. The residue left after removal of n-hexane soluble fraction was noted as Mass L (30 gm).

The combined n-hexane extracts was evaported to dryness at reduced pressure when a light green solid mass designated as Mass M (10 gm) was obtained.

2.4 Decolourization of Mass L

The green solid Mass L (30 gm) was dissolved in methanol (300 ml); 5 gm of activated carbon was added to the solution. The

solution was stirred at room temperature for half an hour. The mixture was filtered to remove charcoal when a yellow filtrate was obtained. The filtrate was evaporated to dryness under reduced pressure at 45°C. The process gave a yellowish solid mass (25 gm) which was noted as Mass L_1.

2.5 Examination of Mass L_1

Examination of Mass L_1 on silica gel TLC plates was performed in different solvent systems. A mixture of carbon tetrachloride:ethyl acetate (1:1) proved to be the best solvent for resolution of the components of Mass L_1. In this system three distinct spots were observed with R_f values 0.76, 0.64 and0.61 respectively; a large amount of the crude did not show any movement along the plate.

2.6 Separation of Mass L_1

A solution of Mass L_1 (10 gm) was dissolved in chloroform and adsorbed in silica gel. The mixture was evaporated to dryness and placed on the top of a column of silica gel made in carbon tetrachloride ; elution was carried out with carbon tetrachloride : ethyl acetate (1:1) and finally washed out with ethanol. Fractions of about 5 ml volume were collected at regular intervals and were examined on TLC plates. The results are given in Table 3.

TABLE 3

Column chromatographic separation of Mass L_1

No. of fraction	Eluting solvent	Observation	R_f values	Yield
1-15	CCl_4:EtOAc 1:1	No spot	-	-
16-24	"	One spot	0.76 CCl_4:EtOAc 1:1	40 mg Compound A_1
29-53	"	Two spots	0.64 ; 0.61 CCl_4:EtOAc 1:1	1.5 gm Compound A_2 & A_3
55-105	Ethanol	One distinct spot along with tailing from the base line.	0.62 for the distinct spot benzene:ethyl acetate (1:1)	150 mg Compound A_4 and Mass L_2 8 gm

Fractions 16-24 and 29-53 were separately combined and solvent was removed from them when the former gave compound A_1(40 mg) and the latter a mixture of compounds A_2 and A_3 (1.5 gm).

Mixture of A_2 and A_3(1 50 mg) was subjected to separation by preparative thin layer chromatography (PTLC) using benzene : diethyl ether (1:1) as developing solvent. Extraction of the relevant regions of the plates with diethyl ether gave compound A_2 (85 mg) and compound A_3 (35 mg).

The fractions 55-105 were combined and concentrated to 150 ml under reduced pressure and allowed to stand overnight. A solid separated out as a colourless crystalline solid which was collected by

filtration. The TLC of the solid showed one spot in benzene: ethyl acetate (1:1) with R_f value 0.62. The yield was (150 mg) and the compound was denoted as A_4. The mother liquor left after removal of compound A_4 was evaporated to dryness under reduced pressure which gave solid Mass L_2 (8 gm).

2.6a Examination of Mass L_2

The crude Mass L_2 was soluble in ethanol, methanol but insoluble in petroleum ether and chloroform. TLC of Mass L_2 was performed in different solvent systems on silica plates. The suitable solvent system for the best resolution of the crude was ethyl acetate : ethanol (8:1) in which two distinct spots were observed with R_f values 0.68 and 0.59 respectively; a small amount of the crude did not show any movement along the plate.

2.6b Column chromatographic Separation of Mass L_2

Solid Mass L_2(8 gm) was dissolved in minimum quantity of ethanol and adsorbed in silica gel. The mixture was made free from the solvent and then the dry material was placed on the top of a column of silica gel made in ethyl acetate. The elution was carried out with ethyl acetate : ethanol (8:1). Fractions of about 5 ml volume were collected at regular intervals and were examined on TLC plates. Finally the column was eluted out with methanol. Results of the chromatography are shown in Table 4.

TABLE 4

Column Chromatographic Separation of Mass L_2

No. of fraction	Eluting solvent	Observation	R_f values	Yield
1-12	EtOAc:EtOH 8:1	No spot	-	-
13-33	"	One spot	0.68 EtOAc:EtOH 8:1	3 gm Compound A_5
36-52	"	One spot	0.59 EtOAc:EtOH 8:1	110 mg Compound A_6
53-83	Methanol	Mixture, no distinct spot.	Tailing EtOAc:EtOH (8:1)	4.1 gm Mass L_3

Fractions 13-33 were combined to give a compound noted as A_5(3 gm). Similarly fractions 36-52 gave compound A_6(110 mg). Fractions 53-83 were also combined and evaporated to dryness which gave solid Mass L_3. No further work was done on Mass L_3.

2.7 Characterization of compound A_1

Compound A_1 separated from Mass L_1 was a white crystalline substance, m.p. 198-201°C. It was highly soluble in chloroform, acetone, diethyl ether, but less soluble in ethanol and methanol. TLC examination of the compound A_1 in carbon tetrachloride : ethyl acetate (1:1) gave a single spot with R_f value 0.76. It was recrystallised from hot ethyl acetate and dried when the crystals melted at 209°C.

2.7a Test for Steroids[27]

Salkowski Reaction

A few mg of compound A_1 was dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. A reddish colour developed indicating the presence of steroidal compound.

Liebermann- Burchard Reaction

A few mg of compound A_1 was dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by 2-3 drops of acetic anhydride. A slightly greenish colour developed indicating the presence of steroidal material

2.7b Test for Diosphenol[28]

An aqueous solution of ferric chloride (2.5%) was slowly added to the solution of compound A_1 in ethanol. The colour of the solution slowly changed yellow to violet which indicated the presence of diosphenolic system in compound A_1.

2.7c Spectral data

UV ($CHCl_3$) λ_{max}:251 and 268 nm (shifted to 247 and 316 nm in NaOH)

IR (nujol) ν_{max}: 3500, 3450, 3360 (weak and sharp, O-H), 1715, 1675, 1620 (strong and sharp, >C=O), 1290, 1255, 1235 and 1125 cm^{-1}(strong and sharp, C-O).

^{1}H NMR (mixture $CDCl_3$ and CD_3OD)δ: 7.00 (d,1H), 6.67 (d,1H), 5.99 (d,1H), 5.80 (s,1H), 6.45 (m,1H), 3.27 (s,2H), 2.10 (s,3H), 1.60 (s,3H), 1.55 (s,3H), 1.48 (s,3H), 1.45 (s,3H), 1.31 (s,3H), 1.25 (s,3H), 1.05 (s,3H), and 0.91(s.3H).

^{13}C NMR(mixture $CDCl_3$ and CD_3OD)δ : 214.16, 203.10, 198.40, 170.17, 150.32, 144.81, 136.49, 120.68, 120.15, 114.90, 79.36, 78.47, 70.39, 57.95, 57.81, 50.02, 48.64, 44.93, 41.49, 38.51, 34.35, 27.13, 25.64, 25.25, 23.52, 23.41, 23.07, 20,89, 19.44, 19.15, 19.09, 17.58.

Mass Spectrum m/e: 496 (M-60), 478, 463, 401, 358, 341, 315, 281, 231, 219, 203, 164, 149, 111, 96 (base peak), 87, 67, 60. and 43. .cw12

2.7d Acetylation of Compound A_1

A solution of compound A_1 (10 mg) in anhydrous pyridine (0.5 ml) and acetic anhydride (2 ml) was stirred overnight at room temperature. The contents of the flask were then poured into 50 ml of ice-water and stirred vigoriously to assist the hydrolysis of unreacted acetic anhydride. After about 30 minutes the solution was extracted with diethyl ether. The dried diethyl ether fraction on evaportion under reduced pressure gave a solid mass (8 mg). It was crystallised from diethyl ether and dried. The purified acetate melted at 119°C.

2.7e Hydrolysis of Compound A_1

A solution of compound A_1 (10 mg) in methanol (2 ml) was treated with aqueous sodium carbonate (0.1M, 0.5 ml) and stirred overnight at room temperature. The mixture was neutralized with acetic acid and extracted with ethyl acetate. Evaporation of the ethyl acetate solution gave a residue (6 mg) which was crystallised from diethyl ether-petroleum ether (4 mg). The crystalline hydrolysed product melted at 149°C.

2.8 Characterization of compound A_2

Compound A_2 separated from Mass L_1 was a white crystalline solid, m.p. 170°C. It was highly soluble in chloroform, acetone, diethyl ether but less soluble in ethanol and methanol. The compound was further purified by recrystallisation from ethyl acetate, m.p. 182°C.

Compund A_2 gave positive test for steroid and triterpenoid.

$\overline{UV}\lambda_{max}$ ($CHCl_3$): 245.5 and 280 nm (shifted to 245 and 310.5 nm in NaOH after 24 hours).

IR (KBr)ν_{max}: 3550, 3475, 3300 (strong and sharp, O-H), 3030(weak and sharp, =C-H), 2970, 2880 (strong and weak, C-H), 1710, 1680 (broad and strong, >C=O) 1610 (strong and sharp, >C=O), 1180, 1125, 1085 cm^{-1} (strong and sharp, C-O)

^1H NMR ($CDCl_3$)δ: 710 (d,1H), 6.44 (d,1H), 5.80 (d,1H, 4.42 (m,1H),4.28 (t,1H), 4.24 (s,2H), 3.60 (d,1H) 3.21 (d,1H), 2.10 (s,3H), 1.60 (s,3H), 1.49 (s,3H), 1.42 (s,3H), 1.29 (s,3H), 1.28(s,3H), 1.25 (s,3H), 1.10 (s,3H) and 1.00 (s,3H)

^{13}C NMR ($CDCl_3$)δ : 213.02, 212.11, 202.47, 170.21, 151.94, 140.34, 120.42, 120.27, 79.29, 78.22, 71.62, 71.25, 58.17, 50.66, 50.21, 48.62, 48.41, 48.08, 45.30, 42.35, 35.98, 33.71, 29.40, 29.34, 25.93, 23.91, 23.85, 21.92, 21.23, 20.03, 19.83 and 18.88.

Mass spectrum m/e: 498 (M-60), 480, 455, 403, 385, 367, 325, 283, 219, 187, 159, 133, 113, 96 (base peak) 87, 67 and 43.

2.8a Acetylation of compound A_2

Compound A_2 (10 mg) was dissolved in acetic anhydride (3 ml) and dry pyridine (1.5 ml) and the mixture was refluxed on a water bath for 12 hours and worked up in the usual manner. The acetate (6 mg) was recrystallised from ethyl acetate, m.p 130°C.

2.9 Characterization of compound A_3

Compound A_3 also separated from Mass L_1 was a white crystalline substance, m.p 223°C. It was highly soluble in chloroform, acetone, diethyl ether and ethyl acetate. Compound A_3 was recrystallised from methanol, m.p 229°C.

UV λ_{max} $CHCl_3$: 245.5 nm

IR (KBr) ν_{max} : 3550, 3475, 3350 (strong and sharp, O-H). 3050 (weak and sharp, =C-H), 2960, 2940, 2895, 2820 (strong and sharp, C-H), 1715, 1680, 1618 (strong and sharp, >C=O) 1180, 1120 and 1100 cm^{-1} (strong and sharp, C-O).

^{1}H NMR ($CDCl_3$) δ: 7.11 (d,1H), 6.45 (d,1H) 5.81 (d,1H), 4.4 (m,1H), 4.25 (t,1H),4.23 (s,2H), 3.61 (d, 1H), 3.20 (d,1H) 2.11

(s,3H), 1.60 (s,3H), 1.49 (s,3H), 1.41 (s,3H), 1.29 (s,3H) 1.27 (s,3H), 1.26 (s,3H) 1.11 (s,3H) and 1.01 (s,3H).

^{13}C NMR ($CDCl_3$) δ: 213.02, 212.11, 202.44, 170.21, 151.49, 140.34, 120.42, 120.27, 79.29, 78.22, 71.62, 71.25, 58.17, 50.66, 50.21, 48.62, 48.41, 48.08, 45.30, 42.35, 35.98, 33.71, 29.34, 26.40, 25.93, 23.91, 23.85, 21.92, 21.23, 20.03, 19.83 and 18.88

Mass spectrum m/e: 498 (M-60), 480, 455, 403, 385, 367, 325, 309, 283, 257, 237, 219, 203, 189, 164, 135, 113, 96 (base peak) 43.

2.9a Acetylation of compound A_3

Compound A_3 (10 mg) was dissolved in a mixture of acetic anhydride (3 ml) and dry pyridine (1.5 ml). The mixture was heated on a water bath for 12 hours and worked up in the usual manner. The acetate (7 mg) was crystallised from methanol m.p 196°C.

2.10 Characterization of Compound A_4

Compound A_4 was a colourless crystalline solid, also separated from Mass L_2, m.p 287°C. It was soluble in chloroform, ethyl acetate but sparingly soluble in ethanol and methanol. Compound A_4 was recrystallised from ethanol, m.p 290°C. The compound on shaking with water gave soapy bubbles indicating it to be a saponin.

2.10a Test for sugar[29]

Molisch's Test

A few mg of compound A_4 was dissolved in methanol- water mixture and 2 drops of a 10% solution of a-naphthol in ethanol were added to it ; 1 ml of concentrated sulfuric acid was poured below the aqueous phase. A red violet ring appeared at the interface which indicated the positive test for sugar.

Resorcinol Test

Compound A_4 (20 mg) was dissolved in 1 ml of a 0.1% solution of resorcinol in water. Then 2 ml of concentrated H_2SO_4 was placed on the top of the mixture. An orange to red zone appeared at the interface which indicated the presence of sugar in compound A_4.

2.10b Spectral data

IR (KBr) ν_{max} :3375 (strong and sharp, O-H), 2925, 2860 (strong and sharp, C-H), 1160, 1105, 1070 and 1030 cm^{-1} strong and sharp, C-O).

^1H NMR (mixture $CDCl_3$ and CD_3OD) δ: 5.14(d. 1H), 4.41 (d,1H), 3.83 (q,1H), 3.70 (m,1H), 3.4 (t,1H), 3.32 (t, 2H), 3.24 (m,1H), 3.21 (t,1H), 0.84 (s,3H), 0.83 (s,3H), 0.82 (s, 3H), 0.81 (s,3H), 0.54 (s,3H) and 0.53 (s, 3H).

Mass spectrum m/e: 414 (M-162), 399, 397, 396, 381, 273, 271, 255, 229, 201, 173, 161, 147, 119, 107, 81, 69, and 55 (base peak).

2.10c Hydrolysis of Compound A_4

50 mg of compound A_4 was dissolved in 8% methanolic hydrochloric acid and refluxed for 12 hours. The mixture was diluted with 100 ml water . On cooling the aglycon separated out as a crystalline solid. The aglycon (30 mg) was separated by filtration and then purified by crystallisation from ethanol, m.p 135°C.

The filtrate was neutralized with dilute ammonia and concentrated to 25 ml under reduced pressure. Paper chromatography of the aqueous part in n-butanol: pyridine: water (10:3:3) indicated the presence of glucose.

2.10d Acetylation of Aglycon

Aglycon (20 mg) was dissolved in acetic anhydride (5 ml) and dry pyridine (2 ml). The mixture was refuxed for 12 hours and worked-up in the usual manner; the acetate (20 mg) was recrystallised from ethanol m.p 129°C.

2.11 Characterization of Compound A_5

Compound A_5 (3 gm) obtained from Mass L_2 was an amorphous solid mass. It was highly soluble in ethanol,methanol but less soluble in chloroform and ethyl acetate.Compound A_5 (100mg) was recrystallised from hot (45°C) ethyl acetate. The crystals were dried under vacuum for 10 hours at 50°C ; melted at 145°C.

Compound A_5 gave positive Salkowski and Liebermann-Burchard tests for steroids.

Compound A_5 gave negative test for phenol with ferric chloride.

Compound A_5 gave positive Molisch's and Resorcinol tests for sugar.

Compound A_5 gave copious foam on shaking with water which indicated the positive test for saponin.

UV (EtOH) λ_{max} : 229 nm (did not show any shift in NaOH).

IR (KBr) ν_{max}: 3400 (strong and broad O-H) 2970, 2910 (strong and sharp, C-H), 1710, 1680 1620 (strong and sharp, >C=O), 1260, 1120 and 1080 cm^{-1} (strong and sharp C-O)

^1H NMR (CD_3OD) δ: 6.95 (d,1H), 6.85 (d,1H), 4.35 (d,1H), 3.2-3.4 (m,6H), 2.6 (t,1H), 2.10 (s,2H), 2.00 (s, 3H), 1.55 (s,3H), 1.54 (s,3H), 1.45 (s,3H), 1.40 (s,3H), 1.35 (s.3H), 1.30 (s,3H). 1.05 (s,3H) and 0.90 (s, 3H).

^{13}C NMR (CD_3OD) δ: 215.99, 213.35, 205.46 172.04, 151.53, 141.60, 122.64, 1212.42, 104.27, 81.11, 80.30, 79.65, 78.16, 77.88, 75.40, 71.82, 71.46, 62.87, 60.21, 52.43, 52.43, 51.74, 49.90, 46.49, 44.11, 35.92, 35.00, 29.37, 26.79, 26.49, 25.54, 24.83, 21.90, 21.90, 21.80, 20.72, 20.09 and 19.44.

2.11a Hydrolysis of compound A_5

Compound A_5 (20 mg) was dissolved in 8% methanolic hydrochloric acid and refluxed for 12 hours. The mixture was diluted with 100 ml water. After cooling the aglycon separated out as crystalline solid. After filtration the aglycon (15 mg) was purified by crystallisation from ethyl acetate.

The aqueous part of the reaction was neutralized by dilute ammonia and concentrated to 25 ml under reduced pressure. Paper chromatography of the aqueous part in n-butanol: pyridine : water (10:3:3) indicated the presence of glucose.

The aglycon was obtained as a white crystalline solid. It was recrystallised from ethyl acetate and melted at 185°C. The aglycon was highly soluble in chloroform, diethyl ether but sparingly soluble in methanol. In solvent system (1:1) benzene : diethyl ether it had R_f value 0.69.

UV ($CHCl_3$) λ_{max} : 280 nm shifted to 310.5 nm in NaOH after 24 hours.

IR (nujol) ν_{max}: 3400 (strong and sharp, O-H) 1710, 1680, 1620 cm^{-1} (strong and sharp, > C=O).

2.12 Characterization of Compound A_6

Compound A_6 (120 mg) was also separated from Mass L_2. It was soluble in methanol, ethanol but insoluble in petroleum ether. Compound A_6 was crystallised from ethyl acetate, m.p 170°C.

Compound A_6 gave positive Salkowski and Liebermann-Burchard test for steroid.

Compound A_6 gave positive Molisch's and Resorcinol test for sugar.

Compound A_6 gave copious foam on shaking with water which indicated it to be a saponin.

Compound A_6 gave negative test for phenol with ferrie chloride.

UV (EtOH) λ_{max} : 211 nm.

IR (nujol) ν_{max} : 3380 (strong and sharp, O-H), 1675, 1625 (weak and broad, >C=O) 1110, 1080 cm^{-1} (weak and broad, C-O).

2.12a Hydrolysis of Compond A_6

Compound A_6 was hydrolysed with methanolic HCl (as compound A_5) to give an aglycon which was crystallised from methanol, m.p 75°C.

Paper chromatography of the aqueous part revealed the presence of glucose.

The aglycon was soluble in chloroform, diethyl ether, ethyl acetate but sparingly soluble in petroleum ether and methanol. TLC examination of the aglycon in petroleum ether : ethyl acetate (1:1) solvent system gave a single spot with R_f 0.65.

UV ($CHCl_3$) λ_{max}: 268 nm, shifted to 315 nm in the presence of NaOH.

2.13 Pharmacological studies of the ethanol extract of Luffa echinata.

The crude ethanol extract of **Luffa echinata** was tested on guinea-pig atria,tracheal chain,ileum and rat uterus tissue preparations. The work was done at the Department of Pharmacology,Faculty of Medicine,Chiang mai University,Thailand.

Effect on isolated guinea-pig atria.

The preparation set up according to method described in "Pharmacological experiments on isolated preparation"[30] Guinea-pigs of either sex,weighing between 300-600 gms,were sacrificed by giving a blow on the head and the heart was rapidly removed. The atria was separated from the ventricles and suspended vertically in organ bath containing 20 ml of Feigens solution at 30°C and bubbled vigorously with 100% oxygen. A resting tension of 1 gm was placed on the atria. The atrial rate and force of contraction was recorded on a Grass model 7D polygraph via a force-displacement transducer(Grass FT 03B).The atria was allowed to equilibrate for one hour.After this period,test drugs were added to the preparation in various doses.The contact time was three minutes and the preparation was washed out at least three times until a constant rate and force were obtained.

Effect on isolated guinea-pig Tracheal chain preparation.

The preparation was formulated by the method of Castillo and De Beer[31]. Guinea-pigs of either sex weighing between 300 - 600 gms, were sacrificed by a blow on the head. Their tracheas were removed and were carefully trimmed of excess fat and connective tissue. They were cut transversely between the segments of cartilage to obtain a number of chains, each consisting of at least 5 rings. Each preparation was placed on a 10 ml organ bath containing oxygenated Kreb's solution maintained at 37°C. Mechanical responses were recorded isometrically via a force displacement transducer (Grss FT 03B, Grass Instruments Co.

Quincy , Mass, USA) and displaced on a polygraph (79D, polygraph, Grass Instrument Co. Quincy, Mass. USA). The chains were maintained under the resting tension of 0.75 gm. and allowed to equilibrate in the organ bath for a period of 2 hours. Histamine(0.1 μg/ml) was used for contraction.

Effect on isolated Guinea- pig ileum.

The preparation of ileum was based on the method described by Blattner[32] . To observe the effects of the test drugs on ileal contractions, various spasmogens at submaximal concentrations, i.e acetye choline (Ach 0.06 μg/ml), histamine (HA, 0.1 μg/ml), serotomin (5-HT, 5 μg/ml) and barium chloride (Ba^{+2} 0.2 mg/ml), were carried out.

Effect on isolated rat uterus.

In this experiment, the method of De Jalone et al.[33] was used. Virgin sprague-Dawley rats, weighing 180-200 gms, were treated with estradiol benzoat (1 mg/kg; i.m) 24 hours before sacrifice by means of spinal shock. The abdomen was opened and the uterine horns were excised into strips of 1 cm long. The strips were mounted on a 10 ml organ bath filled with De Jalone's solution at 30-32°C and aerated with 100% Oxygen. A resting tension of approximately 0.5 mg. was applied to the strip and isometric contractions were recorded by means of a force displacement transducer (Grass FT 03B) and displayed on a polygraph (Grass model 79D). After a equilibration period of 30 minutes, the uterine contractions were induced by acetyl choline

(0.8 μgm/ml). The test and the reference drugs were added and allowed to come in contact with the strips for a period of 3 minutes before the inducer was given.

Luffa cylindrica

2.14 Extraction of the fruits of Luffa Cylindrica.

The fruits of Luffa cylindrica (bitter variety) were collected from Rajshahi, a town about 300 km. north of Dhaka. The deep green fruits were cut into pieces and dried in the sun. The dry sliced fruits were subjected to extraction. The sliced fruits (500 gm) were soaked in chloroform (1 litre) at room temperature for 48 hours and two more such chloroform extracts (2 litres) were collected in the similar fashion. The combined chloroform extract was evaporated to dryness under reduced pressure at 45°C when a light green solid mass (2.5 gm) was obtained which was designated as Mass N_1.

2.15 Examination of Mass N_1

The light green solid Mass N_1 was soluble in chloroform, diethyl ether and acetone. TLC of Mass N_1 in carbon tetrachloride : ethyl acetate (10:1) gave the best resolution of the components of Mass N_1 and two distinct spots with R_f values 0.81 and 0.54 were observed in this system. The compounds corresponding to R_f values 0.81 and 0.54 were disignated as compound B_1 and B_2. A small amount of crude did not show any movement from the starting point and was noted as Mass N_2.

2.16 Column Chromatographic Separation of Mass N_1

A solution of Mass N_1 (2.5 gm) dissolved in chloroform was chromatographed over a column of silica gel made in carbon tetrachloride : ethyl acetate (10:1) and finally washed out with ethyl acetate. Fractions of about 5 ml volume were collected at

regular intervals and were examined on TLC plates. The results are given in Table 5.

Table 5

Column chromatographic Separation of Mass N_1

No.of fraction	Eluting solvent	Observation	Rf values of the Components	yield
1-9	CCl_4:CH_3OAc 10:1	No spot	-	-
10-16	"	one spot	0.81 CCl_4:EtOAc 10:1	30 mg Compd.B_1
17-25	"	Two spots	0.81 & 0.54 CCl_4:EtOAc (10:1)	150 mg Compd.B_1 Compd.B_2
26-35	"	One spot	0.54 CCl_4:EtOAc	280 mg Compd.B_2
38-52	EtOAc	Tailing	-	1.5 gm

Fractions 10-16 and 26-35 were separately combined and solvent was removed from the two. In the process each of them gave one compound which were not identical. These were designated as compound B_1(30 mg) and compound B_2 (280 mg). Fractions 17-25 and 38-52 were also separately combined and concentrated to dryness under reduced pressure. Both of them were allowed to stand in the refrigerator. No further work was done on these fractions.

2.17 Characterization of Compound B_1

Compound B_1 was a colourless amorphous solid, m.p 84-87°C.It was highly soluble in petroleum ether, chloroform, diethyl ether but less soluble in ethanol and methanol. It gave a single spot with R_f value 0.81 in carbon tetrachloride : ethyl acetate (10:1).

IR (KBr) ν_{max}: 3425 (strong and sharp, O-H) 2900, 2850 (strong and sharp, C-H), 1460 (strong and sharp, C-H) 730, 725 (strong and sharp, = C-H) cm^{-1}

^1H NMR ($CDCl_3$) δ: 3.64 (t,2H), 2.30 (t,2H), 1.53-1.60 (m,2H), 1.25 (s,H) and 0.87 (t,3H).

Mass spectrum m/e: 464, 450, 438, 420, 407, 393, 379, 365, 351, 337, 323, 309, 295, 281, 267, 253, 225, 221, 197, 183, 169, 155, 141, 125, 113,111, 99, 97, 85, 83, 71, and 57 (base peak).

2.18 Characterization of Compound B_2

Compound B_2 was soluble in chloroform and diethyl ether but sparingly soluble in ethanol and methanol. Compound B_2 (280 mg) was dissolved in minimum quantity of hot ethanol (50°C) for recrystallisation. Compound B_2 (230 mg) separated out as white crystalline solid which was dried under vacuum for 10 hours at 50°C. The compound melted at 167°C.

Compound B_2 gave positive Salkowski and Liebermann-Burchard tests for steroid.

IR (nujol ν_{max}: 3405 (strong and sharp, O-H), 1640 (strong and sharp, C-C), 1045, 1040 (strong and sharp C-O) cm^{-1}.

^{1}H NMR ($CDCl_3$) δ: 515 (m, 2H), 3.60 (m,1H), 1.05 (s, 3H), 1.03 (s, 3H), 0.85 (,3H), 0.75 (s,3H), 0.70 (s,3H) and 0.55 (s,3H).

^{13}C NMR ($CDCl_3$) δ: 139.57, 138.11, 129.53, 117.48, 71.07, 55.93, 55.15, 51.24, 49.49, 43.31, 40.74, 40.30,39.50, 38,04, 37.18,34.25, 31.83, 39.50, 38.04, 37.18, 34.25, 31.83, 31.52, 29.66, 28.39, 25.39, 23.02, 21.57, 21.35, 20.91, 16.96, 13.04, 12.42 and 12.07.

Mass spectrum m/e: 412 (M^+), 397, 370, 271, 264, 255, 229, 213, 161, 147, 119, 107, 81, 69, 57 and 55 (base peak).

2.18a Acetylation of Compound B_2

Compound B_2 (20 mg) was dissolved in acetic anhydride (3 ml) and dry prydine (1.5 ml). The mixture was heated on a water bath for 12 hours and workedup in the usual manner. The acetate (15 mg) was crystallised from ethyl acetate, which melted at 172°C.

IR (mujol) ν_{max} : 1730 (strong and sharp, >C-O), 1240 (strong and sharp, C-O) cm^{-1}.

CHAPTER 3

RESULTS AND DISCUSSION

The fruits of Luffa echinata were collected from the local market of Dhaka and were used directly for extraction. The dry fruits (800 gm) were extracted with chloroform (3X48 hours) at room temperature. The chloroform extracts yielded a deep green solid mass (40 gm) on removal of the solvent. The chloroform extract was exhaustively triturated with n-hexane at room temperature when a small part of it went into solution.

The n-hexane insoluble green residue (30 gm), designated as Mass L,on decolourization with activated charcoal yielded a yellowish solid mass(25 gm) which was noted as Mass L_1.

Mass L_1 showed the presence of at least six compounds on TLC plates. Column chromatographic separation of the crude over silica gel with carbon tetrachloride : ethyl acetate (1:1) as eluant gave one pure compound (compoundA_1), a mixture of two compounds (compound A_2 & compound A_3) and a fraction which on concentration yielded a crystalline solid (compound A_4). The mother liquor of the last fraction on evaporation gave a residue (Mass L_2).

Compound A_2 and A_3 were separated by preparative TLC.

Mass L_2 was subjected to a second chromatographic separation over silica gel using ethyl acetate : ethanol (8:1) as eluting solvent. This process yielded compound A_5 and compound A_6 and a polar material (4.1 gm) which was not studied further.

The extraction and the isolation of the compounds from the fruits are shown in the flow sheet diagram (Fig.1).

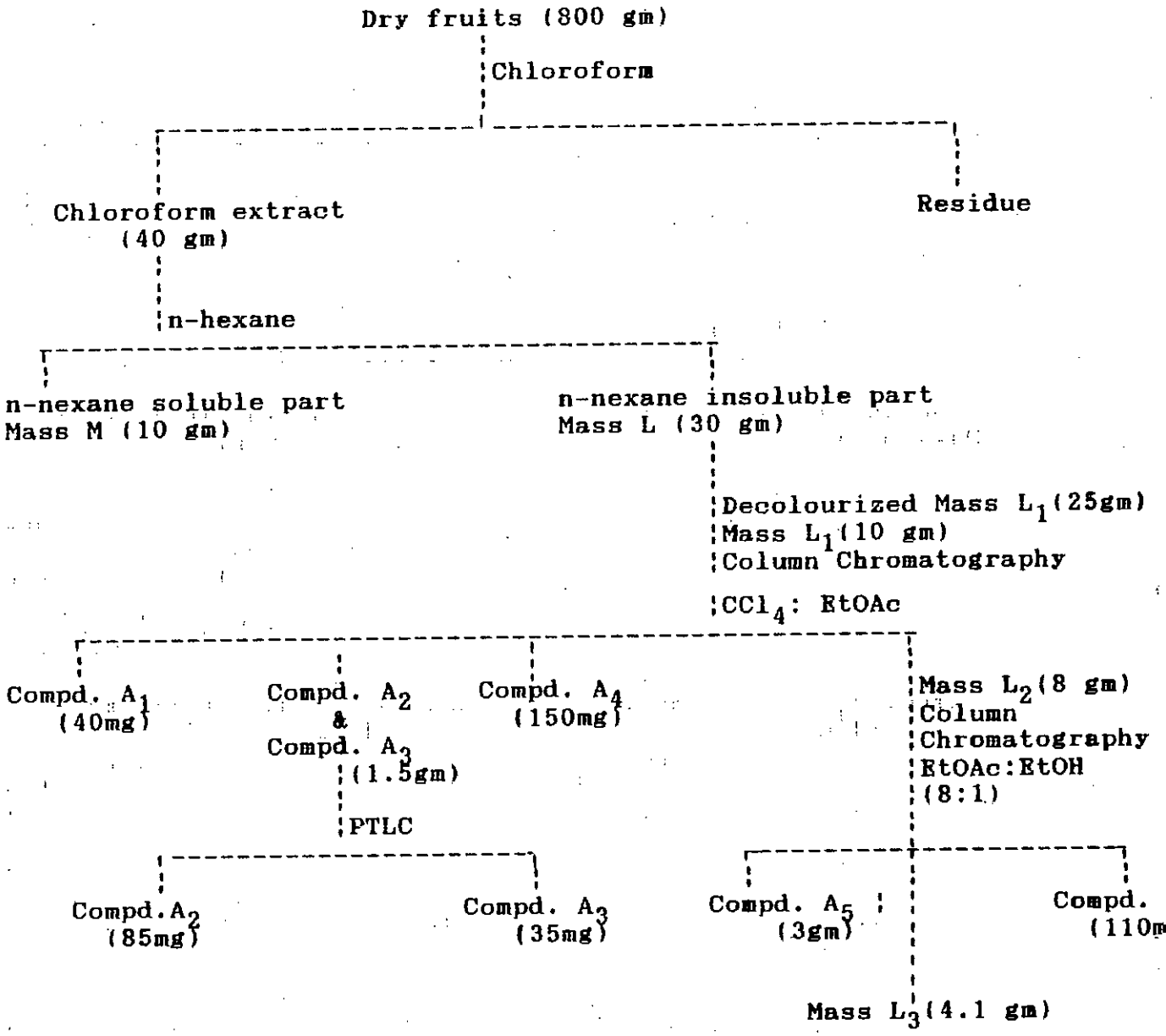


Fig.1 Flow sheet diagram of extracton and isolation process.

