Chapter 2

MICROWAVE ASSISTED ONE POT SYNTHESIS OF PYRAZINE DERIVATIVES OF PENTACYCLIC TRITERPENOIDS AND THEIR BIOLOGICAL ACTIVITY

Introduction

Triterpenoids are distributed widely in nature and recent literature has reported their wide spectrum of biological activities [56]. It is well known and well documented that there exists a connection between the wide spectrum of biological activities and the molecules having pyrazine nucleus [57]. They possess varieties of activities like antimicrobial [57], anti filarial [57], anti leukemia [57] in mice against i.p. P388 and several pyrazines were more active than the corresponding oxazines or thiazines [57]. Also a series of pyrazine-carboxymides has been described as eukalemic agents possessing diuretic and natriuretic properties. Hence pyrazine is a lead compound for designing potential bioactive agents. Thus it is anticipated that incorporation of a pyrazine ring into a molecule like triterpenoids may induce biological activity or may enhance the same if already present in the latter. So a study can be taken up to incorporate a pyrazine ring into the pentacyclic triterpenoids and to study the biological activity of the derivatives. But the conventional method of pyrazine synthesis [58, 59, and 60] involves hazardous, expensive, polluting organic solvents; a prolonged reaction time and tedious working procedures also produce significant amount of side products. Again reports regarding the one pot synthesis of pyrazine derivatives of pentacyclic triterpenoids are very limited. Thus, a one pot method involving milder, more selective, inexpensive and eco-friendly reaction condition is still in demand.

The potential application of microwave (MW) technology in organic synthesis is increasing [61] rapidly because of the reaction simplicity, less polluting and minimum reaction time providing rapid access to large libraries of diverse molecules. This technology has been implemented since the middle of 1980s in the field of organic chemistry. The increasing number of related publications in recent years indicates that this technique is a widely accepted unconventional energy source for performing organic

synthesis due to substantial reduction in reaction time [62, 63], better yields [64] and easier work up procedures [65]. In addition, the high selectivities of the reactions contribute to the prevention of waste formation [66]. Although a very few reports of microwave assisted transformative reactions of terpenoids, flavonoids [67] and steroids [68] are known, current literature is lack about the microwave assisted transformative reaction of pentacyclic triterpenoids.

In continuation of the studies on the transformative reactions of pentacyclic triterpenoids, the author has developed a one pot synthesis of 1, 4-pyrazine derivative of pentacyclic triterpenoids under microwave irradiation. The structures of the compounds have been confirmed by means of spectral data (IR, NMR). Compounds 1b, 2b and 3b have been reported for the first time. The anti microbial potential associated with them has also been investigated.

Results and Discussion

Synthesis of 2, 3- diketo triterpenoids

2, 3-diketo triterpenoids were prepared by auto oxidation of the respective terpenoids (for details please see experimental).

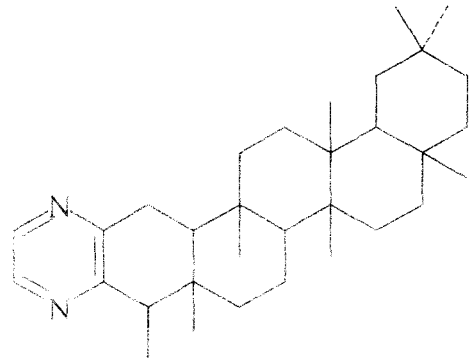
Synthesis of 1, 4-pyrazine derivatives

1,4-pyrazine derivatives of the triterpenoids were prepared (Scheme 1) in a mono-mode microwave oven at 100W(100°C) in only 20 minutes reaction time by adding dry ethylene diamine and Li(for details please see experimental).

Characterization of compound 1b: (1,4-pyrazine derivative of friedelin)

2, 3-diketo friedelin (**1a**) prepared by auto oxidation of friedelin (**1**) was subjected to microwave irradiation (100W, 100°C) for 20 minutes with small pieces of metallic Li and dry ethylene diamine (EDA). The product obtained after usual work up showed a single spot in TLC and was purified over a column of silica gel (60-120 mesh). Crystallization of the compound from $CHCl_3$ – MeOH mixture furnished a compound A, analyzed for $C_{32}H_{50}N_2$, m.p. 228 °C. IR spectrum of the compound showed peaks at 1650 – 70, 1430, 1120 cm^{-1} for pyrazine ring [69]. UV spectrum showed peaks at 272 (ε = 5800)

and 278 (ε = 5450) nm. Mass spectrum of A showed molecular ion peak at m/z 462 as base peak, which is the characteristic feature of pyrazine compounds [69]. The other peaks appeared at m/z 447,420, 271, 241, 227, 163, 149, 125, and 69. The ^{1}H NMR spectrum of A was indicative of the presence of seven tertiary methyls which appeared as sharp singlets (3H each) between 0.82 – 1.22 ppm (7s, 21H, 7t CH_3), the doublet centered at 0.99 ppm was due to the presence of secondary methyl protons (d, J = 6.5Hz), two aromatic protons at 8.40 and 8.27 ppm appeared as a doublet with J = 3Hz. ^{13}C NMR spectrum of the compound A, showed the presence of 32 carbons, two singlets at 150.8 and 150.9 ppm and two doublets at 141.4 and 142.3 ppm were due to heterocyclic ring carbons typical to 2, 3 – disubstituted pyrazine skeleton [70]. All the above facts lead us to assign structure **1b** to compound A. The formulation of structure 1b for compound A is further supported by mass fragmentation pattern that gives similar fragmentation pattern as that of friedelin skeleton as observed earlier [71].

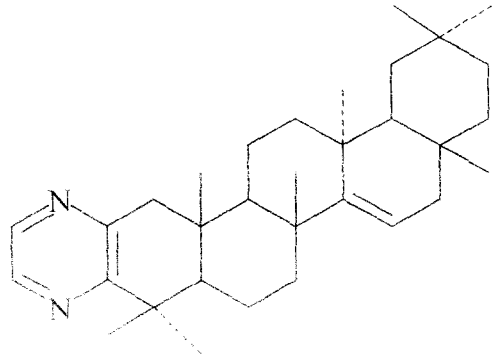


1b

Characterization of compound 2b: (1,4-pyrazine derivative of taraxerone)

2, 3-diketo taraxerone (**2a**) was also treated with Li – dry EDA in the similar way. The single compound obtained was purified over a column of silica gel (60-120 mesh) followed by crystallization from $CHCl_3$ – MeOH mixture to afford a compound, B. It was analyzed for $C_{32}H_{48}N_2$, m.p. 262°C. IR spectrum of B showed peaks at 1600 and 810 cm^{-1} due to the presence of trisubstituted (R_2C = CHR) double bond, the peaks at 1650, 1430 and 1120 cm^{-1}were characteristic for a pyrazine derivative [69]. The UV spectrum of compound B showed absorption at 272 nm (ε = 6150) and at 278 nm (ε = 5200),

characteristic for a pyrazine skeleton. Mass spectrum showed molecular ion peak at m/z 460 as base peak. Its ^{1}H NMR spectrum showed the presence of eight tertiary methyls which appeared as sharp singlets (3H each) in the region 0.81 to 1.25 ppm, the doublet of a doublet centered at 5.54 ppm was due to one olefinic proton at C-15, C-1 protons appeared as a multiplet at 2.30 ppm and two aromatic protons appeared at 8.40 and 8.27 ppm as a doublet with J = 3Hz. The ^{13}C NMR spectrum of B accounted for all 32 carbons and APT experiment indicated the existence of 8 $-CH_3$ as quartets, 9 $-CH_2$ as singlets. The singlet at 156.9 ppm and doublet at 117.4 ppm were due to olefinic carbons at C-14 and C-15 typical of the taraxerone skeleton [72] and the aromatic ring carbons appeared as doublets at 141.4 and 142.3 ppm and singlets at 150.8 and 150.9 ppm. All the above facts lead to the assignment of structure **2b** for compound B.

