Chapter II

Section A

Synthesis of pyrazine derivatives of triterpenoids

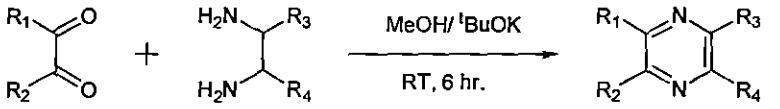
1. Introduction

Heterocyclic compounds occur widely in living organisms and many possess a broad range of biological activities. Researchers have also demonstrated the interesting biological activities of many natural terpenoids.[66] Triterpenoids are widely distributed in nature, and recent reports have demonstrated the interesting biological activities of this class of natural products.[67] However, triterpenoids possessing a nitrogen containing heterocycle condensed to an isoprenoid skeleton are rare. One example of less common isoprenoid is cephalostatin, isolated[66] from the sea worm *Cephalodiscus gilchristie*. This bis-steroidal pyrazine is highly cytotoxic,[68] which sparked interest in the synthesis of similar structures.[69-71] Since compounds containing *N*-heterocyclic moieties have found numerous applications as pharmaceuticals as well as in medicines, it is anticipated that incorporation of a pyrazine ring into the molecule of a pentacyclic triterpenoid may induce or enhance its biological activity. With this view in mind and in continuation of our studies on the transformative reactions of triterpenoids, we report herein the incorporation of pyrazine ring into ring A of the pentacyclic triterpenoids of lupane and friedelan skeleton (Schemes 1 and 2). Additionally, to date no parallel study have been reported that utilizes a green protocol for the synthesis of pyrazine derivatives of triterpenoids, a fact that motivated us to develop a green protocol for the synthesis of pyrazine derivatives from betulinic acid and friedelin. In order to make a series of pyrazine derivatives of triterpenoids and to evaluate their biological activities the present author has developed a novel green protocol for the same. The results of theses investigations have been reported in the following sections.

2. Present Investigation

The present protocol comprises a direct condensation between the respective 1,2-diketo compounds with 1,2-diamines in aqueous methanol catalyzed by potassium tertbutoxide (t-BuOK) at room temperature. This high yielding process did not require any added expensive catalyst or bubbling of oxygen[57] at higher temperature (Scheme 3). Detection of dihydropyrazine along with pyrazines as well as the starting material at an early stage of the reaction indicated that the developed method involved aromatization following a very simple one pot route via the formation of non aromatic dihydropyrazine intermediate, removing any additional steps as reported in literature.

In order to develop the above convenient protocol we first chose the reaction of benzil, a common 1,2-diketone, with ethylenediamine (1:1) as the model case followed by optimisation of the reaction condition (Table 1).



Scheme 24 ^{t}BuOK catalyzed synthesis of pyrazine derivatives in wet methanol

For the one pot synthesis of pyrazine derivatives we studied a good number of bases in various solvents including mixture. On this basis the combination of aqueous methanol-t-BuOK was found to be the best (Table 1) for the said transformation.

Table 1 Optimization of pyrazine synthesis reaction condition

Entry	Solvent	Base	Temperature	Time(hr.)	% Yield	
					%Dihydropyrazine	%Pyrazine
1	Water		RT	18	30	-
2	Water	Et_3N	"	18	30	-
3	Methanol	BuO^tK	"	8	45	76
4	Acetonitrile	"	"	18	65	-
5	DCM	K_2CO_3	"	18	20	-
6	DMSO	"	"	18	45	-
7	DMF	"	"	48	65	-
8	Methanol	"	"	8	24	52
9	Methanol	NaOAc	"	18	78	-
10	Isopropanol	Et_3N	"	18	56	-

% Yield refers to the isolated yield of all the compounds after chromatographic seperation

But in order to establish a greener and cost effective protocol, we have optimized further both the volume of methanol as well as the molar proportion of base (in case of pyrazine preparation) in a reaction of 1:1 feed ratio of benzil and ethylene diamine. After a number of experiments we could able to minimise the proportion of base as well as the volume of solvent in the feed. From figure-1 it is clear that although there is a gradual increase in the % yield of the product but, after a certain voloume (only 3 mL methanol with respect to 1:1 millimolar ratio of diketone and diamine) there is no significant increase in % yield of the product. This observation clearly indicated that the reaction sequence depends upon the amount of the solvent in the reaction mixture taken during the course of the reaction at a given time for a single set of reaction.