3.1 Study on Compound A_1

Compound A_1 was obtained as a white crystalline solid, m.p 198-201°C,(yield 0.013%). On recrystallisation from ethyl acetate followed by drying under high vacuum at 80°C for 10 hours, the m.p of the compound rose to 209°C.

The compound gave Salkowski and Liebermann-Burchard reaction for steroid and triterpenes. The IR spectrum of the compound (Fig.2) showed sharp absorptions for O-H stretching at 3500,3450 and 3360 cm^{-1}. It also showed strong absorptions at 1715 and 1675 cm^{-1}which could be attributed to >C=0 function of an ester group and to that of an α,β- unsaturated ketone respectively. The presence of an α,β- unsaturated keto group was confirmed by UV absorption at λ_{max} 251 nm (Fig.3). Earlier studies on Luffa echinata[26] had revealed that it contains a number of cucurbitacin triterpenes, namely cucurbitacin E, cucurbitacin B, iso cucurbitacin B and glycoside of cucurbitacin E. Attempt was made to correlate compound A_1 an ester showing the highest mass ion at m/e 496 (Fig 4) which can be considered to be the M-60 peak in relation to these of the reported ones. This is in line with the observation that cucurbitacins A, B, and C which contain one acetate group do not show the molecular ion peak but instead the M-60 peak due to the loss of the elements of acetic acid from these molecules[34]. The proposed molecular weight of compound A_1 556(496+60) corroborates with the molecular formula $C_{32}H_{44}O_8$, similar to that of cucurbitacin E.

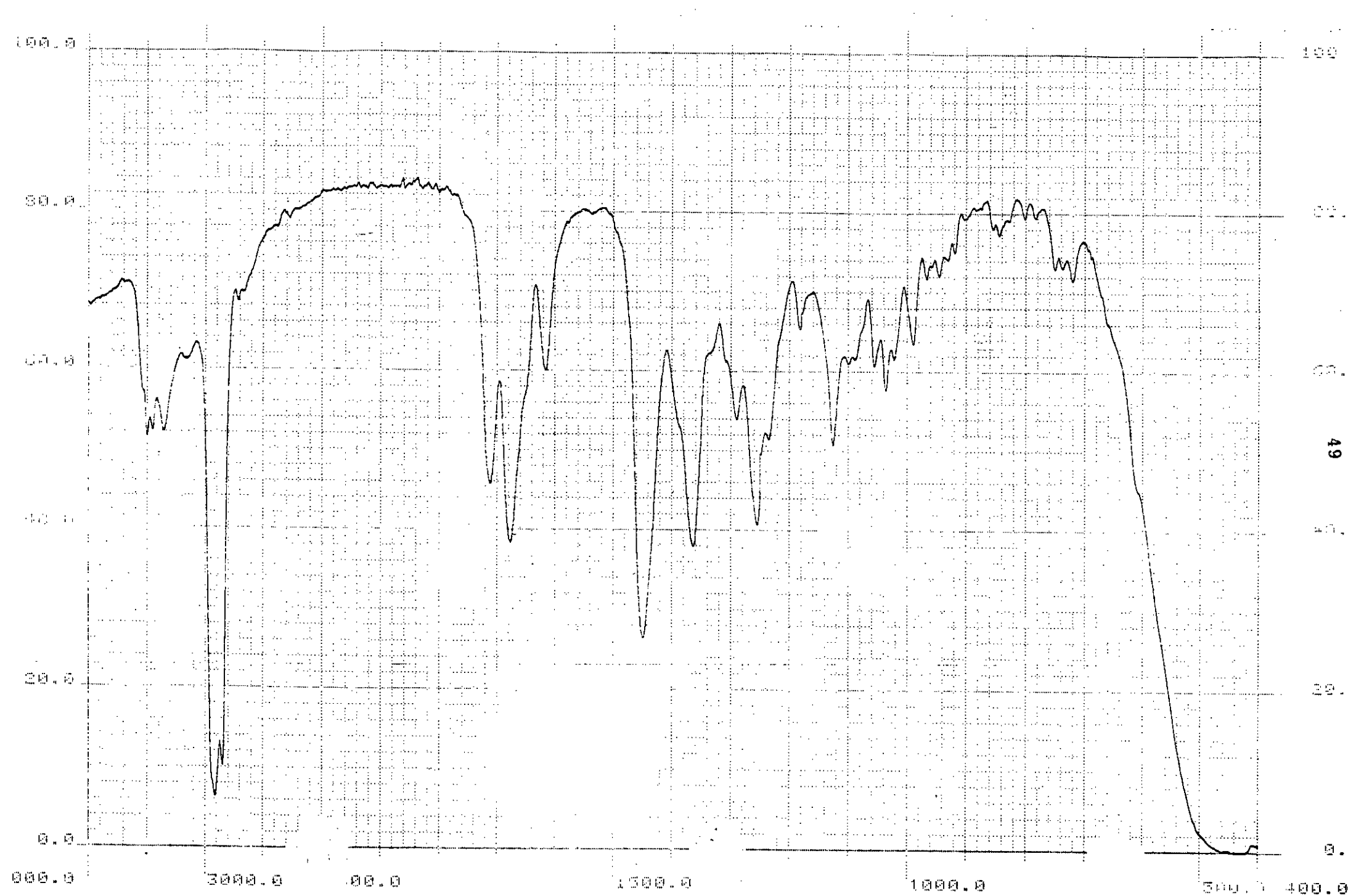
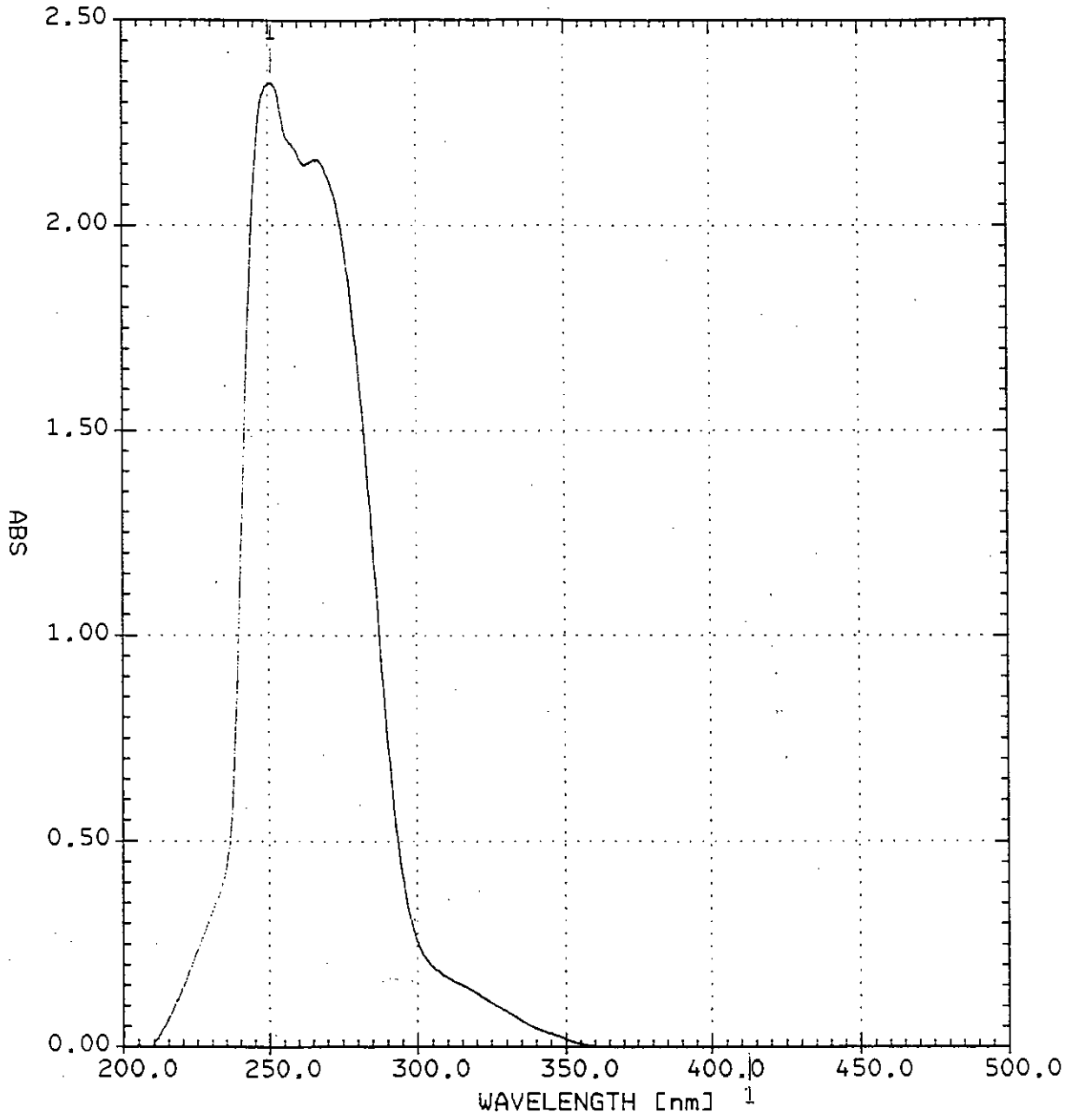


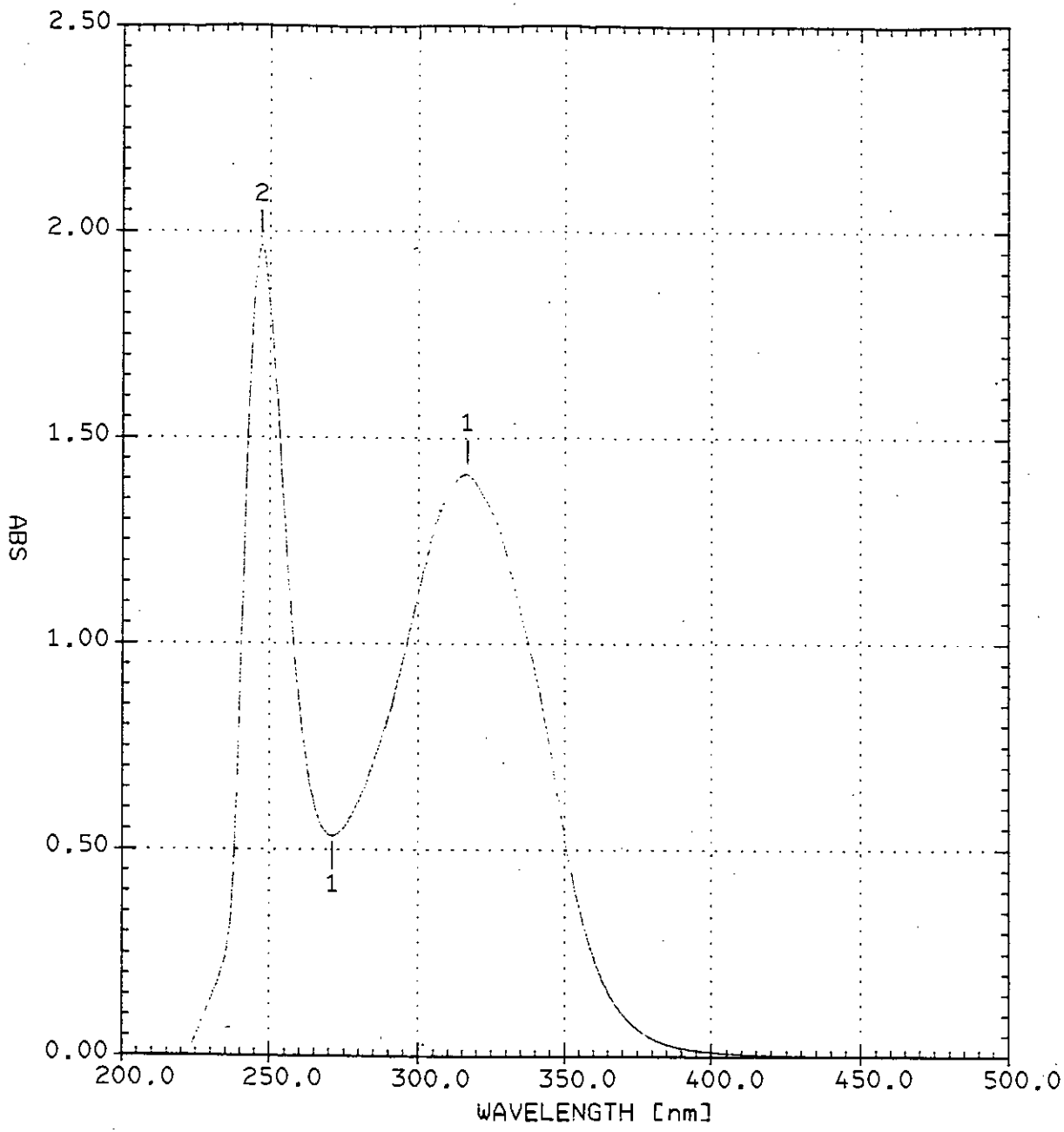
Fig. 2 IR spectrum of Compound A_1 in nujol



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	251.0	2.349	412.5	-0.013

Fig. 3 UV Spectrum of Compound A_1 in $CHCl_3$



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	316.5	1.412	271.0	0.531
2	247.0	1.969		

Fig. 3.1 UV Spectrum of Compound A_1 (in NaOH)

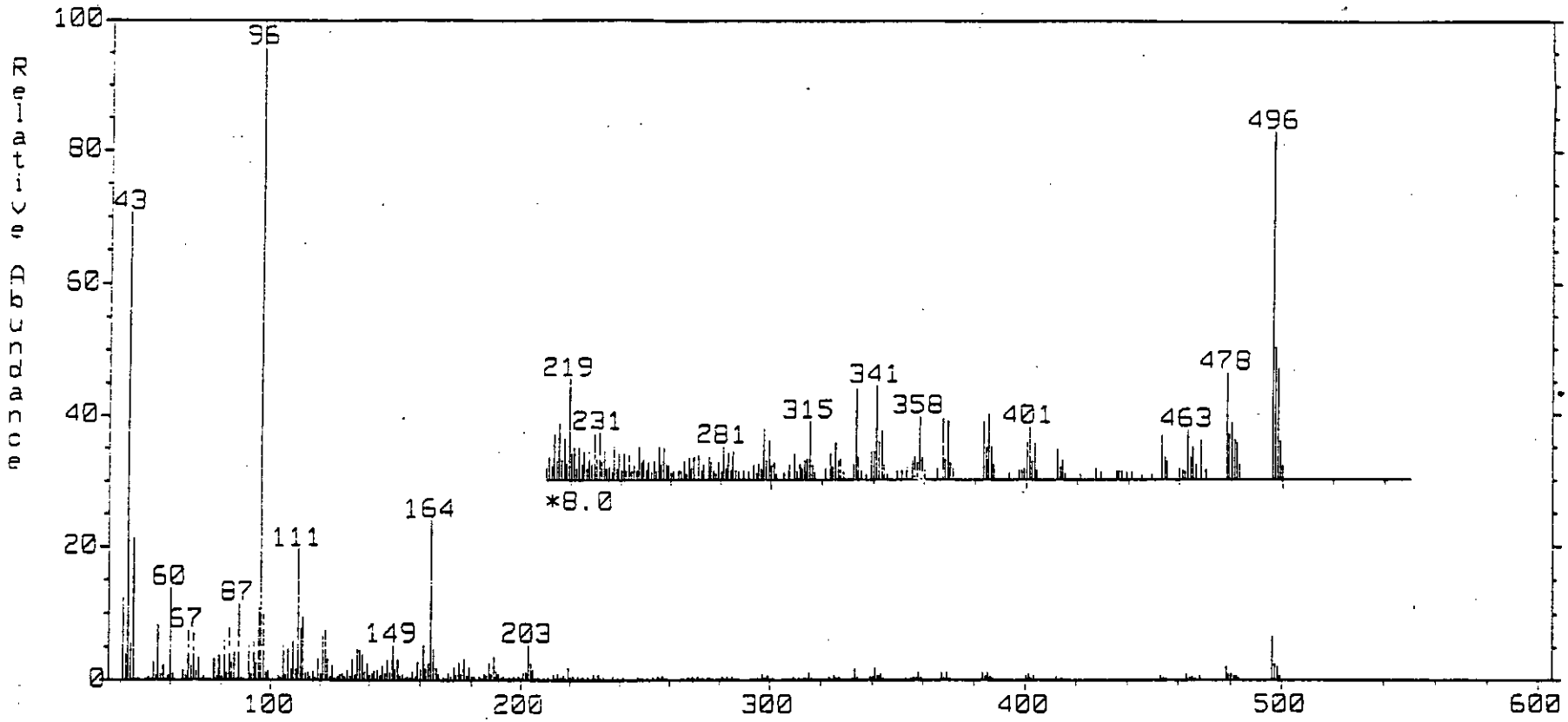


Fig. 4 Mass Spectrum of Compound A_1

The number of carbon atoms is borne out by ^{13}C NMR spectrum,(Fig.5) which exhibited as many as 32 peaks like cucurbitacin E. Compound A_1 gave positive response to ferric chloride test, showed an absorption at 1620 cm^{-1} in the IR and an absorption at 268 nm in the UV spectrum. It also showed a one proton doublet at δ 5.99 in the ^1H NMR spectrum (Fig.6) and a peak at m/e 164 in the mass spectrum like cucurbitacin E. All these data suggest that compound A_1 contains a diosphenol system in ring A like that in cucurbitacin E[28]. The shift of the UV maximum at 268 nm to 316 nm in alkaline solution further confirmed the presence of an easily enolizable a-diketone as in cucurbitacin E[35].

The identical mass spectral fragmentation pattern m/e 96 (base peak), 111,164,219,315,341,358,401,463,478 and 496 of compound A_1 and cucurbitacin E clearly indicated compoun A_1 to be very similar to cucurbitacin E. The reported ^1H NMR data of cucurbitacin E were also similar to those of compound A_1[36]. However cucurbitacin E is known to give molecular ion peak at m/e 556 and morever it has a different melting point (235°C) with that of compound A_1 .Therefore compound A_1 was different from cucurbitacin E. It was then presumed that the difference between compound A_1 (37) and cucurbitacin E(38)might be due to the disposition of the acetate moiety.

The melting point of compound A_1 was however found to be similar to that of datiscacin, a cucurbitacin -20-acetate ester darivative isolated from a chloroform extract of **Datisca glomerata** Baill[28].

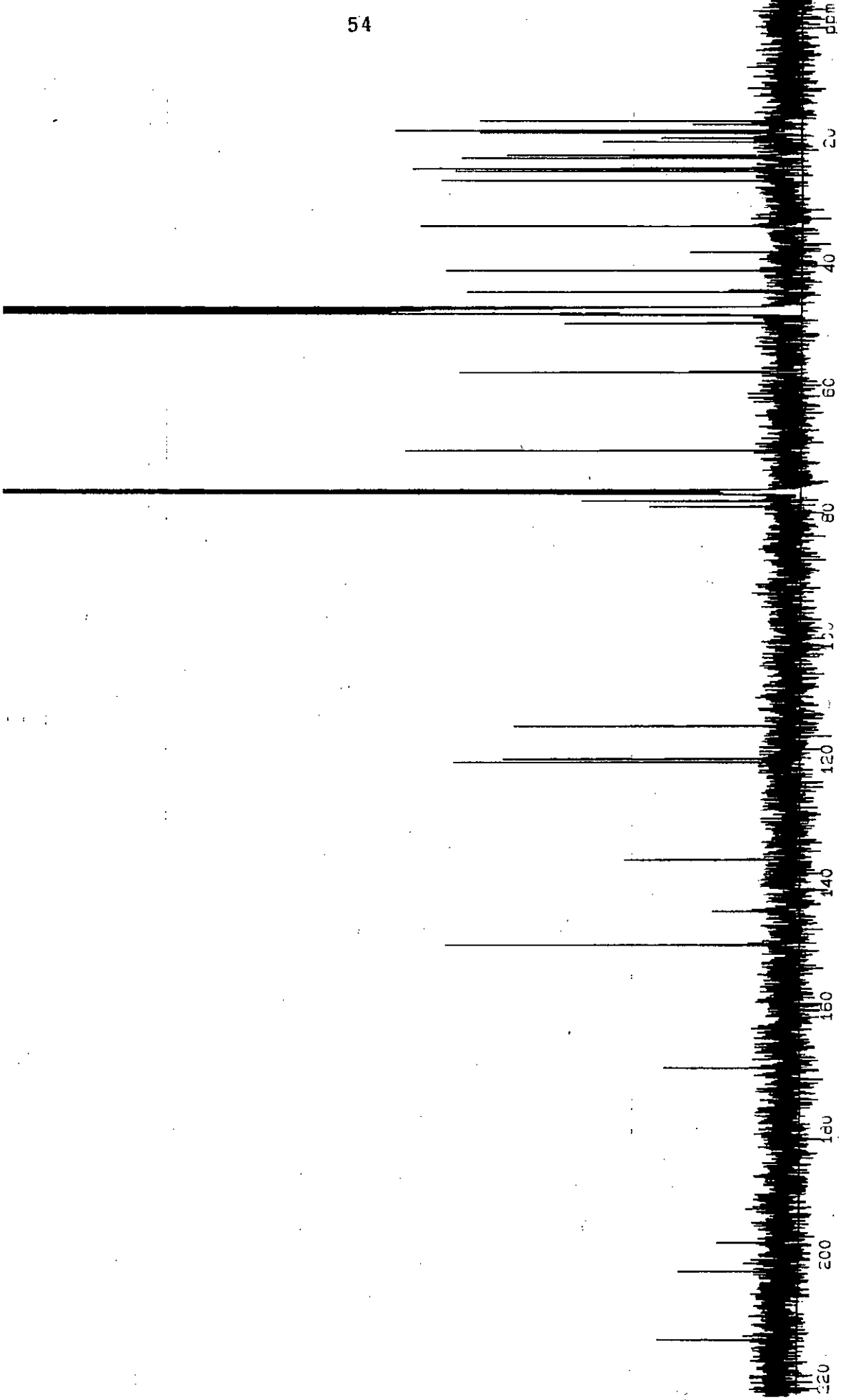


Fig. 5 ^{13}C NMR Spectrum of Compound A_1 in mixture of $CDCl_3$ & CD_3OD

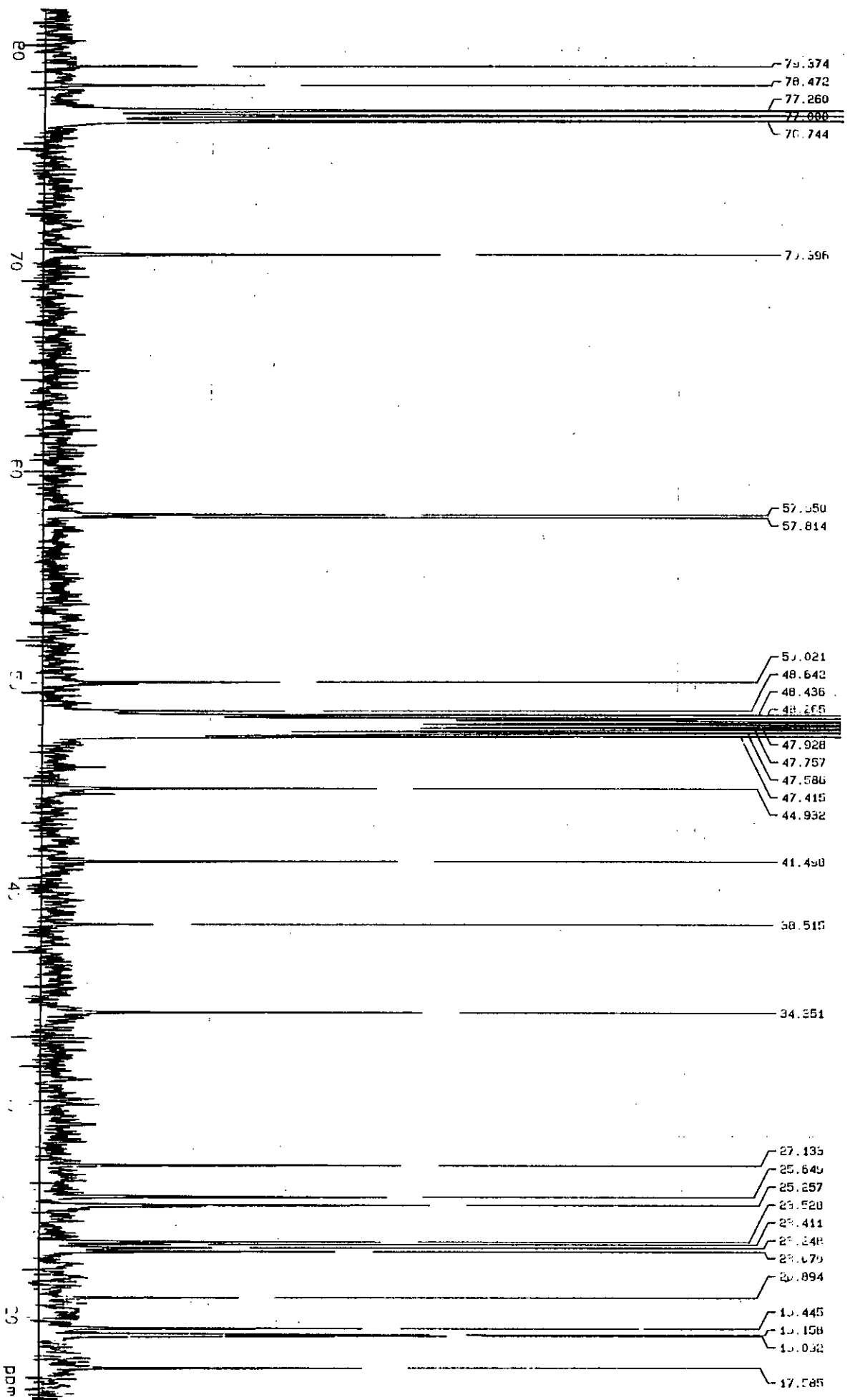


Fig. 5.1 ^{13}C NMR Spectrum of Compound A_1 in mixture of $CDCl_3$ & CD_3OD

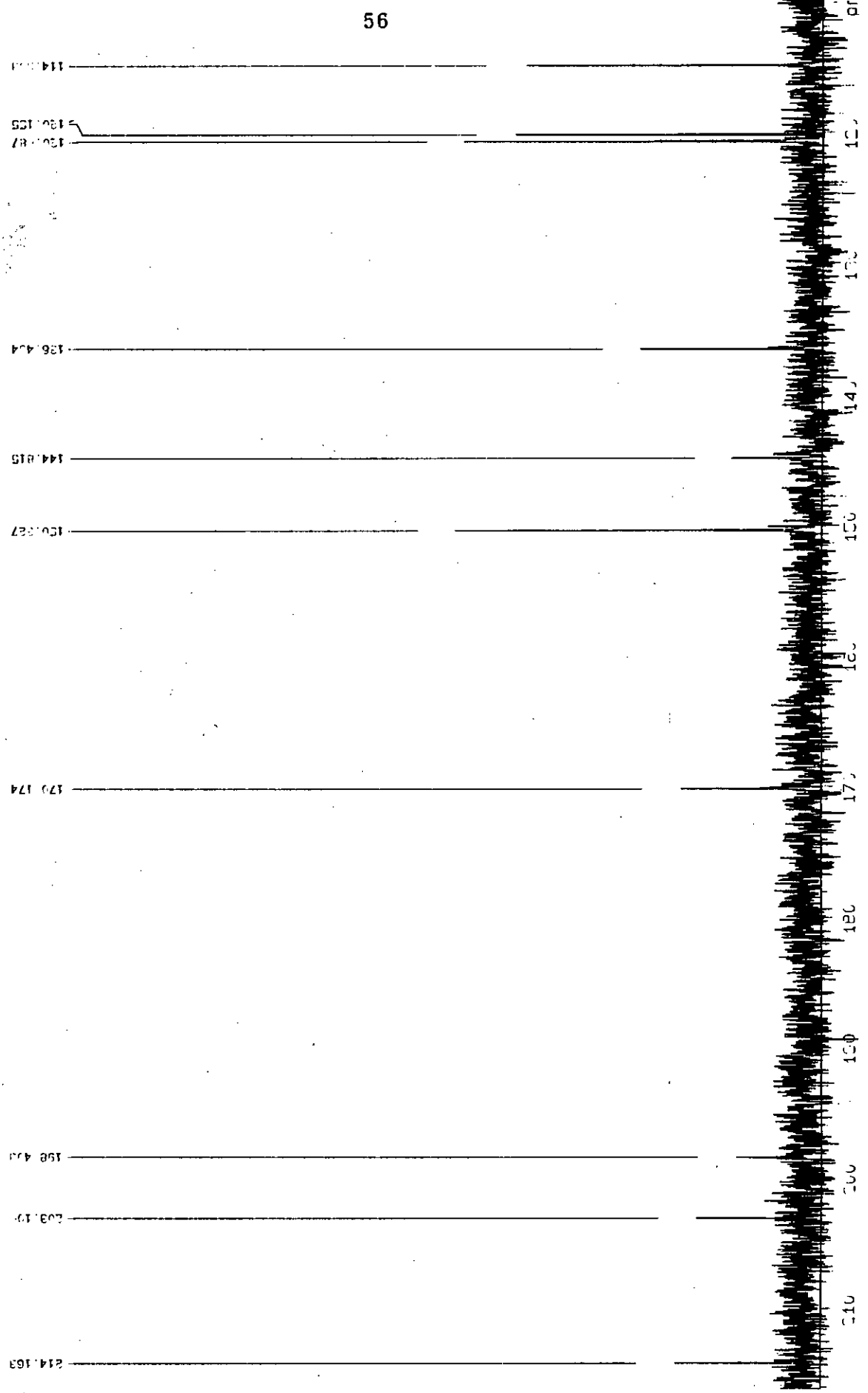
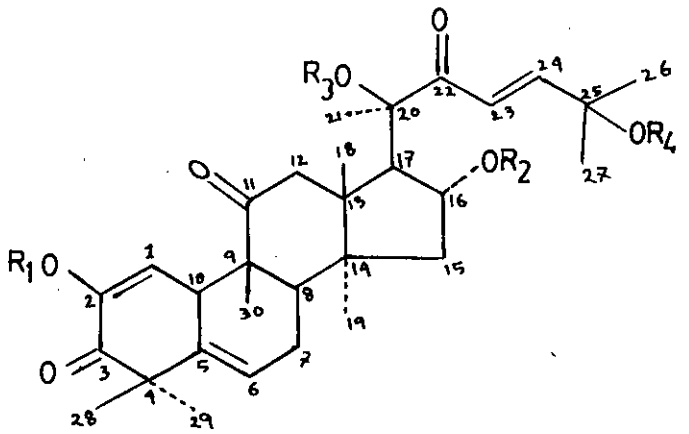


Fig. 5.2 ^{13}C NMR Spectrum of Compound A_1 in mixture of $CDCl_3$ & CD_3OD



37. $R_1=R_2=R_4=H$; $R_3=Ac$
38. $R_1=R_2=R_3=H$; $R_4=Ac$
39. $R_1=R_2=R_3=H$; $R_4=H$
40. $R_1=R_2=R_3=Ac$; $R_4=H$

The proton NMR spectrum of compound A_1 was identical to that of datiscacin[28]. The doublets at δ 7.0, 6.67, 5.99 and a singlet at δ 5.80, each equivalent to one proton are appropriate for olefinic protons at carbon C_{24}, C_{23}, C_1 and C_6 respectively. Protons at C_{16} and C_{12} appeared as multiplet at δ 4.45 (1H) and a singlet at δ 3.27 (2H) respectively. The eight methyl protons appeared as three protons singlets at δ 1.60, 1.55, 1.48, 1.45, 1.31, 1.25, 1.05 and 0.91. The acetate methyl protons appeared as singlet at δ 2.10.

The mass spectrum of compound A_1 was also similar to that of datiscacin where the latter also did not show any molecular ion peak[28]. All these suggested that compound A_1 is in fact datiscacin (37). The ^{13}C NMR spectrum of compound A_1 accommodates the relevant carbon atoms of the molecule, (Table 3).

The identity of compound A_1 was finally confirmed by preparing diacetate of compound A_1 and hydrolysis of compound A_1. The diacetate of compound A_1 (40) possesses similar melting point, 119°C as that reported for diacetate of datiscacin[28].