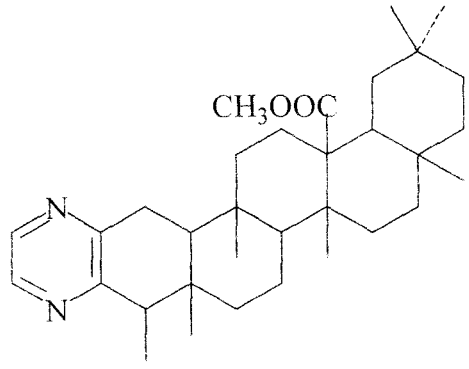


2b

Characterization of compound 3b: (1, 4-pyrazine derivative of methyltrichadenate)

1, 4-pyrazine derivative of methyltrichadenate (**3b**) was also prepared starting from methyltricadenate (**3**). The single compound, C, obtained by the usual procedure was analyzed for $C_{33}H_{50}N_2O_2$, m.p. 198°C. IR spectrum showed peaks at 1730cm^{-1} for carbomethoxy group and at 1650–70, 1430, 1120 cm^{-1} for pyrazine ring [69]. It also showed characteristic UV absorption at 272 nm (ε = 5785) and 278 nm (ε = 5600) for a pyrazine ring. Mass spectrum showed molecular ion peak at m/z 506 as base peak. ^{1}H NMR spectrum of compound C showed the presence of six tertiary methyls that appeared as singlets at 0.81, 0.85, 0.93, 0.99, 1.11 and 1.20 ppm and a secondary methyl as a doublet centered at 0.74 (d, 3H, $CHCH_3$, J = 7Hz) ppm. A singlet at 3.67 ppm indicated the

presence of carboxy methyl group, C-1 protons appeared as a multiplet at 2.30 ppm and two aromatic protons appeared at 8.40 and 8.27 ppm as a doublet with J = 3Hz. Thus from spectral analysis the structure for C has been assigned as **3b**. Encouraged by these findings, and the recent report about the anti HIV activity of triterpenoid lupane skeleton [73] has prompted us to study the same reaction on such skeleton e.g. lupanone and dihydromethyl betulonate and to make a preliminary study on their biocidal activities.



3b

Characterization of compound 4b: (1, 4-pyrazine derivative of lupanone)

Auto oxidation of lupanone yielded 2, 3-diketo lupane which upon treatment with Li – dry EDA in the similar manner followed by purification yielded a single compound. Crystallization of the compound from $CHCl_3$ – MeOH mixture furnished D, analyzed for $C_{32}H_{50}N_2$. $[\alpha]_D$ +19.6°. IR spectrum (Fig. 2) of the compound showed peaks at 1650, 1430 and 1120 cm^{-1}, probably due to the presence of a heterocyclic ring system in compound. UV absorption maxima (Fig. 1) at 272 nm (ε= 5831) and 278 nm (ε= 5792) also suggested presence of aromatic moiety in 4b. The mass spectrum (Fig. 4) of the compound showed molecular ion peak at m/z 462. PMR spectrum (Fig. 3) of the compound D showed the presence of eight tertiary methyl groups resonated at 0.78, 0.83,0.98, 1.11, 1.29, 1.31 ppm (6s, 18H, 6t-CH3), 0.77 and 0,86 ppm (2d, 6H, $CH(CH_3)2$, J=7 Hz): two doublets at 2.47 and 3.04 ppm with germinal coupling of 16 Hz could be assigned to the methylene proton at C-1 that have no protons in the vicinal alpha carbons; two olefinic protons that appeared at 8.405 and 8.27 ppm as doublets with J= 3Hz, the former being further splitted by long rang 1,4 coupling with C-1 proton appearing

at 2.47 ppm; the large downfield shift of these protons indicates that these are present in a pyrazine ring. Hence doublet centered at 8.27 ppm (J=3 Hz) and a doublet of a doublet centred at 8.41 ppm (J= 3 and 1 Hz) were probably due to the aromatic protons (C=C), the nature of (dd) of the peak centred at 8.14 ppm could also be explained by considering the long range coupling of one of the aromatic proton with the methyl proton of the isopropyl group at C-19 position. Thus from the spectral analysis the structure for the compound was identified as pyrazine derivative **4b** of lupanone [74].