Study towards the optimization of the amount of added base did reveal that only a catalytic amount of the t-BuOK in aqueous methanol was sufficient to carry out the reaction efficiently at room temperature within 6 hours in good yield. The present protocol utilized stoichimetric amount (1:1) of reactants, the reagent and catalytic amount of t-BuOK. It is also energetically efficient and did not produce any byproduct and hence it is indeed a green protocol. The generality of the developed process was justified by its successful application on a number of 1,2-diketones effectively[72]

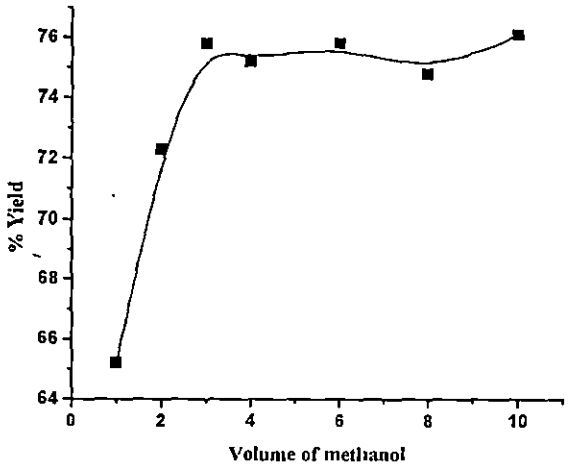
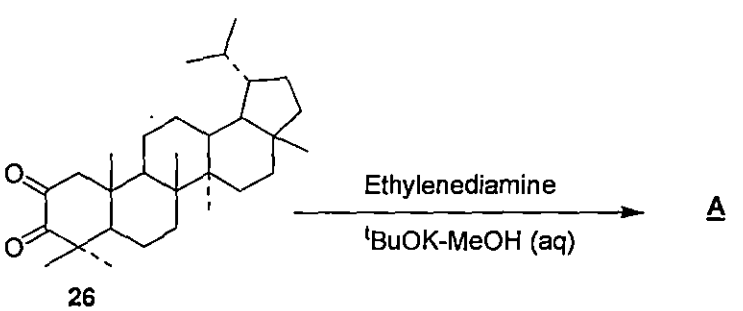


Figure 5 Variation of molar proportion of base in a 1:1 feed ratio of benzil and ethylenediamine

This general optimized protocol was then utilized to prepare some pyrazine derivatives of naturally occurring triterpenoids in the following way.

2.1 Preparation of pyrazine derivative of 2,3-diketo lupane (27)

2,3-diketo lupane (**26**) prepared by the autoxidation[73] of lupanone (see experimental), on treatment with ethylenediamine, EDA in aqueous methanol catalysed by t-BuOK afforded a single compound. Purification of the compound over a column of silica gel followed by crystallization from chloroform-petroleum ether furnished fine needle shaped crystals of compound A, analysed for $C_{32}H_{50}N_2$, mp 220-21°C, $[\alpha]_D$ + 24°.