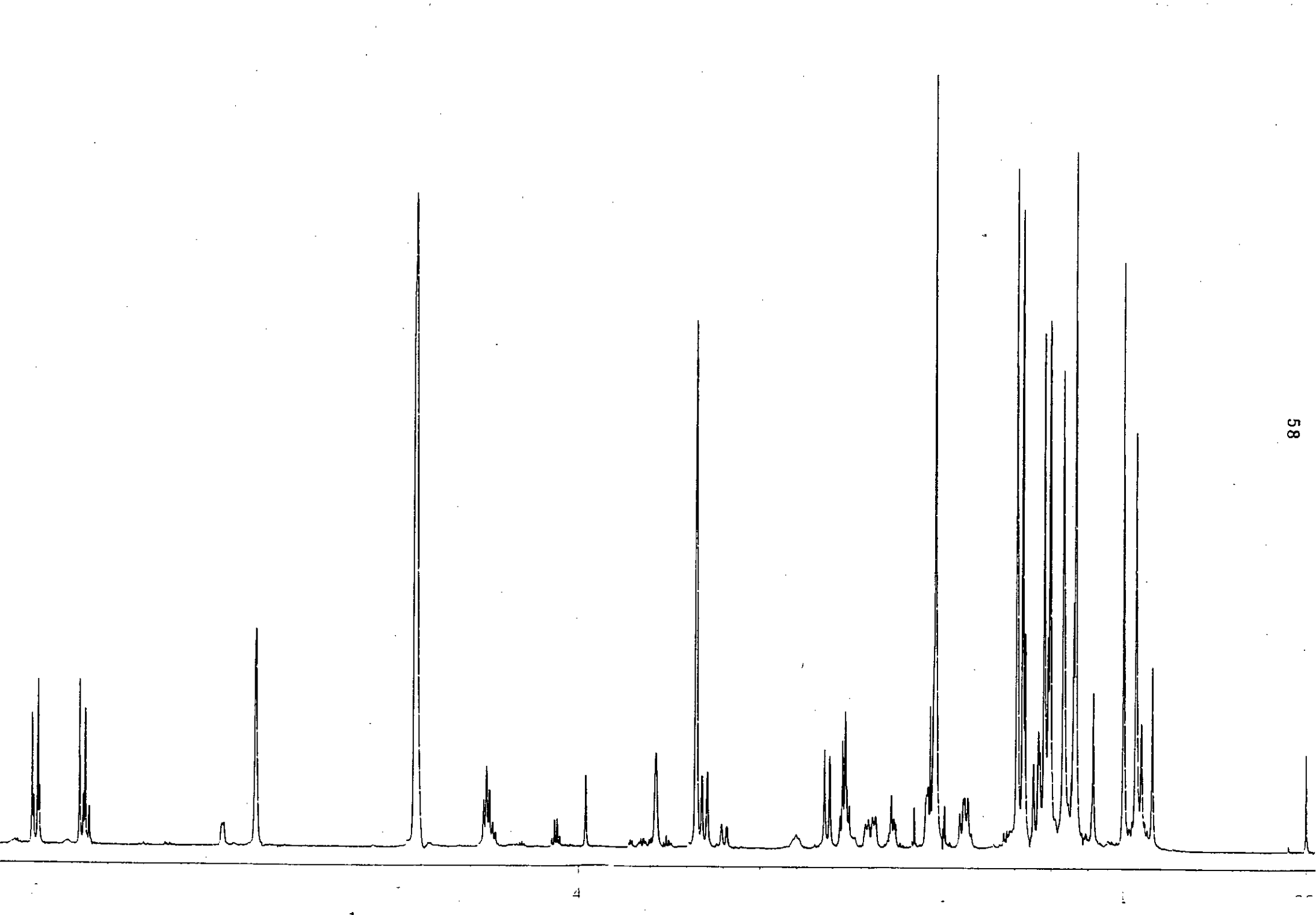


Fig. 6 ^{1}H NMR Spectrum of Compound A_1 in mixture of $CDCl_3$ & CD_3OD

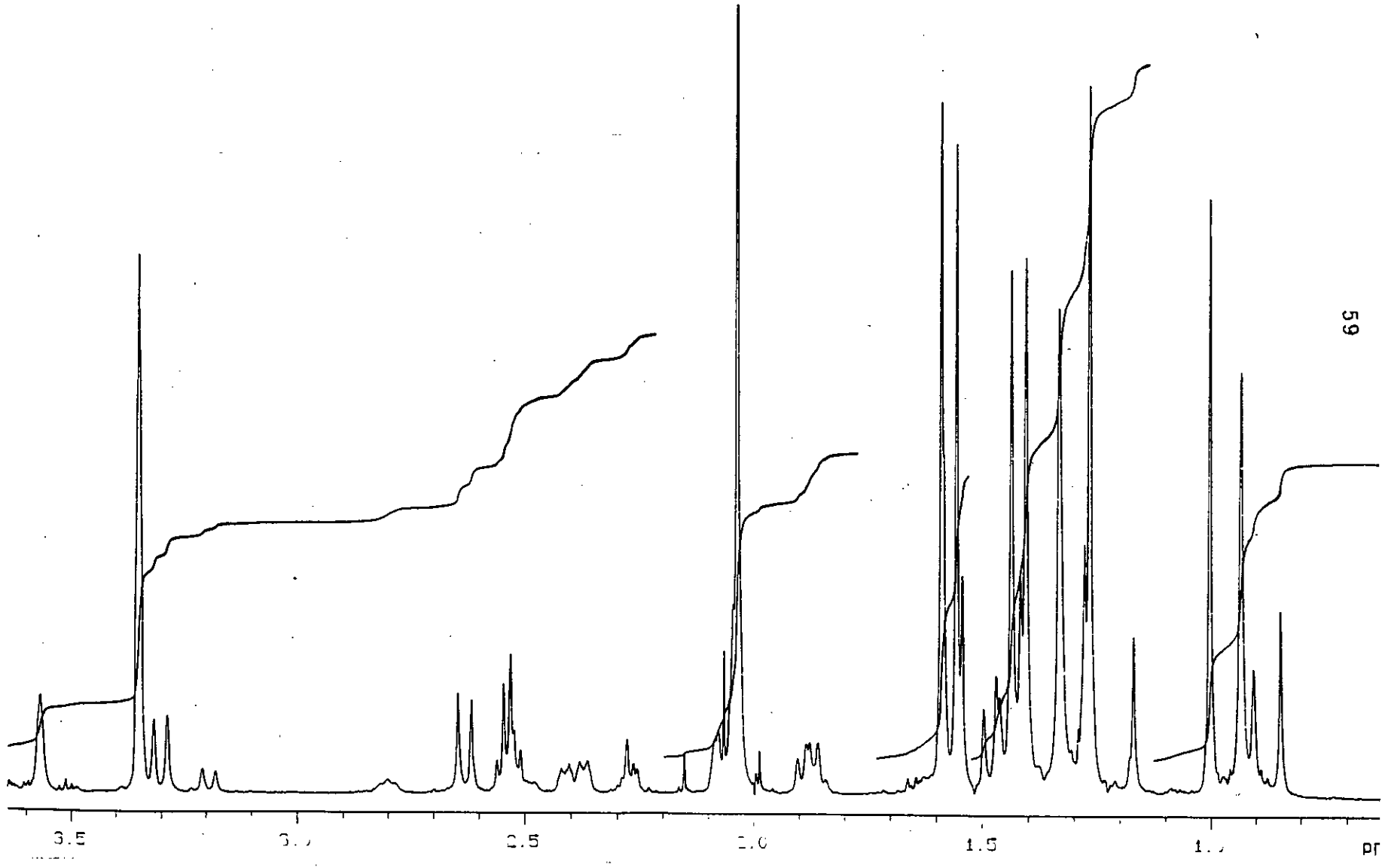


Fig. 6.1 ^{1}H NMR Spectrum of Compound A_1 in mixture of $CDCl_3$ & CD_3OD

On hydrolysis of compound A_1 with aqueous sodium carbonate in methanol, a compound having melting point, 149°C was obtained, which is similar to that of cucurbitacin I **(39)** which is expected from such treatment[28].

Table 6

^{13}C Chemical shifts of Compound A_1(datiscacin).

Carbon nos.	δ-values
1	144.81
2	150.32
3	203.10
4	48.64
5	120.68
6	114.90
7	23.41
8	27.13
9	44.93
10	34.35
11	214.16
12	57.95
13	38.51
14	41.49
15	50.02
16	70.39
17	25.64
18	17.58
19	23.07
20	78.47
21	25.25
22	198.40
23	120.15
24	136.49
25	79.37
26	20.89
27	19.09
28	19.44
29	23.52
30	19.15
31	170.17
32	57.81

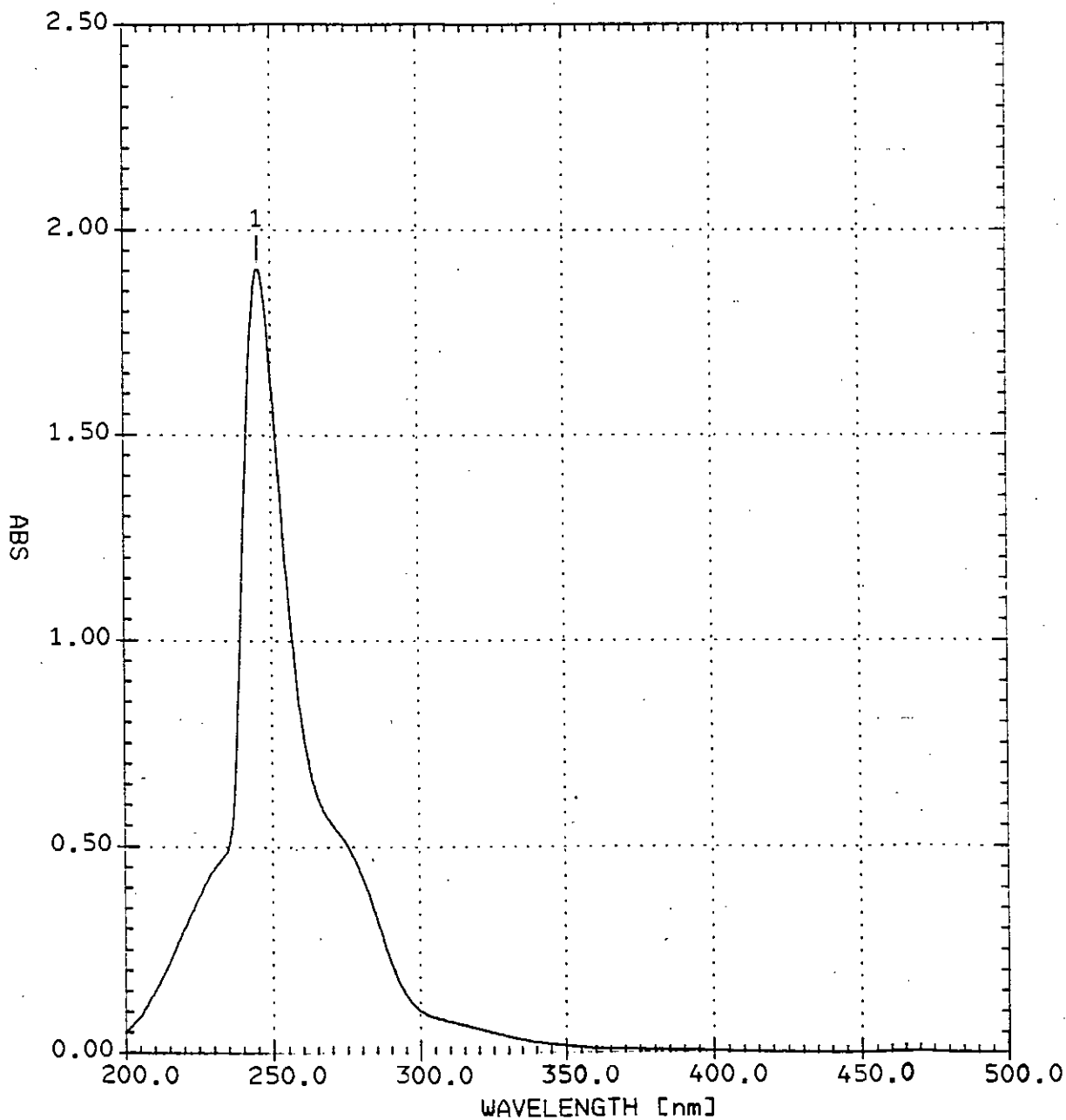
3.2 Study on Compound A_2

Compound A_2 was obtained as a white crystalline solid, m.p 182°C, (yield 0.266%). It gave positive Salkowski and Liebermann - Burchard reaction for steroid and triterpene. Its UV absorption (Fig.7) showed a strong band at λ_{max} 245.5 nm for an α,β-unsaturated ketone which was borne out by the strong IR absorption at 1610 cm^{-1}.

The mass spectrum of the compound,(Fig.8) showed the highest mass ion at m/e 498. Its melting point and mass fragments at m/e 96 (base peak), 113, 385, 403, 455, 480 are identical to that reported for cucurbitacin B. The fragment at m/e 498 is also consistent with the fact that it did not show the molecular ion peak but the M-60 (M-acetate) peak[34].

The IR absorption,(Fig.9) at 3475, 1710 and 1610 cm^{-1} are attributable to OH, ester and carbonyl function of cucurbitacin B respectively. The ^1H NMR spectrum of the compound (Fig.10) was identical to that reported for cucurbitacin B[36]. The presence of three olefinic protons are indicated by doublet at δ 7.10, 6.44 and 5.80, the eight methyl groups appear as singlets at δ 1.60, 1.49, 1.42, 1.29, 1.28, 1.25, 1.11 and 1.00. The acetate showed up as a 3H singlet at δ 2.10.

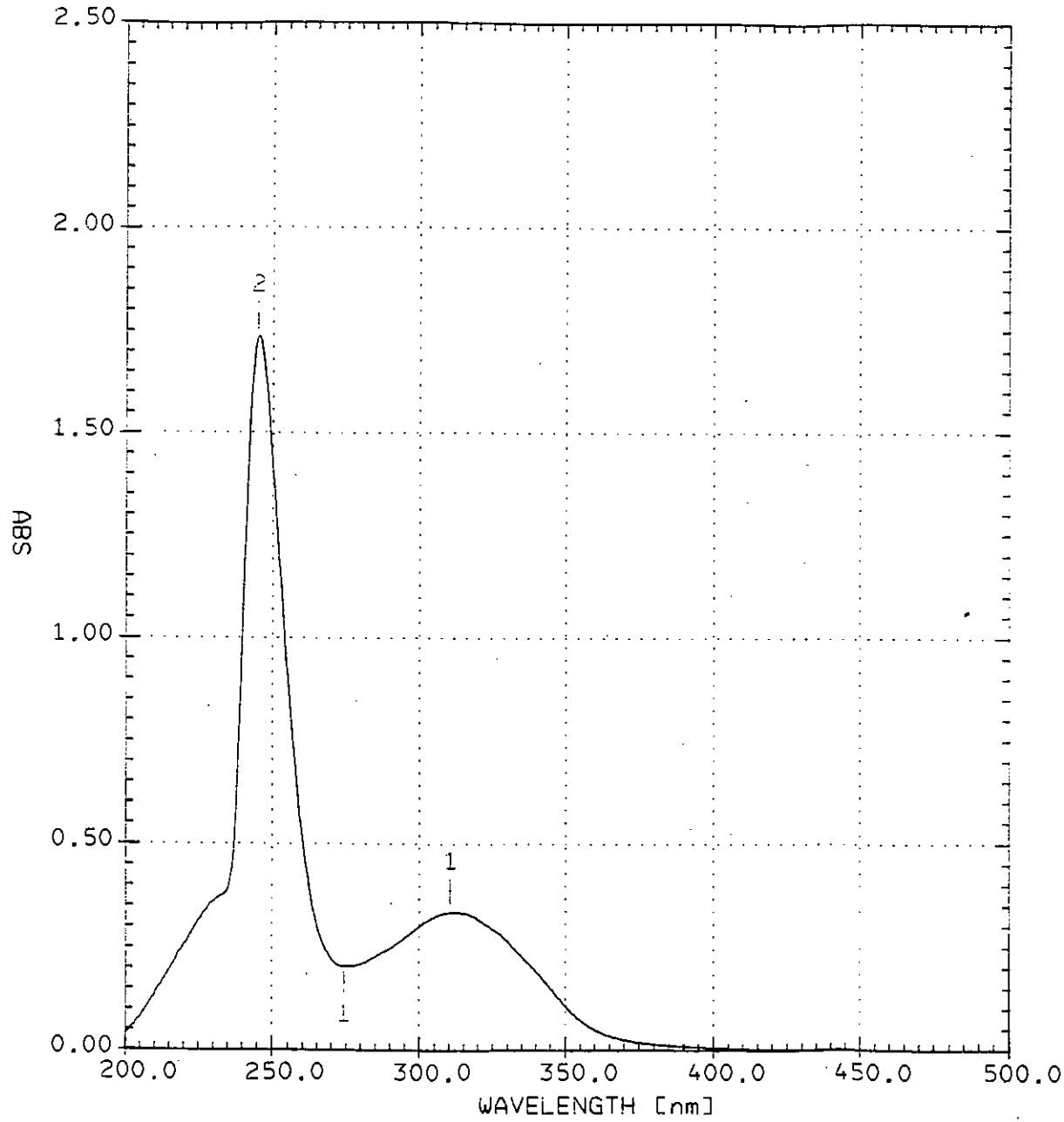
Further confirmation that the compound is identical to that of cucurbitacin B came from ^{13}C NMR spectrum. The compound showed as many as 32 peaks as expected,(Fig.11 , Table.7). Signals at δ 213.02, 212.11 and 202.44 are readily attributable



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	245.5	1.903		

Fig. 7 UV Spectrum of Compound A_2



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	310.5	0.335	274.5	0.204
2	245.0	1.734		

Fig. 7.1 UV Spectrum of Compound A_2 (in NaOH)

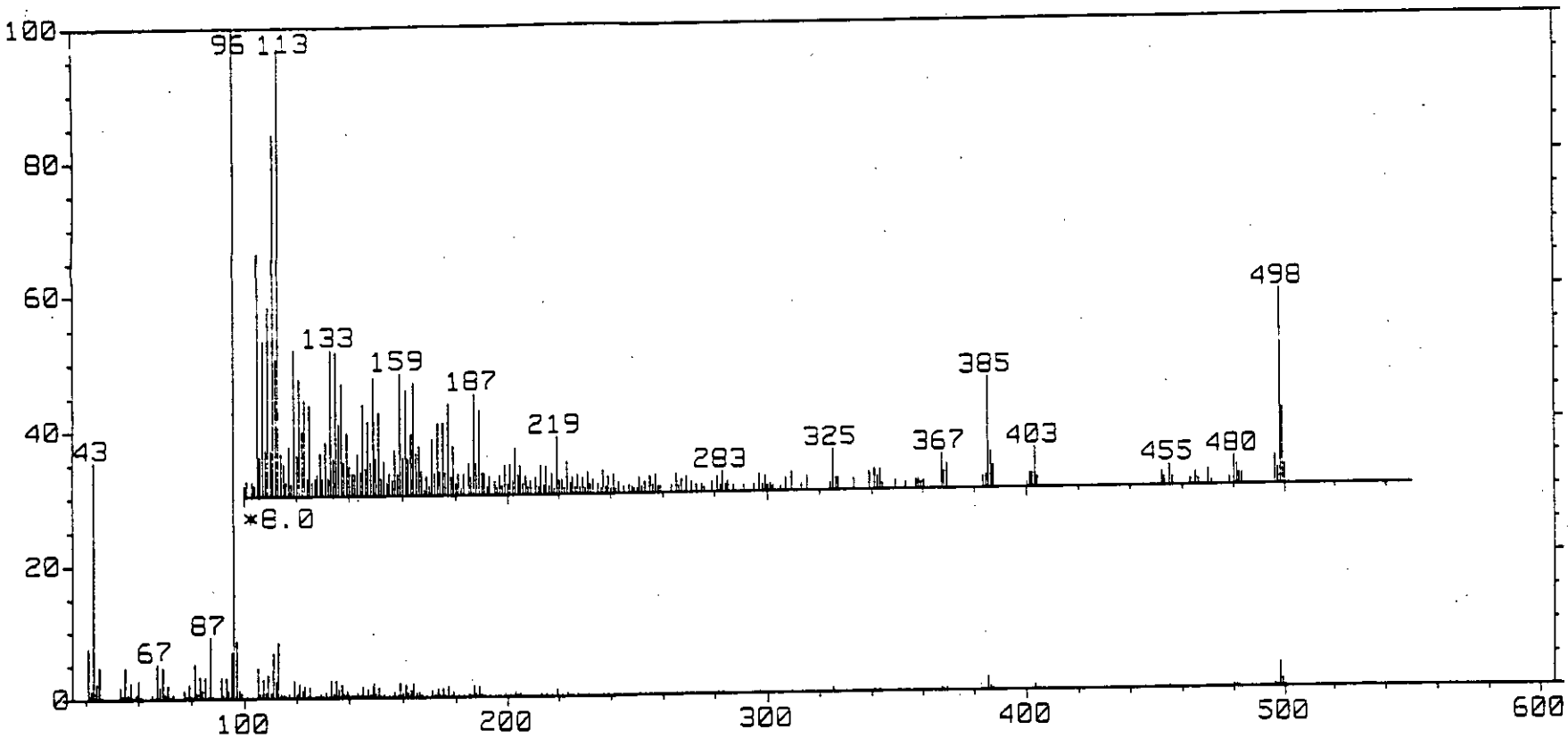


Fig. 8 Mass Spectrum of Compound A_2

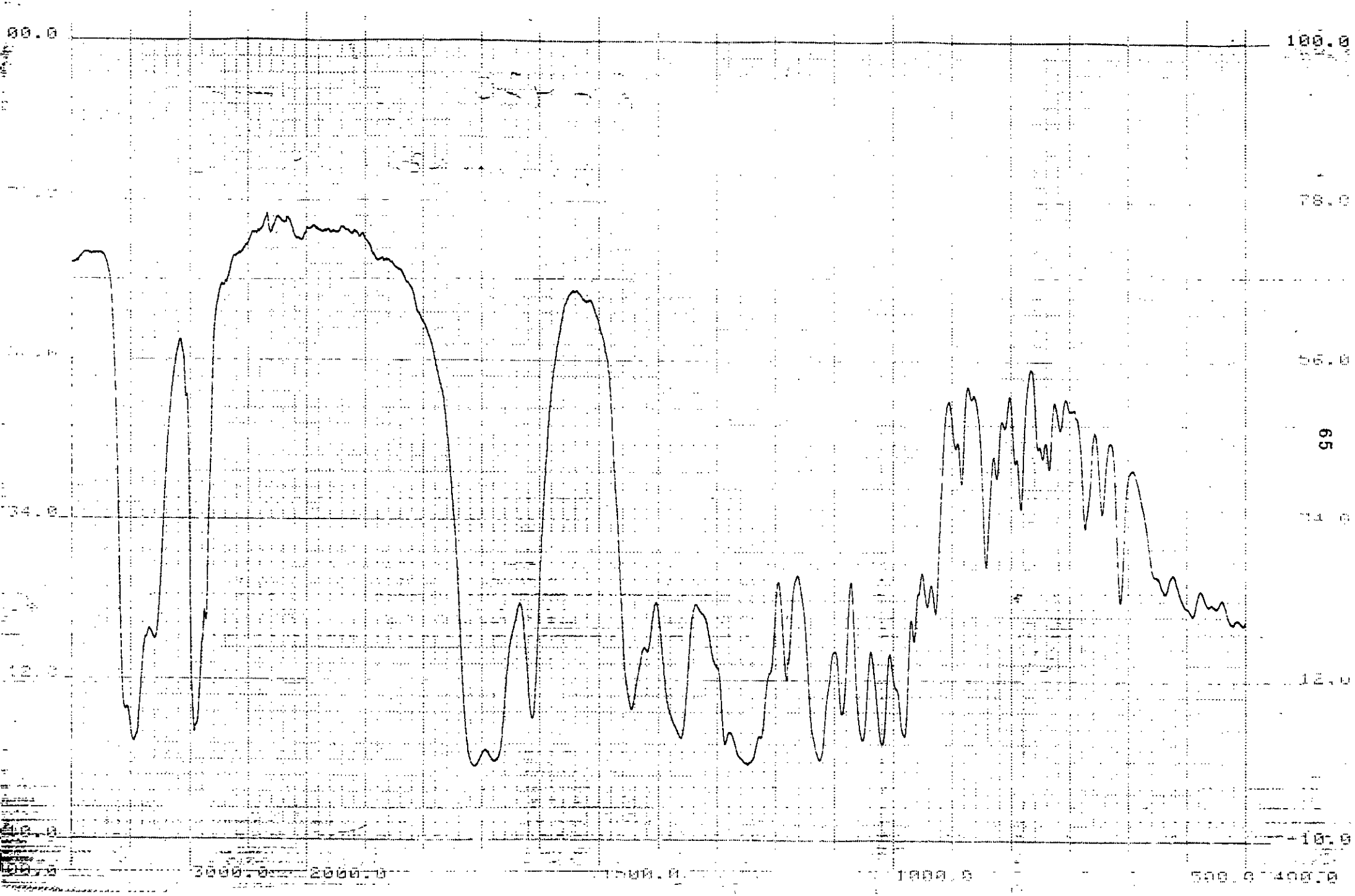


Fig. 9 IR Spectrum of Compound A_2 in KBr

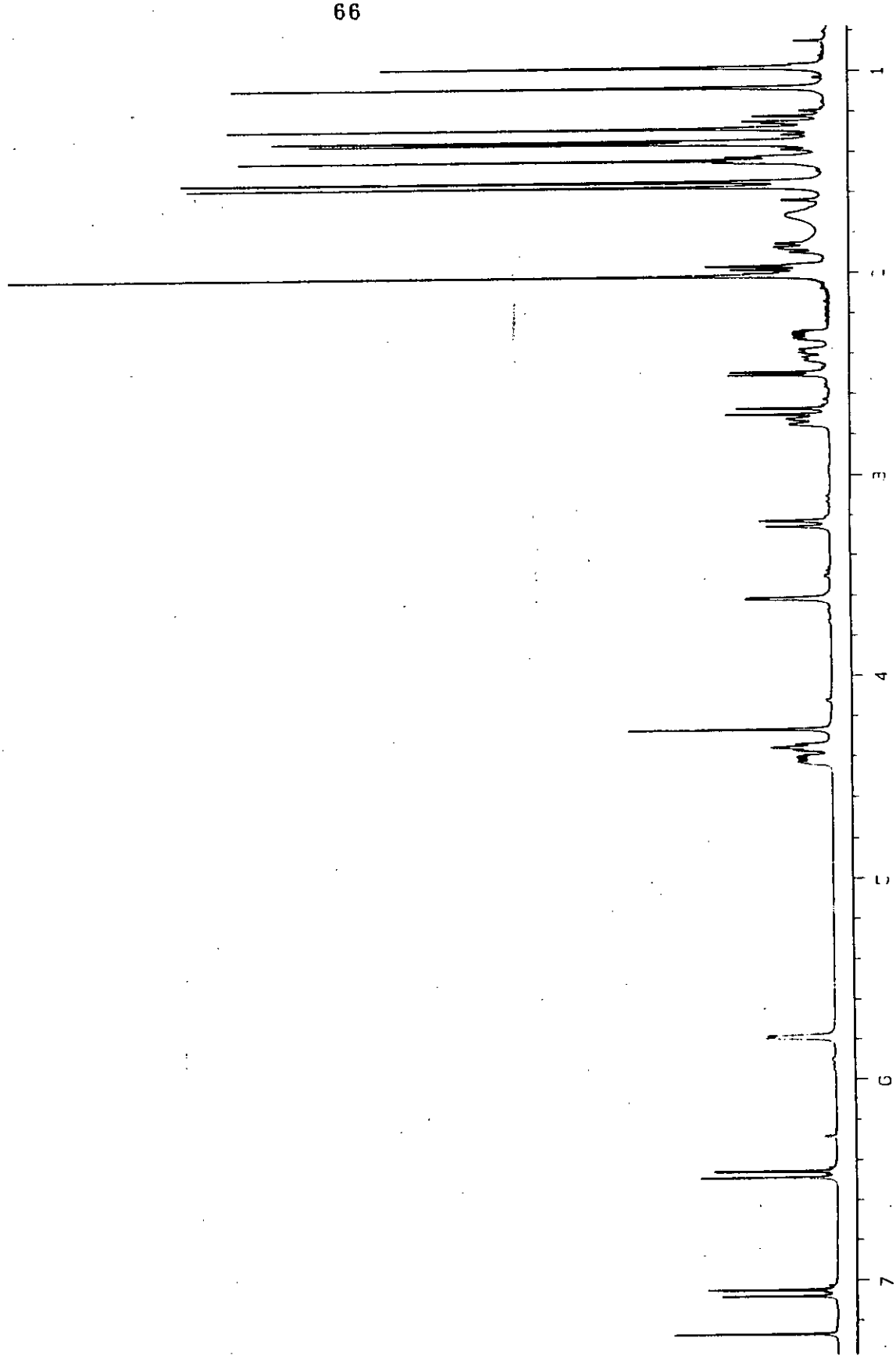


Fig.10 ^1H NMR Spectrum of Compound A_2 in $CDCl_3$

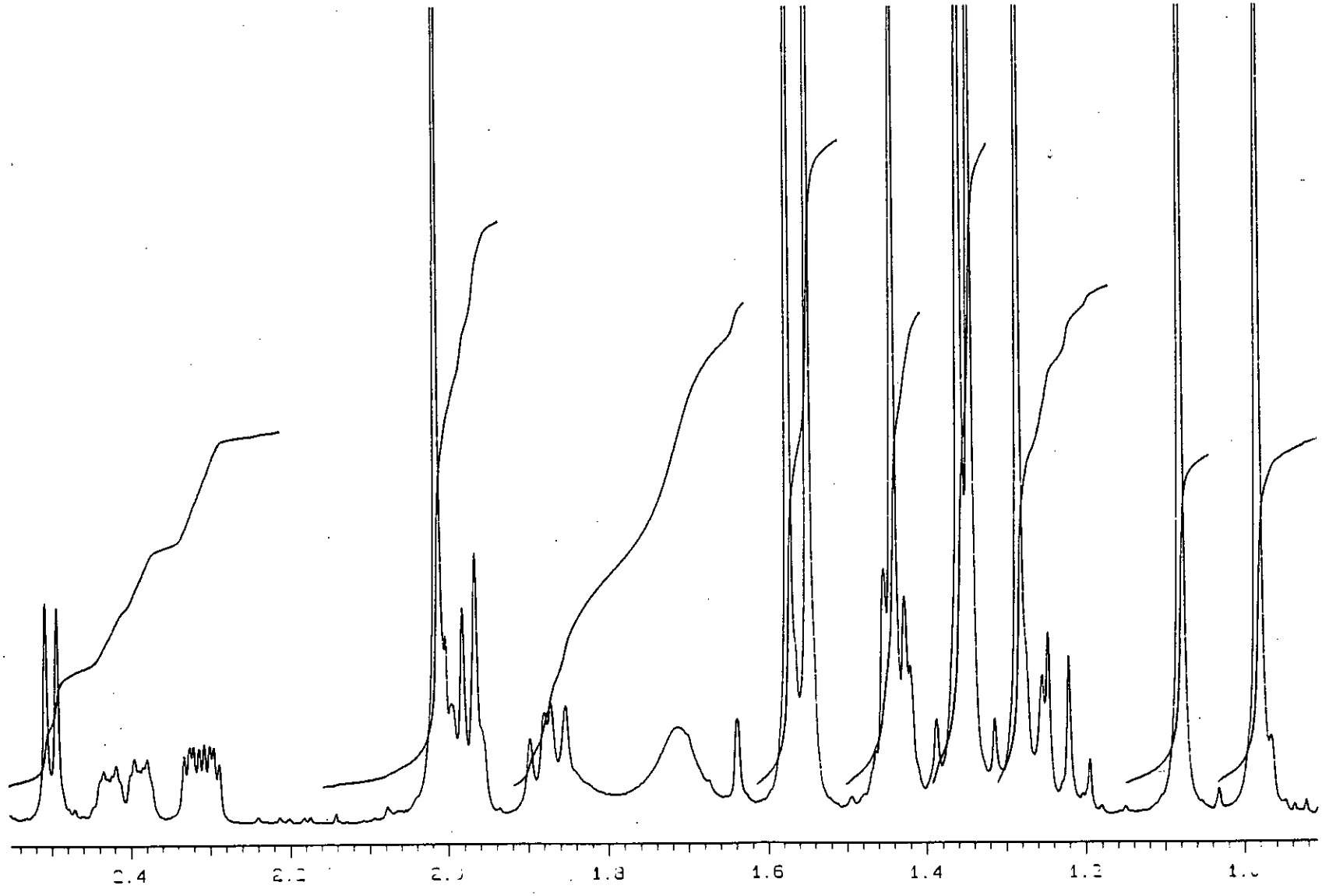


Fig.10.1 ^{1}H NMR Spectrum of Compound A_2 in $CDCl_3$

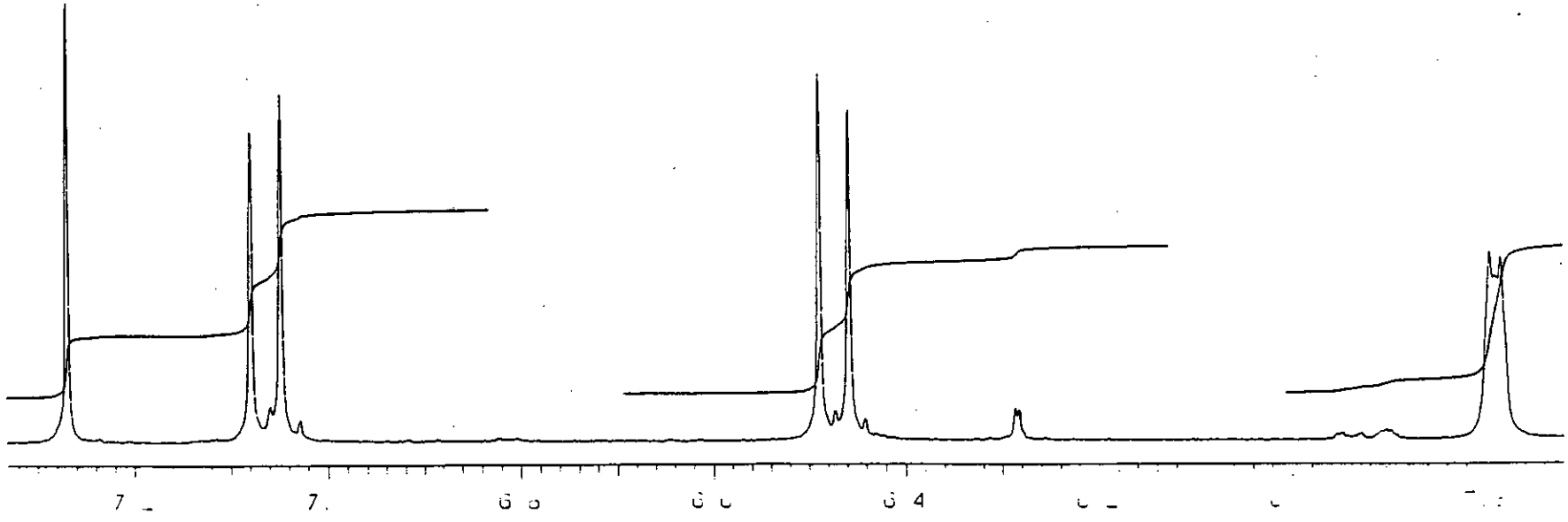
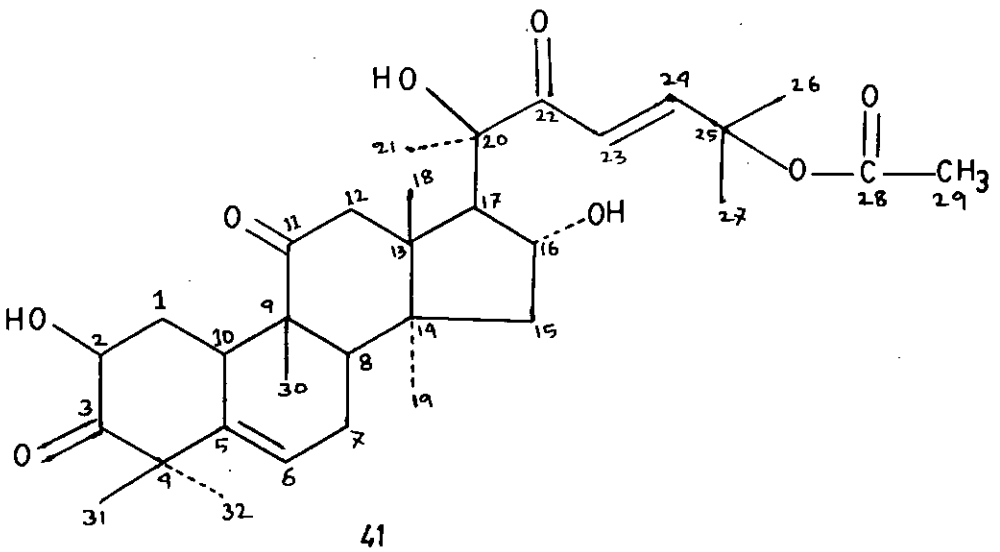