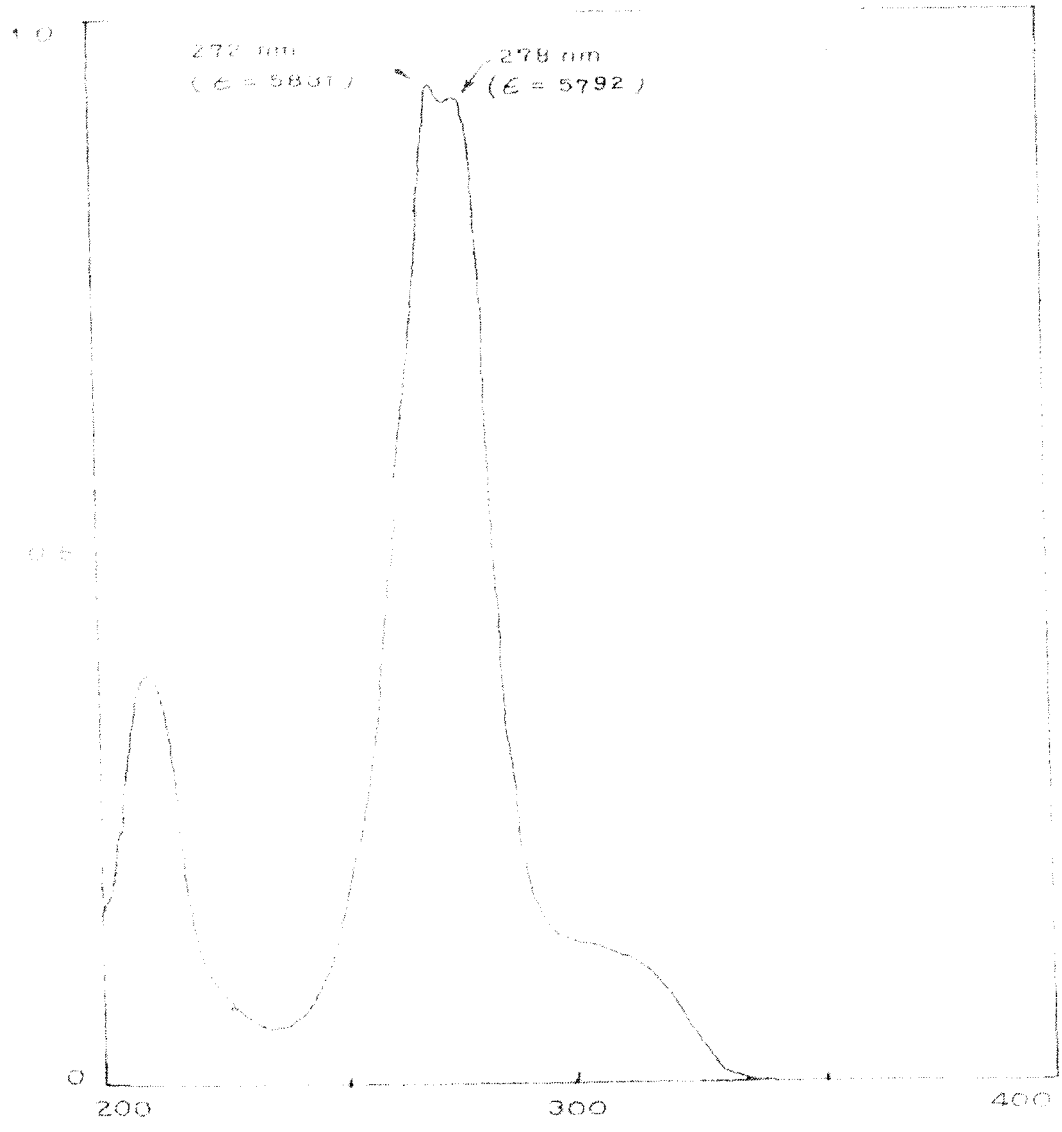


Fig. 1. UV. spectrum of the pyrazine derivative of lupanone

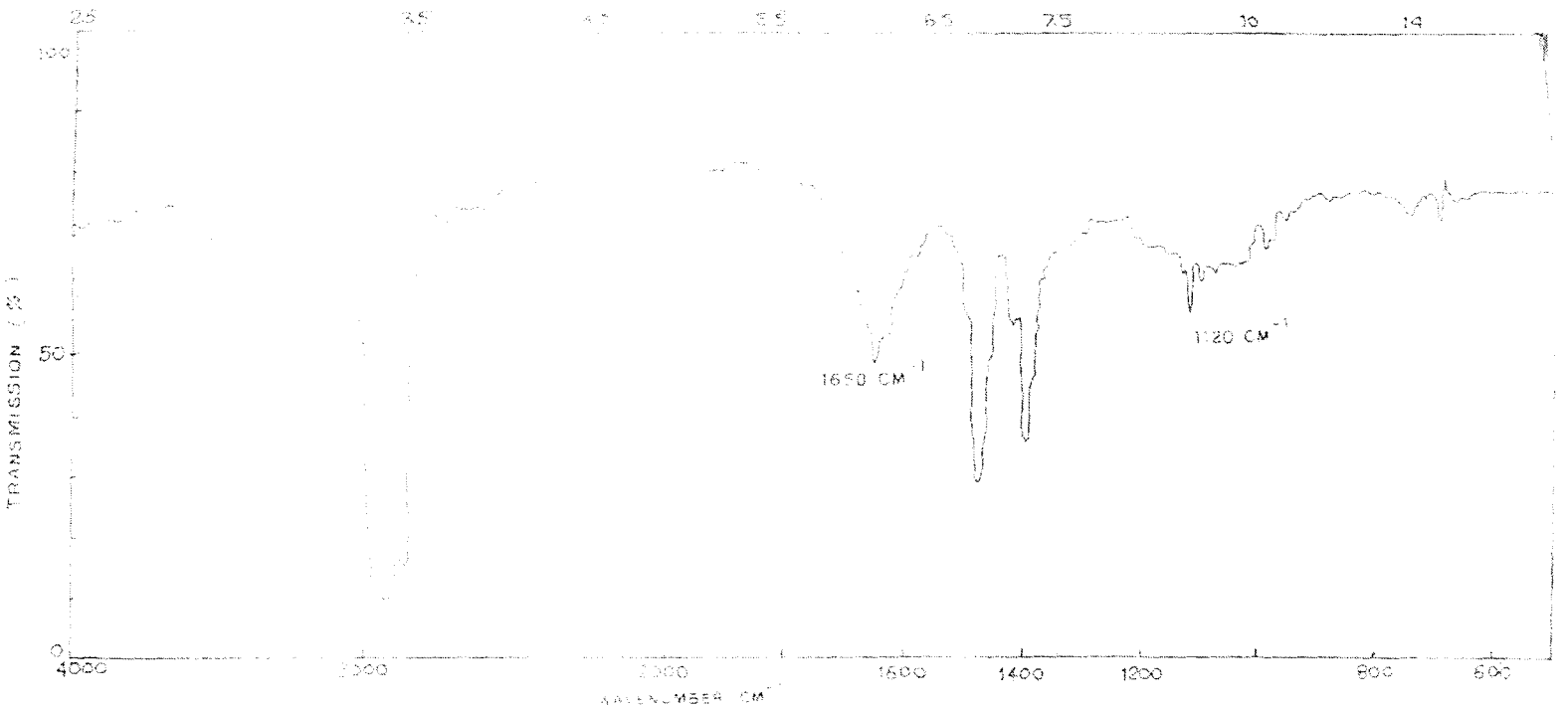


Fig. 2. IR spectrum of the pyrazine derivative of lupanone

