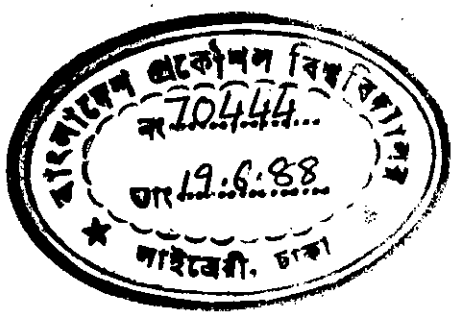
A Thesis

entitled

CHEMICAL INVESTIGATION OF THE AERIAL PARTS OF "PAEDERIA FOETIDA LINN"



Presented by

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TO MY MOTHER

THESIS APPROVAL SHEET

Thesis entitled "CHEMICAL INVESTIGATION OF THE AERIAL PARTS OF PAEDERIA FOETIDA LINN" by Mr. Siddhartha Gupta is approved for the degree of MASTER OF PHILOSOPHY.

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The Author

ABSTRACT

The work is divided into three chapters. The first chapter is entirely devoted to general reviews pointing out the use of the indigenous herbal medicinal plants in our life. This part also deals with the description of the plant Paederia foetida Linn and its medicinal uses. The objective of the project is also included in this chapter.

The interpretation of the results of the isolated products from Paederia foetida Linn has been discussed in the second chapter. The petroleum ether extract was subjected to systematic study and from it the following compounds were isolated and characterized: Epi - friedelanol, a mixture of hydrocarbons, keto-alcohol, embelin, a compound containing carbonyl group and a number of o-butyl group, a keto compound and β -sitosterol. All the above compounds were characterized by ir, pmr and mass spectra. The identities of them were confirmed by melting point and by converting them into known derivatives.

CONTENTS

CHAPTER I

INTRODUCTION

1.1 GENERAL INTRODUCTION

Plant kingdom provides the four basic items for human beings i.e. food, shelter, clothing and medicine. As the existence of human life is dependent to an astounding extent on the plant kingdom, the chemists and the pharmacists all over the world have always been interested in the plant products. Actually the inquisitiveness to investigate into the various parts of the plant by the chemists originates from its use as medicine from time immemorial.

The earliest use of medicinal plants in this subcontinent is evidenced in the Rigveda, Atherveda, Ayurveda sustra. The eight divisions of the Ayurveda were followed by two works written later, i.e., Susruta and Charaka. About the date of Susruta there is a great deal of uncertainty but it could not have been written later than 1000 B.C. In this work surgery is dealt with in detail but there is a comprehensive chapter on therapeutics. Charaka deals more with medicine which was written about the same period[1].

The Arabian system of medicines based on the preparations and decoctions from medicinal plants was prevalent in Indo-Pak-Bangladesh subcontinent during the region of Pathan and Mugal dynasties. The materia medica of plant origin was accepted throughout the middle ages as the only available ways and means of treatment like Kabiraji, Ayurvedic, Unani and folk medicines in this country.

The developed countries also use drugs obtained from the medicinal plants as our country. Now a days about 25%[2] drugs, e.g. antibiotics, vitamins, hormones etc. come from the plant extracts through usual processing. One hundred and seventy drugs from the plants which are or once were, official in USP or NF were used by North American Indians. In 1960, the physicians in the United States prescribed 47% of drugs, mostly antibiotics, from natural sources. In 1967, 25% or 824 out of 3,354 of the trade name or generic name products, which appeared in the 1.05 billion prescriptions filled in the United States contained one or more ingredients derived from higher plants[2].

The first conspicuous success in this field of chemotherapy was obtained in 1910 when Ehrlich discovered the specific action of certain organo arsenic compounds against spirochaetes of syphillies. The discovery of chemotherpeutic drugs has boosted up research works in the field of natural products in the quest of still newer medicines. More potent and effective antibiotics and hormone preparations that we see to-day are results of such activity in the field of natural product chemistry. It is not unlikely that some day in future the chemistry of natural product will afford effective medicines for the therapy of most fatal diseases like cancer which still remains to be conquered.

The useful drugs obtained from the plant origin should be purified from inactive and harmful ones so as to bring

them into use for mitigating the sufferings of the vast masses of humanity. Pure citric acid, for example, was isolated from lemons in 1780, cholesterol from animal fat in 1815, and quinine from the bark of the cinchona tree in 1817[3]. But the financial condition of the people of our country often restricts them to use such expensive chemotherapeutic drugs.

Bangladesh is blessed with vast resources of medicinally important herbs and plants. In this country a large number of medicinal plants grow widely in forests, jungles, hillocks and gardens. Many of these are in wide use in folk medicines as well as in the Ayurvedic and Unani system of treatment.

Some of the more useful medicinal herbs and plants of our country are: Ocimum gratissimum Linn (Beng. Ramtulse) used for the treatment of rheumatism; Cephalanda indica Naud (Telakucha) used for the treatment of glycosuria; Adhatoda vasica Nees (Beng. Vasaka) used for the treatment of chronic bronchitis and asthma and for various affections of the respiratory tract and fevers, Andrographis paniculatae Nees used for the treatment of irregular motions and of appetites; Melia Azadirachta Linn (Beng. Nim, Nimgachh) used for the treatment in fever, thirst, nausea, vomiting and skin diseases, Nyctanthes arbortristis Linn (Beng. Sephalika gachh) used for the treatment of fever rheumatism, Terminalia arjuna Bedd (Beng. Arjun) used in heart diseases, Rauwolfia serpentina (sarpagandha) used for the treatment of high blood pressure,

Eupatorium odoratum Linn (Assamlata) used as fish poison, Jatropha gossypifolia Linn (Lalbharenda) used against urinary complaints, ulcers, Calotropis gigantea Linn (Beng. Akanda) used for the treatment of skin diseases, enlargement of abdominal viscera, cough, ascites.

Various medicinal plants in this country are still awaiting thorough and systematic examination. So an extensive and speedy exploitation of the medicinal plants is deemed necessary.

Paederia foetida Linn (Beng. Gandhabhadulia) is a common climber widely available in the Himalayas and also in Bihar, Orissa, Assam of India and Bangladesh. It has an offensive smell which is attributed to the presence of volatile methyl-mercaptan. However the smell escapes to a great extent during cooking. The plant yields a strong and flexible fibre having a silky appearance[4,5]. Leaves of Paederia foetida Linn are ovate to lanceolate about 5.15 cm long and 2.7 cm broad with long petioles; fruits are ellipsoid and compressed red or black[4]. Almost all parts of the plant are found to have been used as medicines for different diseases. The plant as a whole is employed as a remedy against rheumatism. The species is believed to be helpful for eliminating poisons deposited in the system due to the use of noxious substances such as alcohol and tabacco, or due to defective metabolism. Leaves possess tonic and astringent properties. A soup prepared from the leaves appears to be a good remedy for diarrhoea

and dysentery and in fact is given as a household remedy during convalescence from acute illness. The application of a poultice of the leaves of Paederia foetida Linn to abdomen alleviate distension due to flatulence. The use of the poultice in herpes is also recorded for quick relief. In Philipine boiled and mashed leaves are applied to abdomen in the case of retention of urine. A decoction of the leaves is reported to possess diuretic properties and dissolve vesicle calenli. The protein extracted from the leaf (44.6% dry basis) is composed of amino acids like arginine 4.9; histidine 2.1; lysin 3.8; tyrosin 5.1; tryptophan 1.9; phenyalanine 6.8; cystine 1.4; methionine 2.1 and threonine 4.3[4].

Barks and roots are used as emetics. The juice of the roots is prescribed in piles, inflammation of spleen, and pain in the chest and liver. The hill tribes use the fruits to blacken their teeth for releiving toothache. The claims made by various observers regarding the efficacy of this drug justify further clinical trial.

In 1947 Basu at al[6] at first carried out chemical investigation on Paederia foetida Linn. They reported the presence of free vitamin C (100 mg/100 gm) and carotene (3.6 mg/100 gm). They also observed that the vitamin C content of Paederia foetida Linn increased by 10% when heated. Basu et al extracted the vitamin C from a weighed amount of the sample by thoroughly macerating with a mixture of HCl (4%) and metaphosphoric acid (2%) and sand. It was then estimated by

titration of the extract with Tillman's dye before and after its aliquot portions were treated with hex oxidase. In estimating β -carotene they extracted a weighed amount of Paederia foetida Linn pulp successively with absolute alcohol and petroleum ether. The petroleum ether extract was found to contain β-carotene which was then estimated colorimetrically by matching against a standard solution of pure β -carotene.

In 1953 Bose et al[7] found out the presence of methyl mercaptane which is responsible for the offensive odour of the Paederia foetida Linn. In 1965 Chaturvedi el al[8] showed Paederia foetida Linn is quite effective in reducing swelling of ankle induced in pretreated rats by HCHO which showed its anti-arthritic effects.

Investigation on the dry aerial parts of Paederia foetida Linn was carried out by Khuda et al[9] in 1966. They isolated a crystalline keto alcohol, named Paederolone $C_{44}H_{68}O_2$ m.p. 265-66°C, a keto compound named Paederone $C_{30}H_{50}O$ m.p. 239-41°C and β & δ - sitosterols from the petroleum ether extract. From alcohol extract of the defatted plant body, they isolated two volatile alkaloids named Paederine and Paederenine as their picrates; Paederine picrate $C_{15}H_{16}O_9N_4$, m.p. 145-48°C and Paederinine picrate m.p. 130-31.5°C.

In 1966 Mannan et al[10] reported the presence of appreciable amount of vitamin E in the leaf & stem of Paederia foetida Linn. They also isolated carotene from the plant.

In 1969 Prasad et al[11] carried out research for investigating the effect of Paederia foetida Linn on annanase induced degenerative arthritis. They injected rabbits within 4-6 days of post injection; the joints showed acute hyperemia, increase in local temperature and marked swelling and there was also a significant increase in the blood sialic acid level and decrease in the blood uric acid level. When they injected Paederia foetida extract (10 mg/4 days) for locally the effect of annanase decreased.

1.2 Objective of the project

The plant Paederia foetida Linn (Beng. Gandhabhadulia) is employed as a remedy against rheumatism, diarrhoea, dysentry and as a house-hold remedy during convalescence from acute illness. It has been reported[6,7,8] that the plant Paederia foetida Linn contains vitamin C, β-Carotene, methylmercaptane, a crystalline keto-alcohol Paederolone $C_{44}H_{68}O_2$ m.p. 265-66°C, a keto compound Paederone $C_{30}H_{50}O$ m.p. 239-41°C, β and δ - sitosterols, volatile alkaloids Paederine, Paederenine and vitamin E. The literature survey however revealed that complete spectroscopic analysis has not been done to elucidate the structure for the isolated compounds. With this end in view the plant Paederia foetida Linn is chosen for systematic study.

CHAPTER II

RESULTS AND DISCUSSIONS

2.1 The plant Paederia Foetida Linn (Beng. Gandhabhadulia) is employed as a remedy against rheumatism, diarrhoea, dysentery and as a house-hold remedy during convalescence from acute illness. Uptil now vitamin C, β -carotene[6]; methylmercaptan[7]; a crystalline keto-alcohol Paederolone, $C_{44}H_{68}O_2$ m.p. 265-66°C, a keto compound Paederone, $C_{30}H_{50}O$ m.p. 239-41°C, β and δ -Sitosterols, volatile alkaloids Paederine and Paederenine[9] and vitamin E[10] have been reported to be isolated from the plant Paederia Foetida Linn. However the structures of Paederolone, Paederone, Paederine and Paederenine remained to be elucidated. The work on the isolation and characterisation of the chemical constituents thus appear to be incomplete. It was therefore planned to carryout a systematic examination of the chemical constituents of Paederia Foetida and pinpoint the active principle.

The finely powdered aerial parts (2.0 kg.) of the plant were extracted successively with petroleum ether (40-60°C) and rectified sprit. The petroleum ether extract of Paederia Foetida was concentrated to one tenth of its volume at reduced pressure and allowed to stand in the refrigerator for 48 hours. A white precipitate settled at the bottom of the flask. The supernatant heavy liquid was carefully decanted off and the white solid mass A_1 was collected (1.1 gm). The heavy liquid was further concentrated at reduced pressure at 30°C when a reddish green mass, A_2 was obtained.

2.2 Study on Mass A_1

The white mass A_1 was a complicated mixture of at least three compounds as revealed by tlc. Column chromatography of the substance on silica gel and elution with carbon tetrachloride and a mixture of carbon tetrachloride and ethyl acetate in different proportions yielded four fractions. Only one of these fractions gave a pure compound which was denoted as D_2. The other fractions were found to be mixtures of two or three compounds.

2.2.1 Examination of Fraction D_2

Fraction D_2 (54 mg, 0.003%) melted at 282-84°C. It was partially soluble in petroleum ether, carbon tetrachloride, ethyl acetate, chloroform, acetone on warming and insoluble in ethanol even on being heated. It gave only one spot on tlc plates (R_f 0.4 in carbon tetrachloride - ethyl acetate 9:1 and R_f 0.43 in petroleum ether - ethyl acetate 9:1). It showed ir absorptions at ν_{max} 3450, 1070 cm^{-1} suggesting it to contain a hydroxylic group (Fig. 1). The pmr spectrum (Fig. 2) of the compound showed the presence of eight C-methyl groups appearing as singlets at δ 0.87 (I-CH_3), δ 0.93 (I-CH_3), δ 0.96 (2-CH_3), δ 1.0 (3-CH_3) and δ 1.11 (I-CH_3). The multiplet appearing between δ 1.25-1.8 indicated the presence

of 24 protons attached to saturated carbon atom. A broad singlet for one proton at δ 3.7 could be attributed to a proton attached to C-OH coupled to neighbouring protons.

The compound showed the molecular ion peak (Fig.3) at m/e 428 in agreement with the molecular formula $C_{30}H_{52}O$. The molecular formula of the compound suggested it to be a triterpene. The molecular formula and its melting point (m.p. 282-84°C) further suggested the compound to be epi-friedelanol (friedelan - 3 β -ol, m.p. 282°C)[12]. The mass fragmentation of the compound is also in agreement with that expected from epi-friedelanol. The more important mass peaks observed at m/e 341, 275, 149, 123 and 357 can be nicely explained for epi-friedelanol as shown in the scheme (Fig. 4).