Fig.10.2 ^{1}H NMR Spectrum of Compound A_2 in $CDCl_3$

to the ketonic carbonyl carbons at C_{11}, C_3 and C_{22} respectively; five other low field signals at δ 170.21, 151.94, 140.34, 120.42 and 120.27 are appropriate for the carbon atoms C_{28}, C_{24}, C_5, C_{23} and C_6 respectively. Finally the compound was confirmed to be cucurbitacin B (41) by converting it to its diacetate m.p 135°C, identical to the m.p reported for diacetate of cucurbitacin B[24].



41

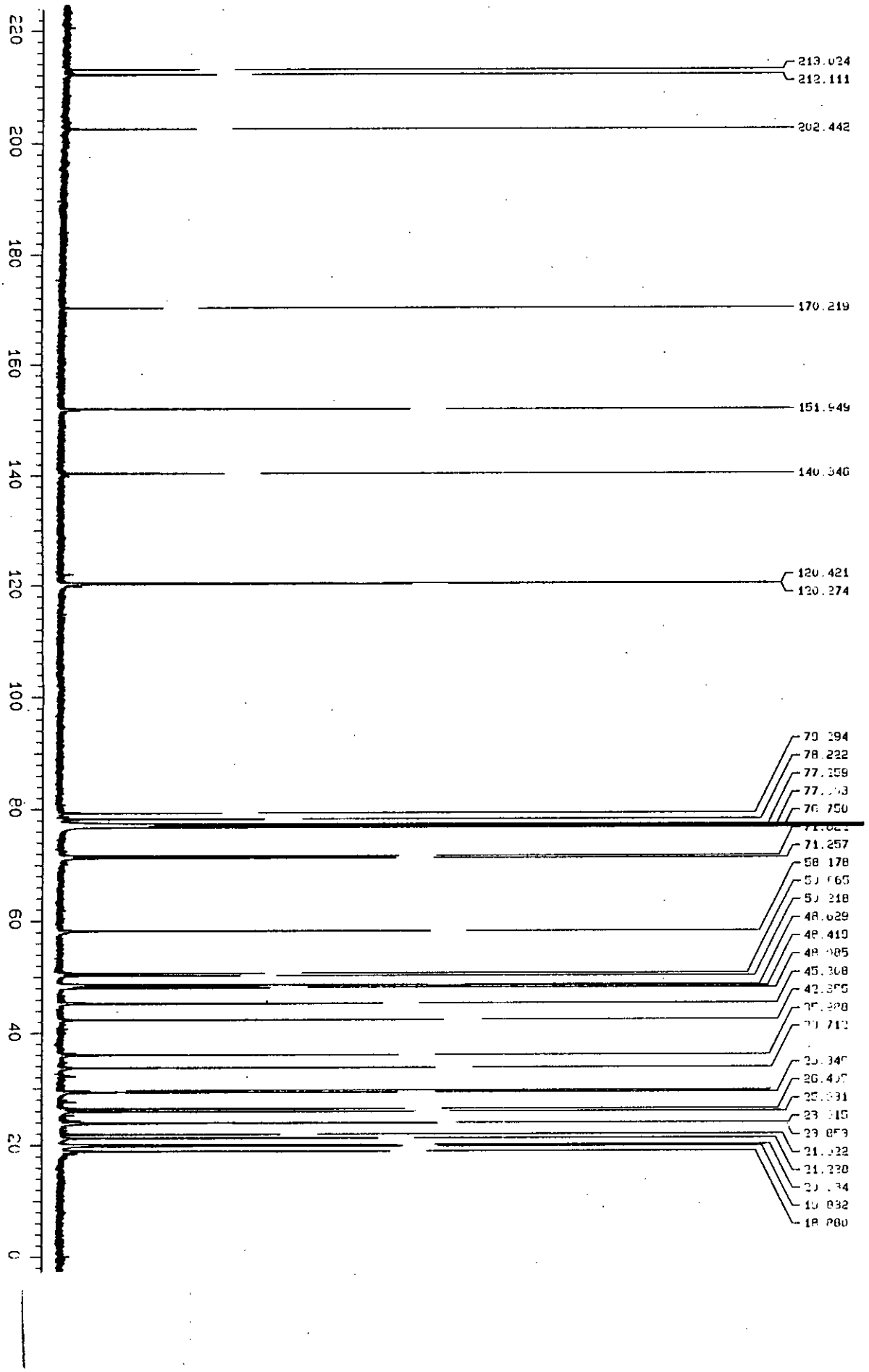


Fig. 11 ^{13}C NMR Spectrum of Compound A_2 in $CDCl_3$

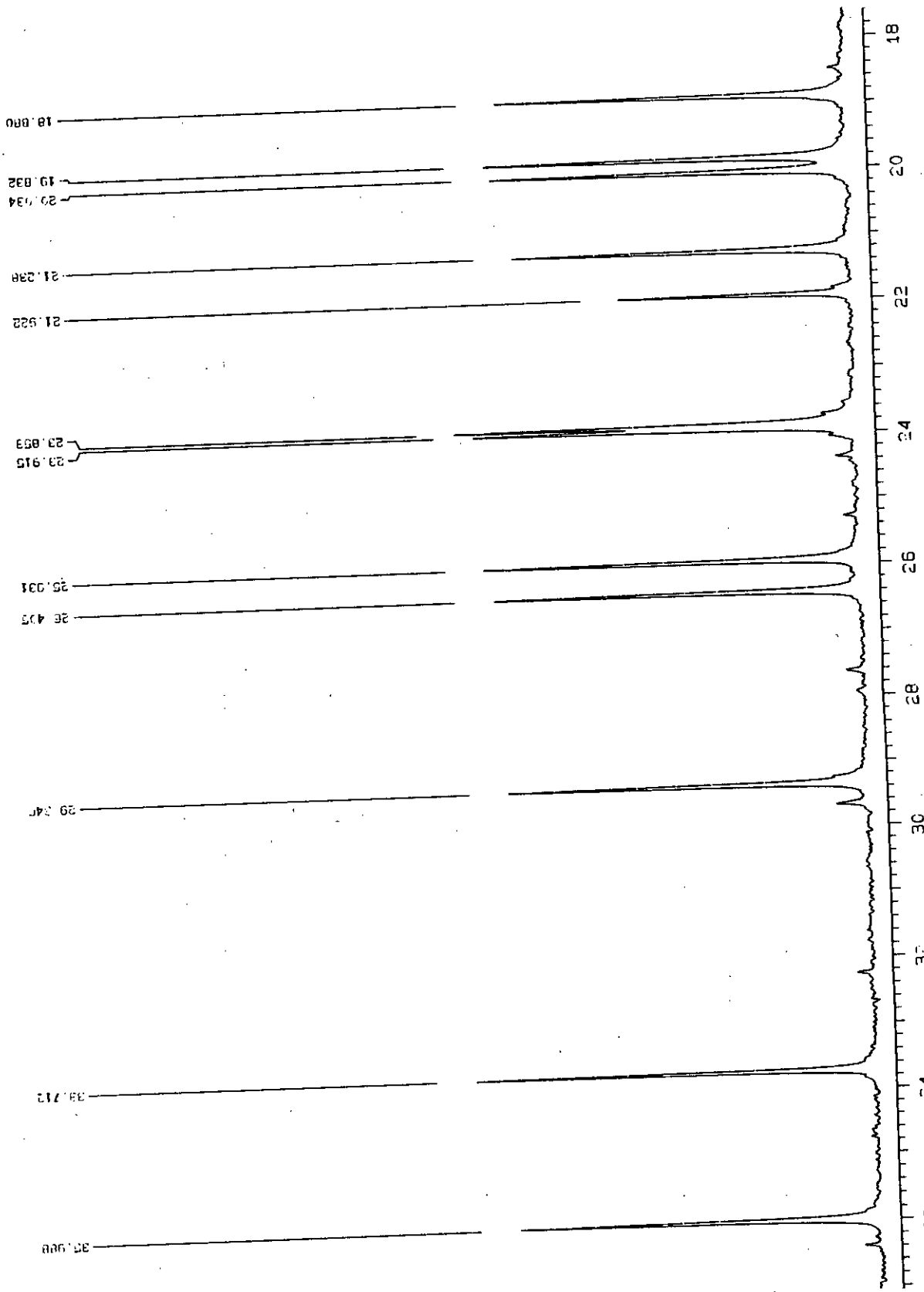


Fig.11.1 ^{13}C NMR Spectrum of Compound A_2 in $CDCl_3$

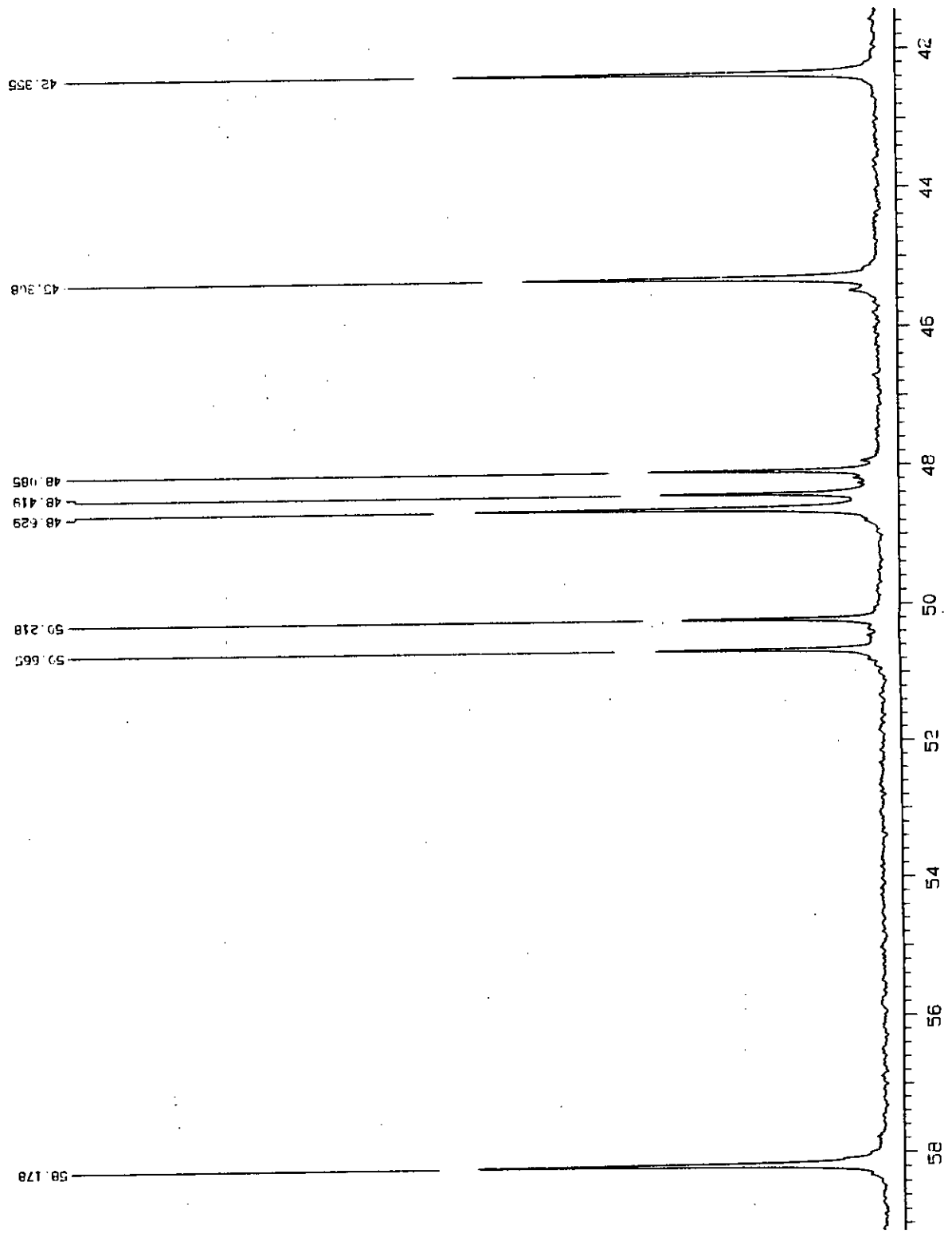


Fig.11.2 ^{13}C NMR Spectrum of Compound A_2 in $CDCl_3$

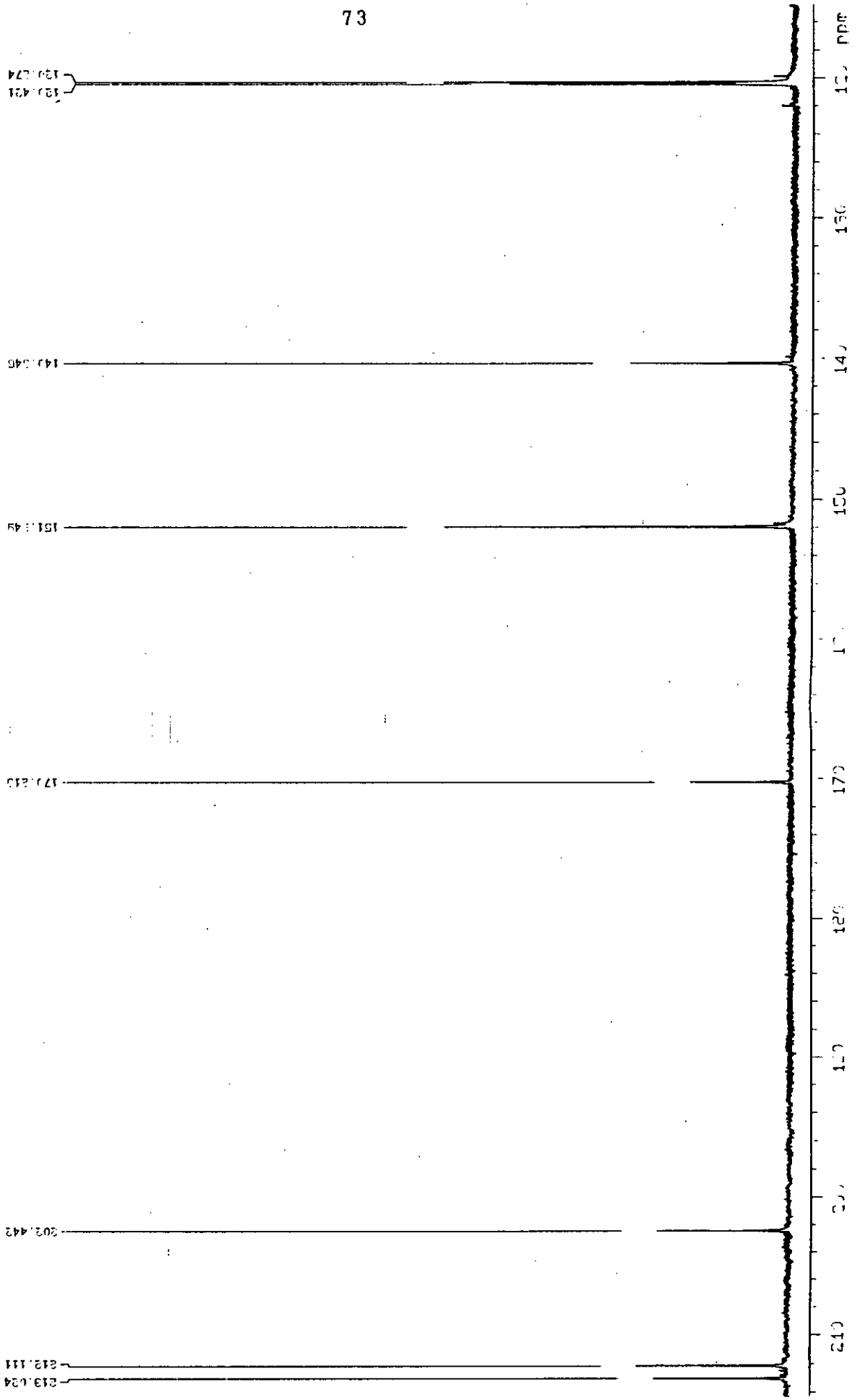


Fig.11.3 ^{13}C NMR Spectrum of Compound A_2 in $CDCl_3$

Table 7

^{13}C Chemical shifts of compound A_2(Cucurbitacin B).

Carbon nos.	δ-values
1	33.71
2	71.62
3	212.11
4	48.08
5	140.34
6	120.27
7	25.93
8	35.98
9	48.41
10	42.35
11	213.02
12	48.62
13	50.21
14	50.66
15	45.30
16	71.25
17	58.17
18	20.03
19	23.85
20	79.29
21	29.34
22	202.44
23	120.42
24	151.94
25	79.24
26	21.92
27	21.23
28	170.21
29	26.40
30	18.88
31	19.83
32	23.91

3.3 Study on Compound A_3

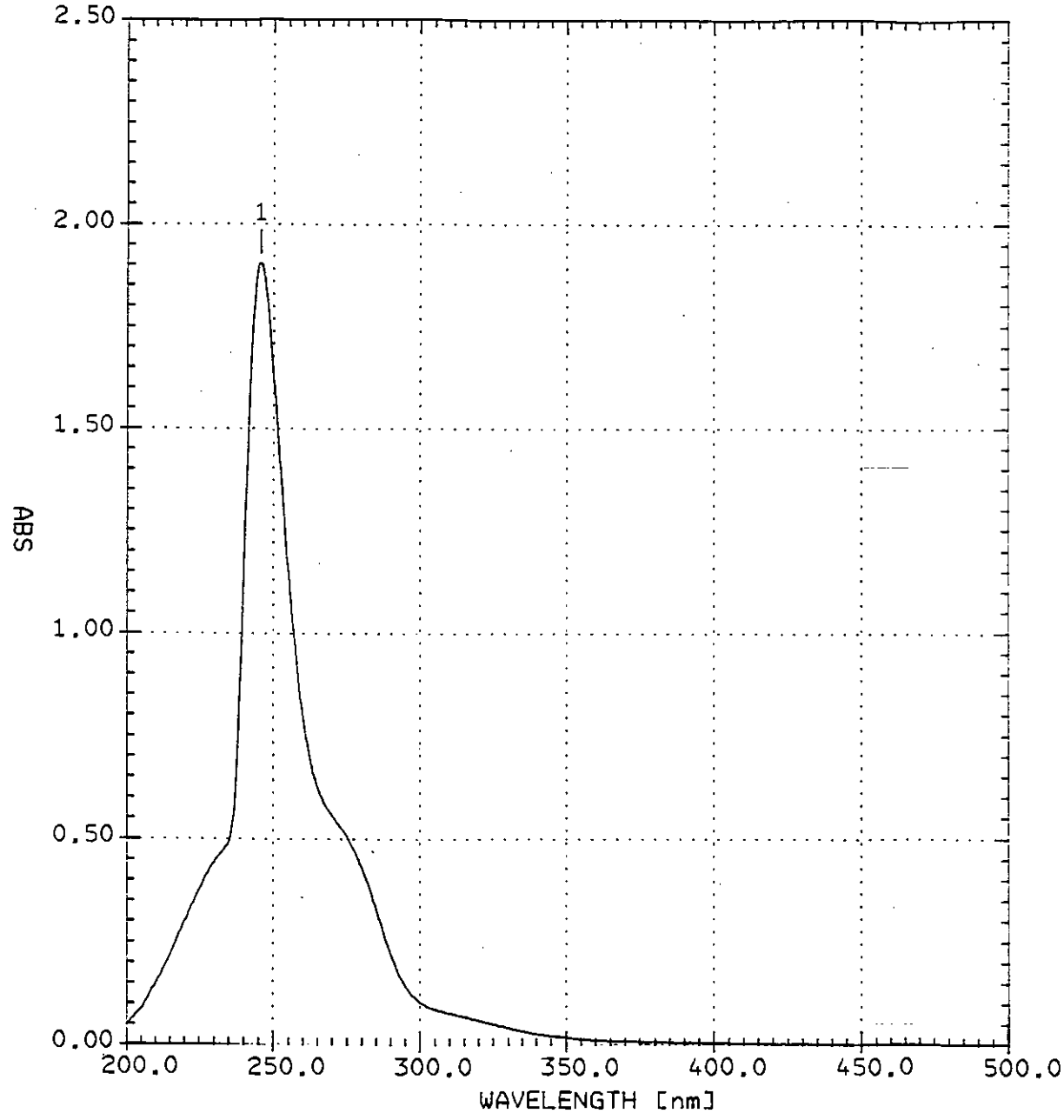
Compound A_3 was obtained as a white crystallinc solid, m.p 229°C, (yield 0.109%). Like compound A_2 (cucurbitain B) it also gave positive test for steroid and triterpene. It was an ,ß-unsaturated ketone λ_{max} 245.5 nm,(Fig.12).

The IR absorption spectrum of compound A_3, (Fig.13) was comparable to that of cucurbitcin B (compound A_2) and showed bands at 3550, 1715 1680 and 1618 cm^{-1} for OH, ester, carbonyl and α,β- unsaturated carbonyl functions respectively. Like compound A_2 compound A_3 showed the highest mass ion at m/e 498 (M-60) as well as other peaks at m/e 96 (base peak), 113, 385, 403, 455 and 480,(Fig.14).

^{13}C NMR spectrum of the compound A_3 (Fig.15) showed the presence of 32 carbon atoms, (Table 8). ^{1}H NMR spectrum of the compound, (Fig.16) was similar to cucurbitacin B.

The spectral data of compound A_3 thus support it to be very similar to cucurbitacin B. The melting point of compound A_3 was however different from that of cucurbitacin B but identical to that of isocucurbitacin B, (m.p 229°C)[37]. It may be noted that the cucurbitacin B and isocucubritacin B (42) differ in the disposition of OH group at C-2.

The identity of compound A_3 as isocucurbitacin B was finally confirmed by preparing its acetate which melted at 196°C, similar to that reported for acetate of isocucurbitacin B[38]. It may be mentioned that both cucurbitacin B and isocucurbitacin B have