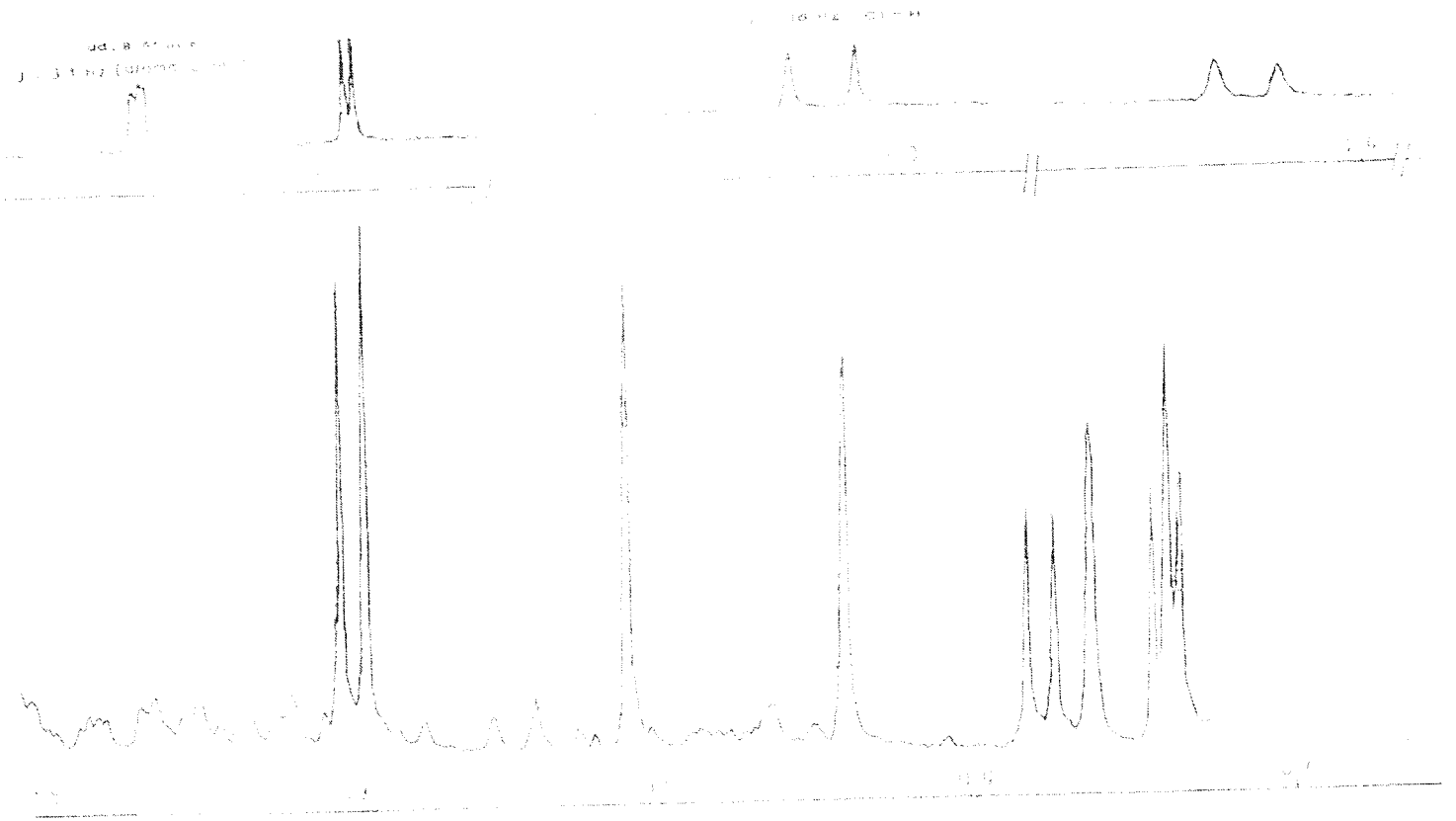


Fig. 3. ^{1}H NMR spectrum of the pyrazine derivative of lupanone

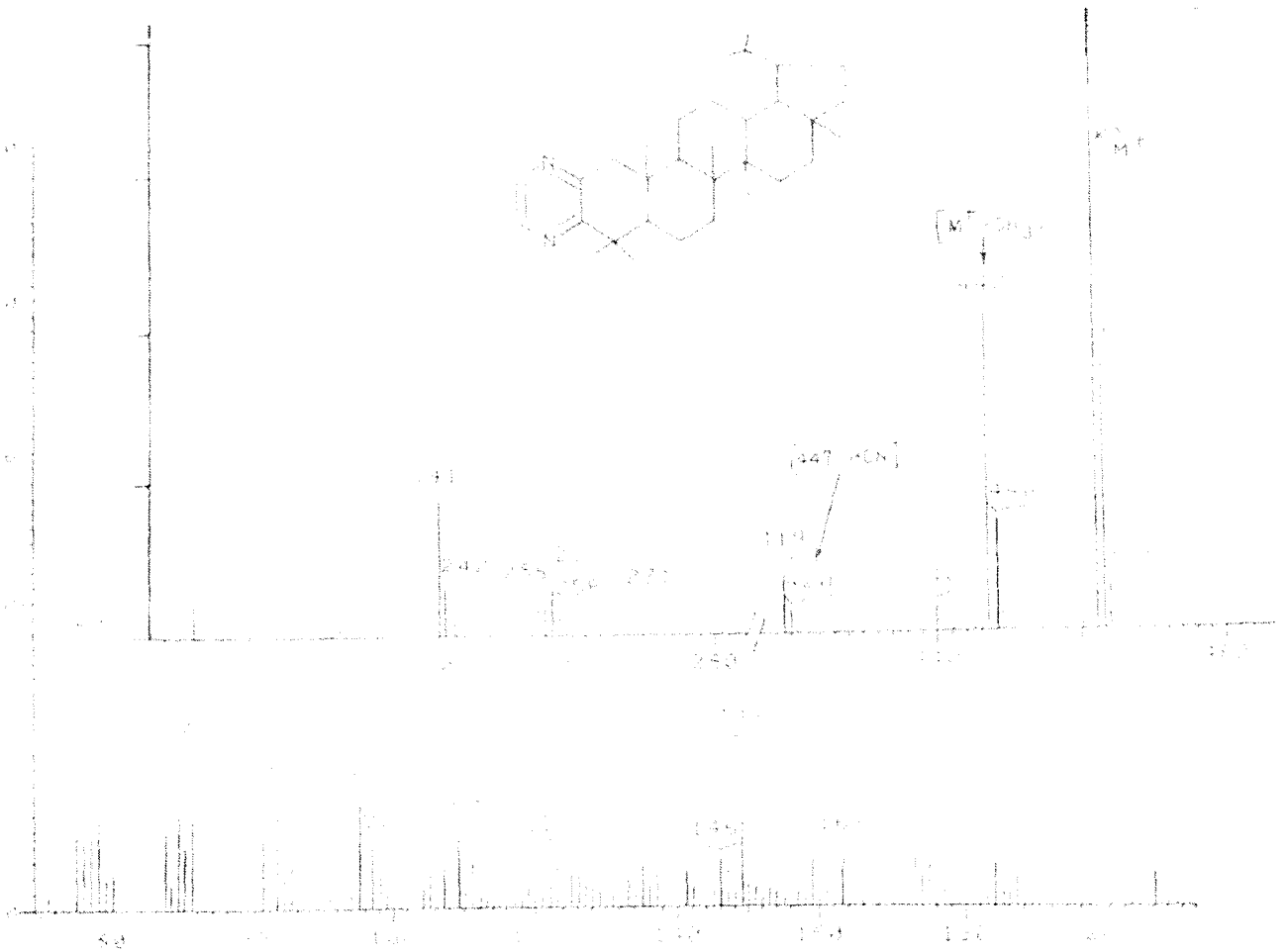
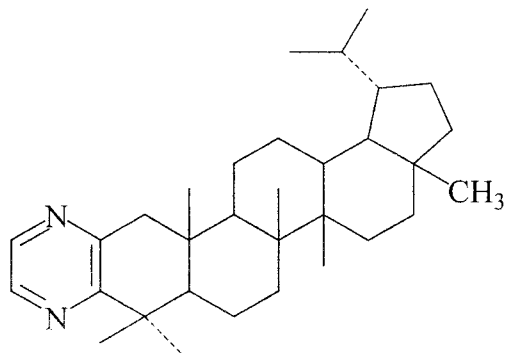