The other peaks at high m/e 413 and 395 could arise from simple elimination of a methyl group and a molecule of H_2O from the molecular ion. It may be noted that such fragmentation pattern is well established for a number of friedelane derivatives[12]. The identity of the compound was further confirmed by acetylating it with a mixture of pyridine and acetic anhydride. The acetate melted at 296-8°C and compared well with the reported value (m.p. 294°C)[12]. The acetate did not show any absorption in the OH stretching region but showed a new sharp band at 1730 cm^{-1} for the ester functional group (Fig. 5). The compound was also oxidised to ketone by using Jone's reagent. The ketone melted at 254-56°C, showed the absence of any band at ν_{max} 3450 cm^{-1} for OH but showed a band at 1715 cm^{-1} for the ketone function (Fig. 6). The melting point of friedelan - 3 - one, the expected oxidation product of epi-friedelanol is reported as 258-59°C[12] and compares well with the oxidised product of compound D_2. The mass spectrum of the oxidised product showed the molecular ion (Fig. 7) at m/e 426 (M^+ $C_{30}H_{50}O$). The mass peaks at m/e 341, 273 and 309 expected from the fragmentation of the molecular ion as shown below are clearly observed in the mass spectrum of the compound. The chemical examination as well as spectral data of the compound confirm the compound D_2 to be epi-friedelanol. A direct comparison of compound D_2 with an authentic sample of epi-friedelanol was not however possible because it was not available with us.

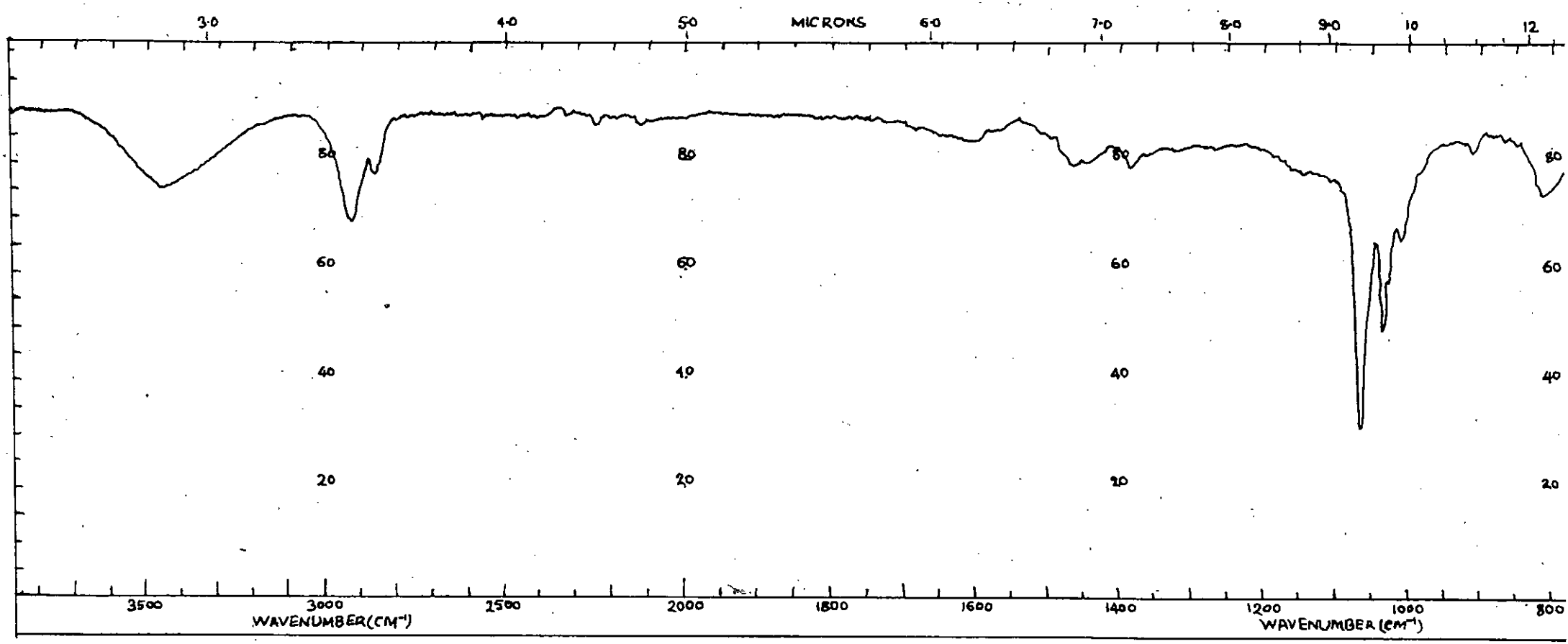
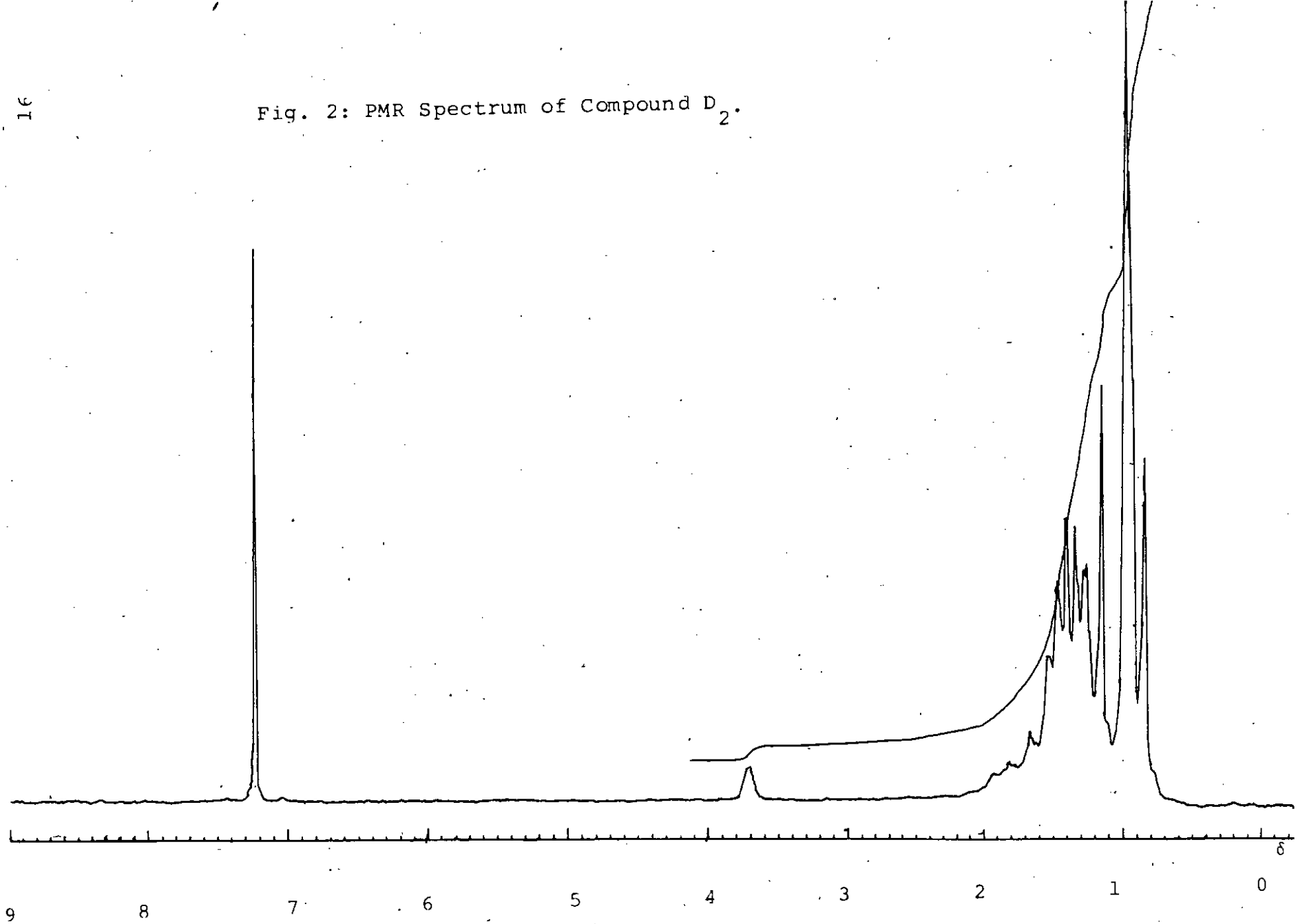


Fig. 1: Infra red spectrum of Compound D_2.

Fig. 2: PMR Spectrum of Compound D_2.



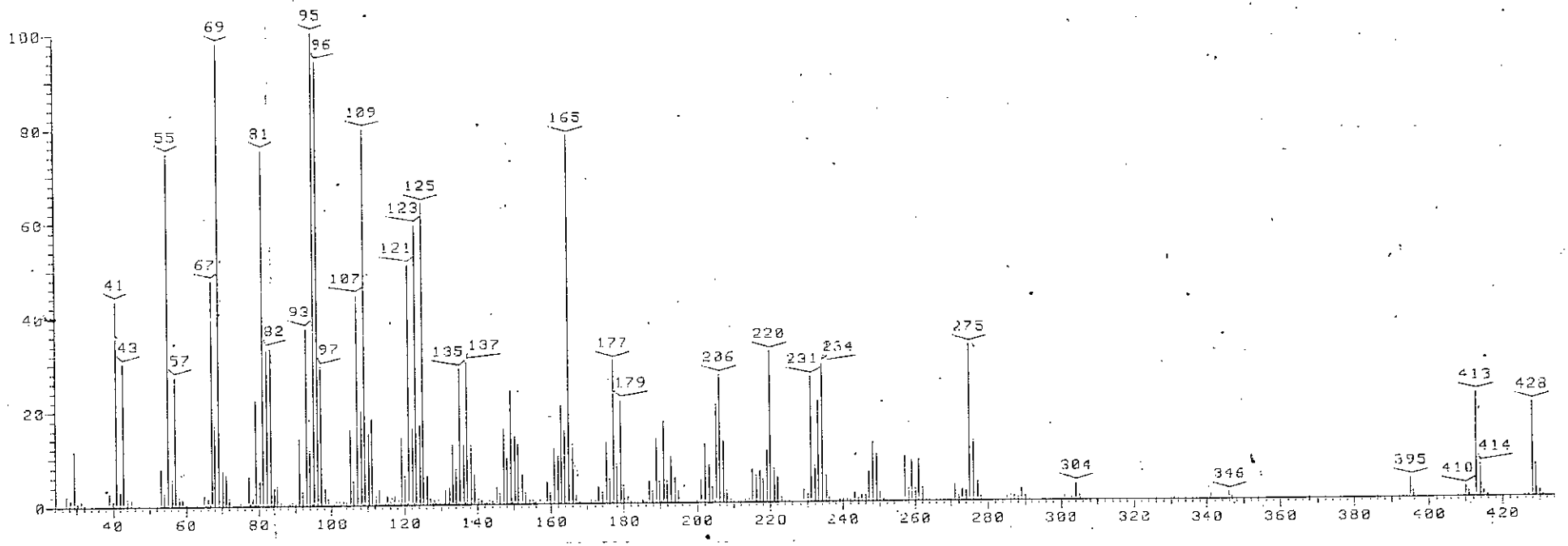


Fig. 3: Mass Spectrum of Compound D_2.

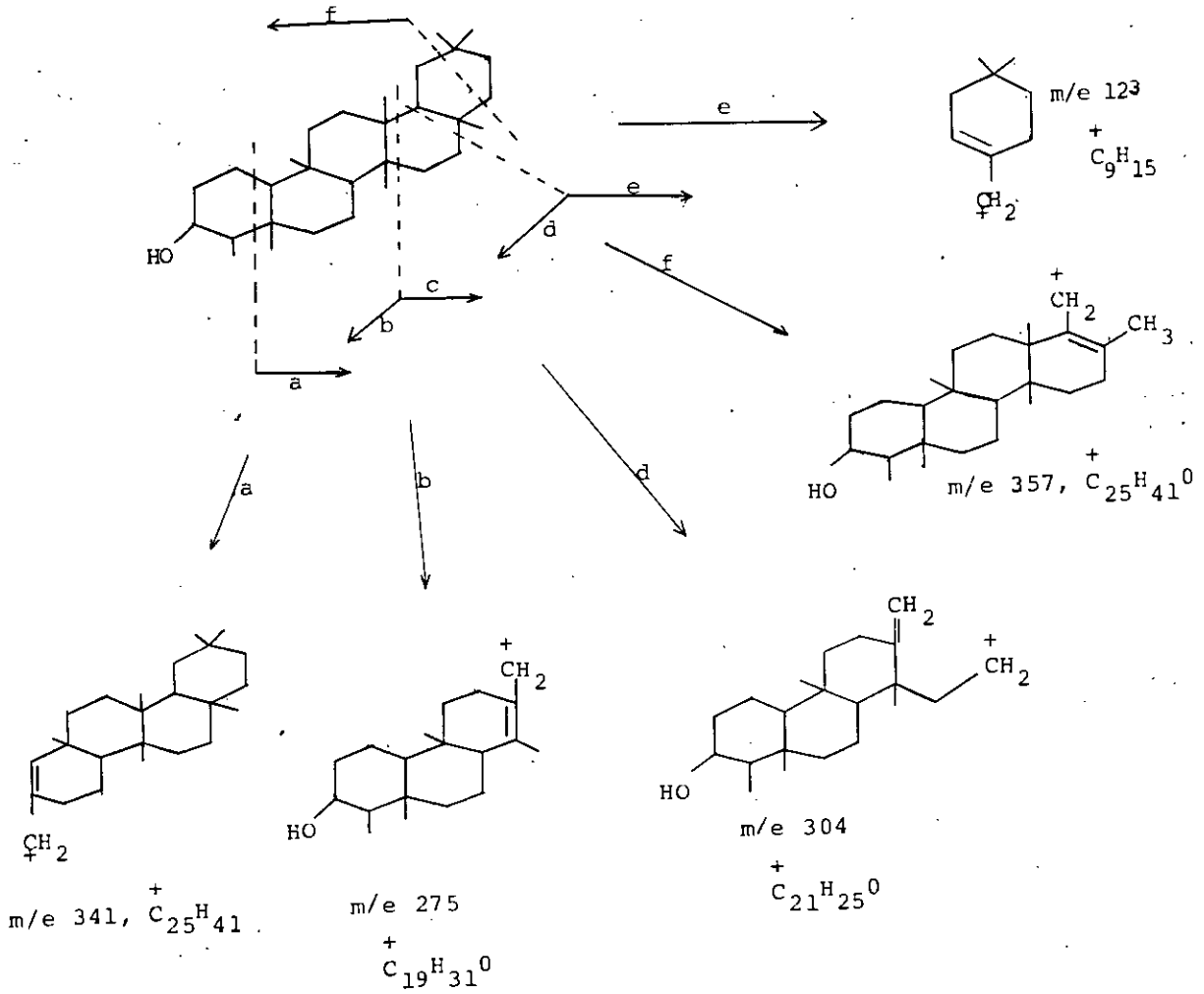


Fig. 4: The positions of cleavage of the ions.