Scheme 25 Treatment of 2,3-diketolupane with EDA in presence of ^{t}BuOK in methanol

IR spectrum of the compound (Figure 6) showed peaks at 1650, 1120 cm^{-1}. It did not respond to the TNM (tetranitro methane) test for unsaturation. Its UV spectrum showed

peaks at 272 nm (ε = 5700) and 278 nm (ε = 5600). In the mass spectrum (Figure 9) of compound **A** it showed a molecular ion peak at *m/z* 462 (M+1). In elemental analysis compound **A** showed 83.10% C, 10.81% H. Thus from elemental analysis and mass spectrum, the molecular formula of compound **A** was established to, $C_{32}H_{50}N_2$. In ^1H NMR spectrum (Figure 7) out of six tertiary methyl groups, each appeared as a sharp singlet at δ_H 0.78 (s, 3H), 0.83 (s, 3H), 0.98 (s, 3H), 1.11 (s, 3H), 1.29 (s, 3H) and 1.31 (s, 3H). Two secondary methyl groups each appeared as a doublet at δ_H 0.77 (d, 3H, J = 7 Hz) and 0.86 (d, 3H, J = 7 Hz). Two doublets at δ_H 2.45 and 3.04 with geminal coupling of 16 Hz could be assigned to the methylene protons at C-1 that has no neighboring protons. Two olefinic protons that appeared at δ_H 8.41 and 8.27 as doublet with J = 3 Hz. The former being splitted by long range 1,4 coupling with C-1 proton appearing at δ_H 2.45. The large downfield shift of these protons indicates that these are the aromatic protons deshielded by the hetero atom nitrogen.[74]