Fig. 4. Mass spectrum of the pyrazine derivative of lupanone



4b

Characterization of compound 5b: (Pyrazine derivative of methyldihydrobetulonate)

Pyrazine derivative **5b** of 28-carbomethoxy lupane was also prepared from 2, 3-diketo 28-carbomethoxy lupane. Crystallization of the compound from chloroform-methanol mixture furnished a compound E, analyzed for $C_{33}H_{50}O_2N_2$ m.p. 220^0. IR spectrum (fig. 6) of the compound showed peaks at 1710-20 (CO_2Me) and 1650-70, 1430, 1120 cm-1 for pyrazine ring. UV spectrum (fig. 5) of compound E showed peaks at 272nm (ε=5712) and 278 nm. It did not respond to the TNM test for active unsaturation. Mass spectrum (fig. 8) of compound E showed molecular ion peak at m/z 506 as base peak which is the characteristic feature of pyrazine compound the others peaks appeared at 491[M – CH_3]$^+$, 463 [M – $CH(CH_3)_2$]$^+$, 447[M – $COOCH_3$]$^+$, 432, 431, 258, 256, 241, 191, 187, 175, 159, 147, 133, 95, 55.

PMR spectrum (Fig. 7) of the compound E showed the presence of eight tertiary methyls resonated at 0.82, 0.985, 0.99, 1.28, 1.305, 0.76 and 88 (2d, 6H, $CH(CH_3)_2$, J = 7 Hz) ppm; two doublet at 2.48 and 3.04 ppm with germinal coupling of 16 Hz which has been assigned to C–1 methylene protons, besides the two doublet at 8.27 and 8.41 ppm with J = 3 Hz for the two aromatic protons and the ester methyl as a sharp singlet at 3.66 ppm. Thus from spectral analysis, the structure for the compound E has been assigned as Pyrazine derivative of methyldihydro betulonate and was found identical with the already reported compound [74].