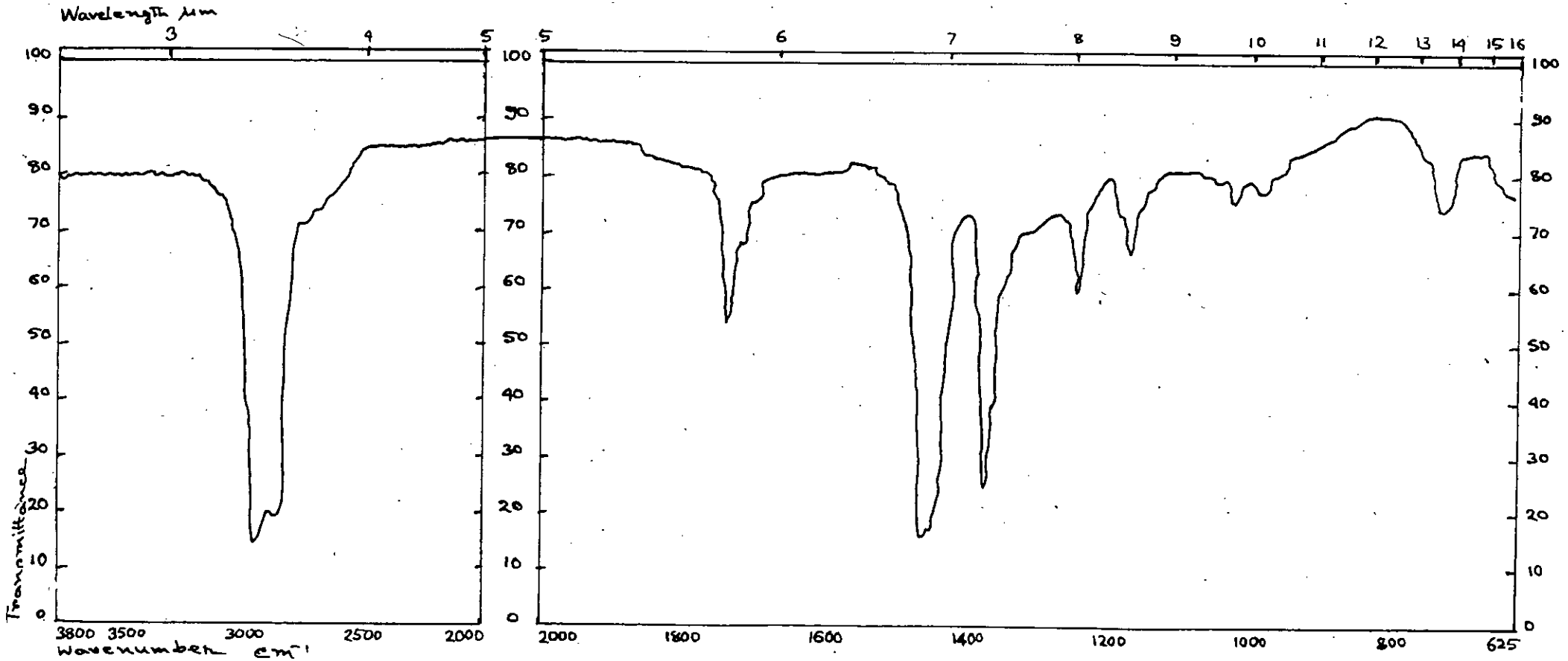


Fig. 5: Infra red spectrum of the acetate of Compound D_2.

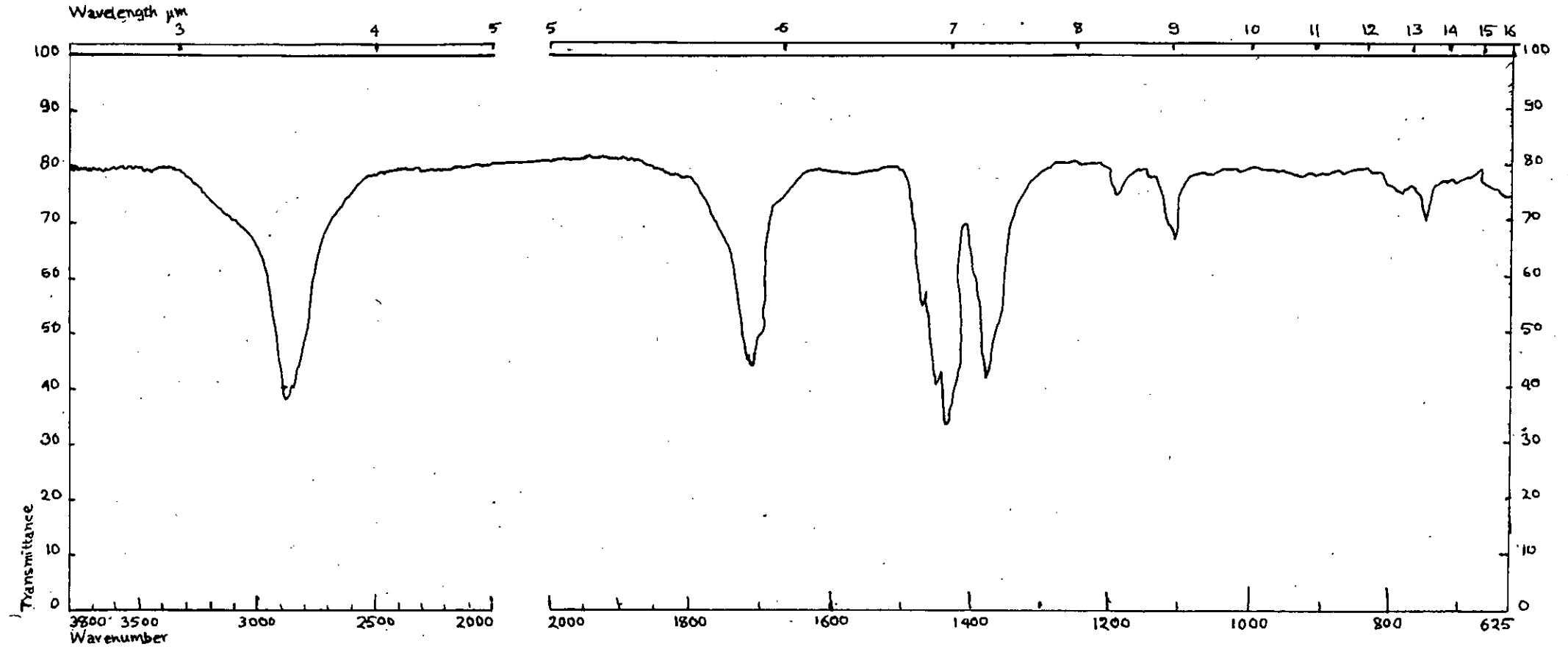
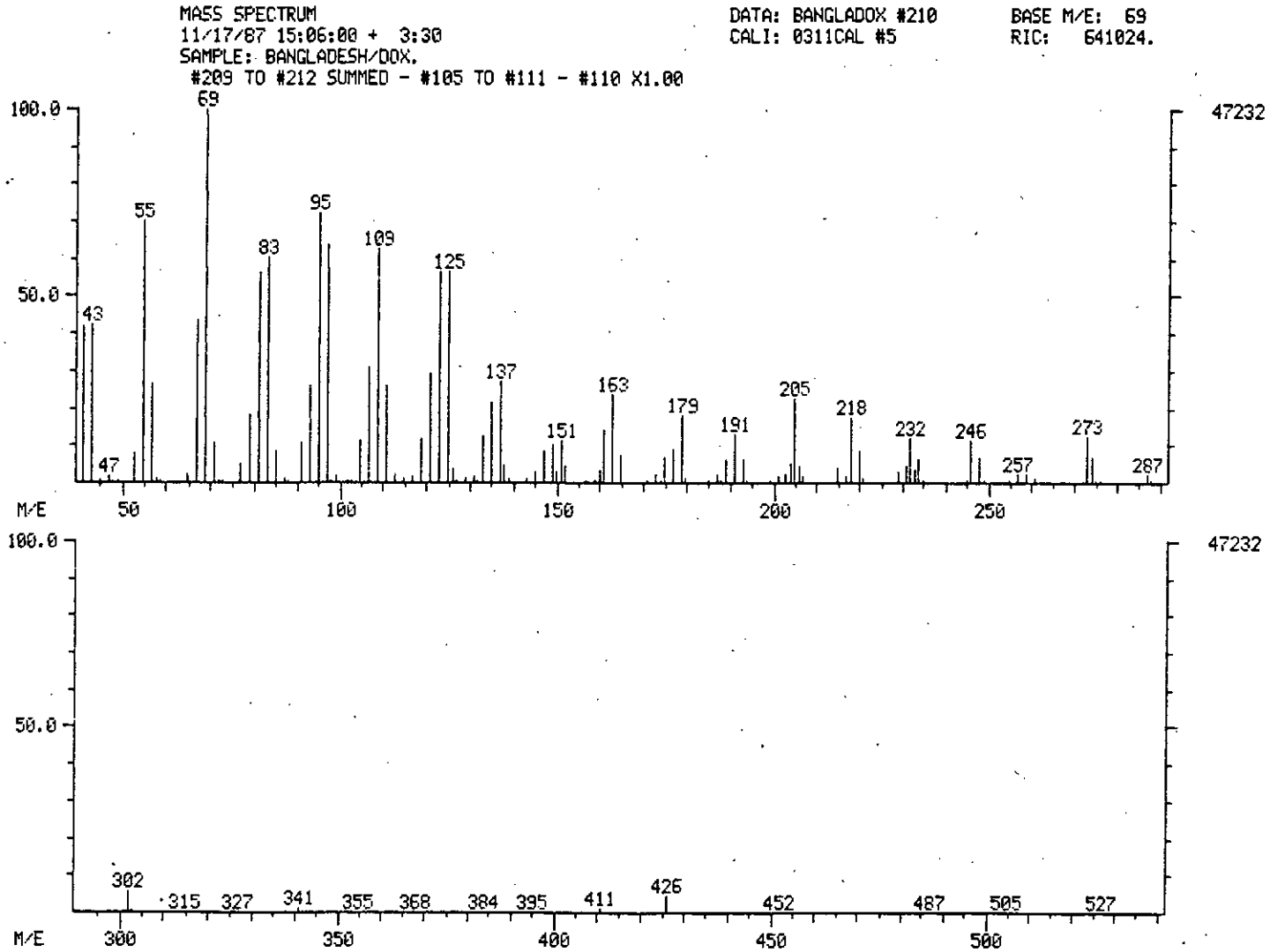


Fig. 6: Infra red spectrum of the Oxidised product (Ketone) of Compound D_2.

Fig. 7: Mass spectrum of the Oxidised product of Compound D_2.



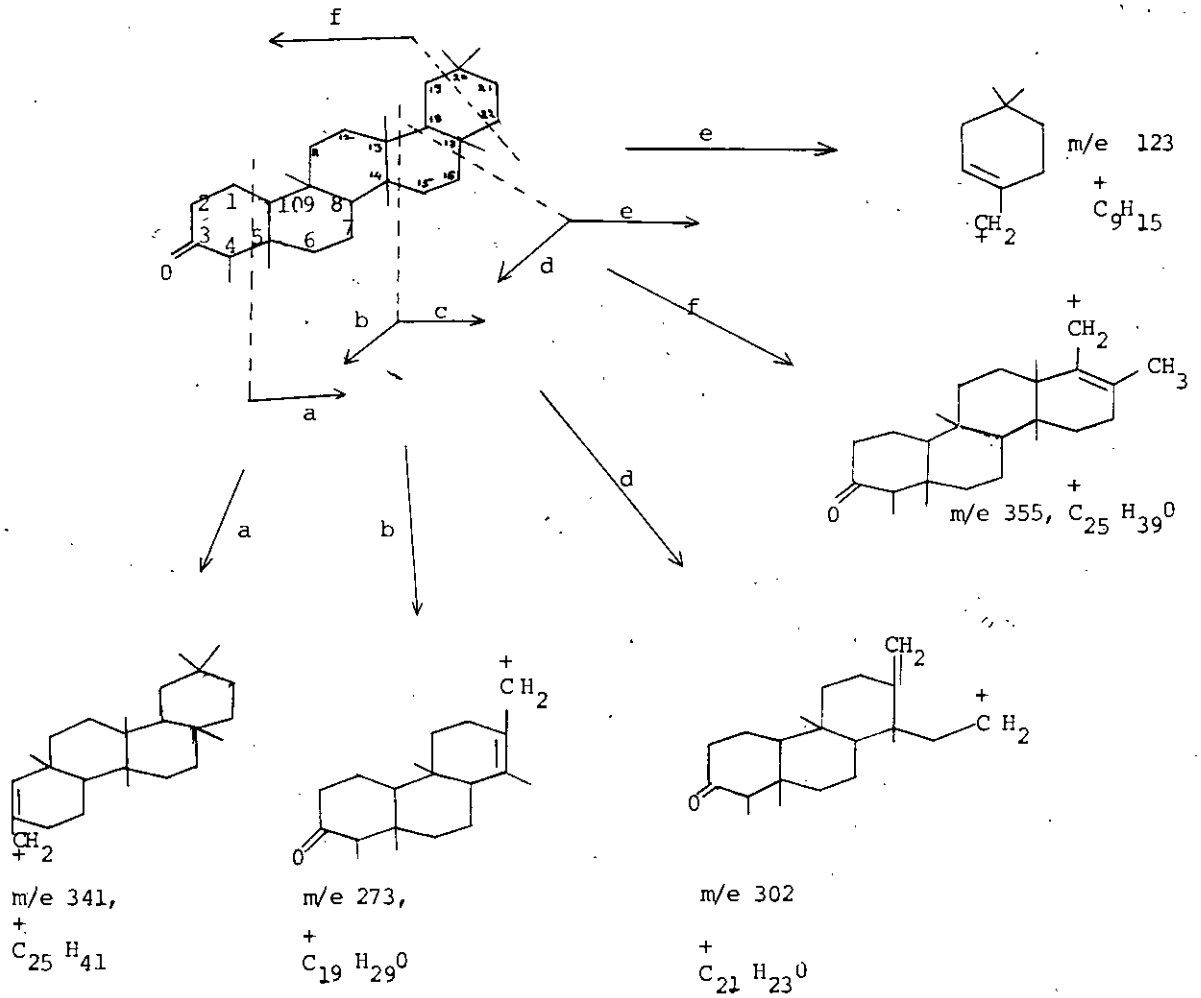


Fig. 8: The positions of cleavage of the ions of epi-fridelanone.

2.3 Study on Mass A_2

Mass A_2, a reddish green viscous substance was chromatographed over a column of silica gel and eluted with petroleum ether (40-60°C) and mixture of petroleum ether - ethyl acetate, 24:1, 5:1 and 1:1 in that order and finally eluted with methanol. As many as seven chromatographic fractions were collected, each of which on tlc plates appeared to give a major compound. These fractions were designated as P_1, P_2, P_3, P_4, P_5, P_6 and P_7 in the order they were collected from the column. Of these fraction P_6 was obtained in meagre quantity and further thorough examination on it could not be carried out.