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	245.5	1.903		

Fig.12 UV Spectrum of Compound A_3 in $CHCl_3$

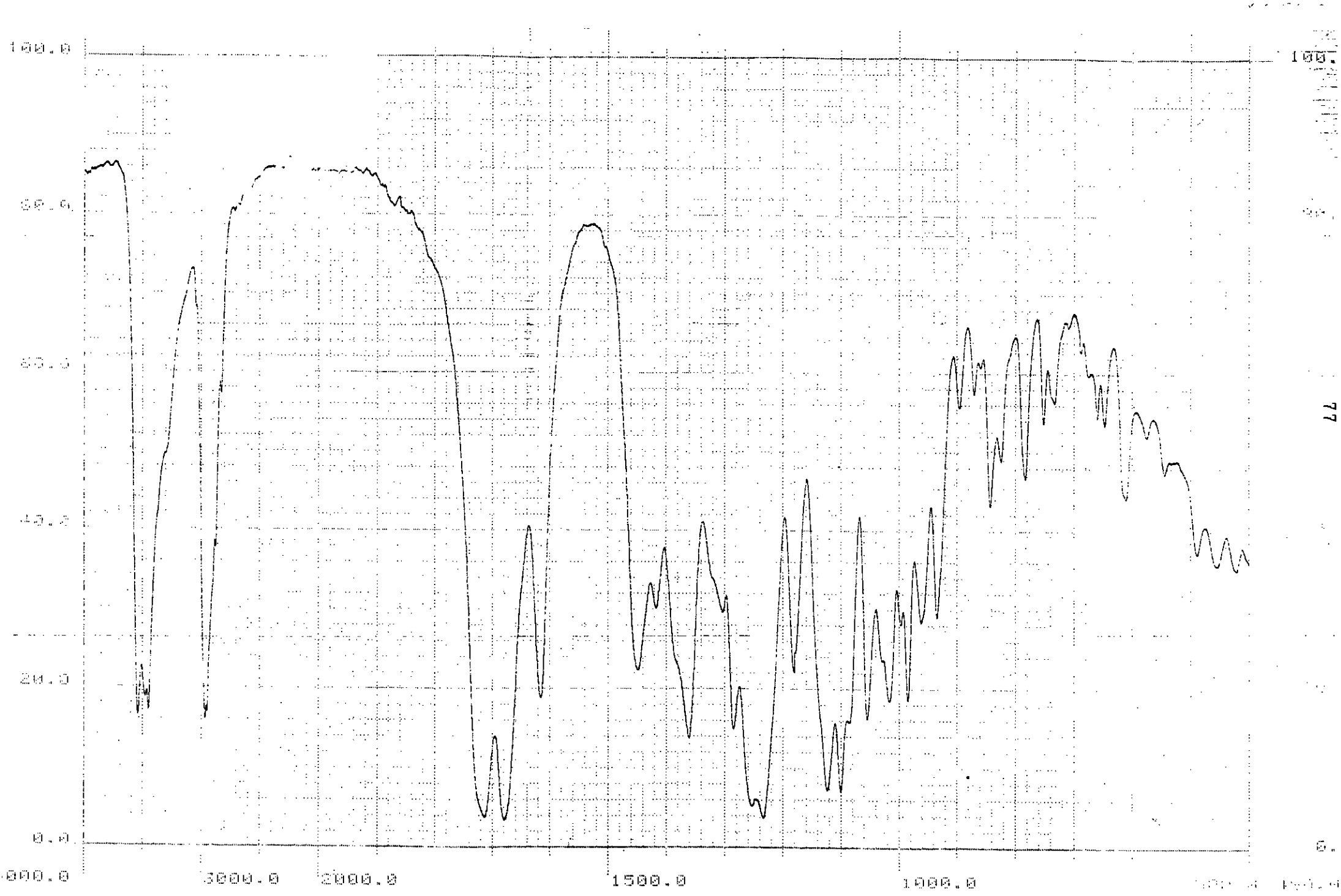


Fig.13 IR Spectrum of Compound A_3 in KBr

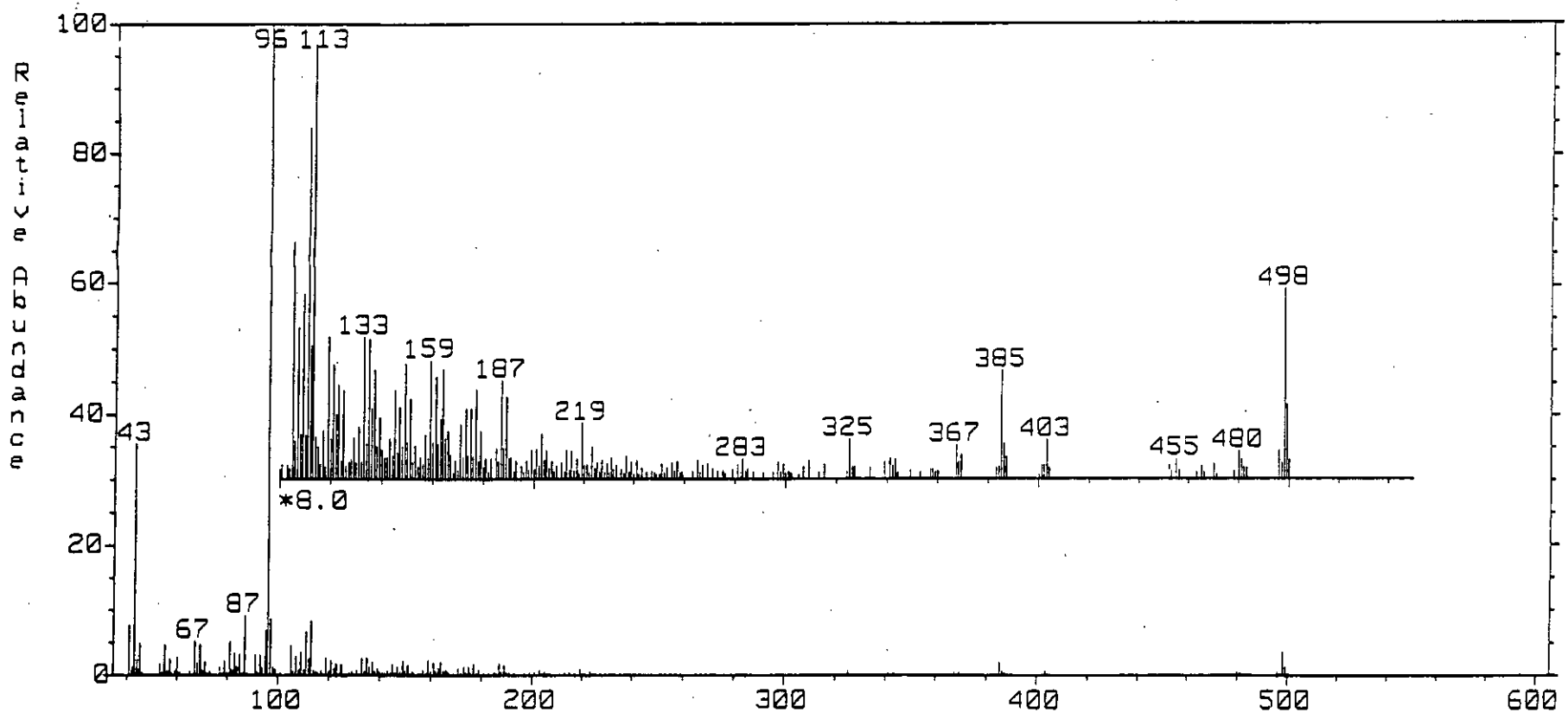


Fig.14 Mass Spectrum of Compound A_3

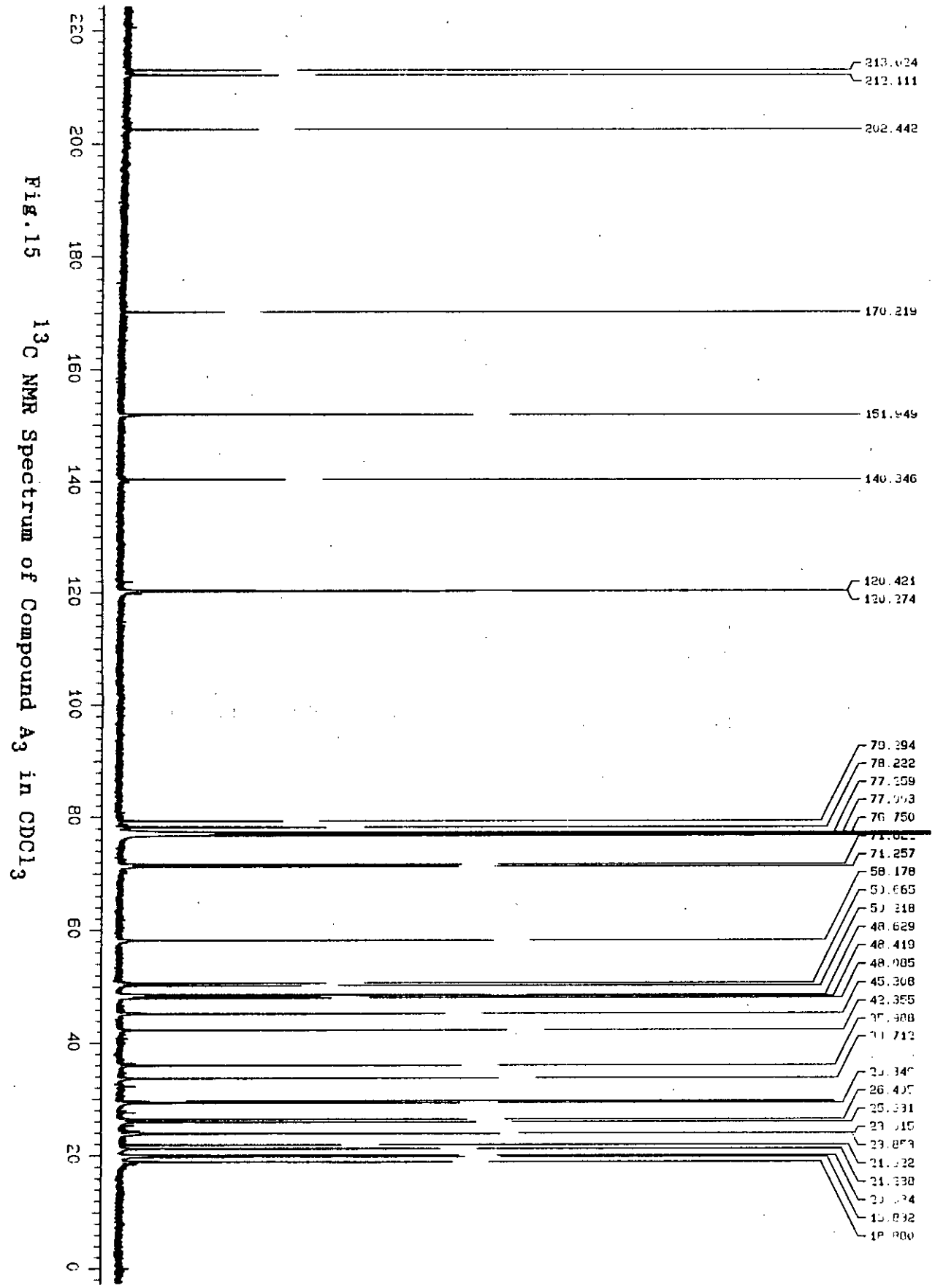


Fig.15 ^{13}C NMR Spectrum of Compound A_3 in $CDCl_3$

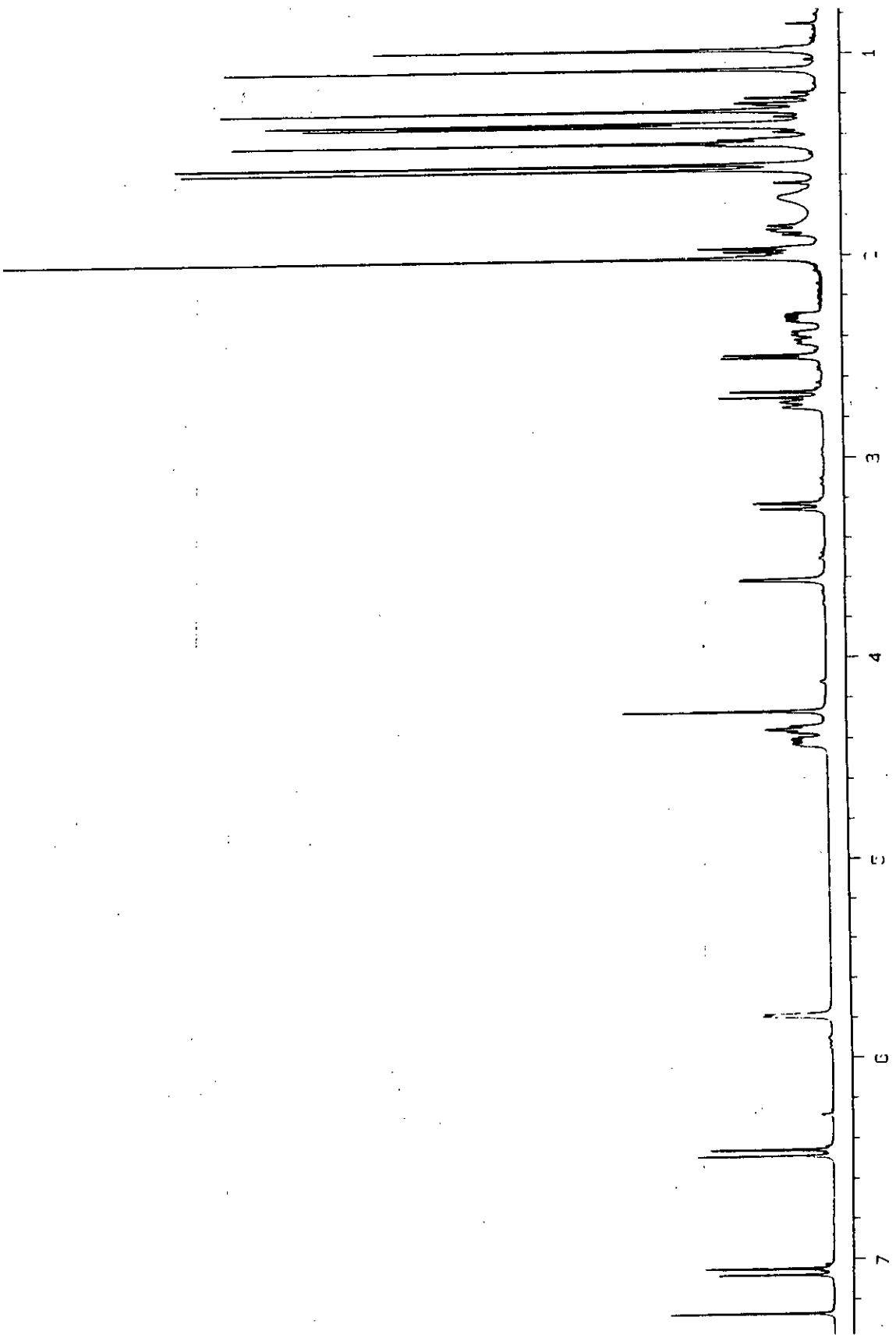


Fig.16 ^1H NMR Spectrum of Compound A_3 in $CDCl_3$

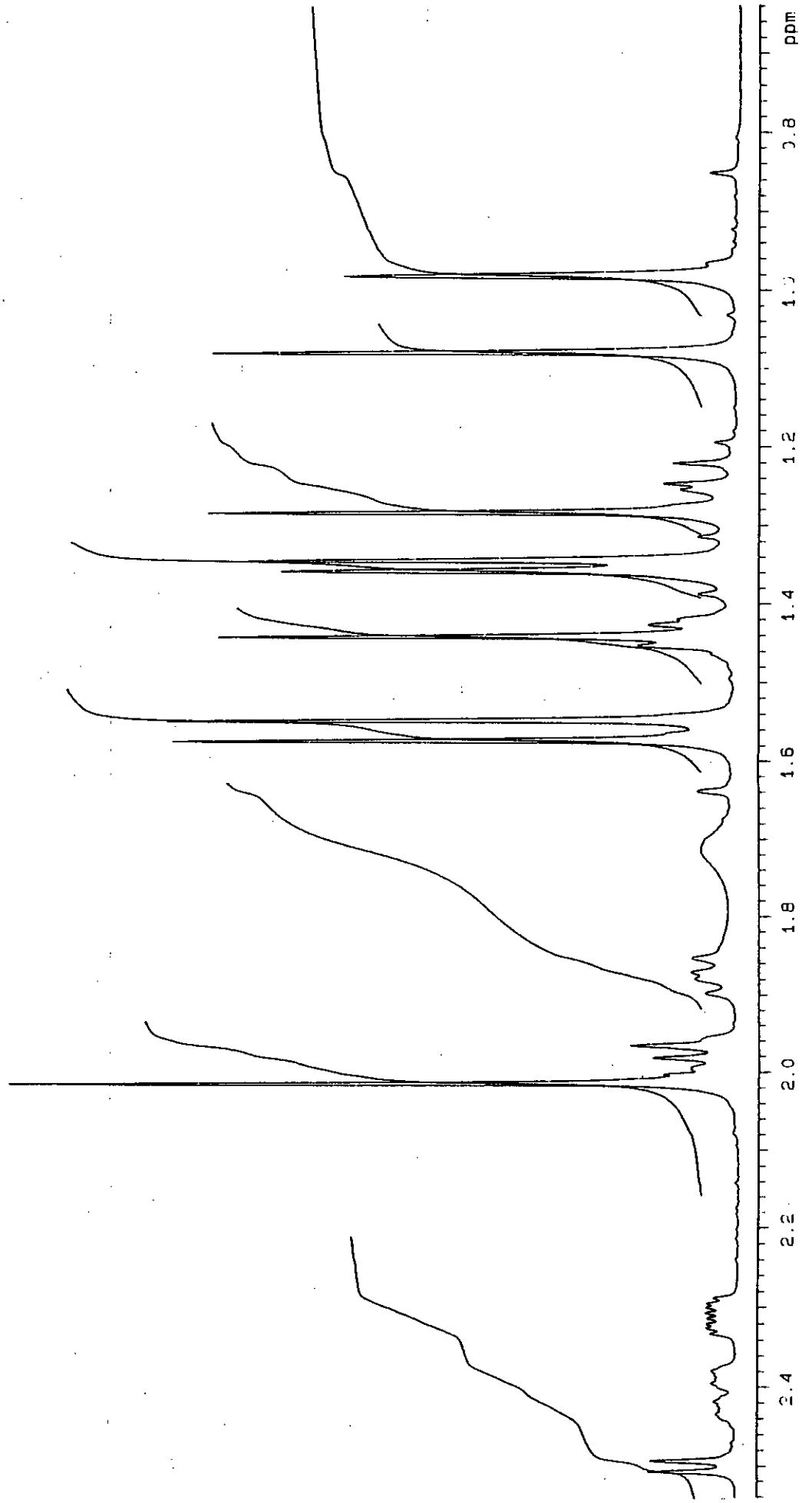


Fig.16.1 ^{1}H NMR Spectrum of Compound A_3 in $CDCl_3$

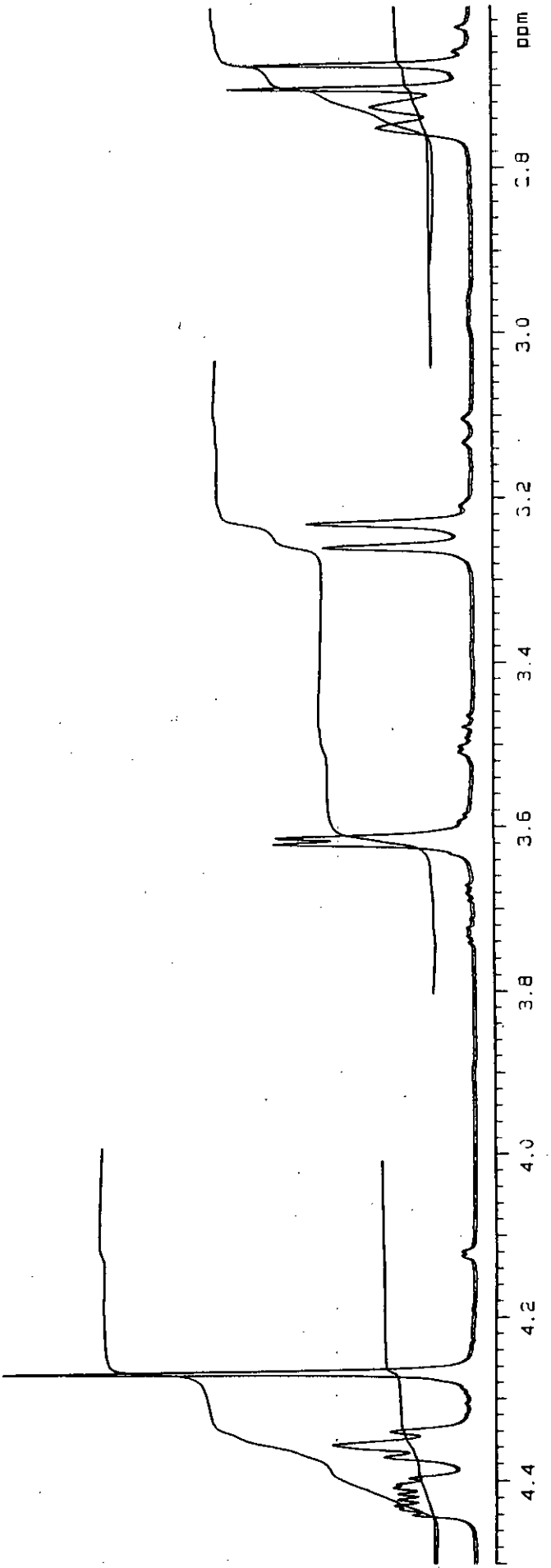
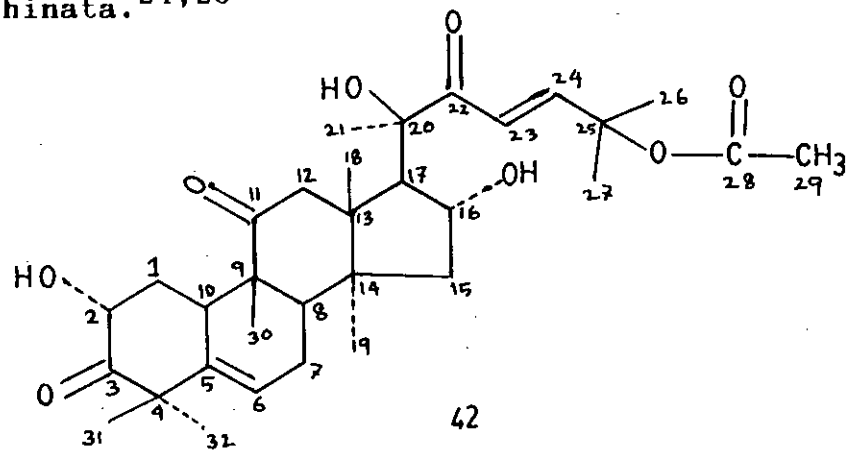


Fig.16.2 ^{1}H NMR Spectrum of Compound A_3 in $CDCl_3$

been found to be the constituents of the seeds and fruits of **Luffa echinata.**[24,26]



42

Table 8

^{13}C Chemical shifts of Compound A_3(isocucurbitacin B)

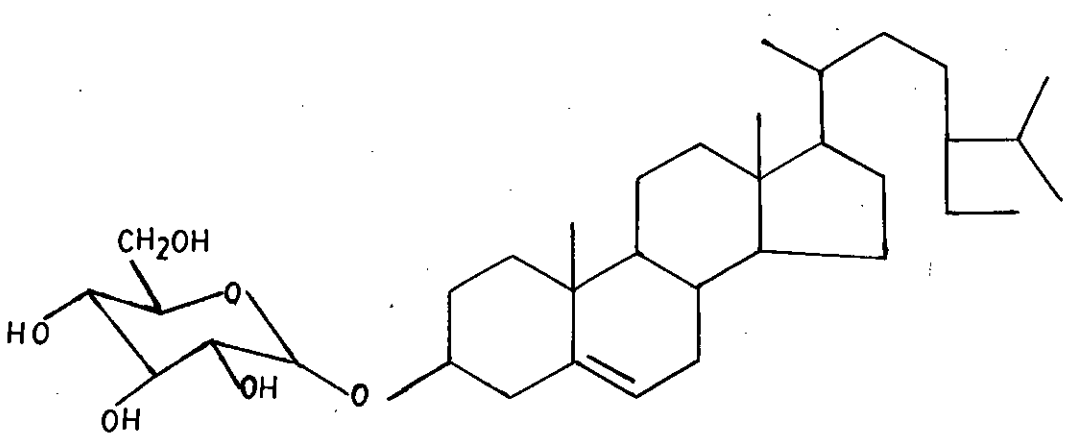
Carbon nos.	δ-values
1	33.71
2	71.62
3	212.11
4	48.08
5	140.34
6	120.27
7	25.93
8	35.98
9	48.41
10	42.35
11	213.02
12	48.62
13	50.21
14	50.66
15	45.30
16	71.25
17	58.17
18	20.03
19	23.85
20	79.29
21	29.34
22	202.44
23	120.42
24	151.94
25	79.24
26	21.92
27	21.23
28	170.21
29	26.40
30	18.88
31	19.83
32	23.91

3.4 Study on Compound A_4

Compound A_4 was obtained as a colourless crystalline solid, m.p 291°C, (yield 0.047%). It gave weak Salkowski and Liebermann - Burchard tests for steroid and triterpene. It also gave positive Molisch's and Resorcinal tests for sugar. The compound was thus a steroid glycoside. As expected compound A_4 gave copious foam on shaking with water.

Hydrolysis of compound A_4 by 8% methanolic hydrochloric acid gave an aglycon, m.p 135°C and a sugar. The aglycone was confirmed to be β- sitosterol by mixed m.p, co-TLC and preparing acetate, m.p 129°C. The sugar was identified as glucose by paper chromatography. Compound A_4 is thus **β- sitosterol glucoside(43).**

It may be recalled that the isolation of β- sitosterol glucoside from the fruits of **Luffa echinata** has been reported by Seshadri[26].



43

The spectral data, IR, ^1H NMR and mass, are in agreement with compound A_4 being β- sitosterol glucoside. The IR spectrum (Fig.17) showed O-H absorptions at 3375 and 1030 cm^{-1}. The ^1H NMR spectrum of compound A_4 (Fig.18) showed the presence of four C-methyl signals appearing at δ 0.84, 0.83, 0.82 and 0.81. The two angular methyl groups of the molecule appeared as singlets at δ 0.54 and 0.53. The olefinic proton appeared as a one proton doublet at δ5.14 whereas the proton attached to the carbon atom bearing glucose moiety appeared as a multiplet at δ 3.4. The five ^1H NMR absorptions each for one proton attached to carbon C_{30}, C_{31}, C_{32}, C_{33} and C_{34} appeared at δ 4.41 as doublet, 3.21 as triplet, 3.24 as multiplet, 3.70 as multiplet and 3.83 as quartet respectively. The protons attached to primary alcohol group of the sugar unit appeared as an unresolved singlet at δ 3.32.

The compound showed the highest mass peak at m/e 414 (M^+-162) indicating the removal of the glucose moiety during fragmentation. The mass spectrum, (Fig.19) also showed other important mass peaks at m/e 399, 396, 271, 255, 229 which are characteristics for steroids.