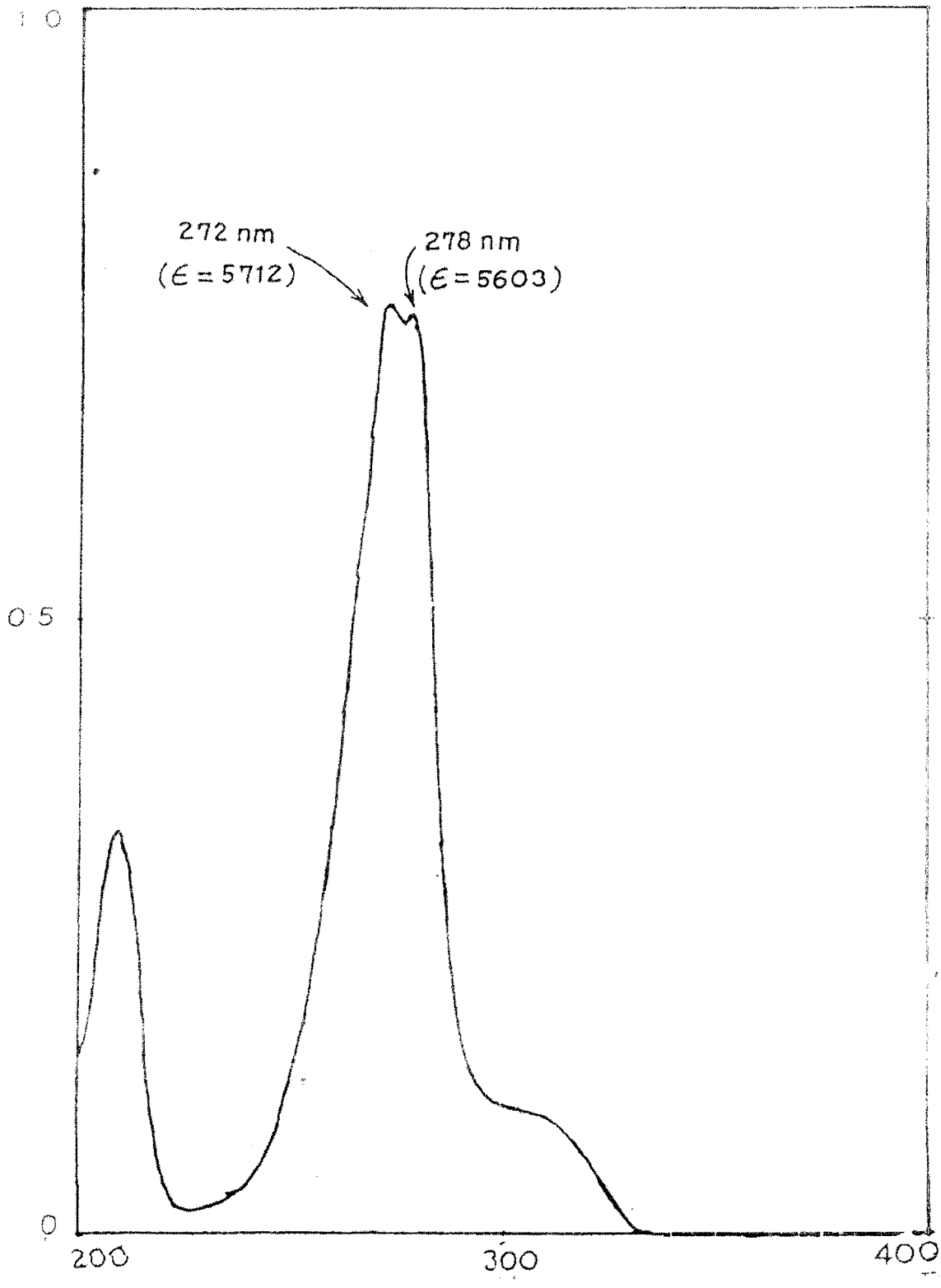


Fig. 5. UV spectrum of the pyrazine derivative of methyldihydrobetulonate

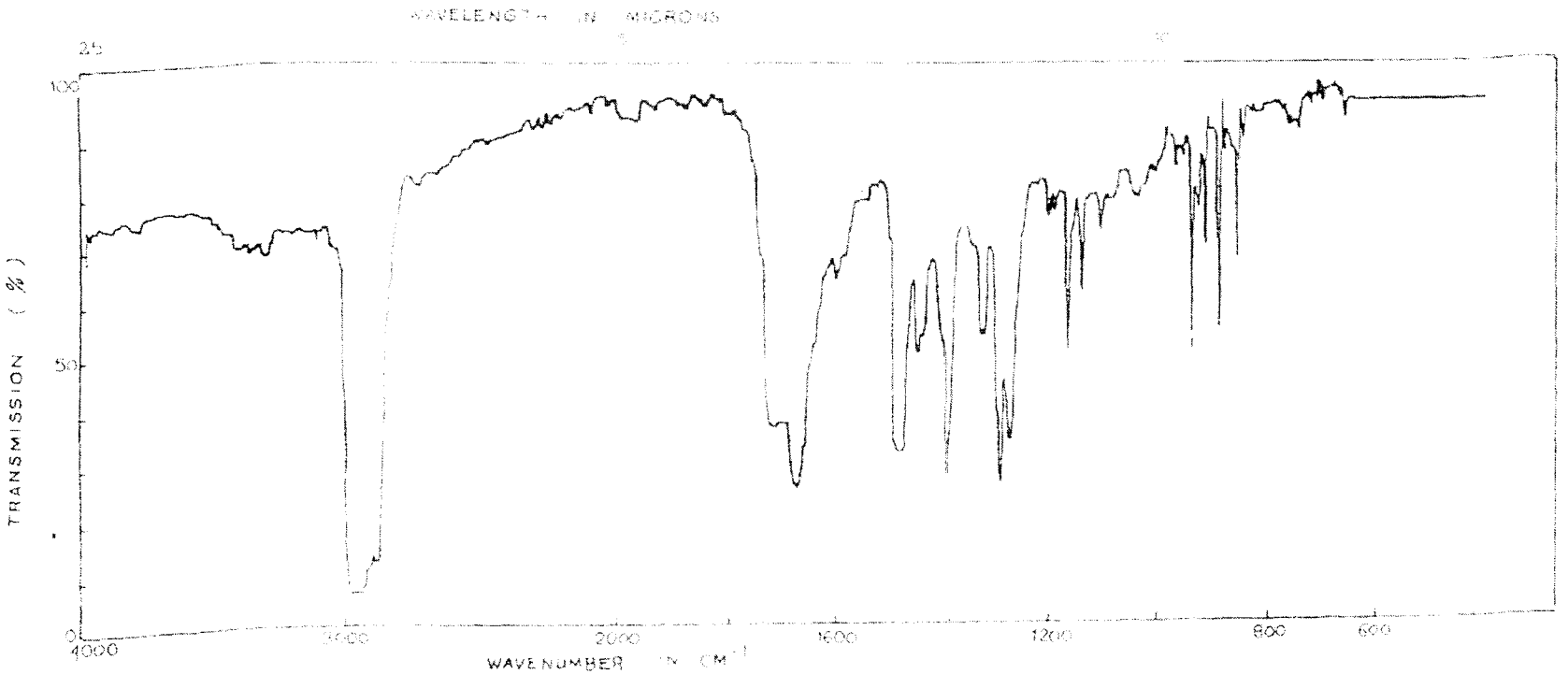


Fig. 6. IR spectrum of the pyrazine derivative of methyldihydrobetulonate