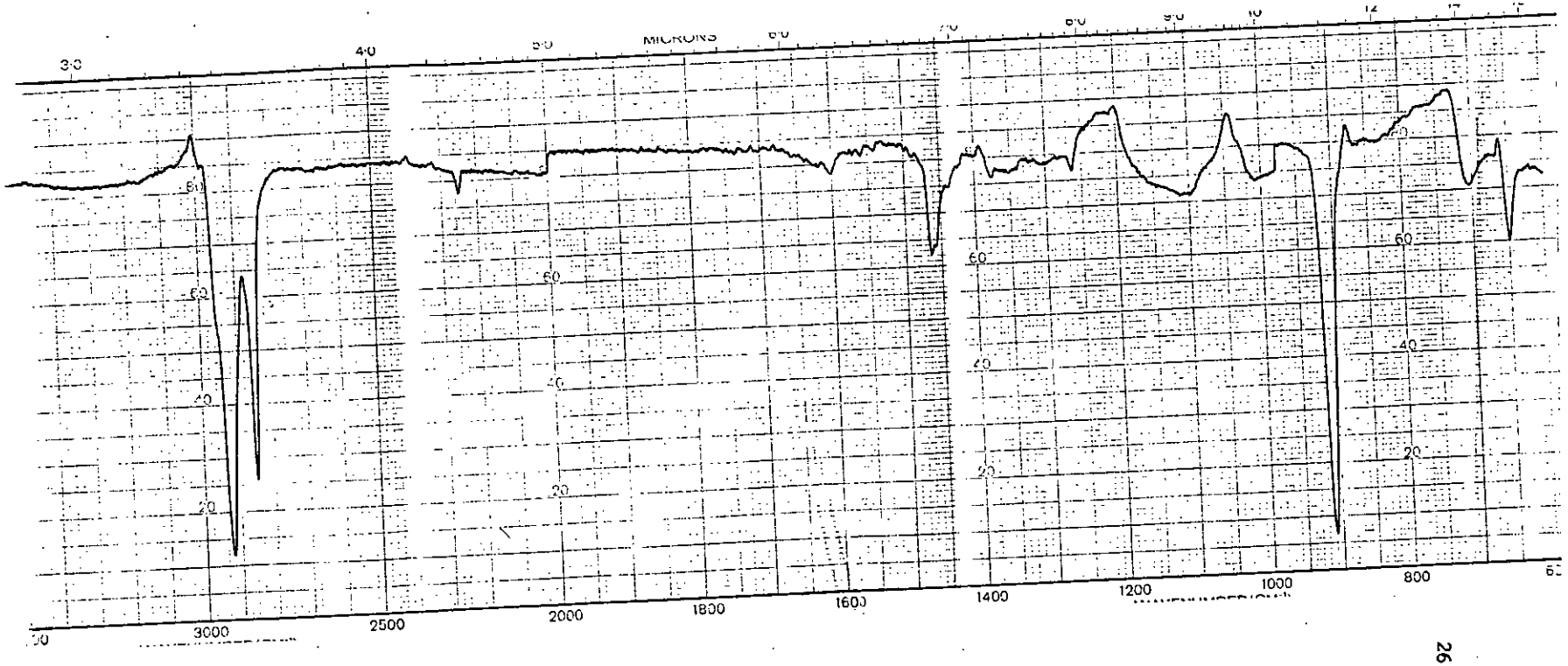
2.3.1 Examination of Fraction P_1

Fraction P_1 yielded a light brown solid which on crystallisation from petroleum ether at ice-bath temperature gave a low melting waxy white solid (100 mg, 0.005%), m.p. 66-68°C. The compound was highly nonpolar as shown by its behaviour on tlc plates (R_f 1.0 in petroleum ether). The ir spectrum of the compound (Fig. 9) in $CHCl_3$ did not show any absorption characteristic of any functional group. However

the strong band at ν_{max} 910 cm^{-1}, characteristic for C-H bending suggested it to contain a long chain alkyl group. The pmr spectrum of the compound showed absorptions only in the saturated C-H region. The tlc behaviour of the compound and the nature of ir and pmr spectrum of the compound suggested it to be a hydrocarbon. The melting point of the compound is close to those for hentriacontane ($C_{31}H_{64}$, m.p. 68°C)[14] and dotriacontane ($C_{32}H_{66}$, m.p. 69.7°C)[14]. The mass spectrum (Fig. 10) of the compound showed the highest mass peak at as high as m/e 464. The mass spectrum exhibited fragmentation pattern expected for long chain alkanes i.e. mass peaks are observed at successive loss of 14 and 28 mass units with lowering of intensity, with increase in m/e. The pattern, however indicates that if m/e 464 is considered as the molecular ion of the hydrocarbon, it is also mixed with a hydrocarbon having molecular ion m/e 436. On this basis the compound which is pure on tlc plates is in fact a mixture of at least two hydrocarbons namely hentriacontane ($C_{31}H_{64}$, m.p. 68°C)[14] and tritriacontane ($C_{33}H_{68}$, m.p. -). Of course it is rather rare to have hydrocarbons with odd number of carbon atoms in nature. That it is really a mixture of two hydrocarbons was confirmed by running a GLC (Fig. 11) of the compound on a Silar 100 column at 200°C which showed two peaks having much higher retention time than C_{18}, C_{19} and C_{20} alkanes. Finally the identification of the hydrocarbon mixture was

resolved by running a GC-Mass spectra of the hydrocarbon on a OV-275 column at programmed temperature range of 100-215^{o}C with 5^{o}/minute rise. The presence of four hydrocarbons namely $C_{30}H_{62}$, $C_{31}H_{64}$, $C_{32}H_{66}$ and $C_{33}H_{68}$ were clearly established by this technique (Fig. 12). The molecular ion of the hydrocarbons were established by running a CI mass spectrum of the hydrocarbon mixture. The results are in complete accord with the mass spectrum (Fig. 10) of the compound which showed the hydrocarbons $C_{31}H_{64}$ and $C_{33}H_{68}$ to be the major components of the hydrocarbon mixture.

Fig. 9: Infra red spectrum of Compound P_1.



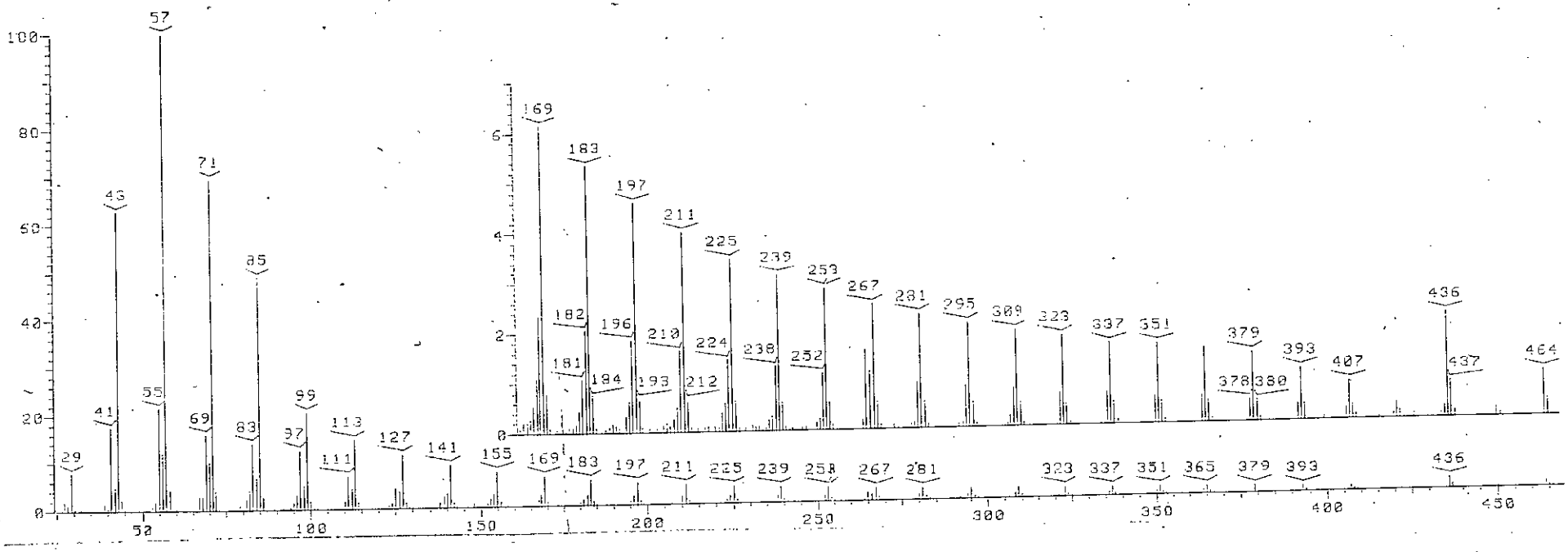


Fig. 10: Mass Spectrum of Compound P_1.

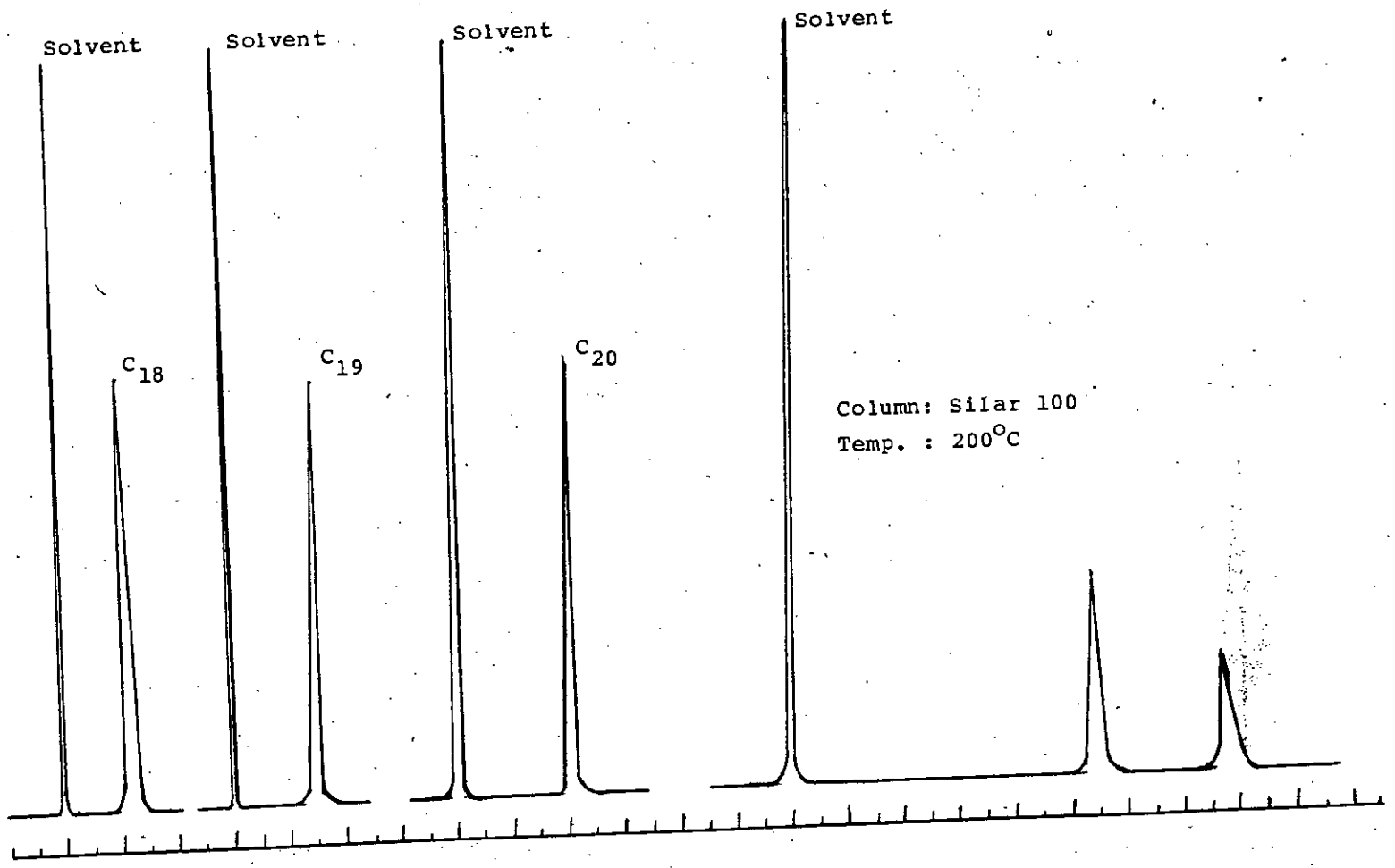


Fig. 11: Gas Liquid Chromatograph of Compound P_1.

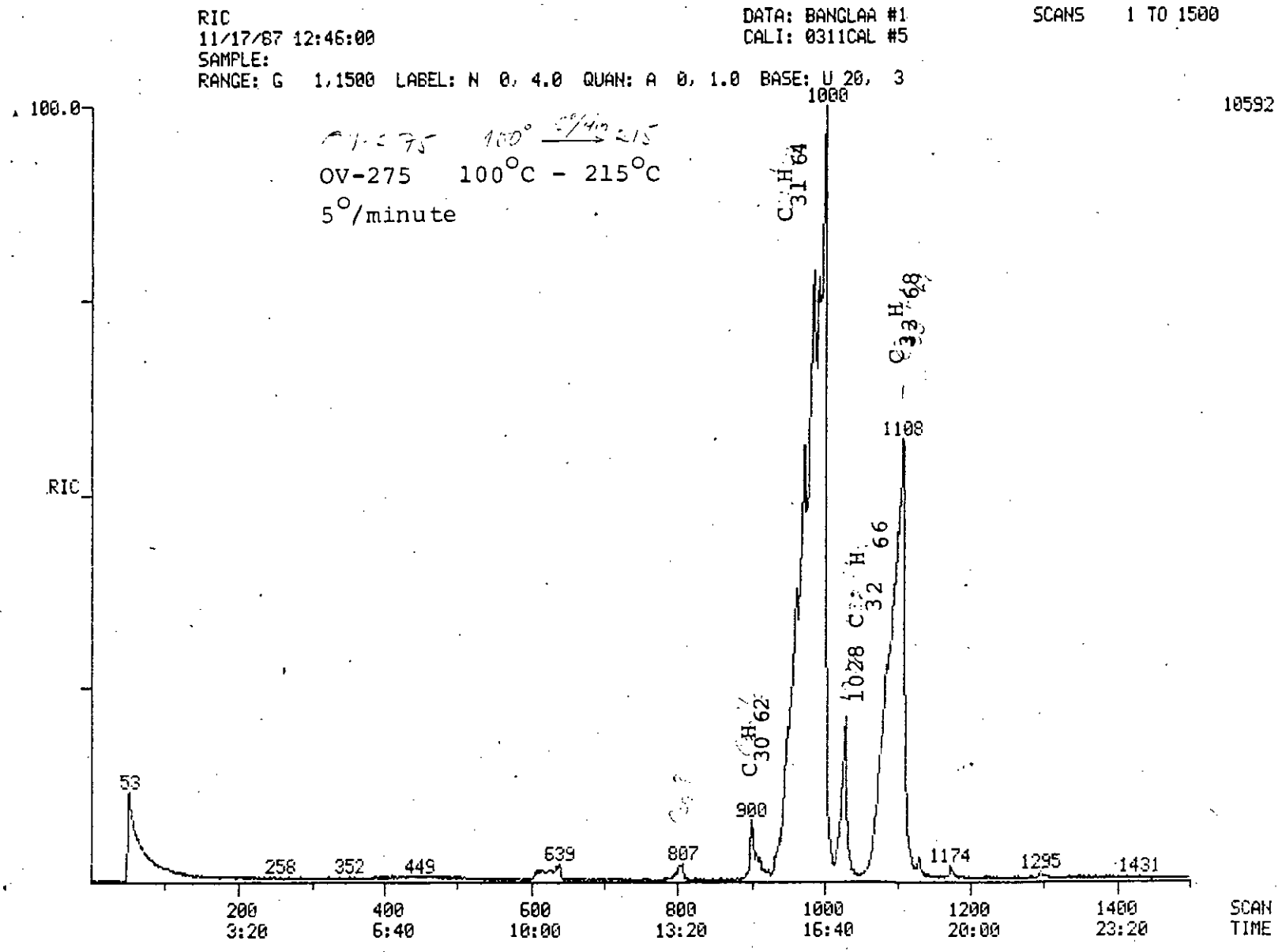


Fig. 12: Gas Chromatogram of Compound P_1.