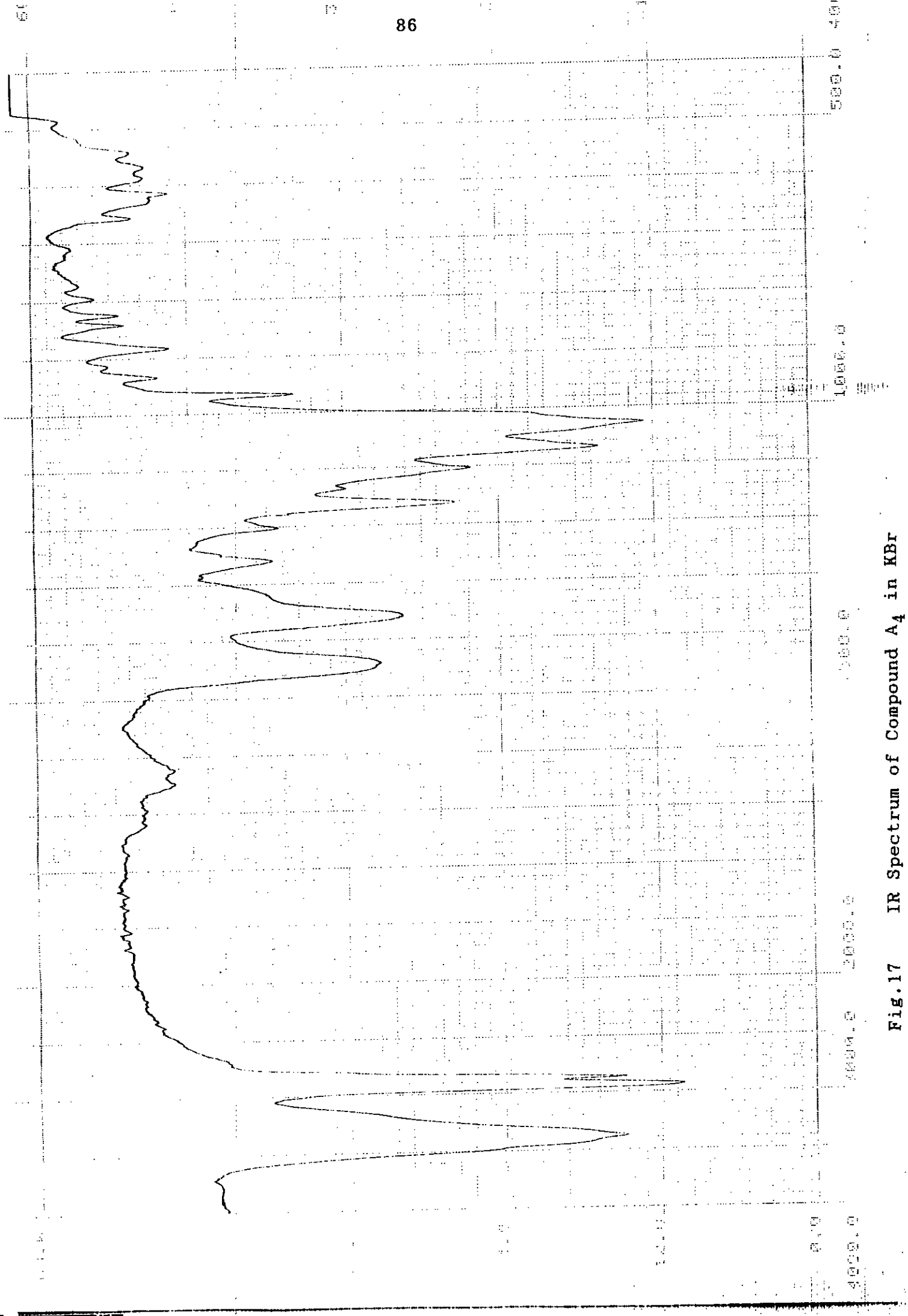


Fig.17 IR Spectrum of Compound A_4 in KBr

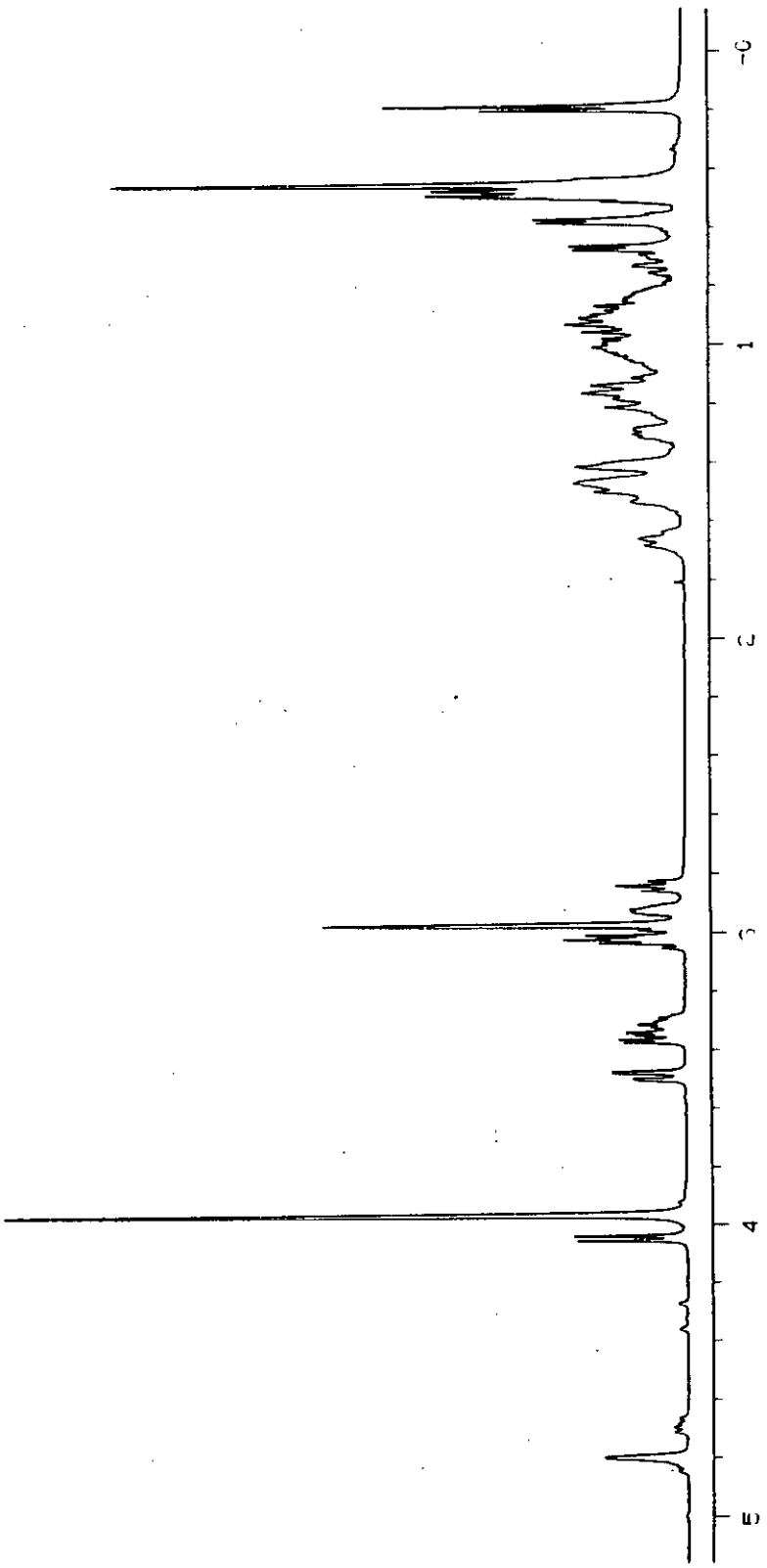


Fig.18 ^{1}H NMR Spectrum of Compound A_4 in mix. of $CDCl_3$ & CD_3OD

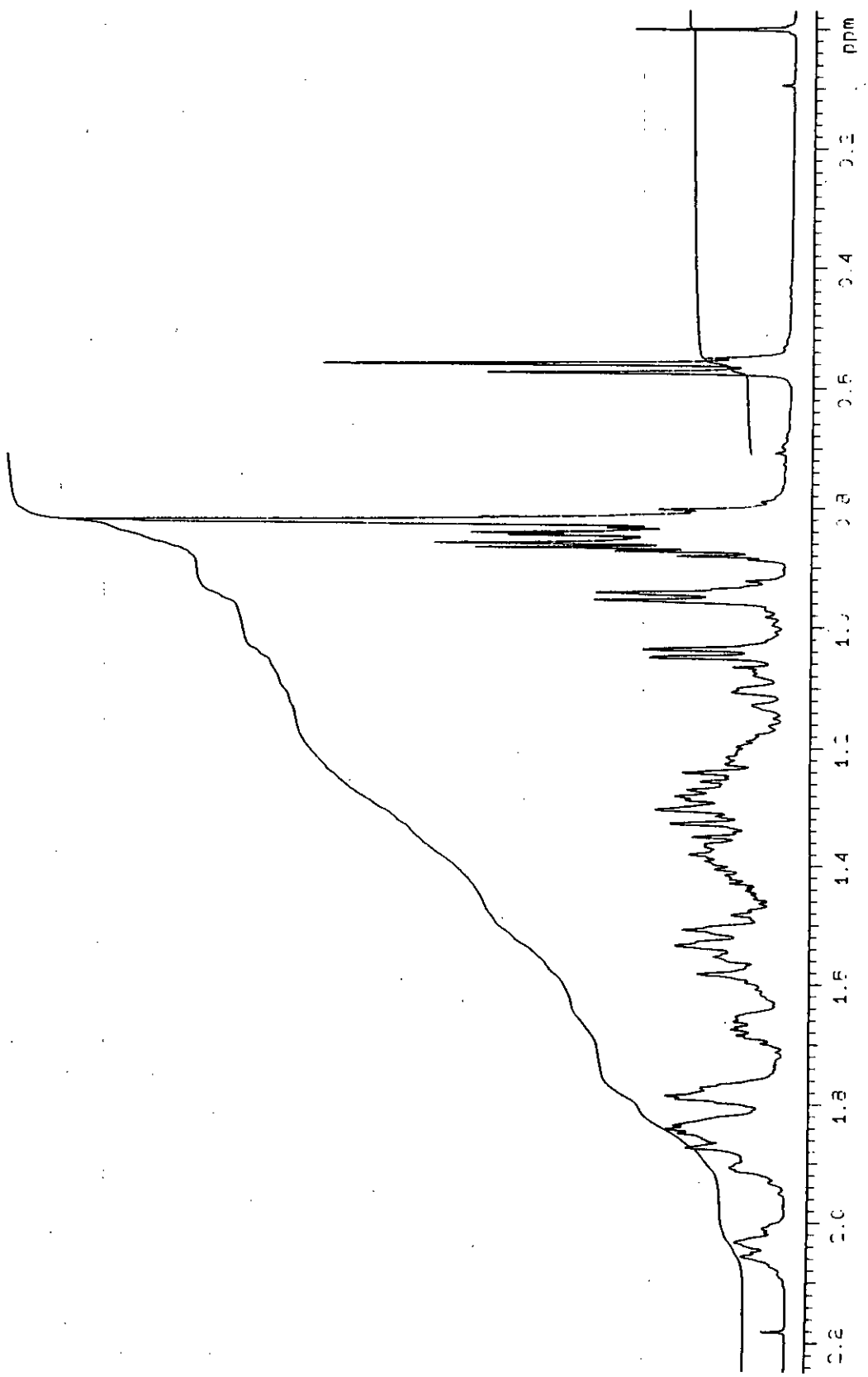


Fig.18.1 ^1H NMR Spectrum of Compound A_4 in mix. of $CDCl_3$ & CD_3OD

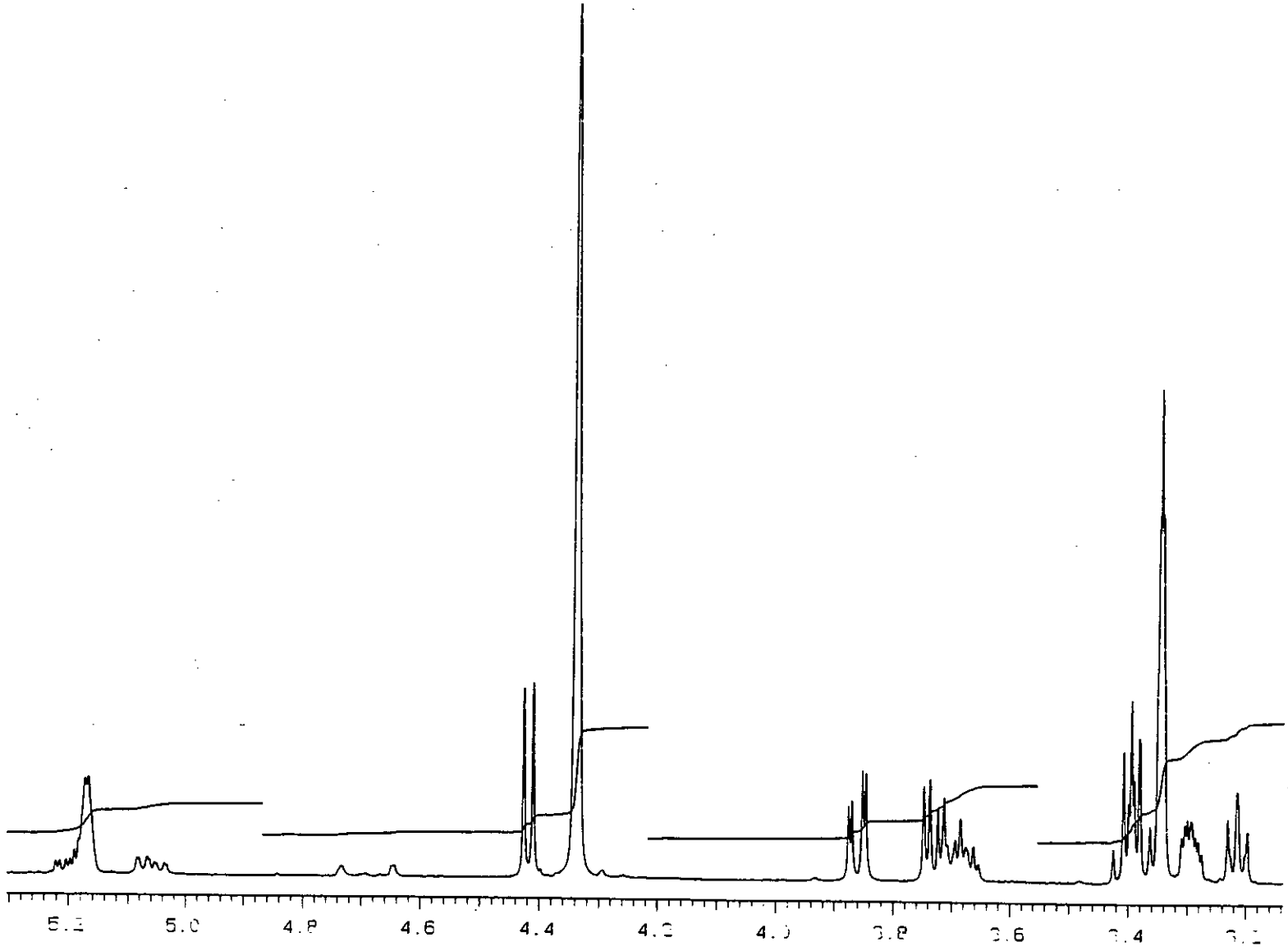


Fig.18.2 ^{1}H NMR Spectrum of Compound A_4 in mix. of $CDCl_3$ & CD_3OD

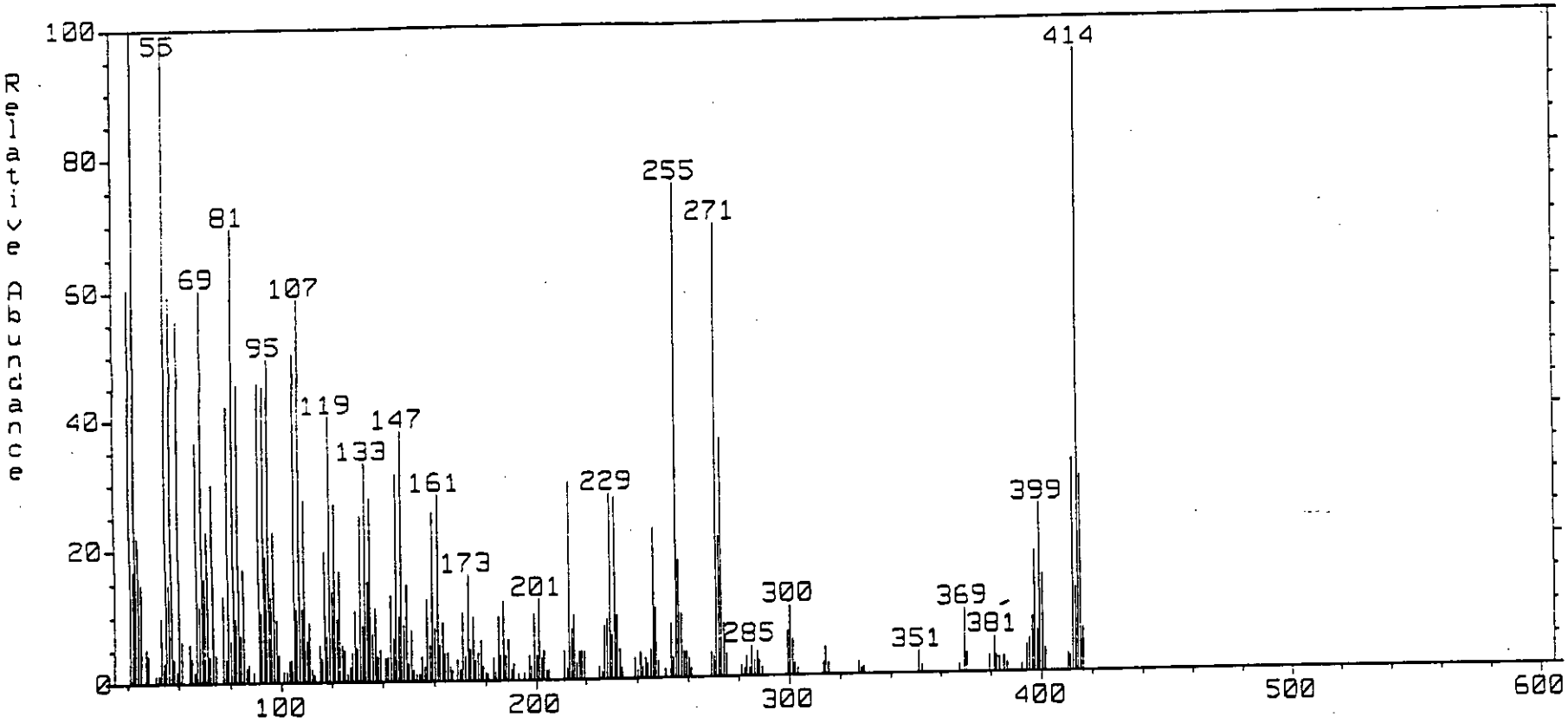


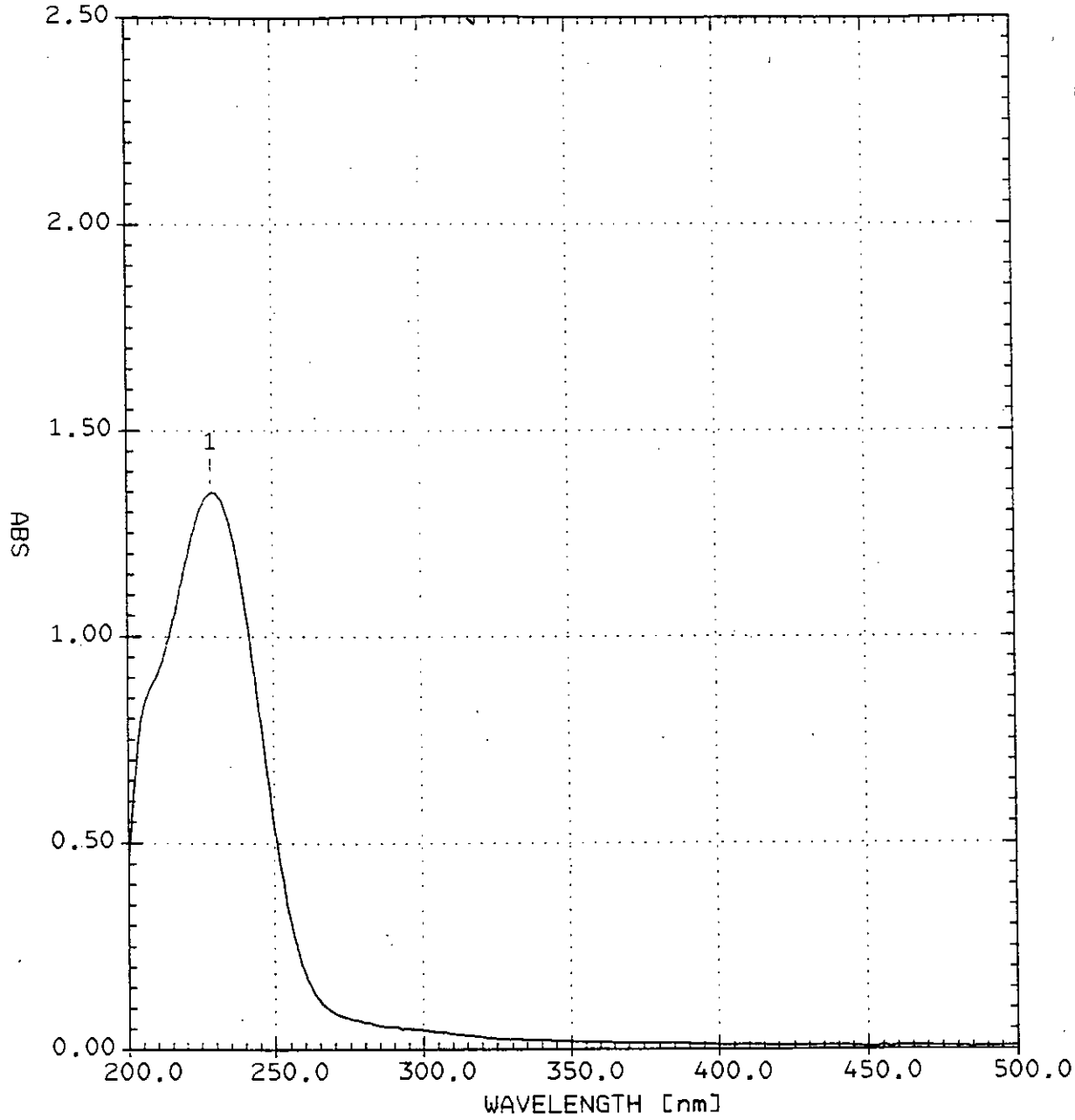
Fig.19 Mass Spectrum of Compound A_4

3.5 Study on compound A_5

Compound A_5 was obtained as an amorphous solid, m.p 145°C, (yield 0·936%). It gave weak Salkowski and Liebermann- Burchard tests for steroid and triterpene. It also gave positive Molisch's and Resorcinol tests for sugar. Compound A_5 gave copious foam on shaking with water. All these indicate compound A_5 to be a steroid glycoside.

Hydrolysis of compound A_5 by 8% methanolic hydrochloric acid gave an aglycon, m.p 182°C and a sugar. The aglycon showed similar melting point and similar TLC behaviour as compound A_2, (cucubritacin B). The aglycon was confirmed to be cucurbitacin B by co TLC, mixed melting point and superimposable IR spectra. The confirmation was also done by preparing acetate and comparing its properties with that of compound A_2. The sugar was identified as glucose by paper chromatography. Compound A_5 is thus cucurbitacin B glucoside.

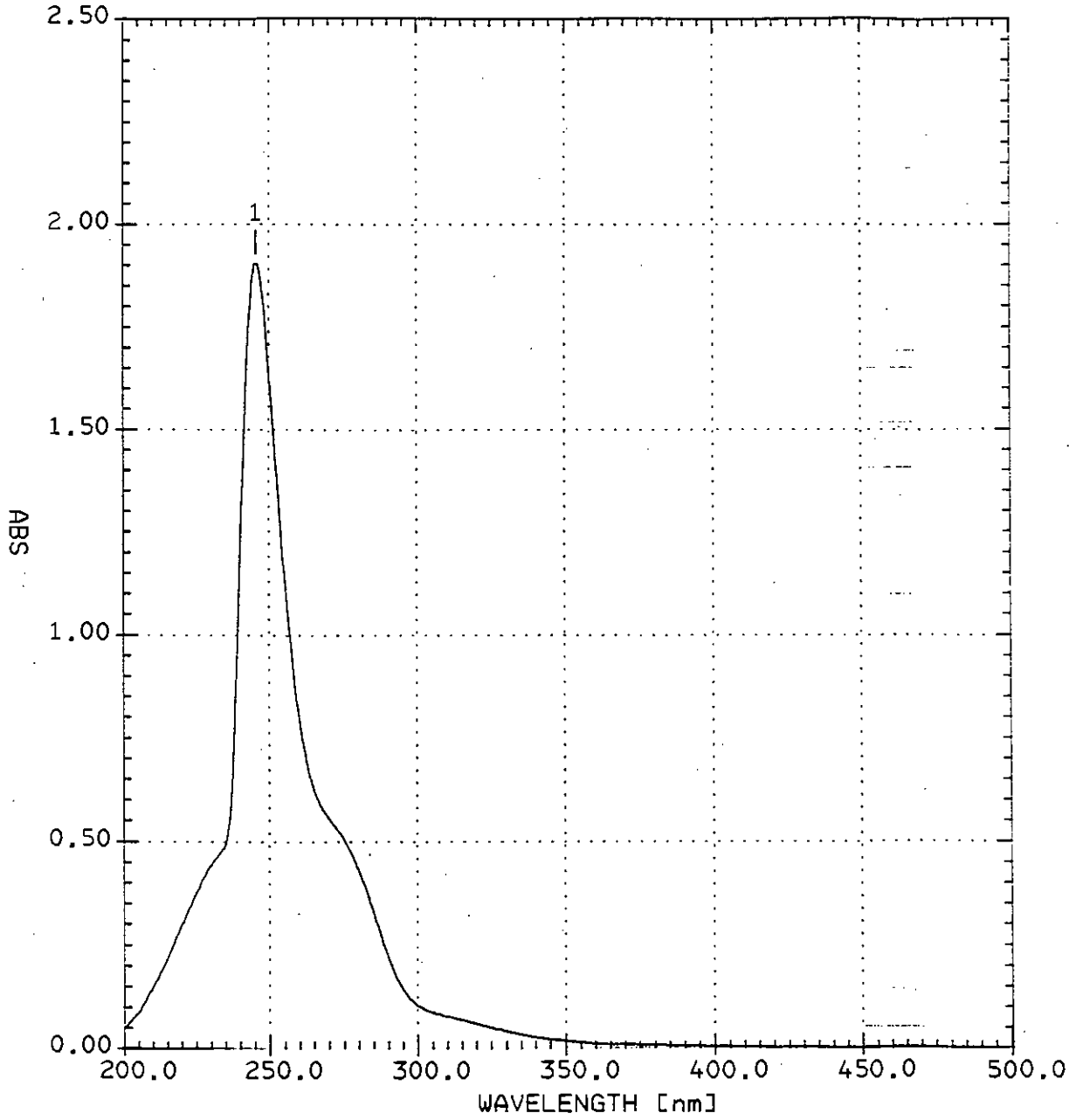
The UV absorption of compound A_5 (Fig.20) showed λ_{max} 229 nm for a α, β- unsaturated keto group. The band did not show any shift on standing in alkaline solution. On the other hand the aglycon showed λ_{max} 280 nm (Fig.20.1) for enolizable α-diketonic system which shifted to 310.5 nm in alkaline solution after 24 hours. Since the UV absorption spectra of glucoside show no change in alkaline solution, the sugar moiety must be attached to the enol hydroxyl group at C_2[35]. This is in conformity with 2-O-β-D-glucopyranosyl cucurbitacin B isolated from the herbs of Anagallis arvensis by Yamada[39].



*** PEAK-PICK ***

	-- PEAK --		-- VALLEY --	
No.	λ	ABS	λ	ABS
1	229.0	1.348		

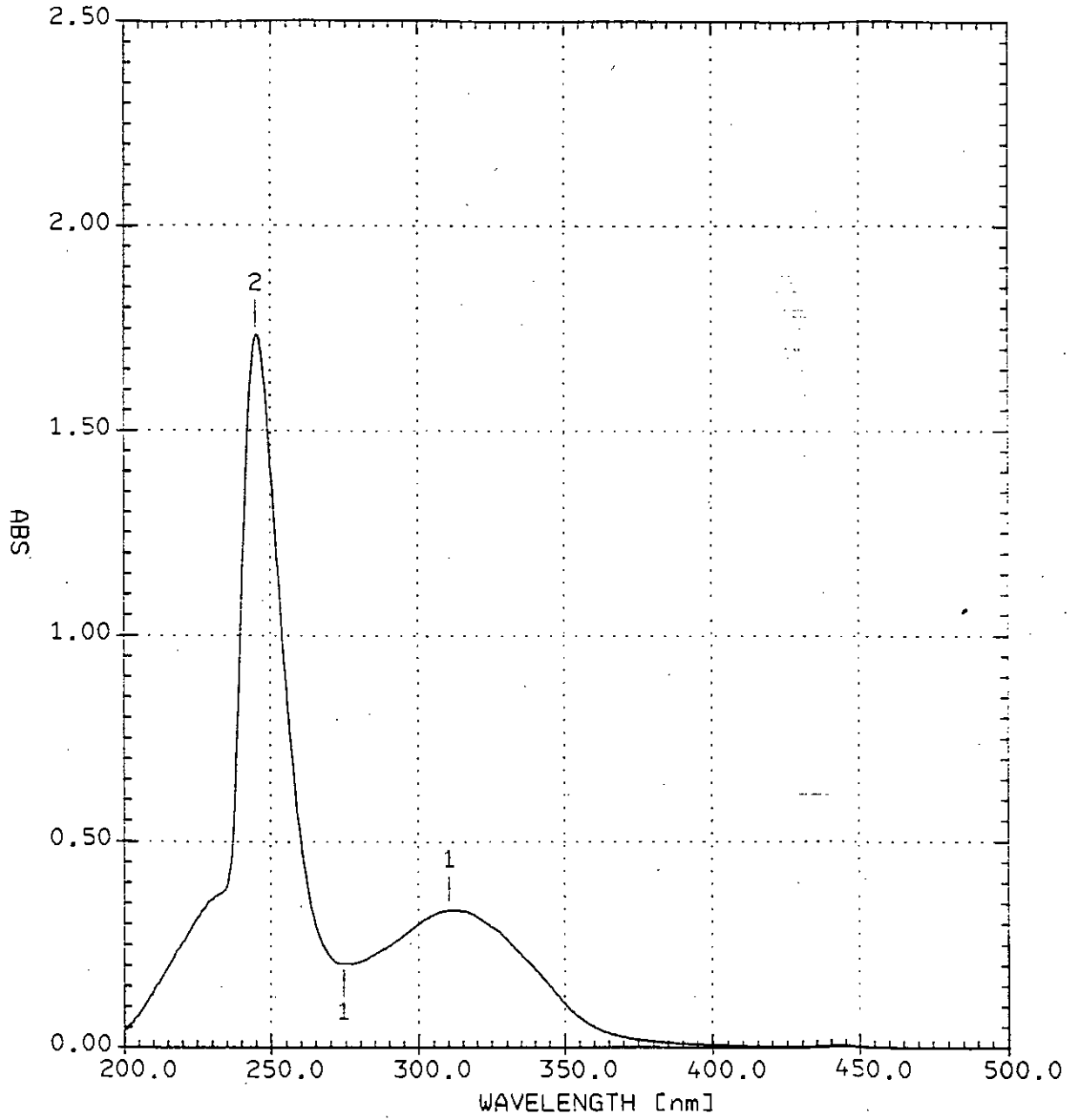
Fig.20 UV Spectrum of Compound A_5 in EtOH



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	245.5	1.903		

Fig.20.1 UV Spectrum of the aglycon in $CHCl_3$



*** PEAK-PICK ***

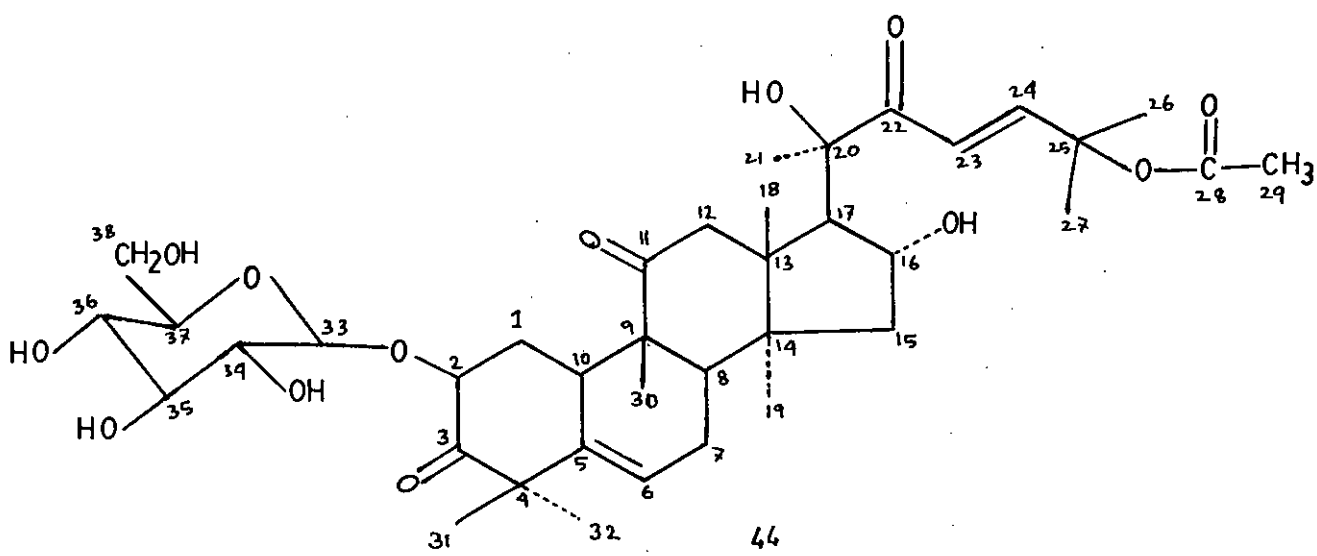
No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	310.5	0.335	274.5	0.204
2	245.0	1.734		

Fig.20.2 UV Spectrum of the aglycon (in NaOH)

The IR spectrum of compound A_5 (Fig.21) accounts for all the functional groups of 2-O-ß-D-glucopyranosyl cucurbitacin B. The bands at 3400, 1710, 1680 and 1620 cm^{-1} are attributable to OH, ester, ketone and α,β- unsaturated ketone respectively.

As expected the ^1H NMR spectrum (Fig.22) of the compound showed three olefinic protons at δ 6.95, 6.85 and 5.85 as doublets. The protons of the eight methyl groups appeared as singlets at δ 1.55, 1.54, 1.45, 1.40, 1.35, 1.30, 1.05 and 0.90. The protons of the acetate group appeared as a singlet at δ 2.00. The sugar protons appeared between δ 3.00 - 4.00. ^{13}C NMR spectrum (Fig.23) showed 38 signals and their identification is summarized in Table 9.

The compound A_5 is thus shown to be **2-O-ß-D-glucopyranosyl cucurbitacin B(44)**. This is the first report on isolation of this compound from a Luffa plant.



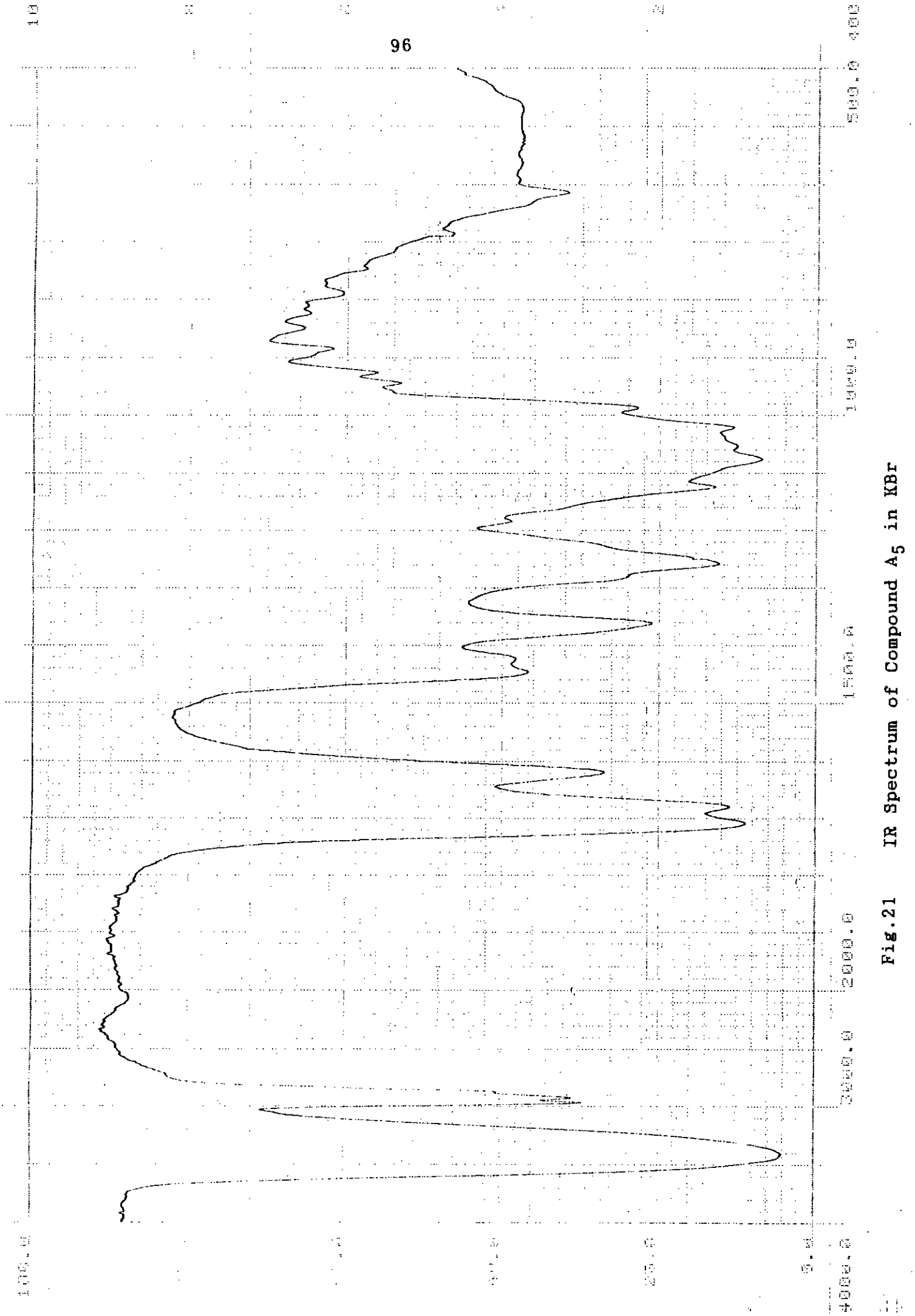


Fig.21 IR Spectrum of Compound A_5 in KBr

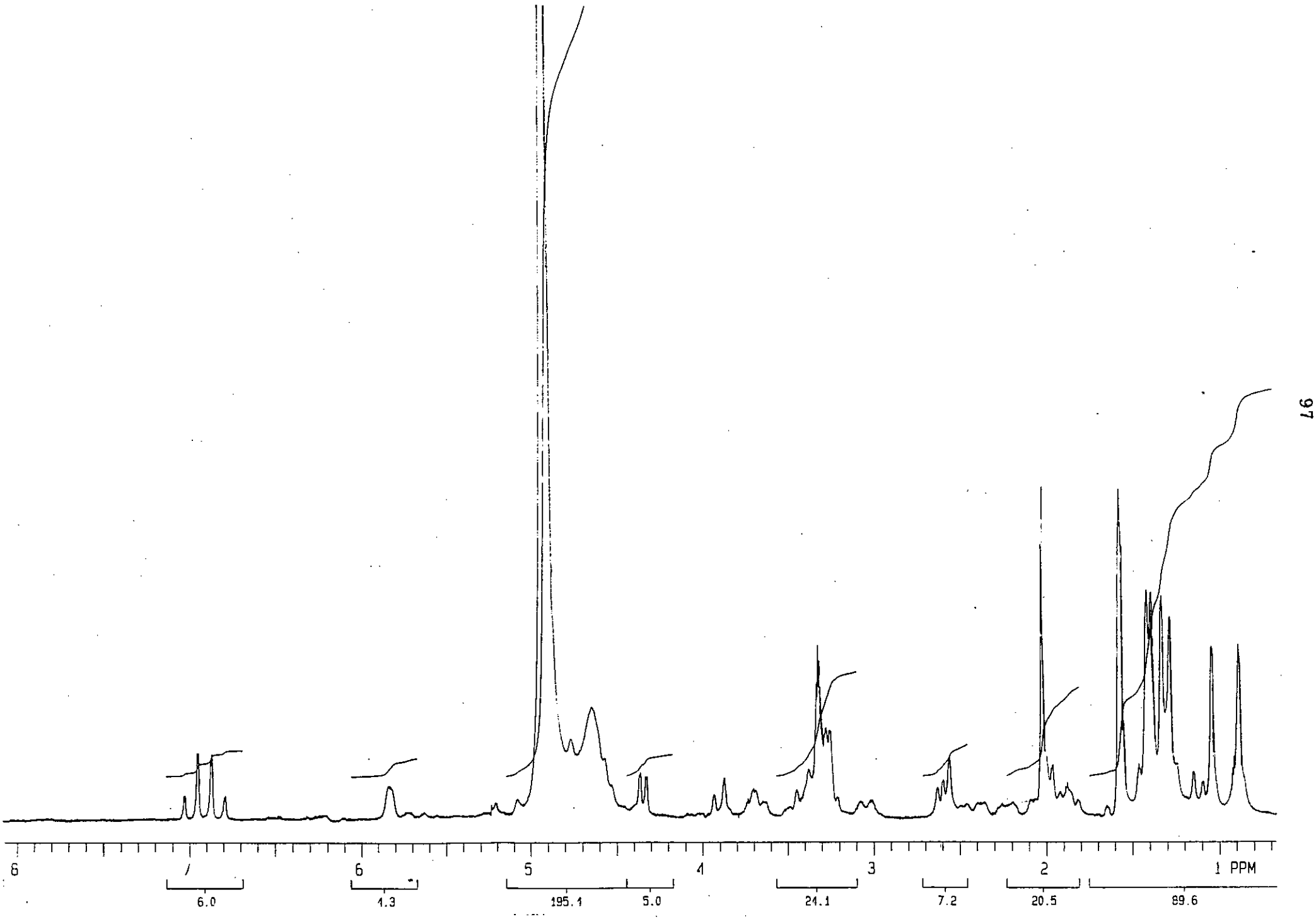


Fig.22 ^{1}H NMR Spectrum of Compound A_5 in CD_3OD

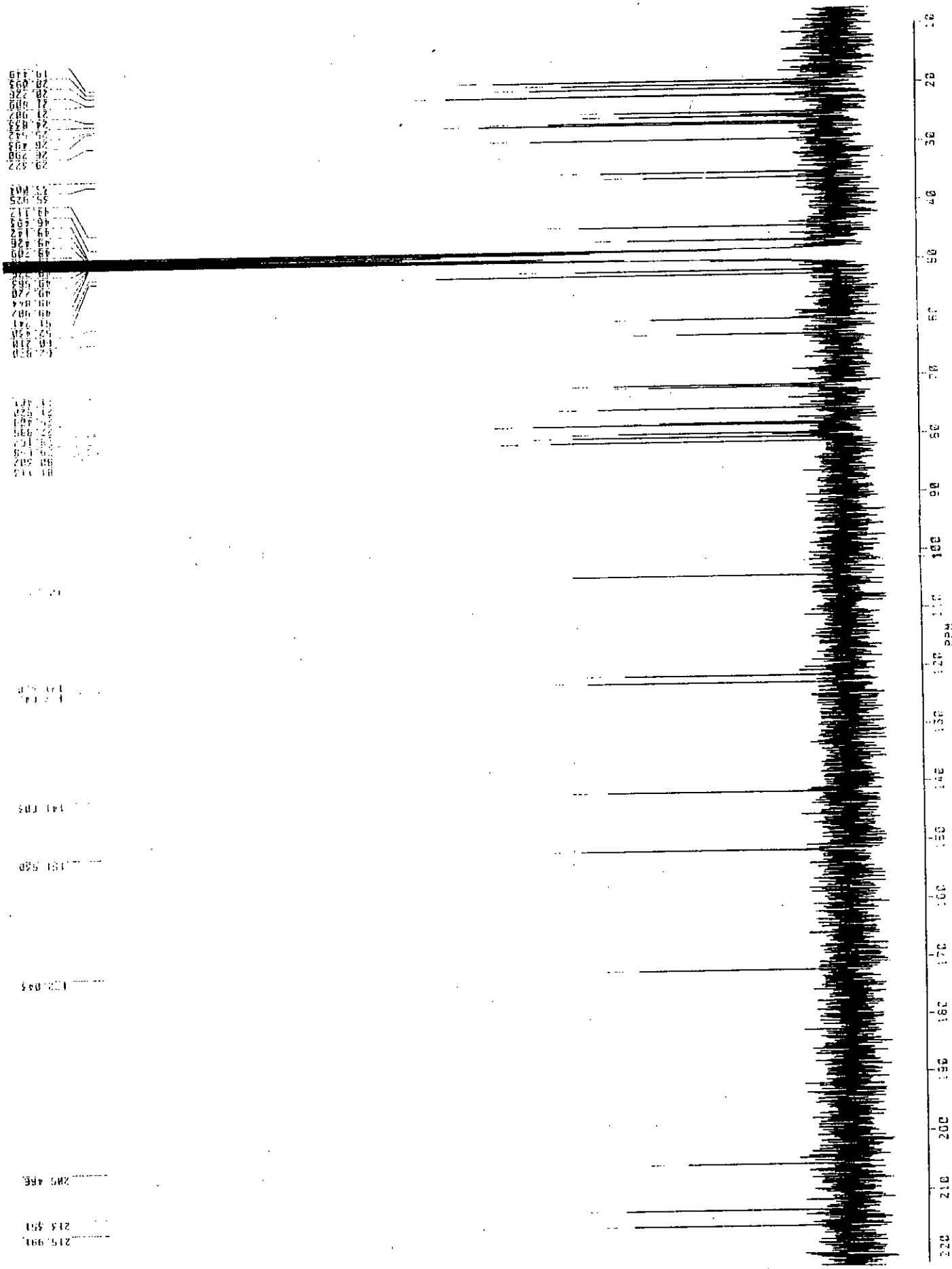


Fig.23 ^{13}C NMR Spectrum of Compound A_5 in CD_3OD

Table 9

^{13}C Chemical shifts of Compound A_5(Cucurbitacin B glucoside).