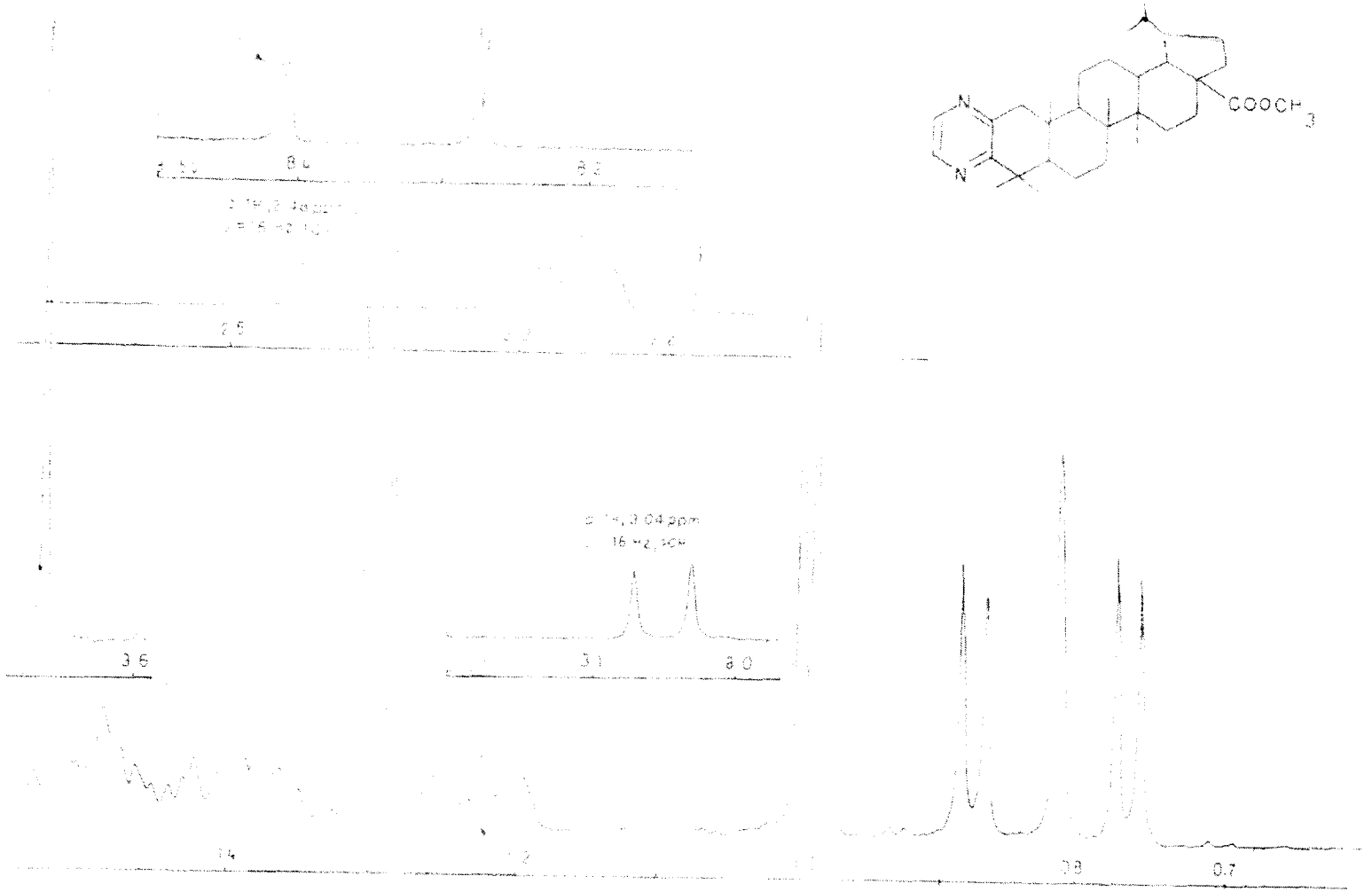


Fig.7. ^{1}H NMR spectrum of the pyrazine derivative of methyldihydrobetulonate

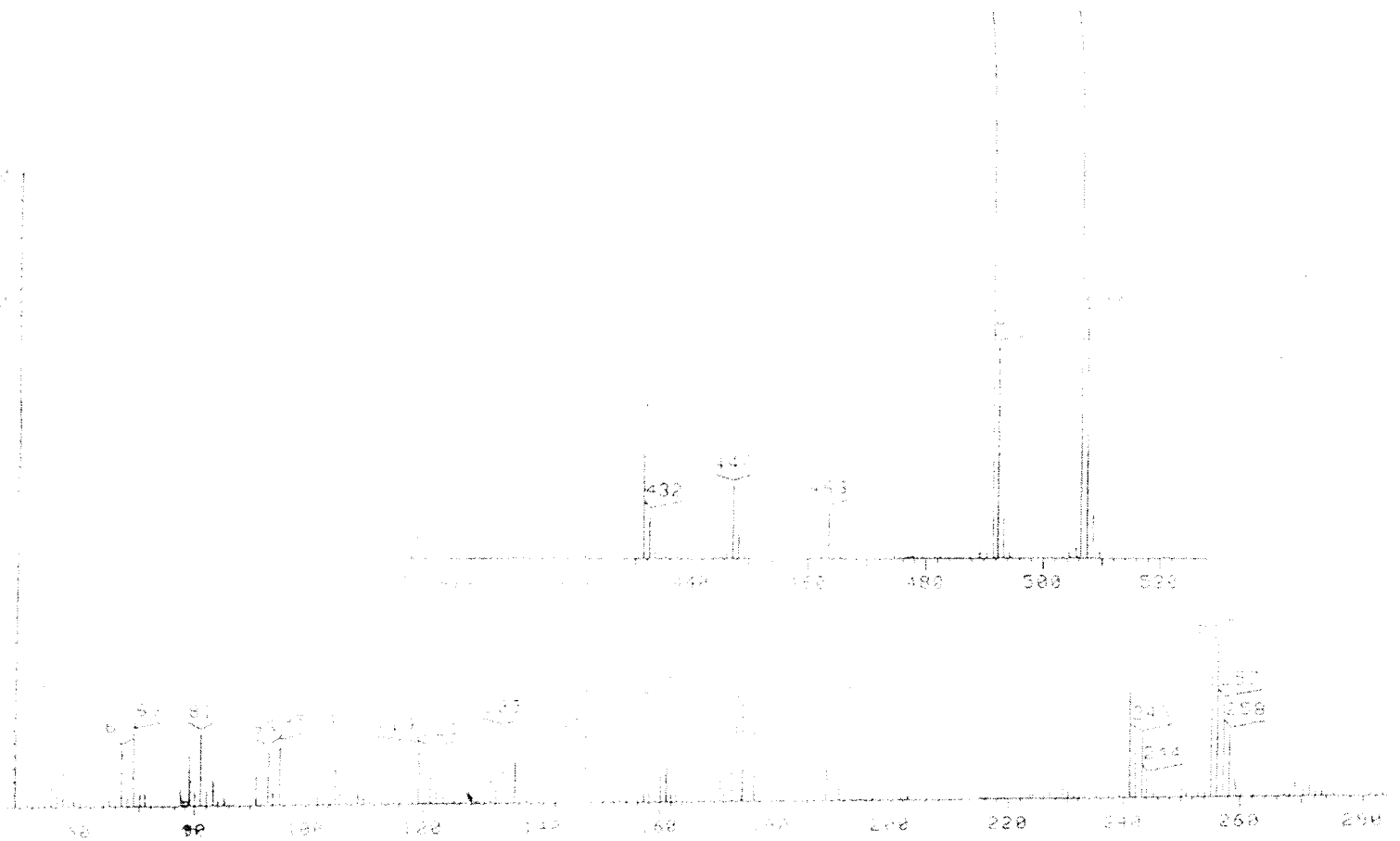
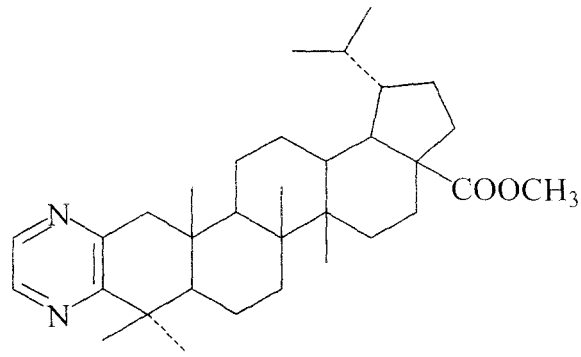
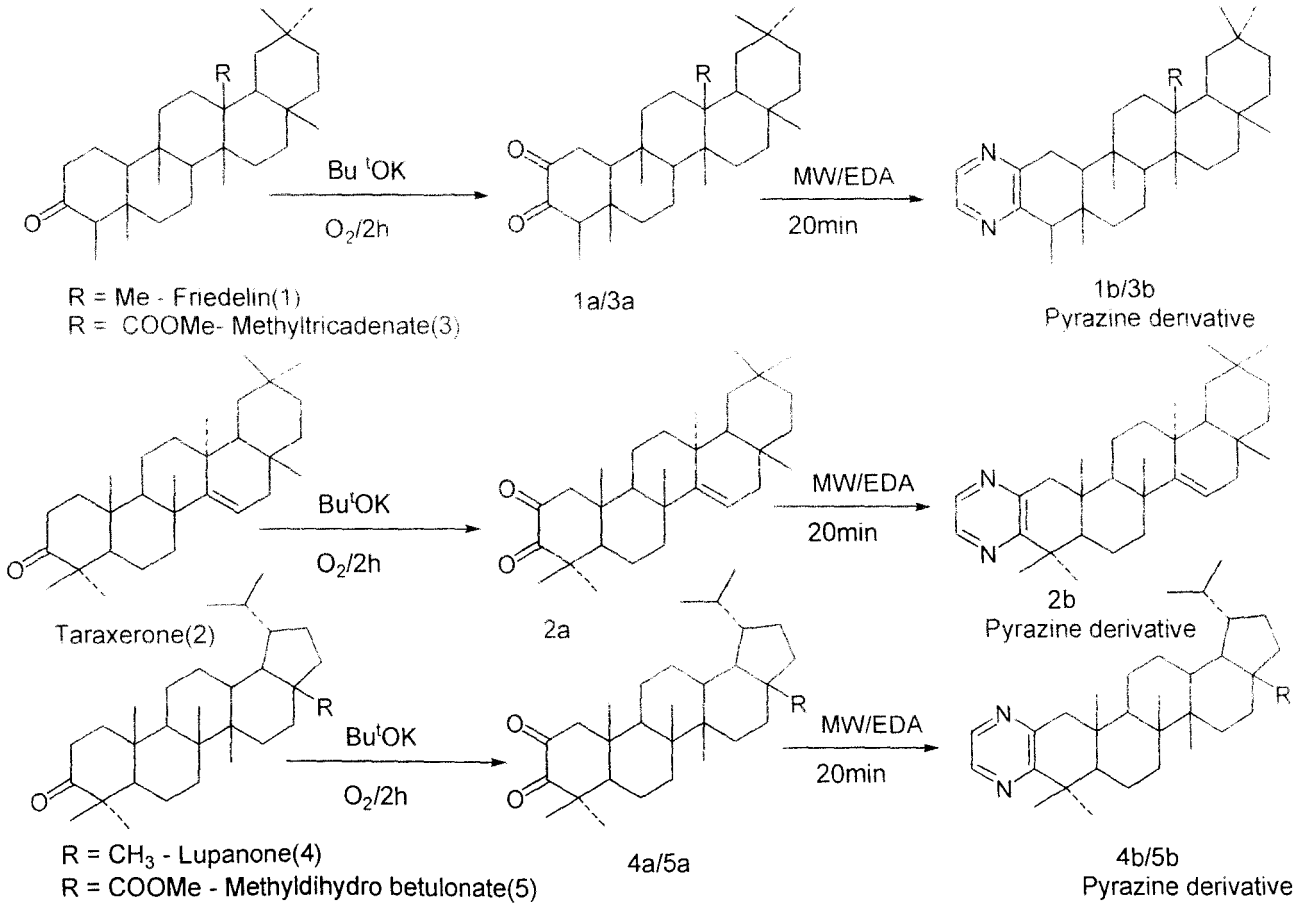