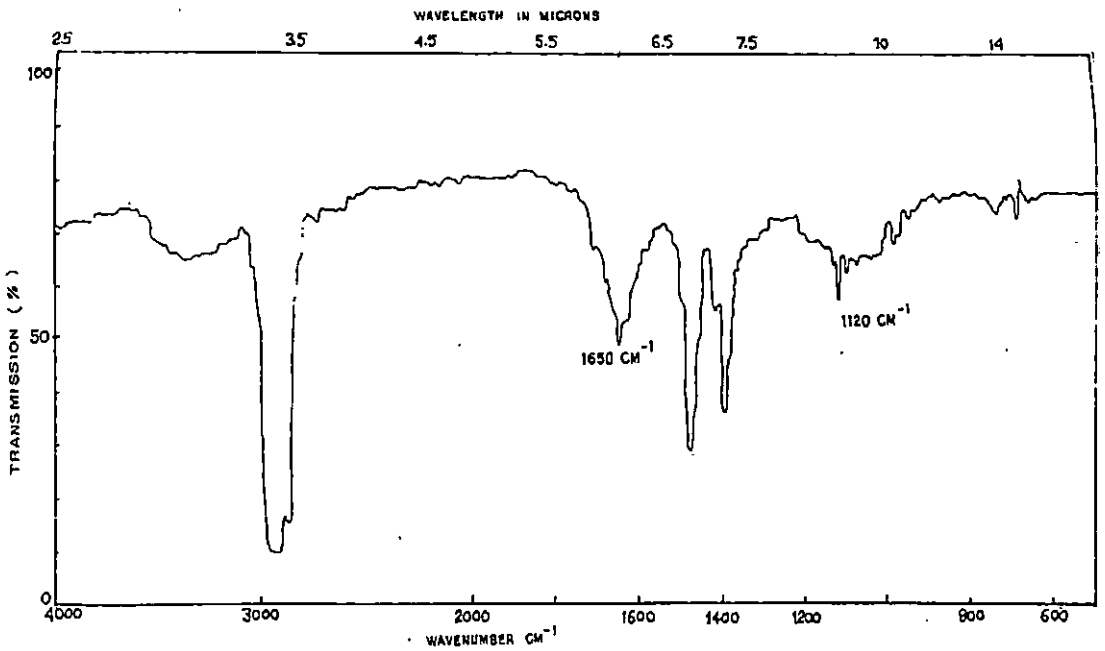


Figure 6 IR spectrum of pyrazine derivative of lupane

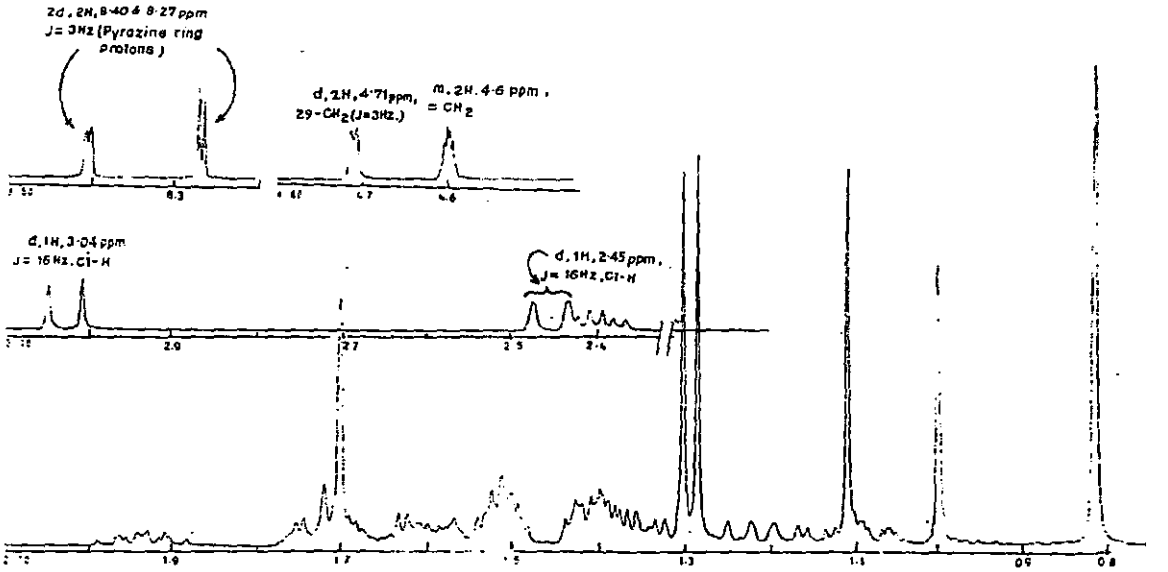


Figure 7 ^{1}H NMR spectrum of pyrazine derivative of lupane (**27**)