Carbon nos.	δ-values
1	35.00
2	79.65
3	213.35
4	46.49
5	141.60
6	121.42
7	26.79
8	29.37
9	49.90
10	35.92
11	215.99
12	51.74
13	52.43
14	52.43
15	44.11
16	71.46
17	60.21
18	21.80
19	24.83
20	81.11
21	25.54
22	205.46
23	122.64
24	151.53
25	80.30
26	21.90
27	21.90
28	172.04
29	26.49
30	20.09
31	19.44
32	20.72
33	104.27
34	77.88
35	78.16
36	71.82
37	75.40
38	62.87

3.6 Study on Compound A_6

Compound A_6 was obtained as an amorphous solid, m.p 170°C, (yield 0·034%) It gave positive Salkowski and Liebermann Burchard tests for steroid and triterpene. Compound A_6 also gave positive test for sugar and copious foam on shaking with water. All these indicated compound A_6 to be a steroid glycoside.

Hydrolysis of compound A_6 by 8% methanolic hydrochloric acid gave an aglycon, m.p 75°C and a sugar. The sugar was identified as glucose by paper chromatography. The aglycon showed UV absorption at λ_{max} 268 which shifted to 315 nm,(Fig.24) on treatment with alkali indicating it to possess a diosphenolic ring as in cucurbitacin E and datiscacin[28]. Unfortunately no other spectral data are available at the moment for further characterization. The melting point of the aglycon is however similar to that of cucurbitacin S which also possesses a diosphenolic ring. The bands at 3380, 1675 and 1625 cm^{-1} in IR spectrum, (Fig.25) of the glycoside indicate the presence of OH, ketone and α,β- unsaturated ketone respectively, which are also in all probability retained in the aglycon. It may be noted here that such IR absorptions are characteristics of cucurbitacin S[40].

The UV absorption of the glycoside showed λ_{max} 211 nm (Fig.24.1) which did not show any shift in alkali solution but such shift is exhibited by the aglycon as mentioned above. This clearly suggested that the sugar was attached to the enolic OH group of a diosphenolic ring like that as in cucurbitacin B (compound A_5). With the available data compound A_6 is proposed to

λ_{max} = 268 nm

λ_{max} = 315 nm (in NaOH)

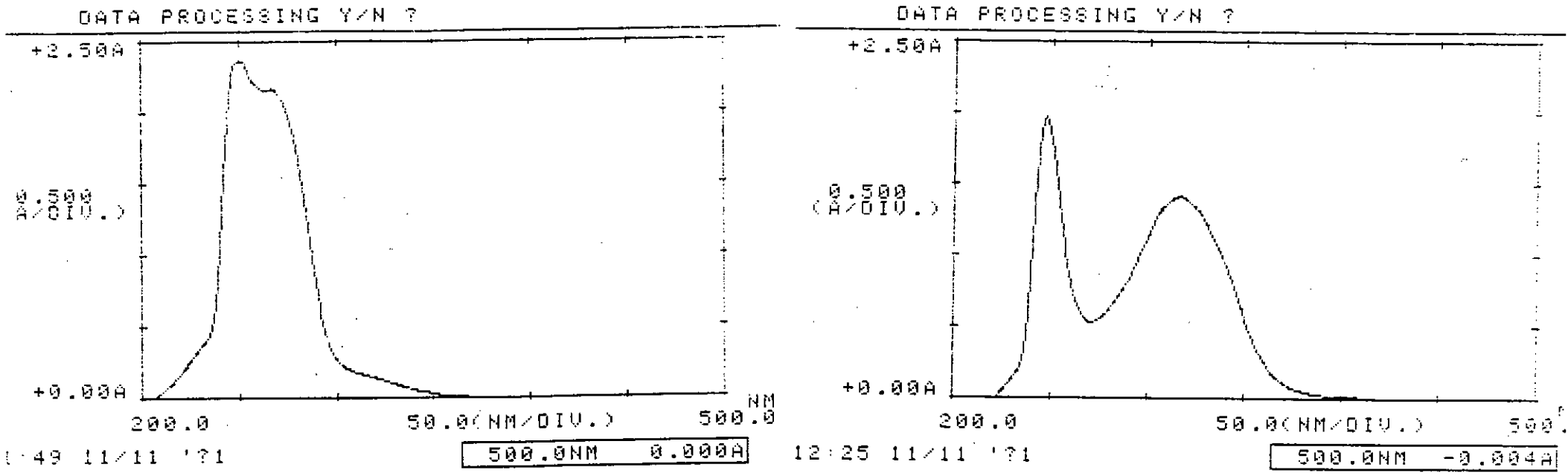


Fig.24 UV Spectrum of the aglycon of Compound A_6 in $CHCl_3$

λ_{max} = 268 nm

λ_{max} = 315 nm (in NaOH)

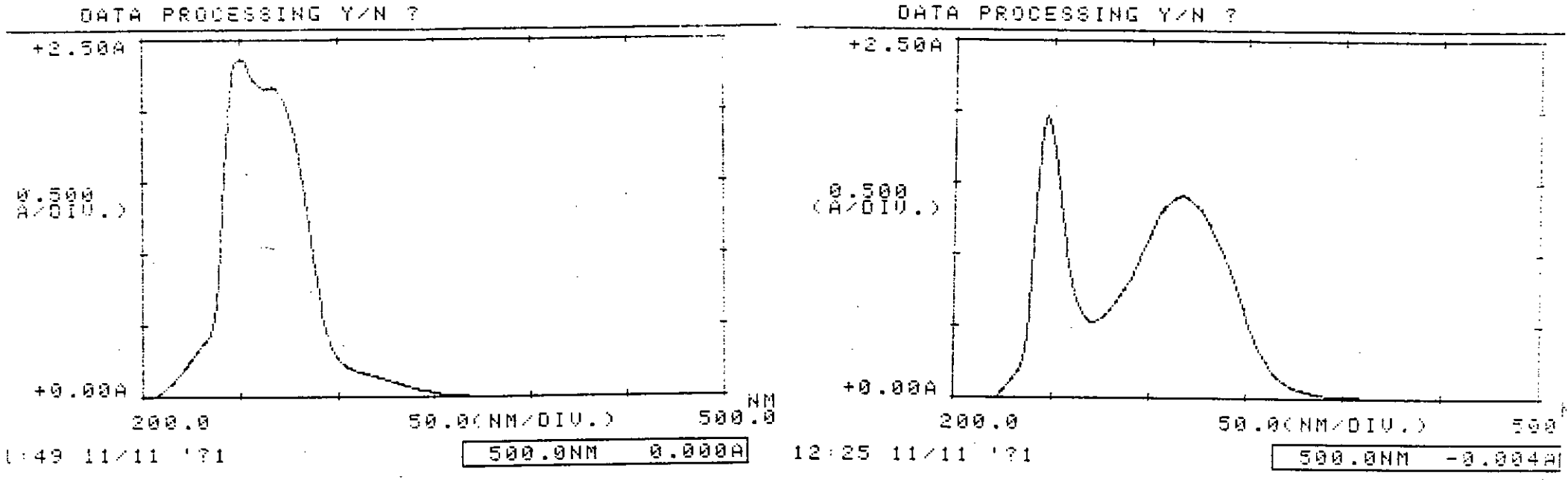
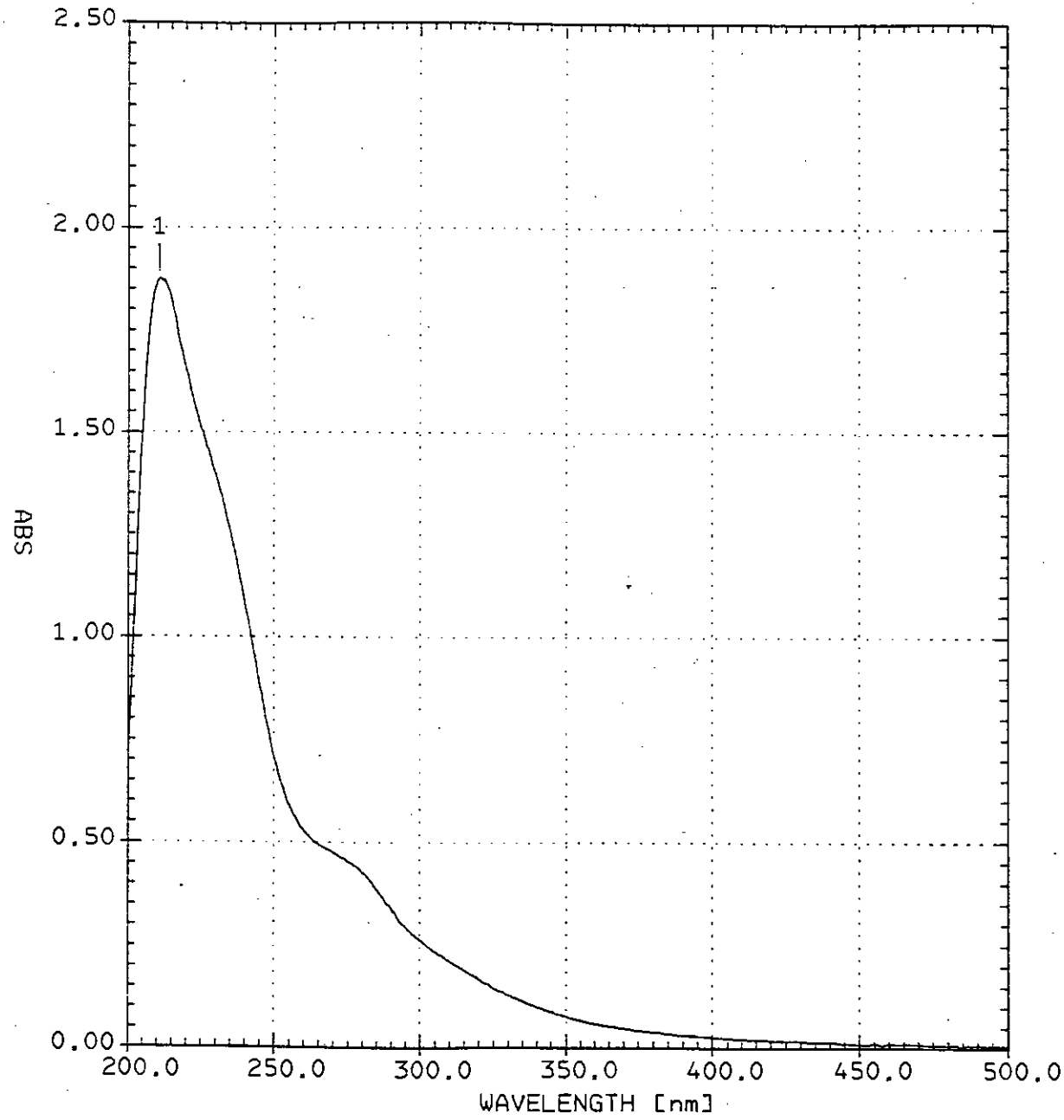


Fig.24 UV Spectrum of the aglycon of Compound A_6 in $CHCl_3$



*** PEAK-PICK ***

	-- PEAK --		-- VALLEY --	
No.	λ	ABS	λ	ABS
1	211.0	1.876		

Fig.24.1 UV Spectrum of Compound A_6 in EtOH

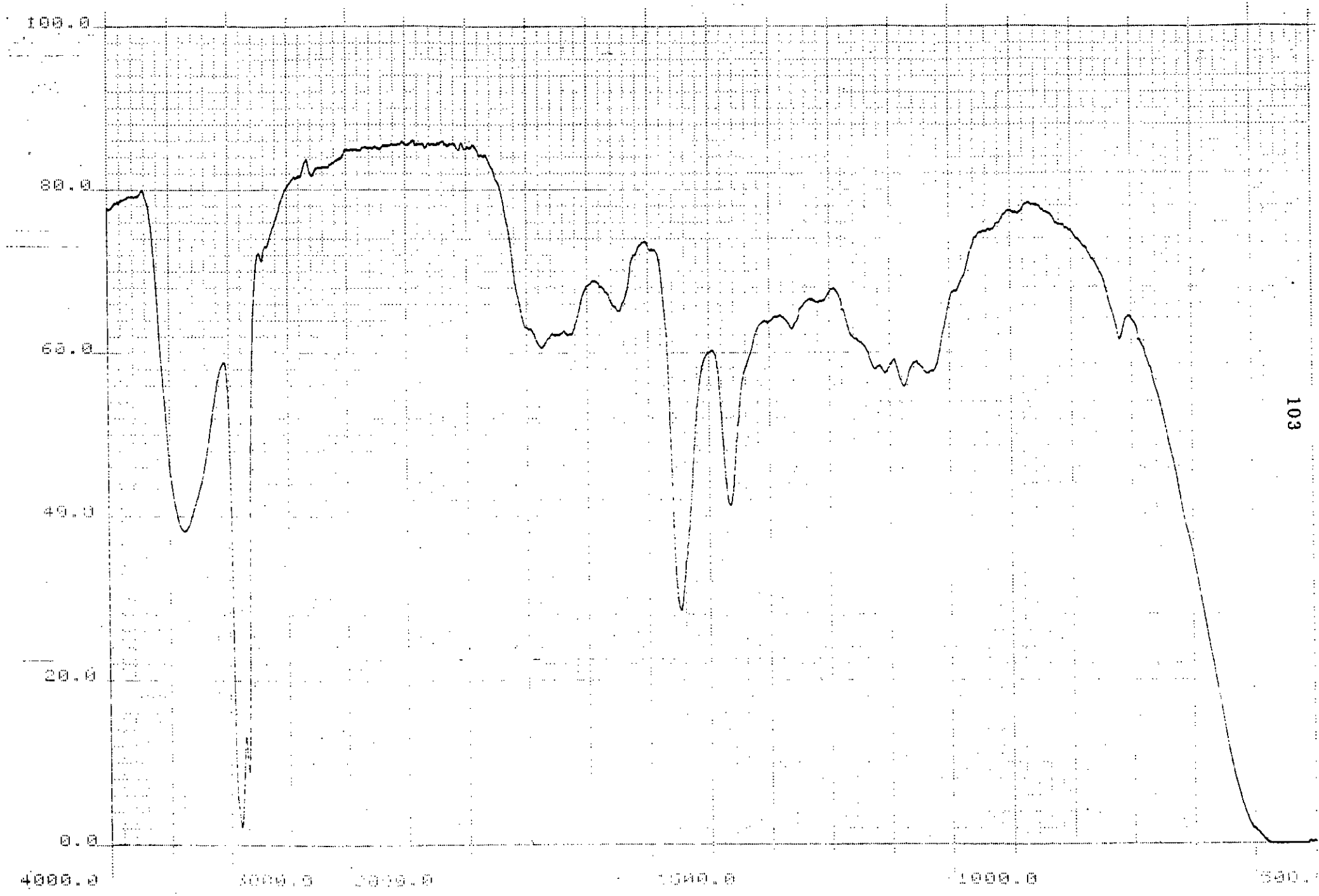
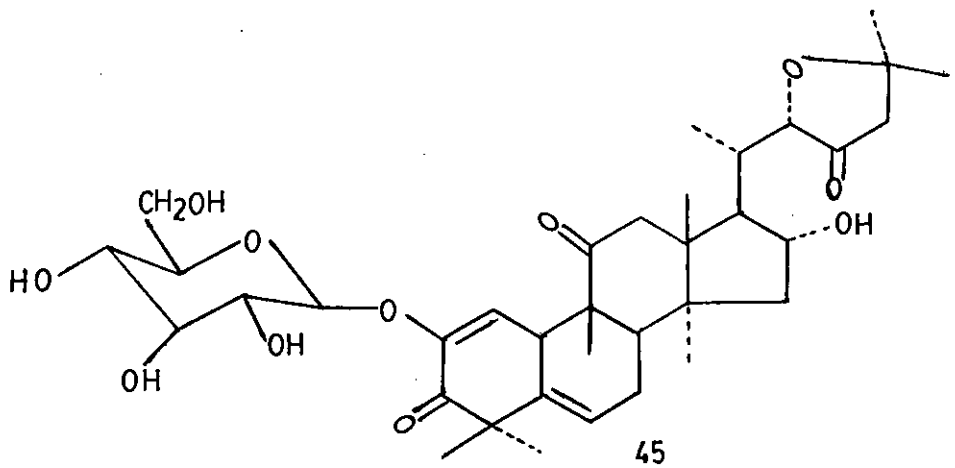


Fig.25. IR Spectrum of Compound A_6 in nujol

be 2-O-β-D-glucopyranosyl cucurbitacin S (45); however the structure of the aglycon remains to be more rigorously established.



45

3.7 Pharmacological Studies.

The plant **Luffa echinata** is reported to be active against jaundice.The ethanol extract was tested on guinea-pig atria, tracheal chain, ileum and rat uterus[30,31,32,33]. No work on liver disorder could be carried out.

Ethanol extract showed poor activity on the atria by decreasing both the rate and also the force of contraction.

Ethanol extract proved to be inactive to antagonize the histamine induced contraction of the Guinea- pig tracheal chain. Ethanol extract exhibited potent antispasmodic activity against spasmogens (e.g acetyl choline, histamine, serotomin and barium chloride).

Ethanol extract was found to have poor activity against oxytocin induced contraction in rat uterus.

Luffa cylindrica

The fruits of Luffa cylindrica (bitter variety) were collected from Rajshahi Bangladesh. The deep green fruits were cut into small pieces and dried in the sun. The dry sliced fruits were extracted with chloroform (3X48 hours) at room temperature. The combined chloroform extract (3 litre) was evaporated to dryness when a light green solid mass (2.5 gm) was obtained which was denoted as Mass N_1

The solid Mass N_1 showed the presence of at least two compounds on TLC plates. These were isolated in the pure state by column chromatography over a silica gel column using CCl_4 : EtOAc as the eluting solvent. Compounds were noted as B_1 and B_2. The polar material which did not move along the column was washed out with ethyl acetate and was collected as Mass N_2. No further work was done on Mass N_2.

3.8 Study on Compound B_1

Compound B_1 was a colourless amorphous solid, m.p 84-87°C, (yield 0.006%). It did not respond to Salkowski and Liebermann-Burchard reaction for steroids and triterpenes.

The mass spectrum of the compound (Fig.26) exhibited fragments at m/e 57 (base peak), 71 85, 99, 113,127, 141,155, 169, 183, 197, 211, 225, 139,253,267, 281, 295, 309, 323, 337, 351, 365, 379, 393, and 407 with decreasing intensity and a loss of 14 mass unit from higher fragment to the next lower fragment indicating the compound to possess a long alkyl chain. The compound was hydroxylic in nature as revealed by IR absorption at

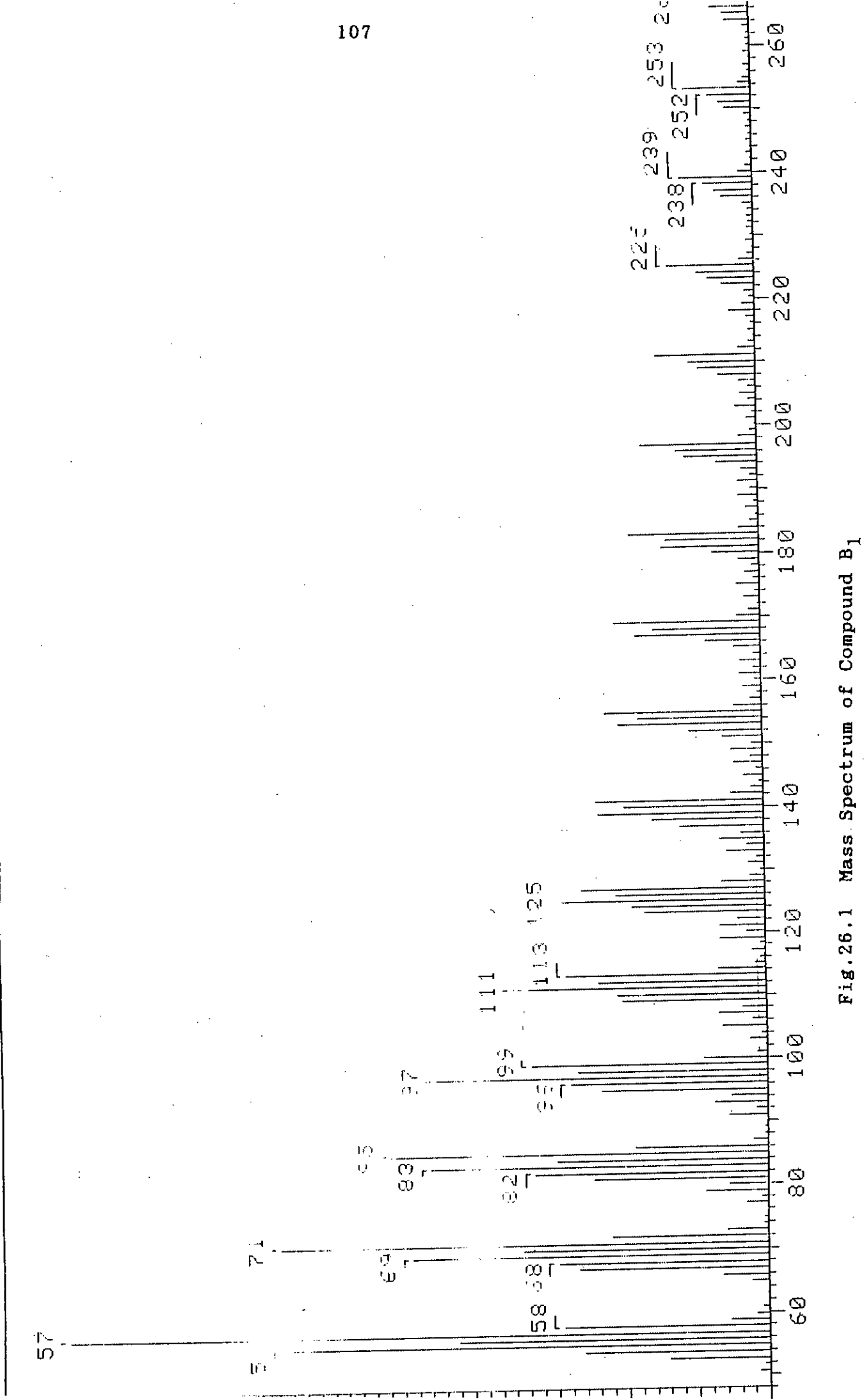


Fig.26.1 Mass Spectrum of Compound B_1

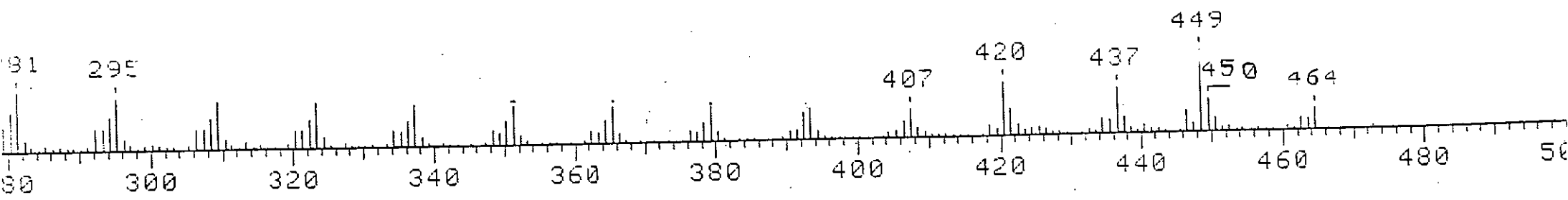


Fig.26.2 Mass Spectrum of Compound B_1

3425 cm^{-1} (Fig.27), thus suggesting the compound to be a long chain alcohol. The mass spectrum also showed mass peaks at m/e 438, 420 and 407 indicating successive loss of a water molecule and CH unit from the long chain alcohol. If m/e 438 is considered to be the molecular ion peak for the compound, the molecular fromula of the compound is in agreement with $C_{30}H_{62}O$ and the alcohol being $CH_3-(CH_2)_{27}-CH_2-CH_2-OH$ (1-triacontanol). The ^1H NMR spectrum of the compound (Fig.28) is in agreement with this structure for the alcolhol. The triplet at δ 0.88 is attributable to the terminal methyl group whereas the triplet at δ 3.64 is appropriate for the methylene protons attached to the hydroxylic group. The multiplet at δ 2.30 may be assigned to the methylene protons next to CH_2-OH group. The methylene protons appear as a huge peak at about δ 1.25. 1- Triacontanol, it may be noted has been isolated from plant bodies and reported to have m.p 86°C[41]. Compound B_1 has also similar m.p as 1- triacontanol but not exactly the same. This may be due to slight impurity present in B_1 as revealed by mass fragments at m/e 450 and 464.

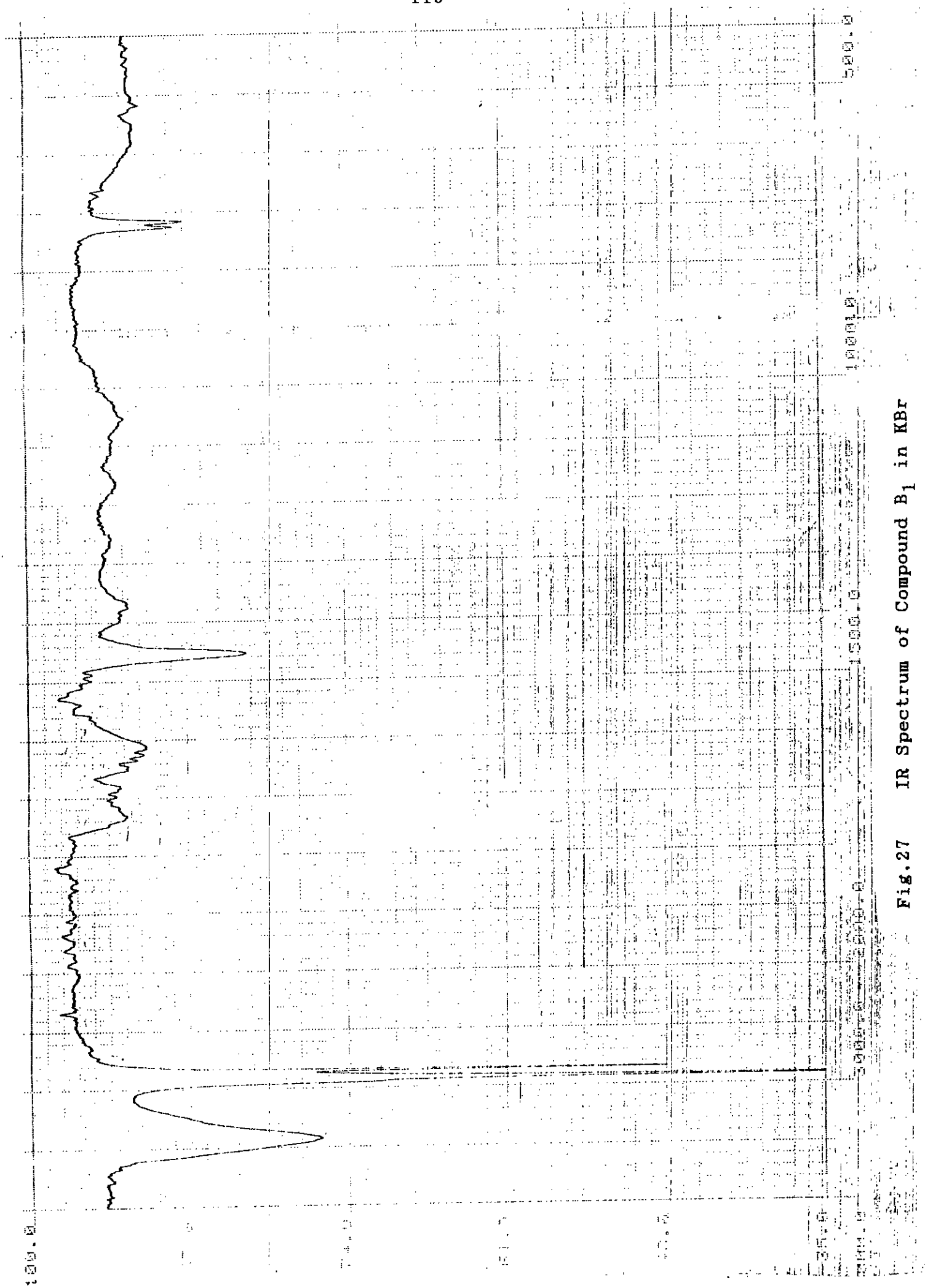


Fig.27 IR Spectrum of Compound B_1 in KBr

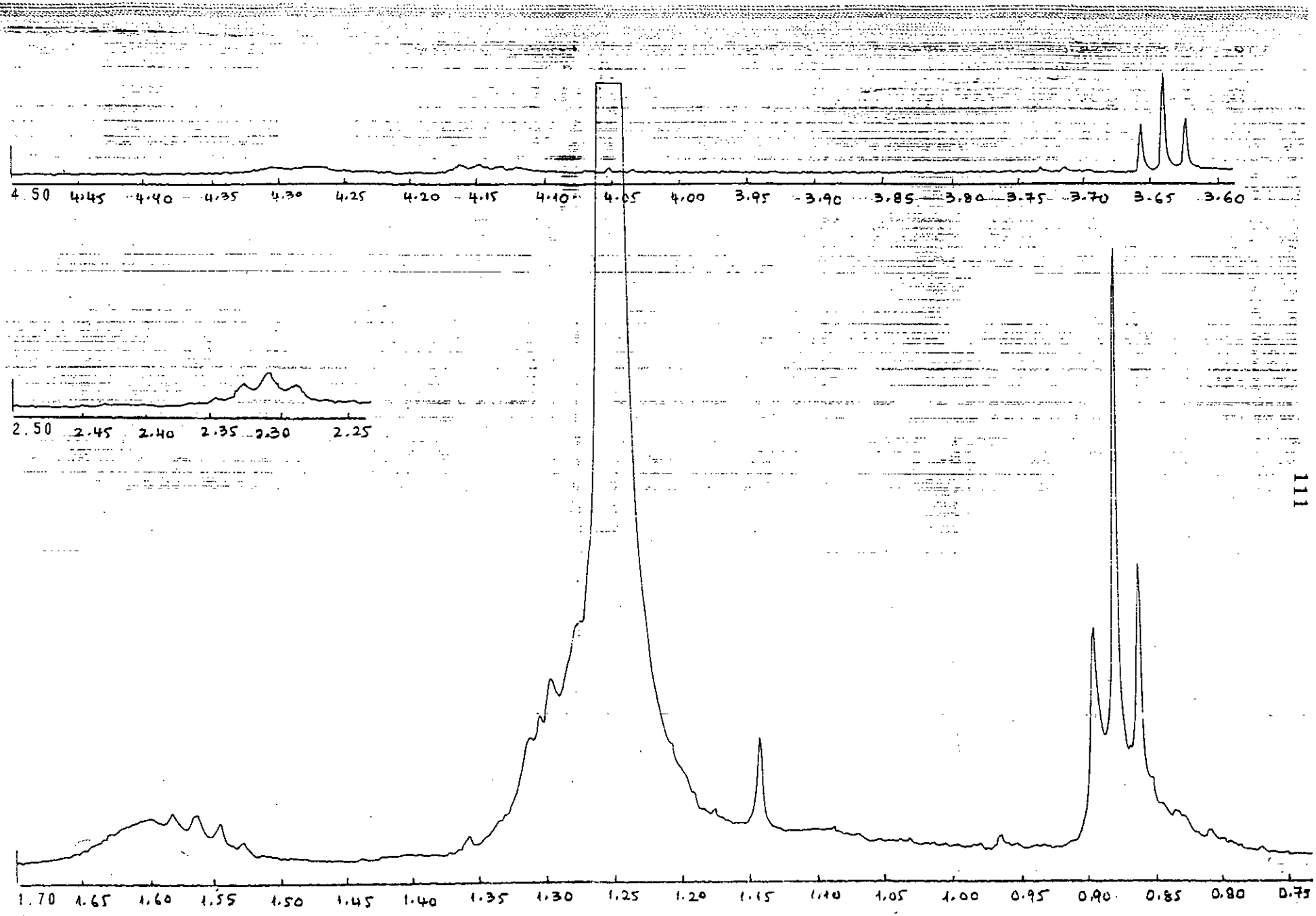


Fig.28 ^{1}H NMR Spectrum of Compound B_1 in $CDCl_3$

3.9 Study on Compound B_2

Compound B_2 was isolated as white crystalline needles, m.p 167°C (yield 0.056%). It gave positive Liebermann-Burchard test for sterol. The IR spectrum,(Fig.29) showing bands at 3405, 1045 and 1040 cm^{-1} indicated the presence of hyroxyl group. The olefinic double bond was suggested by a weak absorption band at 1640 cm^{-1}.