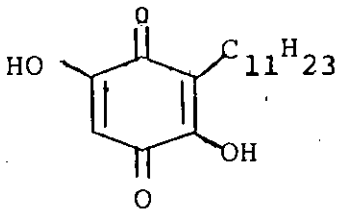
2.2.3 Examination of fraction P_2

Compound P_2 yielded a transparent liquid (49 mg, R_f 0.46 in petroleum ether) and gave only one spot on tlc plate. It showed an ir absorption at ν_{max} 3450 cm^{-1} indicating it to contain a hydroxylic group and another ir absorptions at ν_{max} 1715 cm^{-1} suggesting it to contain a ketonic group. The pmr spectrum of the compound showed a multiplet in the methyl absorption region δ 0.85 and a sharp singlet at δ 1.2 for methylene protons. There was another sharp doublet at δ 1.55-1.65. The compound showed highest mass peak at 240. The more important mass peaks are observed at m/e 225, 211, 197, 183 169, 155, 141, 127, 113, 99, 85, 71, 57 and 43 with consecutive loss of 14 mass units along with decreasing intensity, with rise in m/e values indicate the presence of alkyl chain. Thus it appears to be a compound containing carbonyl group, hydroxyl group attached to an alkyl chain. However the actual structure of the compound could not be established on the basis of the available data. Further work is necessary to elucidate its structure.

2.3.3 Examination of Fraction P_3

Fraction P_3 yielded an orange red solid which on crystallisation from methanol at 60°C gave orange crystals m.p. 140-145°C (20 mg, 0.0001%). It gave only one spot on tlc plates (R_f 0.62 in petroleum ether). It was soluble in petroleum ether, diethyl ether, carbontetrachloride, n-hexane and sparingly soluble in benzene, chloroform, ethyl acetate, acetone, ethanol and methanol. The UV spectrum (Fig. 13) of the compound showed absorption at λ_{max} 275 nm and an end absorption at 215 nm. The colour of the compound and the nature of UV absorption suggested it to be a quinone. The IR spectrum (Fig. 14) of the coumpound confirmed the idea which showed unresolved bands with inflections at 1660, 1645 and 1630 cm^{-1} for $\nu_{C=O}$ and $\nu_{C=C}$ of quinone type structure. The IR spectrum of the compound also showed a broad band at 3350 cm^{-1} which indicated the presence of hydroxyl group. The pmr spectrum (Fig. 15) of the compound showed broad singlet at δ 6.82 for a proton in quinoid nucleus. The other peaks in the pmr spectrum were observed at δ 0.92 as a distorted triplet and a singlet at δ 1.3 and a broad absorption at δ 2.27. They could be assigned to a terminal methyl group next to a methylene group, methylene groups of an alkyl chain and CH_2 attached to an unsaturated carbon atom respectively. The mass spectrum (Fig. 16) of the compound clearly showed the presence of an alkyl chain which showed mass peaks at 57, 71, 85, 99, 113, 127, 141 with successive loss of 14 mass units. The highest

mass peak was observed at m/e 294 and if it is considered as the molecular ion, one of the possible molecular formula for the compound is $C_{17}H_{26}O_4$. This incidentally is the molecular formula of a known natural quinone named Embelin which has the following structure.



Embelin

Such structure nicely accommodated all the spectral data exhibited by the above compound. Moreover the melting point of the compound, 140-45°C is also very similar to that reported for embelin (142-43°C). All these evidences lead us to believe that the compound is in fact embelin. Because of the meagre quantity of the sample it has not been possible to do more chemistry on the compound and to compare the compound with an authentic sample of embelin.

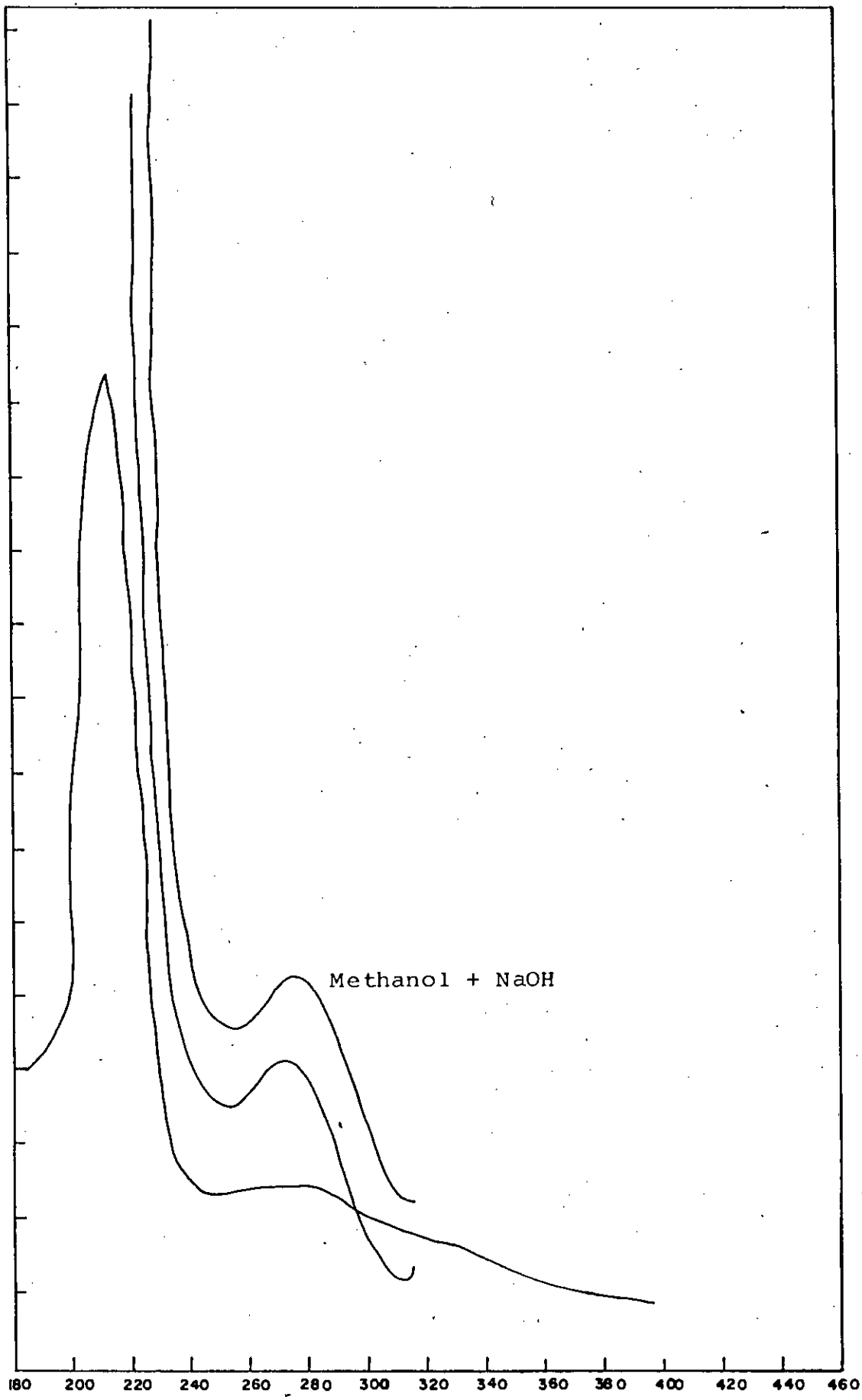


Fig. 13: Ultra-Violet Spectrum of Compound P_3.

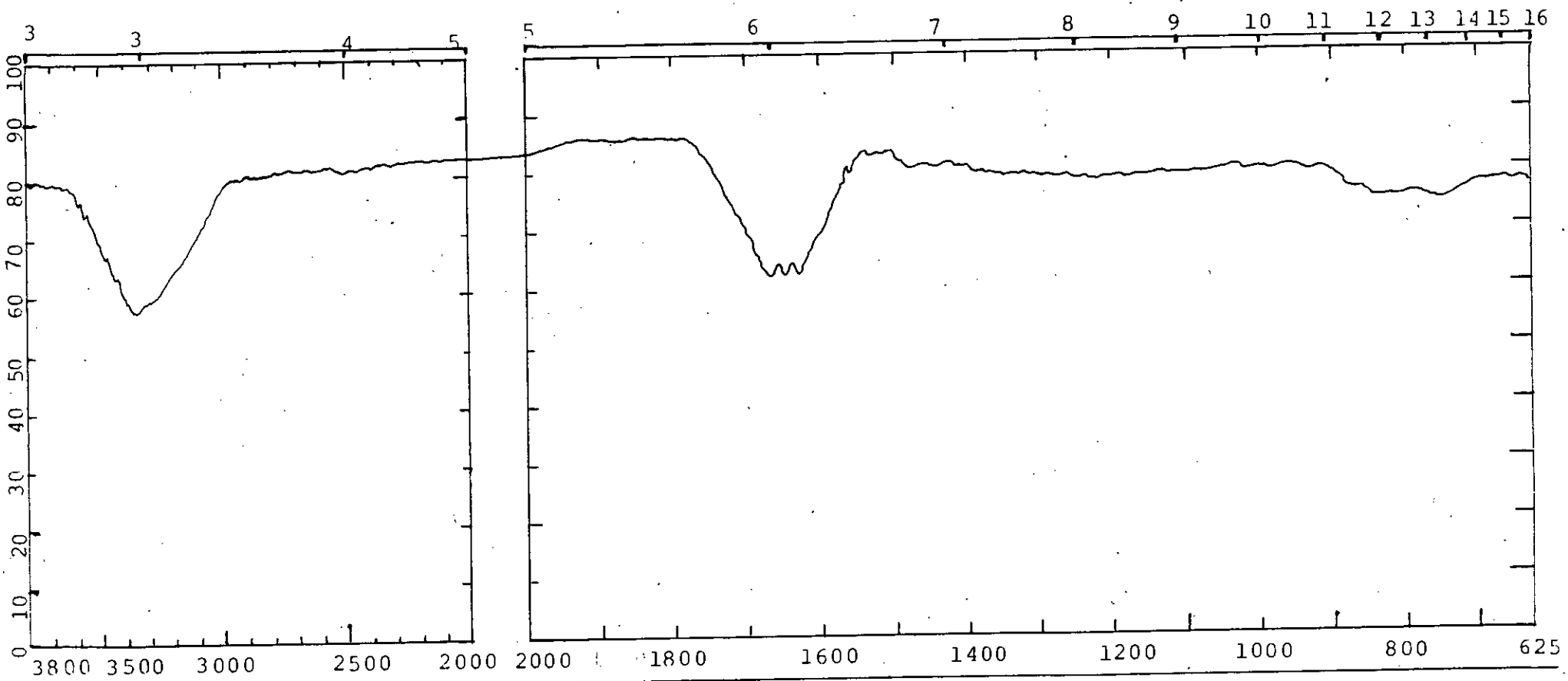


Fig. 14: Infra red Spectrum of Compound P_3.

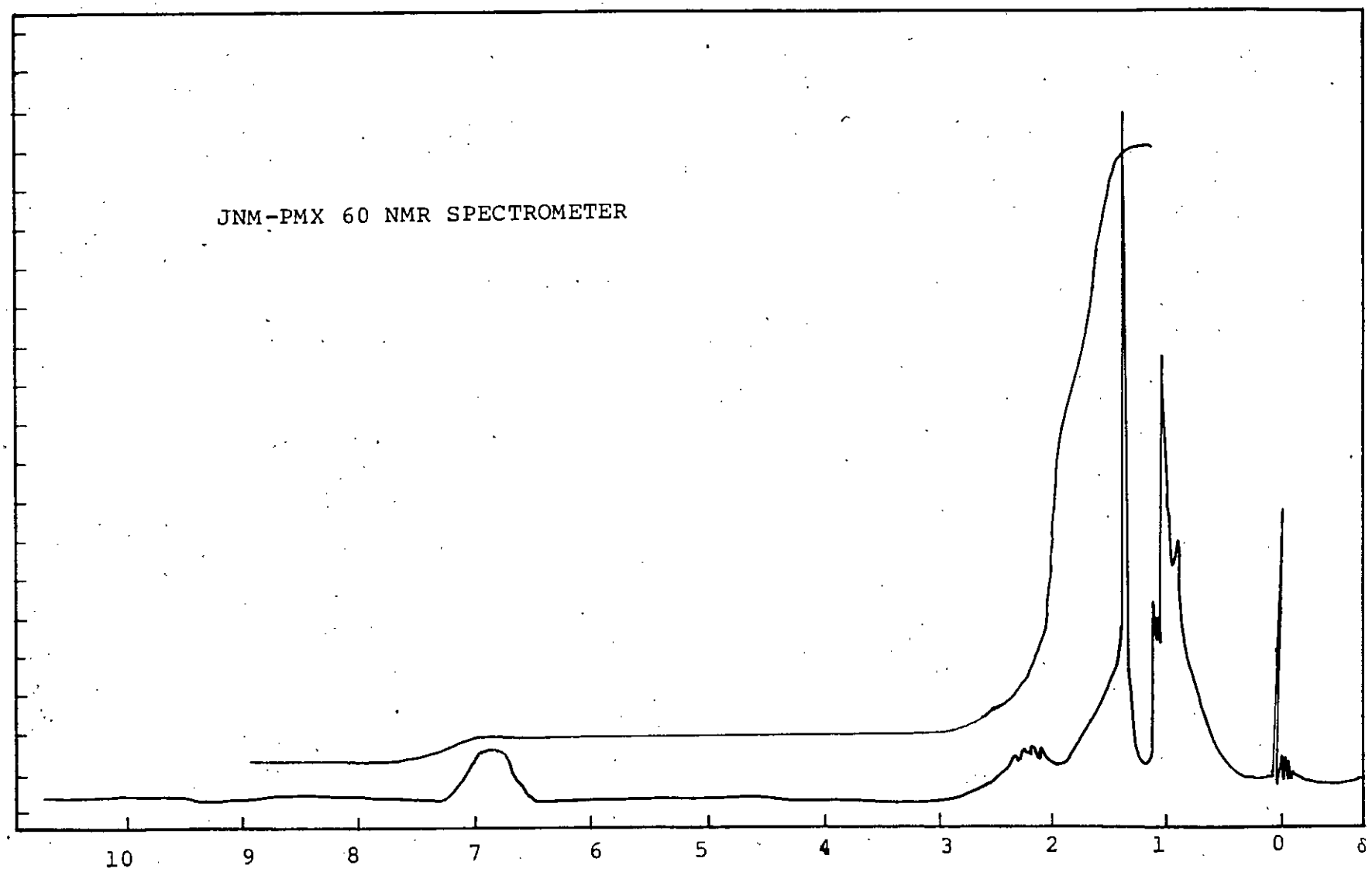


Fig. 15: PMR Spectrum of Compound P_3.