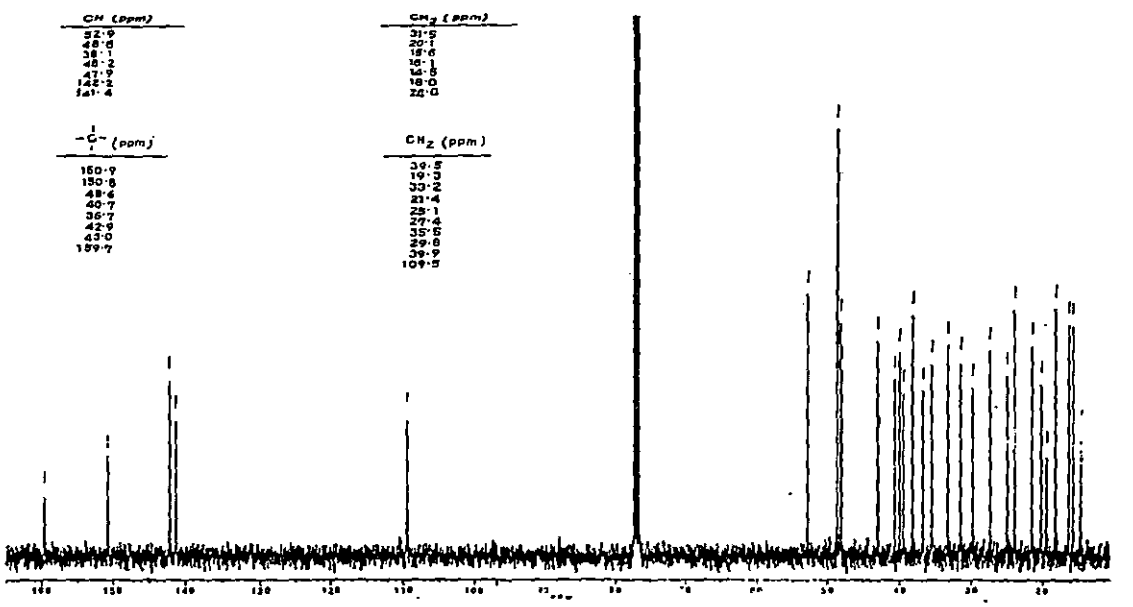


Figure 8 ^{13}C NMR spectrum of pyrazine derivative of lupane (**27**)

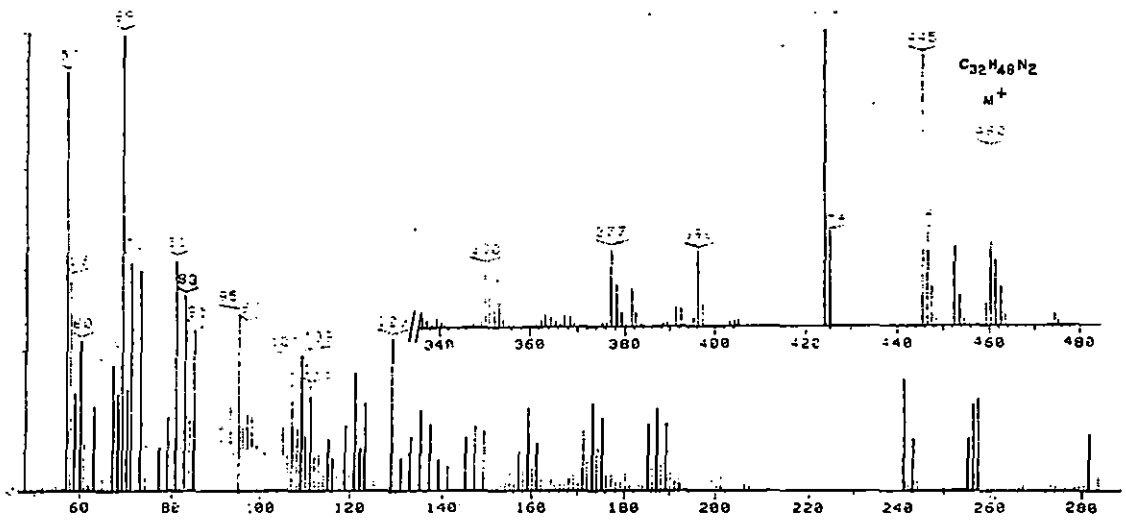
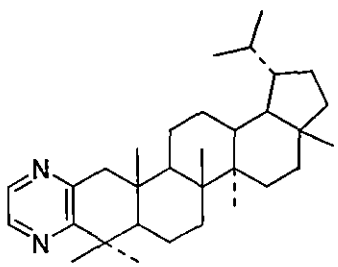


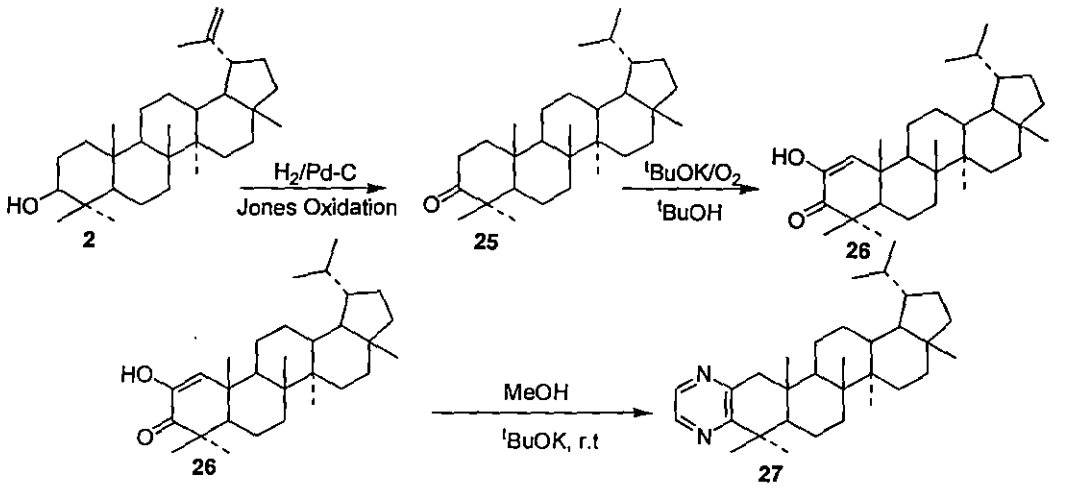
Figure 9 Mass spectrum of pyrazine derivative of lupane (27)