Fig.8. Mass spectrum of the pyrazine derivative of methyldihydrobetulonate


COOCH3
N
N

5b

Scheme 1


R
Bu tOK
O2/2h
MW/EDA
20min
R = Me - Friedelin(1)
R = COOMe- Methyltricadenate(3)
1a/3a
1b/3b
Pyrazine derivative
Taraxerone(2)
BuᵗOK
O2/2h
2a
MW/EDA
20min
2b
Pyrazine derivative
R = CH3 - Lupanone(4)
R = COOMe - Methyldihydro betulonate(5)
4a/5a
4b/5b
Pyrazine derivative

Biological activity

The parent compounds isolated from plants and their pyrazine derivatives were tested against the bacterial species *E. coli* (EC), *S. dysenteries* (SD) (gram negative) and *S. aureus* (SA), *B. subtilis* (BS) (gram positive). Similarly the antifungal activity against *Aspergilus niger* (AN), *Candida albicans* (CA) were also determined. Suitable strains of these organisms were procured from the microbiology laboratory of our institute. MICs (Minimum inhibitory concentration) of the compounds against bacterial and fungal pathogen are reported in table 1. All experiments were performed in Petri dishes and were incubated at 37°C for 48h.The bacterial growth was confirmed by a change of yellow to purple colour. Bacterial nutrient media was prepared by using agar, beef extract and bacto peptone in distilled water and the pH of the solution (6.8 - 7.0) was adjusted. Culture media for fungal strains were prepared by mixing in suitable proportions of potato extract, dextrose and agar powder. All glass apparatus, culture media were autoclaved before use. The whole process was carried out in inoculation chamber. Additionally slide germination method was also used for determination of antifungal activity [77].

Table 1. ***In vitro*** **Antimicrobial screening results of the compounds.**

Microorganis-	MIC in g/mL of the compounds									
ms	1	1b	2	2b	3	3b	4	4b	5	5b
SA	25	<25	100	25	100	25	200	50	200	25
BS	50	25	100	25	50	50	100	25	200	25
EC	200	25	100	<25	200	25	100	<25	100	25
SD	200	50	200	<25	200	50	200	<25	100	50
AN	100	50	100	25	100	>100	100	<25	100	<25
CA	50	<50	200	25	50	50	50	25	100	<25

SA- *S. aureus*, BS- *B. subtilis*, EC- *E. coli*, SD- *S. dysenteriae*, AN- *Aspergillus niger*, and CA- *Candida albicans*, MIC- Minimum inhibitory concentration.

Discussion

All the compounds showed prominent antimicrobial activities against the tested fungal specimens (*Aspergillus niger*, *Candida albicans*) and bacterial pathogens (*E. coli*, *S. dysenteries* *S. aureus*, *B. subtilis*) as evident from table 1.

MIC record of the compounds (Table 1) clearly indicates that in comparison to the parent compounds, derivatives 2b and 5b were highly active against *S. dysenteries*, *B. subtilis* and *E. coli*. Compound 3b showed high activity against *S. aureus* and *E. coli* and moderate activity against *B. subtilis* except *S. dysenteries* 5b showed high activity against all the bacterial organisms studied in comparison to the respective parent compounds 1, 2, 3 and 4. In addition, all the derivatives showed high to moderate activity against the fungi *Aspergilus niger* and *Candida albicans*.

Srikrishna *et al.* [78] carried out antibacterial activity using cup plate method. They observed that pet. ether, chloroform, methanol and water extract of the bark of *Aporosa lindleyana* (Euphorbiaceae) showed moderate to very good activity against bacteria such as *Bacillus subtilis*, *Escherichia coli*. They studied antifungal activity such as *Candida albicans*, *Aspergilus niger* and compared with the standard drug fluconazole. The pet. ether extract showed considerable activity towards all the four fungal organisms.

Audu *et al.* [79] extracted components from *Annona senegalensis* (root), *Nauclea latifolia* (stem bark) and *Ziziphus abyssinica* (root bark) using methanol, diethyl ether and cold water as solvent. They studied their activity on *Candida albicans*, *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* at different concentrations and found that all these components inhibited the growth of microbes.

Ragasa *et al.* [80] extracted the air dried leaves of *Vitex negundo* which afforded vitexilactone and casticin by silica gel chromatography. They studied their activity and found that the compounds inhibited the growth of the fungi: *Candida albicans* and *Aspergillus niger* and the bacteria: *Staphyllococcus aureus* and *Pseudomonas aeruginasa*.

Kumar *et al.* [81] carried out antimicrobial properties of a series of 61 medicinal plants belonging to 33 different families used in various infectious disorders at 1000 and 500 microg/ml concentration by agar dilution method against *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Bordetella bronchiseptica, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa , Streptococcus faecali, Candida albicans, Aspergillus niger and Saccharomyces cerevisiae.* They found that 28 plant extracts showed activity against at least one of the test organisms used.

Mbwambo *et al.* [82] were extracted compounds from stem bark, wood and whole roots of *Ternimalia brownii* using solvents of increasing polarity, namely, pet ether, dichloromethane, dichloromethane: methanol (1:1), methanol and aqua, respectively and the extracts were tested for antifungal and antibacterial activity. They observed that the extracts of the stem bark, wood and whole roots of *T. brownii* exhibited antibacterial activity against standard strains of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi* and *Bacillus anthracis* and the fungi *Candida albicans* and *Cryptococcus neoformans*. They found that aqueous extracts exhibited the strongest activity against both bacteria and fungi.