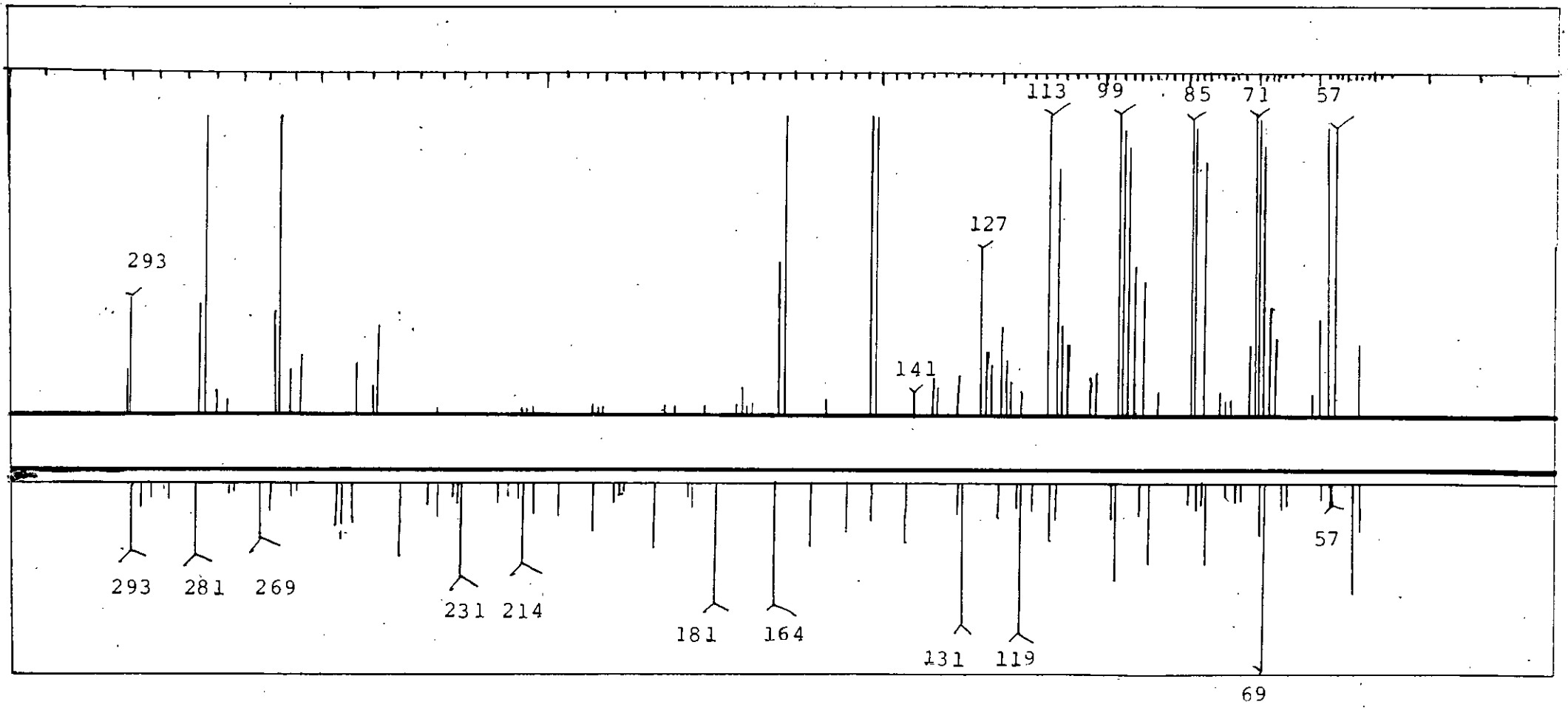


Fig. 16: Mass Spectrum of Compound P_3.

2.3.4 Examination of Fraction, P_4

Fraction P_4 (1.4 g), on tlc plate showed a long tailing with its centre at R_f 0.38 in petroleum ether - ethyl acetate. It was chromatographed through silica-gel using petroleum ether, and mixture of petroleum ether - ethyl acetate as eluant when a fraction fairly pure, K_1 (0.21 g, R_f 0.44 in petroleum ether - ethyl acetate 4.9:0.1) was obtained. The fraction K_1 was rechromatographed through silica-gel using petroleum ether and a mixture of petroleum ether - acetone as eluants. The process yielded a pure compound (60 mg, 0.003%), m.p. 57°C. It was soluble in petroleum ether, n-hexane, carbon tetrachloride, diethyl ether, chloroform, ethyl acetate and acetone. The ir spectrum of the compound showed absorptions at ν_{max} 1720 cm^{-1} suggesting it to contain a carbonyl group. Absorption peak at ν_{max} 910 cm^{-1} indicated it to be for C-H bending of a long chain alkyl group. The pmr spectrum showed a multiplet in the methyl absorption region δ 0.85 and a sharp singlet at δ 1.25 for methylene protons. There were other small peaks in the spectrum which most probably are due to impurities. The compound showed highest m/e peak at 577 (Fig. 17). The more important mass peaks are observed at m/e 503, 429, 355, 281 and 207 with successive loss of 74 mass units. The loss of 74 mass unit indicates the compound to contain at least 5-OC_4H_9 group. The mass peaks at m/e 43, 57, 71, 85, 99, 113 and 127 with consecutive loss of 14 mass

units along with decreasing intensity, with rise in m/e values indicate the presence of alkyl chain in the compound. The compound thus appears to be a compound containing carbonyl group and a number of O-butyl group attached to an alkyl chain. The actual structure of the compound could not be established on the basis of the available data. Further work is necessary to elucidate its structure.

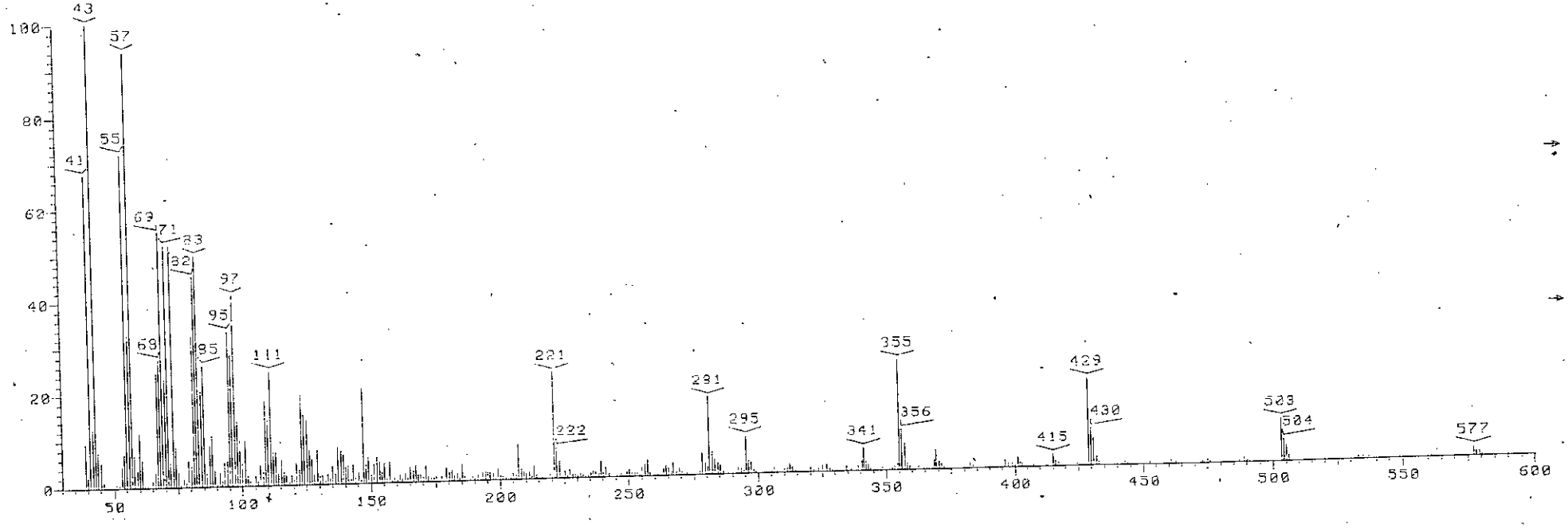


Fig. 17: Mass Spectrum of Compound K_1.

2.3.5 Examination of Fraction P_5

Fraction P_5 gave colorless needle like crystals embedded in a red viscous liquid. The crystals were separated by dissolving out the red liquid with petroleum ether. The crystals thus obtained were chromatographed through a silicagel column using petroleum ether and mixtures of petroleum ether and ethyl acetate. The crystals collected from the relevant chromatographic fraction were further recrystallized three times from methanol. The crystals were fine needles (40 mg, 0.002%) and melted at 233-34°C. It gave only one spot on tlc plates (R_f 0.53 in petroleum ether - ethyl acetate 9:1). It was soluble in petroleum ether, carbon tetrachloride, diethyl ether, chloroform, ethyl acetate, acetone; formed emulsion with benzene and was slightly soluble in ethanol and methanol on warming. The ir spectrum (Fig. 18) of the compound showed a carbonyl absorption at ν_{max} 1715 cm^{-1}. The pmr spectrum (Fig. 19) of the compound showed absorptions between δ 2.4-0.66 indicating the presence of only proton attached to saturated carbon atom. Neither the ir spectrum nor the pmr spectrum indicated the presence of olefinic protons. The mass spectrum (Fig. 20) of the compound showed the highest mass peak at m/e 412. The mass peaks at m/e 341, 302, 273, 246, 232, 218, 205 were consistent with friedelane type of structure. The mass peaks at m/e 273, 302 and 205 further indicated it to be a 3-keto-friedelane type of structure.

The compound was reduced to alcohol by using lithium-aluminium-hydride. The alcohol melted at 250-52°C and showed an ir band at ν_{max} 3450 cm^{-1}. The melting point of the compound suggested it to be same as Paederone isolated by Khuda et al. With the available data it is not possible to suggest the actual structure of the compound.

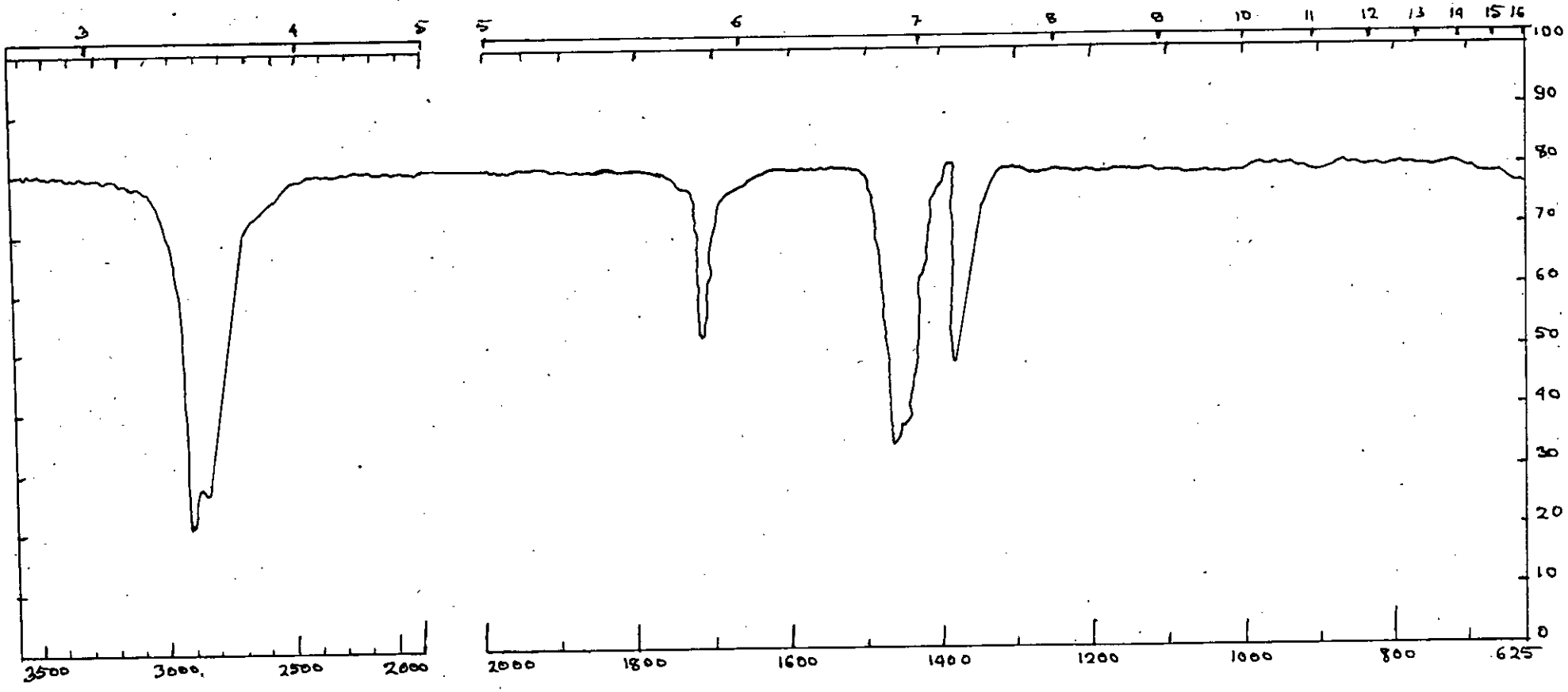


Fig. 18: Infra red Spectrum of Compound P_5.