The mass spectrum,(Fig.30) of the compound B_2 showed the molecular ion peak at m/e 412 which also provides evidence for the presence of a $C_{10}H_{21}$ saturated side chain by showing intense peaks at m/e 271 ($M^+-C_{10}H_{21}$) and 229 ($M^+-C_{10}H_{21}-42$). The peak at m/e 229 is chacteristic of a normal tetracyclic steroidal skeleton with two double bonds and one hydroxyl function on the tetracyclic moiety; the saturated side chain being attached at position C_{17}[42].

The steroidal structure of the compound was confirmed by ^{13}C NMR spectrum,(Fig.31) which exhibited as many as 29 peaks. The presence of six methyl groups in the molecule is clear from the ^1H NMR spectrum (Fig.32) which showed six 3H singlets at δ 1.05, 1.03, 0.85, 0.75, 0.75 and 0.55. Intergration of the proton signals of the ^1H NMR spectrum of compound B_2 is computed for 48 protons in the molecule. It clearly shows the presence of two ethylenic protons as a complex multiplet centred about δ 5.15. The diffused multiplet centred about δ 3.60 is approprate for proton on the carbon attached to the hydroxyl function (>CH-OH).

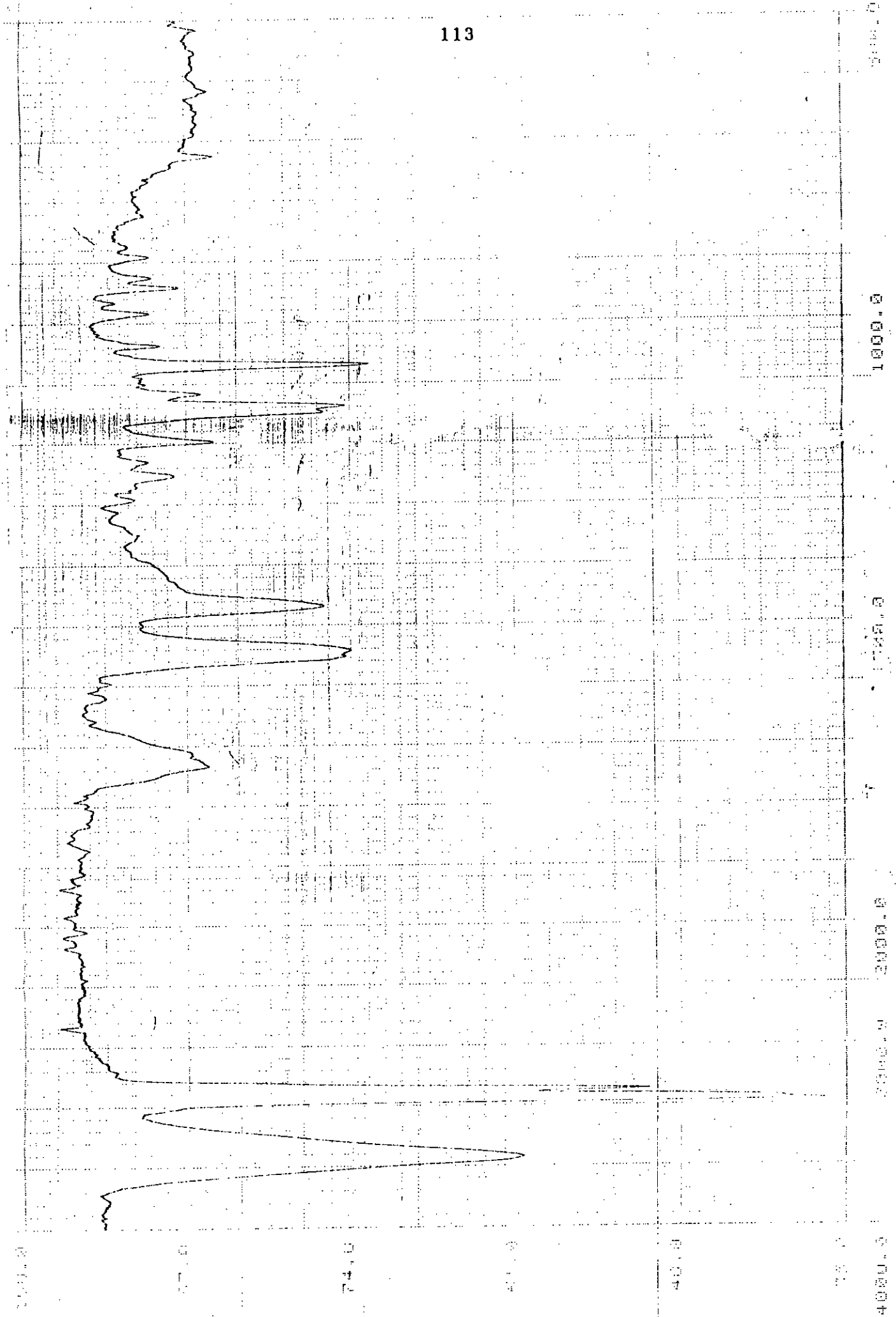


Fig.29 IR Spectrum of Compound B_2 in nujol

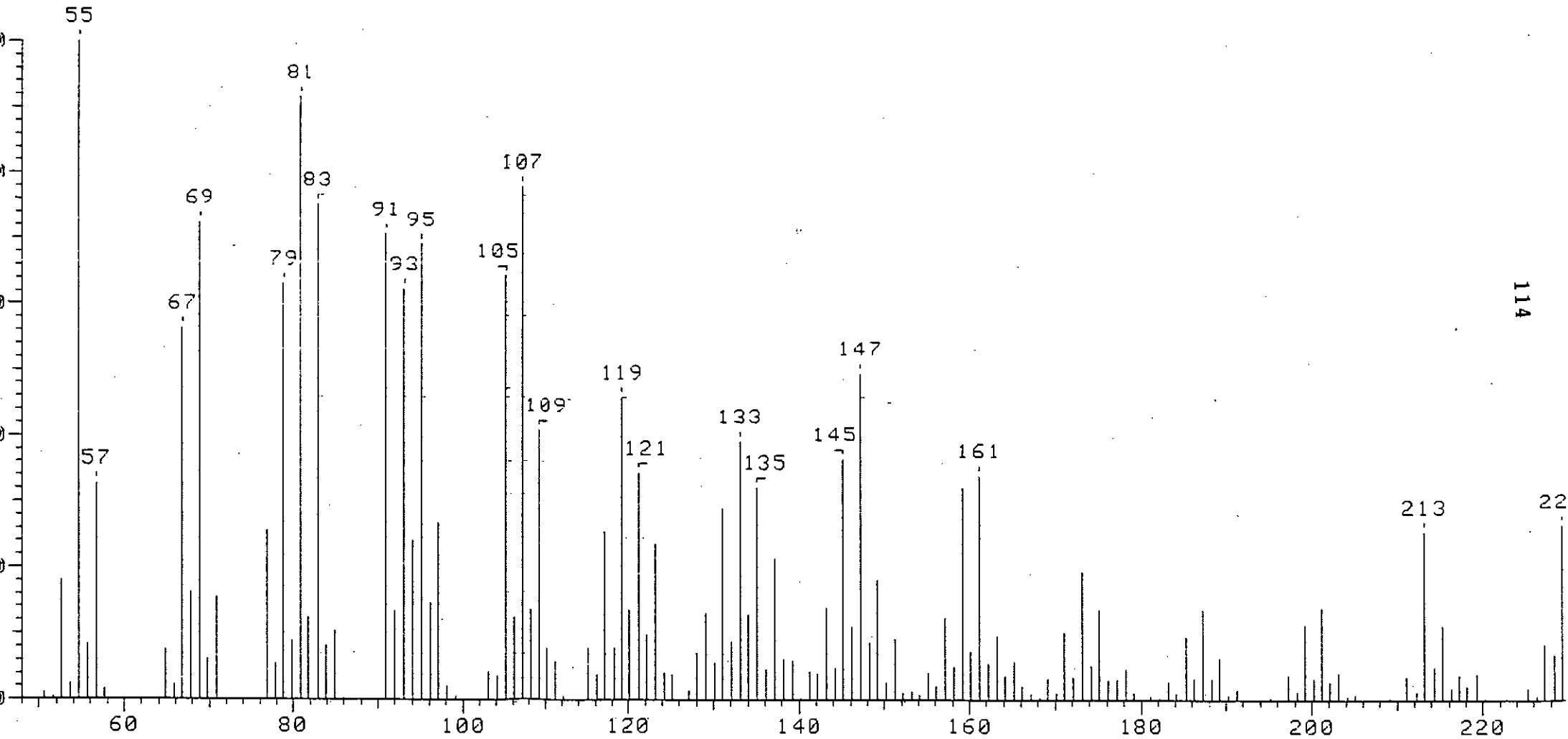


Fig.30.1 Mass Spectrum of Compound B_2

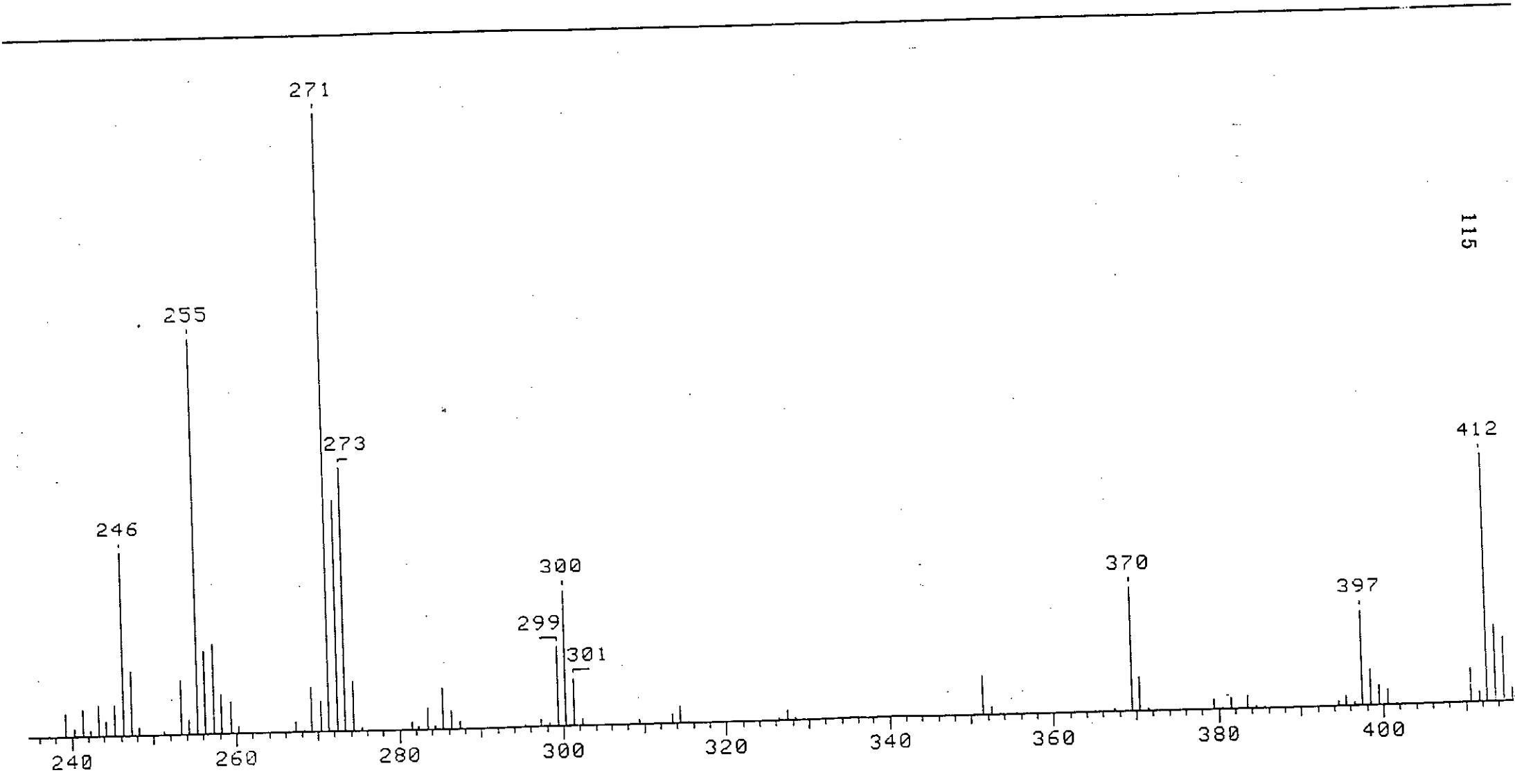


Fig.30.2 Mass Spectrum of Compound B_2

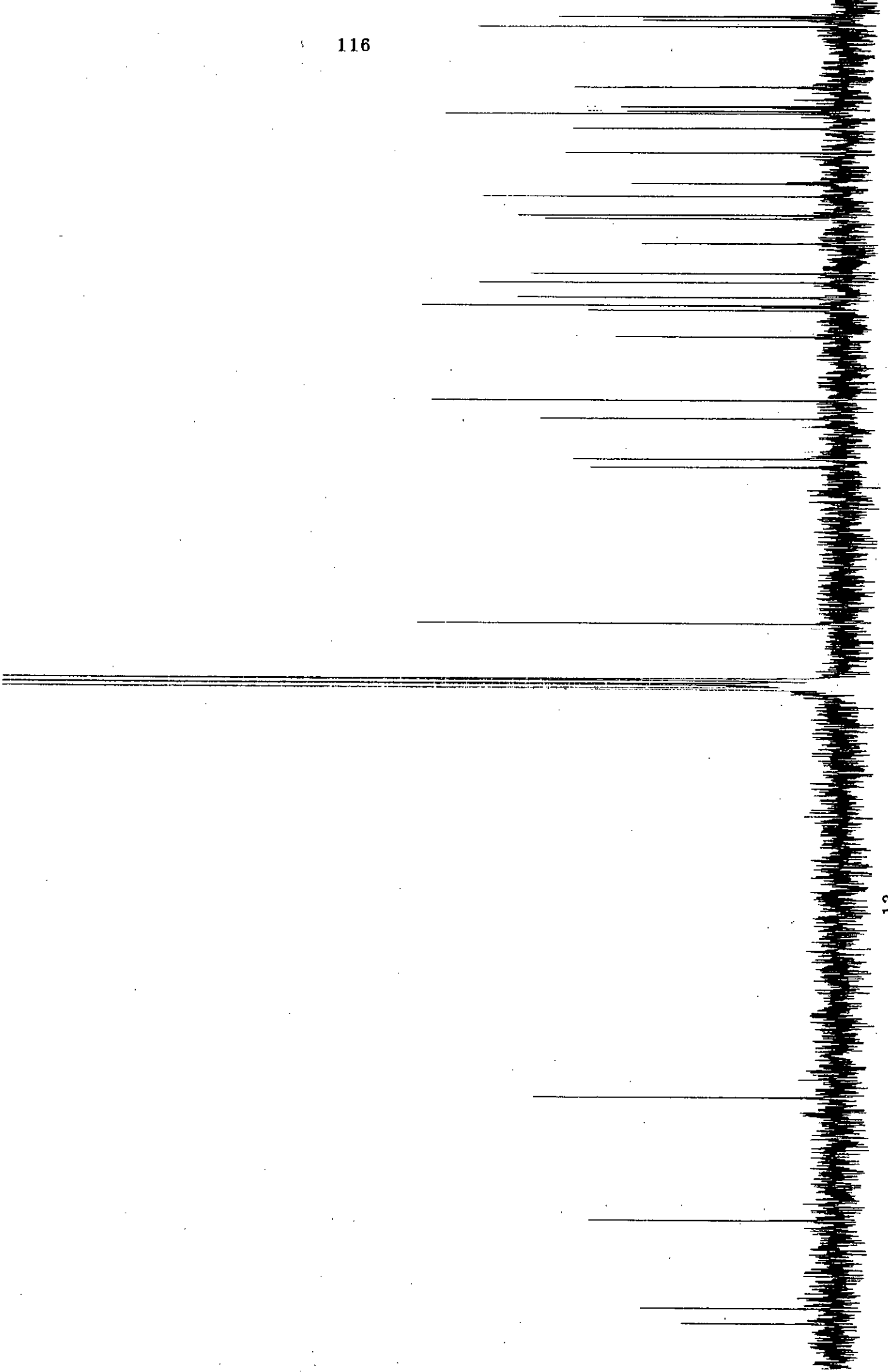


Fig.31 ^{13}C NMR Spectrum of Compound B_2 in $CDCl_3$

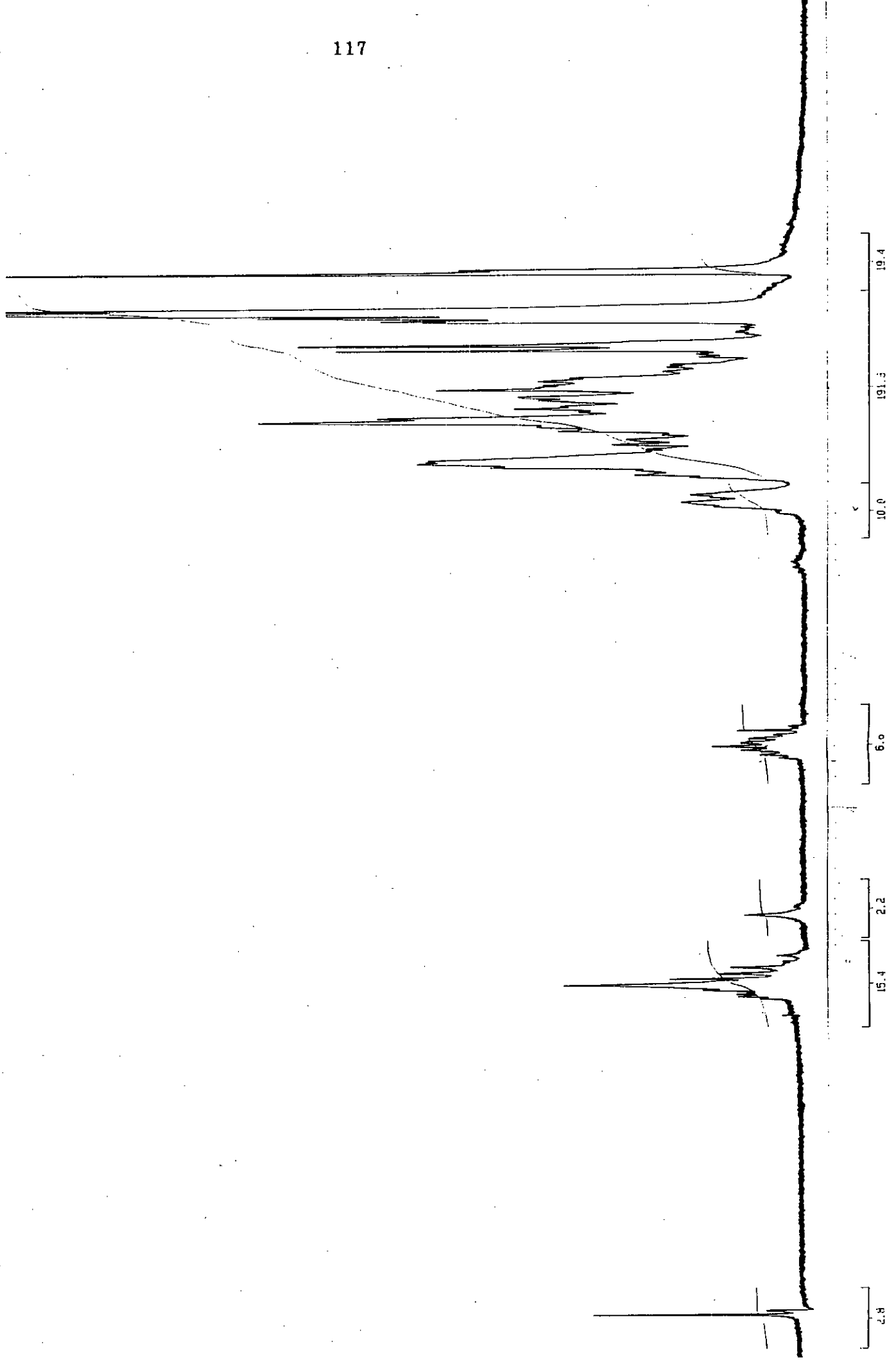
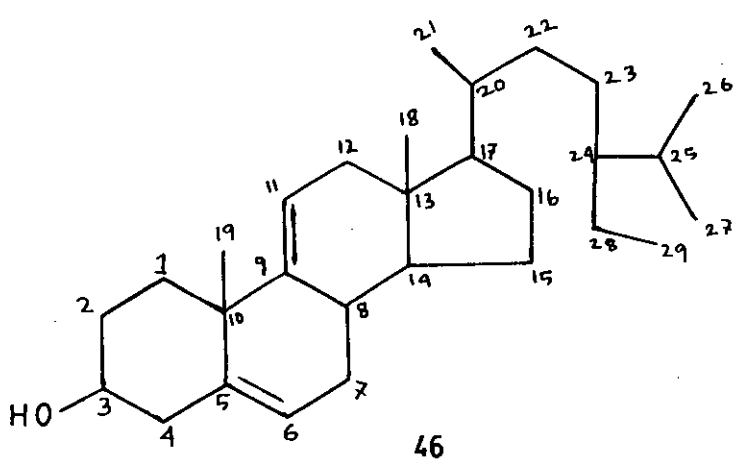


Fig.32 ^1H NMR Spectrum of Compound B_2 in $CDCl_3$

The ^{13}C NMR signal of the compound contains four comparatively low field ones at δ 139.57, 138.11 129.53 and 117.48 which confirms not only the presence of ethylenic carbon atoms but also the number being four suggesting the presence of two carbon-carbon double bonds. The molecular formula of the compound is computed to be $C_{29}H_{48}O$ (M^+m/e 412) .The molecular formula, melting point and the spectral characteristics suggest the compound to be stigmasta $\Delta^{5,9(11)}$-diene-3β-ol which was named as celsianol(46) by Sen and Chowdhury who for the first time reported the isolation of the compound from plant bodies[42].

The mass spectral fragment of the compound B_2 at m/e 55 (base peak), 397, 370, 271, 255, 229, 213, 161, 147 and 107 is nicely accommodated by this structure and is similar to that reported for celsianol by Sen and Chowdhury[42]. Furthermore all the ^{13}C signals can be logicallly assigned to the carbon atoms of the molecule,(Table 10).

The identity of compound B_2 as celsianol was further confirmed by preparing its acetate (ν_{max} 1730 cm^{-1} for ester) which melted at 172°C, same as that of the m.p 172°C, reported for celsianol acetate[42].



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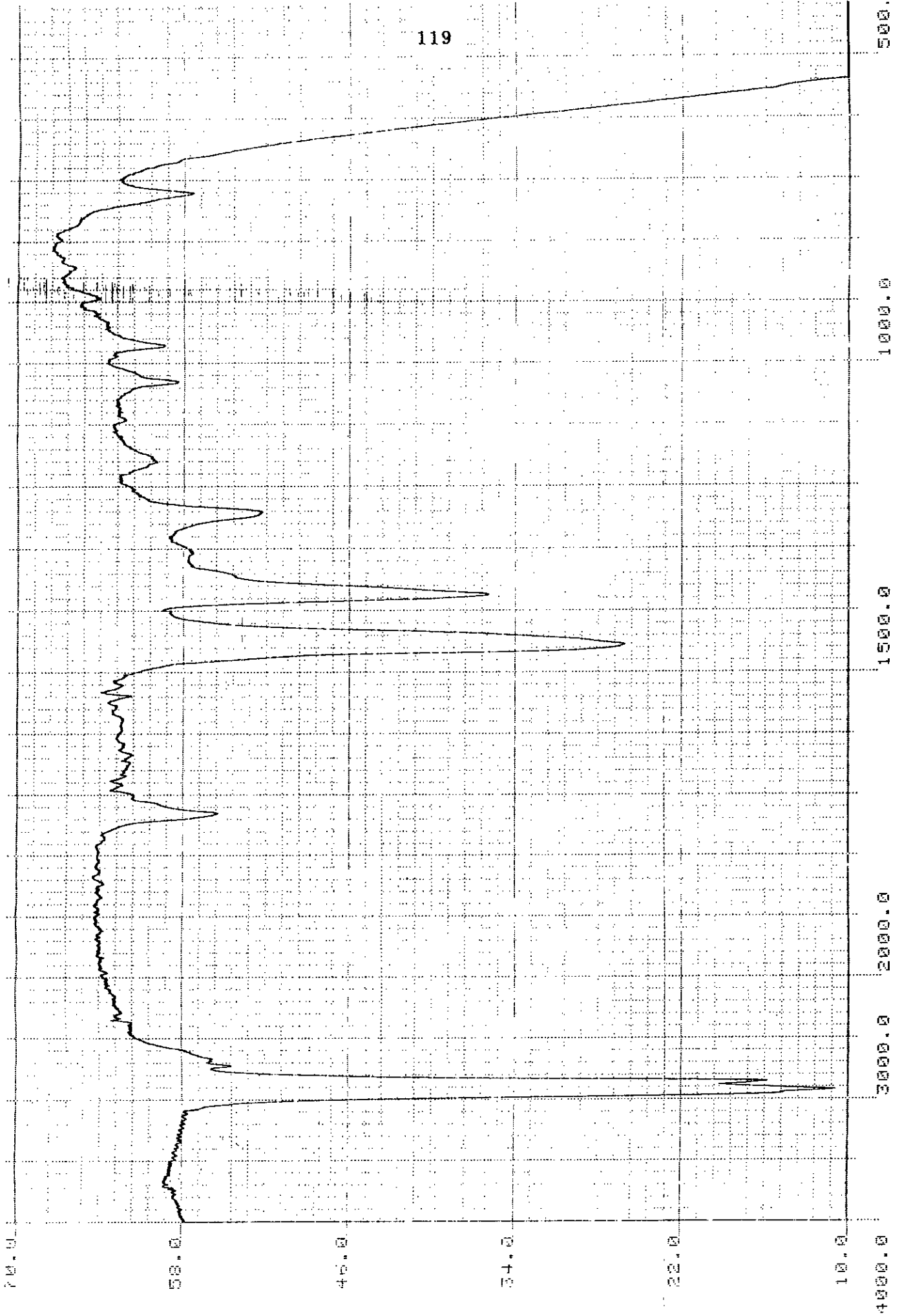

Fig.33 IR Spectrum of the acetate of Compound B_2 in nujol

Table 10

^{13}C Chemical shifts of compound B_2 (celsianol).

Carbon nos	δ-values
1	37.18
2	28.39
3	71.07
4	34.25
5	138.11
6	117.48
7	40.30
8	40.74
9	139.57
10	29.66
11	129.53
12	38.04
13	39.50
14	43.31
15	31.83
16	31.52
17	49.49
18	16.96
19	20.91
20	51.24
21	21.35
22	21.57
23	23.02
24	55.15
25	55.93
26	12.67
27	12.42
28	25.39
29	13.04

CHAPTER 4

ABSTRACT

The fruits of Luffa echinata (800 gm) were extracted with chloroform (3X48 hours) at room temperature. The chloroform extracts yielded a deep green solid mass (40 gm) on removal of the solvent. The chloroform extract was fractionated with n-hexane. The n-hexane insoluble part gave a green solid Mass L (30 gm). On decolourization with activated charcoal Mass L yielded a yellowish solid mass (25 gm) which was noted as Mass L_1.

Mass L_1 showed the presence of at least six compounds on TLC plates. Column chromatographic separation of Mass L_1 on silica gel with CCl_4 : EtOAc (1:1) as eluant gave one pure compound A_1, a mixture of two compunds (A_2 & A_3) and a fraction which on concentration gave a crystalline solid, (compound A_4). Compound A_2 and compound A_3 were separated from the mixture by preparative TLC. The mother liquor on removal of compound A_4 gave a solid mass, (Mass L_2). Mass L_2 on further chromatography gave two more pure compounds, compound A_5 and compound A_6.

Compound A_1, A_2, A_3, A_4 and A_5 were characterized by extensive use of UV, IR, ^1H NMR, ^{13}C NMR and mass spectral analyses. The compounds are datiscacin(37)(a cucurbitacin-20-acetate ester darivative),(A_1, yield 0.013%), cucurbitacin B(41), (A_2, yield 0.266%), isocucurbitacin B(42), (A_3, yield 0.109%), β-sitosterol glucoside(43), (A_4, yield 0.047%) and 2-O-β-D-glucopyranosyl cucurbitacin B(44), (A_5, yield 0.936%). UV and IR spectra of compound A_6 suggested it to be cucurbitacin S glucoside(45), (A_6, yield 0.043%) but remains to be confirmed.

The present investigation has led to the isolation of three more compounds namely datiscacin(37), 2-O-β-D-glucopyranosyl cucurbitacin B(44) and cucurbitacin S glucoside(45) besides cucurbitacin B(41),isocucurbitacin B(42)and β-sitosterol glucoside(43) from Luffa echinata. Of course the structure of cucurbitacin S glucoside requires to be more regorously established.

The ethanol extract of the plant Luffa echinata shows potent activity against spasmogen induced contraction in guinea-pig ileum, poor activity on guinea-pig atria and poor activity against oxytocin induced contraction in rat uterus.

The fruits of **Luffa Cylindrica**, (bitter variety), (500 gm), were extracted with chloroform (3X48 hours) at room temperature. The combined chloroform extracts was evaporated to dryness under reduced pressure at 45°C. This gave a light green solid mass (2.5 gm) which was noted as Mass N_1. TLC of Mass N_1 showed the presence of two compounds in CCl_4:EtOAc (10:1) along with one which did not move from the starting point. Column chromatography of the crude extract over a silica gel column using CCl_4 :EtOAc (10:1) gave two pure compounds, compound B_1 and compound B_2. Extensive use of IR, ^1H NMR, ^{13}C NMR and mass spectra confirm them to be 1- triacontanol,(B_1,yield 0.006%) and celsianol(stigmasta $\Delta^{5,9(11)}$-diene-3β-ol),(B_2, yield 0.056%) respectively.

Preliminary chemical investigation on **Luffa cylindrica** (bitter variety) has been performed for the first time. The two compounds isolated from the fruits of **Luffa cylindrica** (bitter variety) are different from those isolated from the sweet variety which were lucyosides A to H (11-18), ginsenosides-Re (19), ginsenosides-Rg_1(20), lucyoside I (21) and lucyosides J to M (22-25).

CHAPTER 5

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