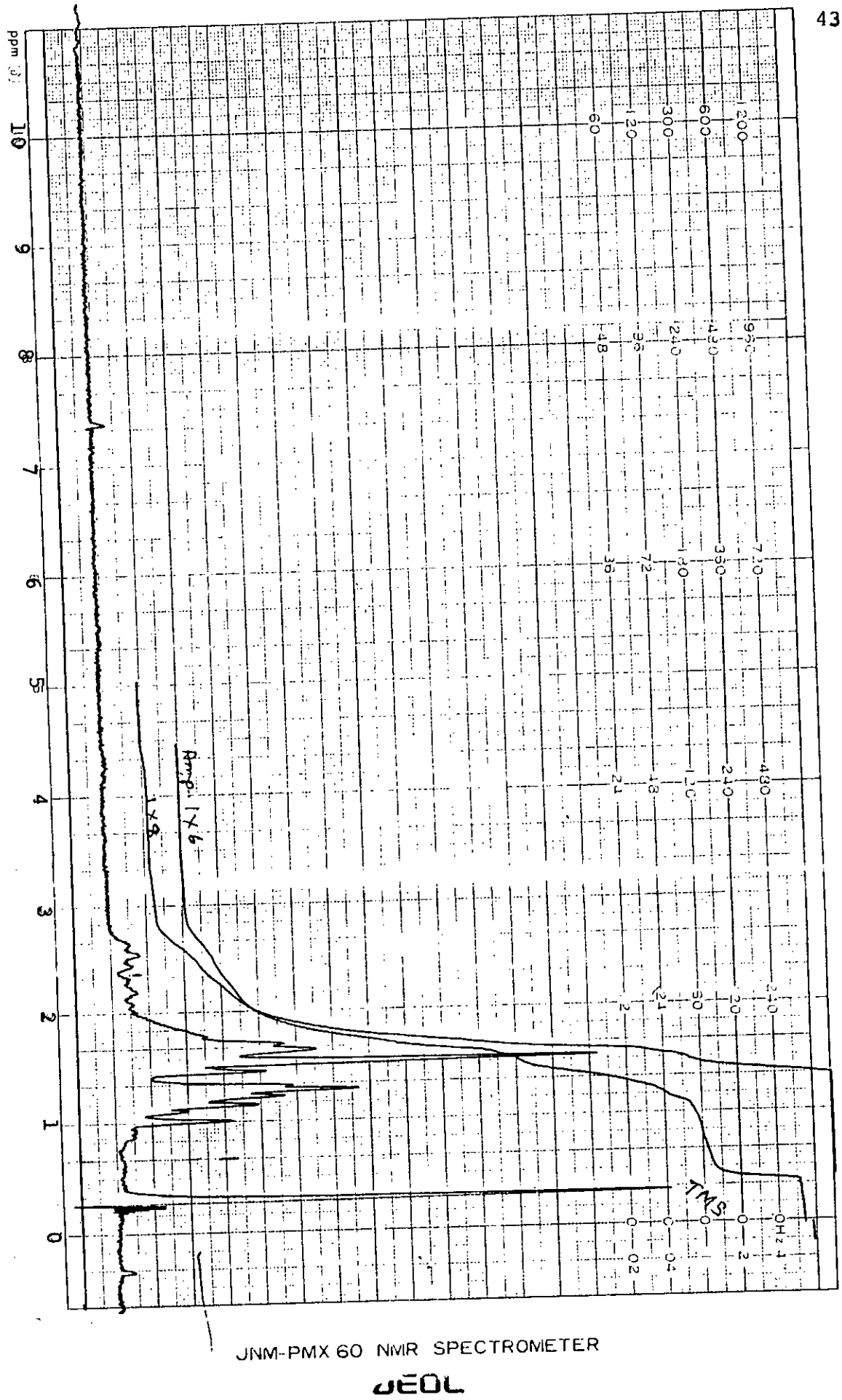


Fig. 19: PMR Spectrum of Compound P_5

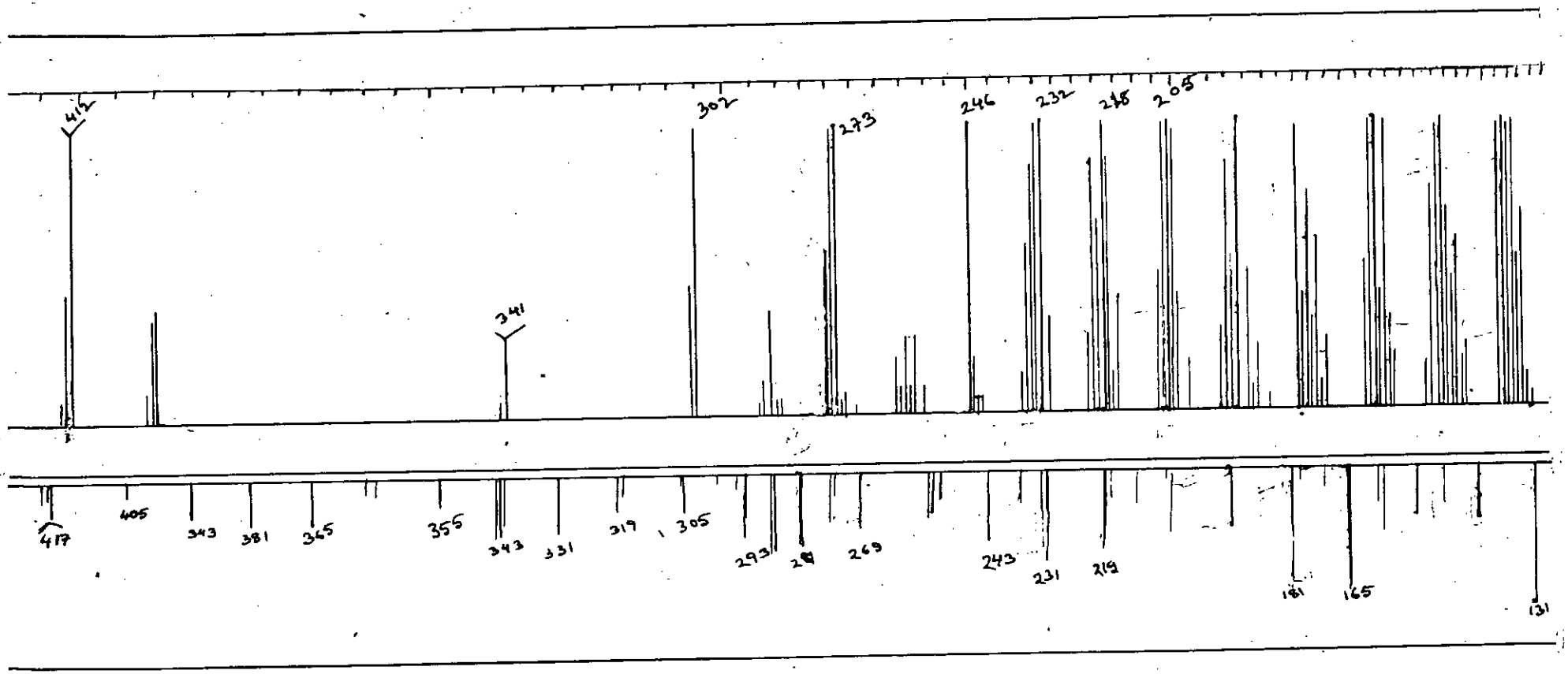


Fig. 20: Mass Spectrum of Compound P_5.

2.3.6 Examination of Fraction P_7

Fraction P_7 yielded greenish needle shaped crystals. The crystals were treated with petroleum ether when the solvent dissolved out the greenish material. The crystals thus obtained were recrystallized three times from methanol. It gave only one spot on tlc plate (R_f 0.65 in 3:2, petroleum ether - ethyl acetate) and melted at 136-137°C (0.30 gm). It gave positive Liebermann-Burchard and Salkowski test for sterol. The ir spectrum (nujol, chloroform) showed O-H absorptions at ν_{max} 3430 and 1040 cm^{-1}. The pmr spectrum of the compound showed the presence of seven C-methyl signals appearing between δ 0.7-1.07. Out of these signals the one at δ 1.02 (3H, S) and δ 0.7 (3H,S) are characteristic of the two angular methyl groups (C_{19} and C_{18} respectively) in a steroid nucleus. The other five C-methyl groups were observed at δ 0.8, 0.85, 0.9, 1.0 and 1.10. A broad doublet (probably a doublet of a doublet) for one proton at δ 5.35 revealed the presence of an olefinic proton. The multiplate at δ 3.5 may be attributed to a proton attached to a carbon bearing hydroxyl group. The compound showed the molecular ion peak at m/e 414 in agreement with the molecular formula of β-sitosterol $C_{29}H_{50}O$. The mass spectrum also showed other important mass peaks at m/e 400 ($M-CH_2$), 396 (M-18), 382 ($M-H_2O-CH_2$), 371 (M-isopropyl group), 329 (M- -CH-CH_2-CH_3-isopropyl group), 273 (M-side chain $C_{10}H_{21}$), 255 (M-side chain $C_{10}H_{21}-H_2O$), 213 (M-side

chain $C_{10}H_{21}$- -ring D C_3H_5-H_2O-H^+) which are characteristics for a hydroxy steroid. The above spectral data and the observed melting point of the compound suggested its identity with β -sitosterol. The identity of the compound was further confirmed by acetylating the compound with a mixture of pyridine and acetic anhydride. The acetate melted at 124-26°C and compared well with the reported melting point (125-26°C) for β -sitosterol acetate. The acetate did not show any absorption in the OH region of the ir spectrum but showed a new sharp band at ν_{max} 1730 cm^{-1} for the ester function.

CHAPTER III
EXPERIMENTAL

3.1 General Experimental

Melting points (mp)

Melting points were determined on a Reichert hot-stage microscope and on a Fisher John's electro thermal melting point apparatus. Melting points were not corrected.

Infra-red Spectra (ir)

Infra-red spectra were recorded on a Perking-Elmer 237 spectrometer and on a PVE UNICAM SP3-200 spectrometer using either chloroform or nujol mull or liquid film.

Nuclear Magnetic Resonance Spectra (pmr)

Nuclear magnetic resonance spectra were recorded on a Parkin-Elmer R12A or Varian HA 100 instrument, using either deuterochloroform ($CDCl_3$) or carbon tetrachloride (CCl_4) as solvent with tetra methyl silane (TMS) as internal standard.

Mass Spectra (ms)

Mass spectra were recorded on a DS-55 Mass Spectrometer, RF I EASF 3.20.

Thin Layer Chromatography (tlc)

The material used for thin layer chromatography was kiesel gel 60 GF_{254} (MERCK). The plates (7.5X2.5 cm) were

prepared by drawing a suspension of kiesel gel 60 GF_{254} (8 g. in 16 ml water) over the thoroughly cleaned plates. The plates were left in position at room temperature until the surface becomes completely dry. The plates were then allowed to stand for twenty four hours for activation and were ready for use.

Preparative Thin Layer Chromatography (plc)

Preparative thin layer chromatography was carried out on plates coated with kieselgel 60 GF_{254} (MERCK). The plates were prepared in the same manner as described above but using larger glass plates (23X20 cm) and a larger spreader allowing thicker coating (0.75 cm). The plates were air dried at room temperature over night and then further activated by warming them at 110^{o}C for half an hour.

Column Chromatography

The column was prepared by slurry method, silica gel (60-120 mesh, BDH, England) being the stationary phase. The column was made half filled with the appropriate solvent (the best running solvent was established by tlc), and the slurry was poured into it so that the packing was compact and uniform. Air bubble was avoided by making the column as quickly as possible and allowing the solvent to fall drop by drop through the stopcock of the column. The solvent

was allowed to pass through the column for sufficient time and then the column was allowed to settle for about one hour. The mixture of compounds was then taken as a solution and was allowed to fall on the surface of the column. The column was then eluted previously purified desired solvent system.

Gar Liquid Chromatography (glc)

The gas chromatographic analysis of the samples reported in the dissertation was performed on a Schimadzu GC-3BF gas chromatograph with dual flame ionization detector using 2.1 m X 3 mm glass column coated with 5% silicone GE SE-30 on AW-DMCS(60-80 mesh).

Drying of pyridine

Laboratory grade pyridine was taken in a quick - fit round bottom flask with a reflux condenser which in turn 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. Pyridine was then distilled twice from phosphorous pentoxide to yield pure and dry pyridine, b.p. 114-115^{o}C.

Drying of acetic anhydride

Acetic anhydride was dried in the same way as described

for pyridine. Acetic anhydride distilling over at 135-140^{o}C was collected and used.

Preparation of mercuric Chloride-Potassium iodide (Meyer reagent)[15]

Mercuric chloride (1.35 g) was dissolved in 2 ml of water in a conical flask. The resulting solution was designated as 'solution-X'. In another conical flask potassium iodide (4.98 g) was dissolved in 2 ml of water and the solution was designated as 'solution-Y'. 'Solution-X' and 'solution-Y' were mixed together and the total volume was diluted to 100 ml by water.

Preparation of Dragendorff reagent (Munier and Machebocuf modification)[15]

A mixture of glacial acetic acid (40 ml) and water (40 ml) was taken in a conical flask and 0.85 g of bismuth subnitrate was dissolved in the mixture. The resulting solution was designated as 'solution-A'. In another conical flask potassium iodide (8 g) was dissolved in 20 ml of water and the solution was designated as 'solution-B'. 'Solution-A' (5 ml) and 'solution-B((5 ml) were mixed together and then 20 ml of glacial acetic acid was added to this mixture and the total volume was diluted to 100 ml by water.

The following abbreviations were used in describing spectra:

IR : s, strong ; m, medium;
w, weak ; b, broad.

Pmr : s, singlet; d, doublet;
t, triplet; q, quartet;
m, multiplet; dd, doublet of
doublets ; b, broad ;
sh, sharp.

3.2 Extraction of the plant Paederia Foetida Linn

Paederia Foetida Linn (Beng. Gandhabhadulia) plant was collected from Ramna area of the Dhaka University Campus, Dhaka, Bangladesh. The plant was identified by the botany department of the University of Dhaka. The aerial parts were dried in the sun, powdered (2 kg) and were extracted exhaustively with petroleum ether (40-60^{o}C) and rectified sprit at room temperature respectively. The petroleum ether extract of Paederia Foetida Linn was concentrated to one tenth of its volume at reduced pressure and allowed to stand in the refrigirator for 48 hours. A white precipitate settled at the bottom of the flask. The supernatant heavy liquid was carefully decanted off and the white solid mass A_1 was collected (1.1 gm). The heavy liquid was further concentrated at reduced pressure at 30^{o}C when a reddish green mass, A_2 was obtained. The rectified sprit extract was concentrated at reduced pressure at 30^{o}C when a semisolid mass was obtained. Distilled methanol was added to the concentrated mass several times and concentrated at 35^{o}C at reduced pressure. A greenish black gummy mass was obtained, devoted as 'R'.

3.3 Isolation of the Components of Mass A_1

The white mass, A_1 was a complicated mixture of at least three compounds as revealed by tlc (R_f 0.70, 0.48 & 0.37 in carbon tetrachloride - ethyl acetate 9:1). It was soluble in carbon tetrachloride on worming, sparingly soluble in petroleum ether, diethyl ether, ethyl acetate, chloroform, acetone and insoluble in ethanol and methanol. Mass A_1 was chromatographed over a column of silica gel and eluted with carbon tetrachloride and mixture of carbon tetrachloride & ethyl acetate in the ratio of 24:1. Fractions of about 7 ml volume were collected and every fraction was examined on tlc plates. The fractions showing similar tlc behaviour were combined. The results are shown in Table I.

Table I: Column Chromatographic separation of mass A_1

Fraction No.	Eluting solvents	Yield	Remarks
Fraction D_1 (collection nos. 17-18)	CCl_4: ethyl acetate(24:1)	49 mg	Two spot R_f(CCl_4: ethyl acetate 9:1) 0.70 & 0.48 white crystal.
Fraction D_2 (collection nos. 19-20)	CCl_4: ethyl acetate(24:1)	94 mg	One spot R_f(CCl_4: ethyl acetate 9:1) 0.48 white crystal.
Fraction D_3 (collection nos. 21-22)	CCl_4: ethyl acetate(24:1)	47 mg	Two spot R_f(CCl_4: ethyl acetate, 9:1) 0.48 & 0.37 white crystals
Fraction D_4 (collection nos. 23-27)	CCl_4: ethyl acetate(24:1)	6 mg	One spot with tailing R_f(CCl_4: ethyl acetate, 9:1) 0.37 yellowish white shining crystals

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3.4 Examination of Mass A_2

Tlc of the reddish green heavy viscous mass A_2 was performed in different solvent systems in different ratios on silica plate. The suitable solvent systems were petroleum ether and petroleum ether-ethyl acetate mixture in different ratios in which the different components of the crude mass A_2 were best resolved. In petroleum ether the crude mass A_2 exhibited two different distinct spots at R_f values 1 and 0.62. In petroleum ether - ethyl acetate (9:1) mixture the crude mass A_2 exhibited five different distinct spots at R_f values 1, 0.91, 0.70, 0.48 and 0.24 respectively with an unresolved zone at the base line.

Separation of the Components of mass A_2

The concentrated mass A_2 was diluted in minimum quantity of petroleum ether and was chromatographed over a column of silicagel using petroleum ether as the initial eluting solvent. Fraction of about 7 ml volume were collected at regular intervals and were examined on tlc plates. The polarity of the eluting solvent was developed by using ethyl acetate as a distinct proportion. The fractions showing similar tlc behaviour were combined. The chromatographic results are given in Table II.

Table II: Column Chromatographic separation of mass A_2

Fraction No.	Eluting solvents	Yield	Remarks
Fraction P_1 (collection nos. 4-7)	Petroleum ether	200 mg	One spot with a unresolved zone at the base line. R_f(petroleum ether) = 1.0 Brown viscous mass.
Fraction P_2 (collection nos. 16-24)	Petroleum ether	51 mg	One spot R_f(petroleum ether) = 0.76 Colourless gummy.
Fraction P_3 (collection nos. 52-76)	Petroleum ether	41 mg	One spot R_f(petroleum ether) = 0.62 Yellowish red. (Orange)
Fraction P_4 (collection nos. 129-134)	Petroleum ether - ethyl acetate (24:1)	1.4 gm	One spot with long tail R_f(petroleum ether - ethyl acetate 4.8:0.2) 0.38 Red liquid plus white crystals

Contd..

Table II: Column Chromatographic separation of mass A_2 (Contd.)

Fraction No.	Eluting solvents	Yield	Remarks
Fraction P_5 (collection nos. 135-153)	Petroleum ether -ethyl acetate (24:1)	444 mg	One spot with tailing R_f (petroleum ether - ethyl acetate 4.8:0.2) = 0.34 Red liquid + White Crystals.
Fraction P_6 (collection nos. 154-196)	Petroleum ether -ethyl acetate (24:1)	887 mg	One spot with long tailing R_f (petroleum ether- ethyl acetate 4.5:0.5) = 0.35 Red liquid plus White Crystal.
Fraction P_7 (collection nos. 252-279)	Petroleum ether -ethyl acetate (5:1)	287 mg	One spot with long tailing R_f (petroleum ether- ethyl acetate, 3:2) = 0.65 Green Crystals.

3.5 Study on Fraction P_4

Fraction P_4, was reddish semisolid mass (1.4 g), showed single spot (R_f 0.38 in petroleum ether - ethyl acetate, 4.8:0.2) with unresolved zone on the base line. It was chromatographed over a column of silicagel using petroleum ether and mixture of petroleum ether - ethyl acetate as eluent. The fractions showing similar tlc behaviour were combined. The chromatographic results are given in table III.

Table III: Column Chromatographic separation of mass P_4

Fraction No.	Eluting solvents	Yield	Remarks
Fraction K_1 (collection nos. 5-25)	Petroleum ether (60-80°C)	207 mg	One spot R_f (petroleum ether-ethyl acetate 4.9:0.1) = 0.44 Visco solid White.
Fraction K_2 (collection nos. 27-45)	Petroleum ether -ethyl acetate (99:1)	217 mg	Long tailing Yellowish red semi liquid.

3.6 Study on Mass R

The blackish green gummy mass R (72 g) obtained from the rectified sprit extraction of the aerial parts of Paederia foetida Linn was soluble in chloroform and partly soluble in benzene, petroleum ether and ethyl acetate. Mass R was subjected to tests for alkaloids.

a. Test with Mayer's Reagent

A few mg of crude Mass R was dissolved in dilute sulfuric acid and two drops of Mayer's reagent were added to the solution. A white precipitate was formed.

b. Test with Dragendorf Reagent

Chloroform solution of Mass R was spotted on a TLC plates of silica gel, when Dragendorf reagent was sprayed on the dry plate, orange colour spots were found on the plate.

3.7 Extraction of Alkaloids from Crude Mass R

The crude Mass R (27.7 g) was dissolved in a minimum volume of chloroform (30 ml) and the solution was extracted with 2% sulfuric acid (10 X 3 ml). The acid extracts were combined and basified to P^H-8 with 2% NH_4OH solution and was again extracted with chloroform (15X4 ml). The chloroform extracts were combined and dried over anhydrous sodium sulfate. After filtration the solvent was removed by vacuum pump and finally dried in a vacuum desiccator. A yellowish gummy crude alkaloid mixture was obtained (270 ml).

3.8 Characterisation of Fraction D_2

Fraction D_2 (0.054 g, 0.003%) gave only one spot on tlc plate (R_f 0.48) when developed in carbon tetrachloride - ethyl acetate (9:1) mixture and (R_f 0.43) when developed in petroleum ether - ethylacetate (9:1) mixture. It melted at 282-84°C.

IR($CHCl_3$) : ν_{max} 3450, 1070 cm^{-1}

PMR($CDCl_3$): δ0.87, 0.93, 0.96, 1.0,1.11, 1.25-1.8 and 3.7.

Mass spectrum: m/e 341, 275, 149, 123 and 357, 413, 395.

Acetylation of the Compound

A small quantity of the compound (11 mg) was taken in a round bottom flask and freshly distilled acetic anhydride (1.0 ml) was added to it followed by addition of 4-5 drops of distilled dry pyridine. The mixture was refluxed under anhydrous condition for three hours and then allowed to cool. The reaction mixture was poured into ice-water (20-25 ml). The residue which separated out was filtered and the product was crystallised from methanol. The acetate was dried in a vacuum desiccator. It melted at 293-94°C and showed one spot

on tlc plate having R_f 0.55 in petroleum ether-ethyl acetate (9:1) mixture.

IR(nujol) : ν_{max} 1730 cm^{-1}

Oxidation of the Compound D_2

A small quantity of the compound (11 mg) was taken in a small flask, dissolved by distilled acetone with stirring on ice-bath. To the stirred and cooled solution (10-15°C) of the compound in acetone (7 ml), Jone's reagent was added dropwise during 1½ hours. After stirring for a further 30 minutes two layers, green at the bottom and red on the top were formed. The product was extracted with diethyl ether twice, dried over anhydrous sodium sulphate & filtered. Removal of solvent gave a white solid mass, which was dried in a vacuum desiccator. It melted at 252-54°C and showed one spot on tlc plate having R_f 0.53 in petroleum ether-ethyl acetate (9:1) mixture.

IR (nujol) : ν_{max} 1730 cm^{-1}

Mass spectrum: m/e 341, 309 and 273.

3.9 Characterisation of Fraction P_1

Fraction P_1 yielded a white low melting solid (0.08 g, m.p. 66-68°C). It gave one spot on tlc plate when developed in petroleum ether (40-60°C), R_f 1.0.

IR($CHCl_3$) : ν_{max} 910 cm^{-1}

PMR($CDCl_3$) : δ 1.0-1.5,
shows absorptions only in the saturated C-H region.

Mass spectrum: m/e 449, 436.

GLC analysis of Fraction P_1 was carried-out on silicone silar 100 column at 120-200°C. It showed two peaks having very higher retention time than C_{18}, C_{19} and C_{20} alkanes.

GC-Mass spectra of the Fraction P_1 on a OV-275 column at 100-215°C with 5°/minute rise showed four peak, of which two were prominent. The molecular ion of the sub-fractions of fraction P_1 were established by running a CI mass spectrum.

3.10 Characterisation of Fraction P_2

Fraction P_2 yielded a transparent gummy liquid mass, (0.05 g, R_f 0.46 in petroleum ether).

IR($CHCl_3$) : ν_{max} 3450 and 1715 cm^{-1}

PMR($CDCl_3$) : δ 0.88, 1.2 and 1.55-1.65

Mass spectrum : m/e 225, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71 and 57.

3.11 Characterization of Fraction P_3

Fraction P_3 (20 mg, 0.001%) yielded orange red crystals. It gave only one spot on tlc plates (R_f 0.62) when developed in petroleum ether on Silica gel plate. It melted at 140-45°C.

IR(Liquid film) : ν_{max} 1660, 1645, 1630 and 3350(b) cm^{-1}.

PMR(CCl_4) : δ 0.92(t-distorted), 1.3(s), 2.27 and 6.82

Mass spectrum : m/e 294, 57, 71, 85, 99, 113, 127 and 141.

3.12 Characterisation of Fraction K_1

Fraction K_1 gave a yellowish coloured solid mass, fairly pure (0.21 g, R_f 0.44 in petroleum ether - ethyl acetate 4.9:0.1) was rechromatographed through silica gel using petroleum ether, petroleum ether-acetone as eluant. The process yielded a pure compound, m.p. 57°C.

IR($CHCl_3$) : ν_{max} 1720, 910 cm^{-1}

PMR($CDCl_3$) : δ 0.85, 1.25

Mass spectrum: m/e 503, 429, 355, 281, 207, 127, 113, 99, 85, 71, 57, 43.

3.13 Characterisation of Fraction P_5

Fraction P_5 gave colourless needle like crystals embedded in a red viscous liquid. Crystals were separated out by dissolving the red liquid with petroleum ether. The crystals thus obtained were chromatographed through a silica-gel column using petroleum ether followed by mixtures of petroleum ether & ethyl acetate. The crystals collected from the chromatographic fraction was further recrystalized three times from methanol. The crystals were fine needles (0.03 g) and melted at 233-34°C. It gave only one spot on tlc plates (R_f 0.53 in petroleum ether-ethyl acetate 9:1).

IR(nujol) : ν_{max} 1715 cm^{-1}

Reduction of the Compound

A small quantity of the compound (14 mg) and dry diethyl ether were taken in a two-necked little flask fitted with an efficient condenser, cooled on a ice-bath by stirring. Lithium-aluminium hydride (56 mg) was added and the solution was stirred overnight at room temperature. Wet ether (water + diethyl ether) was added to decompose an unreacted lithium aluminium hydride. After ten minutes, ice was added to the mixture. Normal work up, extracting twice with ether, dried over anhydride sodium sulphate. Removal of the ether yielded the expected alcohol (10 mg, 75%) which was dried in a vacuum

desiccator. It melted at 250-52°C and showed one deep spot on tlc plate having R_f 0.4 in petroleum ether-ethyl acetate (9:1).

IR(nujol): ν_{max} 3450 cm^{-1}.

3.14 Characterisation of Fraction P_7

Fraction P_7, eluted with petroleum ether-ethyl acetate (2:3) mixture yielded greenish needle shaped crystals. The crystals were treated with petroleum ether when the solvent dissolved out the greenish material. The crystals thus obtained were recrystalized three times from methanol (0.30 gm). It gave only one spot on tlc plate (R_f 0.65) when developed in petroleum ether-ethyl acetate (3:2) mixture and melted at 136-37°C.

IR($CHCl_3$) : ν_{max} 3430 and 1040 cm^{-1}

PMR($CDCl_3$): δ 0.7, 0.8, 0.85, 0.9, 1.0, 1.1, 1.02, 3.5 and 5.35

Mass spectrum: m/e 400 (M^+), 396, 382, 371, 329, 273, 255, 213.

Salkowski Reaction

A few mg of the compound was dissolved in chloroform and a few drops of concentrated sulphuric acid was added. A deep red colour was observed which indicated the positive test for sterol.

Liebermann-Burchard Reaction

A few mg of the compound was dissolved in chloroform and a few drops of concentrated sulphuric acid were added to it. Then 2-3 drops of acetic anhydride were added to the mixture. A green colour was observed which indicated it to be a sterol.

Acetylation of the Compound

A small quantity of the compound (17 mg) was taken in a round bottom flask and freshly distilled acetic anhydride (1.5 ml) was added to it followed by addition of 4-5 drops of distilled dry pyridine. The mixture was refluxed under anhydrous condition for three hours and then allowed to cool. The reaction mixture was poured into ice-water (20-25 ml). The residue which separated out was filtered and the product was crystallised from methanol. The acetate was dried in a vacuum desiccator. It melted at 124-26^{o}C and showed one

spot on tlc plate having R_f 0.80 in petroleum ether-ethyl acetate (2:3) mixture.

IR(nujol) : ν_{max} 1730, 1040 cm^{-1}

A mixed melting point of the compound with β - sitosterol (136-37°C) remained undepressed. The ir spectrum of the compound was superimposable on that of an authentic sample of β-sitostrol. The R_f value of the compound was also similar to that of β-sitosterol.

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