^{13}C NMR spectrum of compound A (Figure 8) accounted for all the 32 carbon atoms. The ^{13}C NMR data of compound A is represented along with those of lupanone in table 1. Thus from spectral analysis the exact structure of compound A has been assigned as structure **27**.



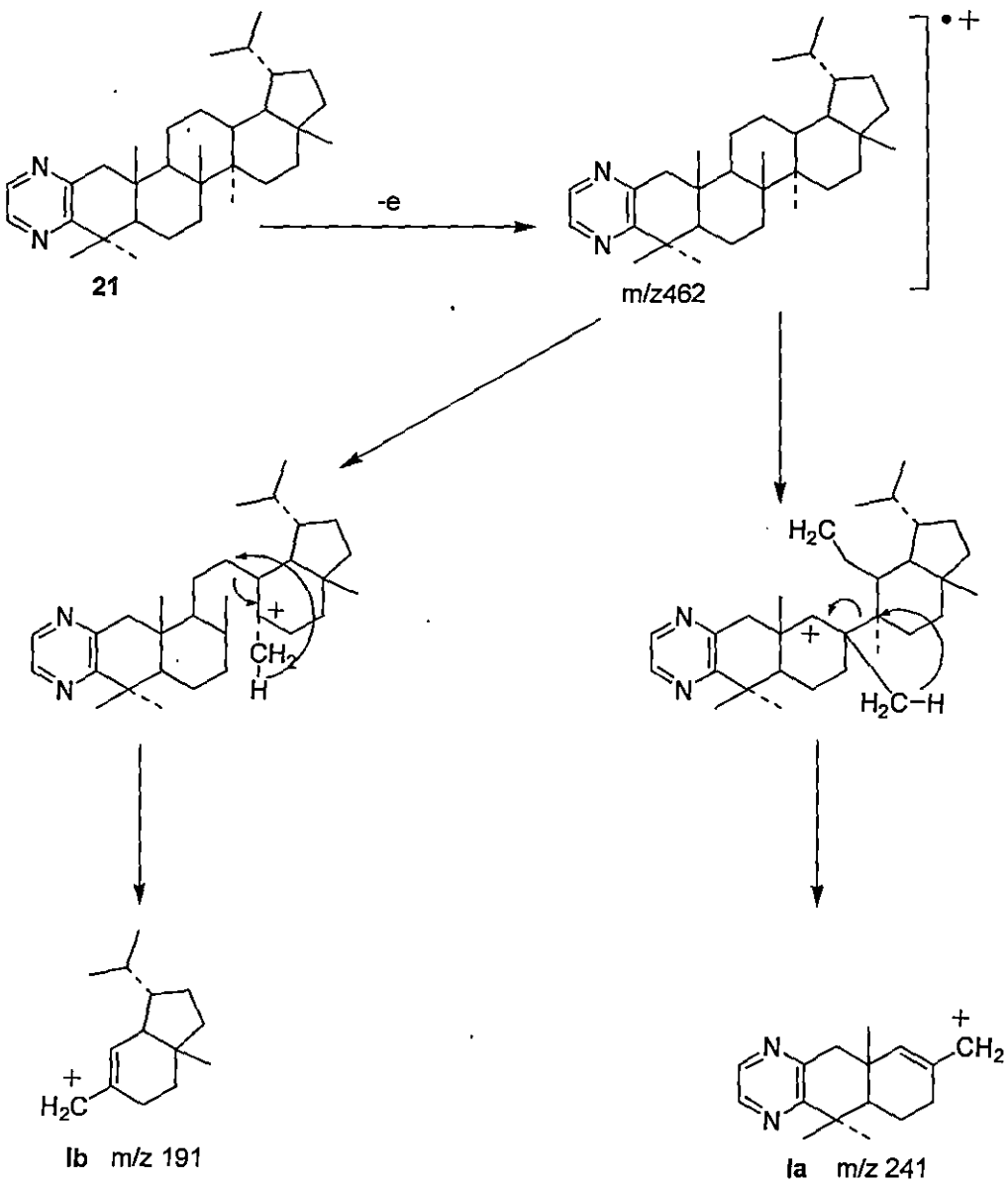
27

The total schematic representation of the synthetic strategy for the preparation of compound **27** is shown below. For a total description of experimental procedures please see the experimental section of this part. The parent compound for the synthesis of compound **27** was isolated from the outer bark of *Xanthoxylum budrunga* through soxhlet apparatus using toluene as the solvent.



Scheme 26 Synthesis of pyrazine derivative of lupane (**27**)

The structure of **27** for compound **A** was further established by a through mass spectrum analysis. Besides molecular ion peak (*m/z* 462), the mass spectrum (Figure 27) of compound **A** showed other peaks at *m/z* 445 (base peak), 377, 256, 241, 191 etc. The peak at *m/z* 445 was due to the elimination of methyl group from the molecular ion. The peak at *m/z* 241 was probably due to the fragment[75] **Ia** and that at m/z 191 was due to the existence of the fragment[75] **Ib** (Scheme 27).

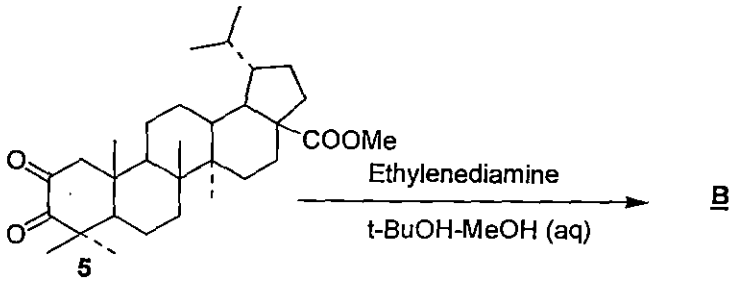


Scheme 27 Probable mass fragmentation pattern of compound **27**

2.2 Preparation of pyrazine derivative of 2,3-diketo methyldihydrobetulonate (6):

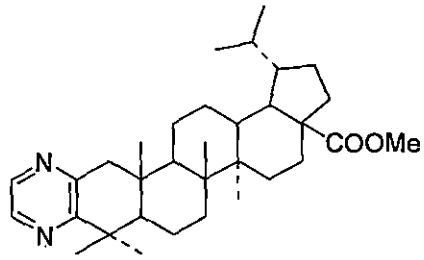
2,3-diketo methyldihydrobetulonate (**6**) prepared by the autoxidation[73] of methyldihydrobetulonate, **5** (see experimental), on treatment with ethylenediamine in aqueous methanol catalyzed by t-BuOK for 6 hours afforded a single compound. Purification of the compound over a column of silica gel followed by crystallization from

chloroform-petroleum ether furnished fine needle shaped crystals of compound **B**, analyzed for $C_{33}H_{50}O_2N_2$, mp 220-21 °C.



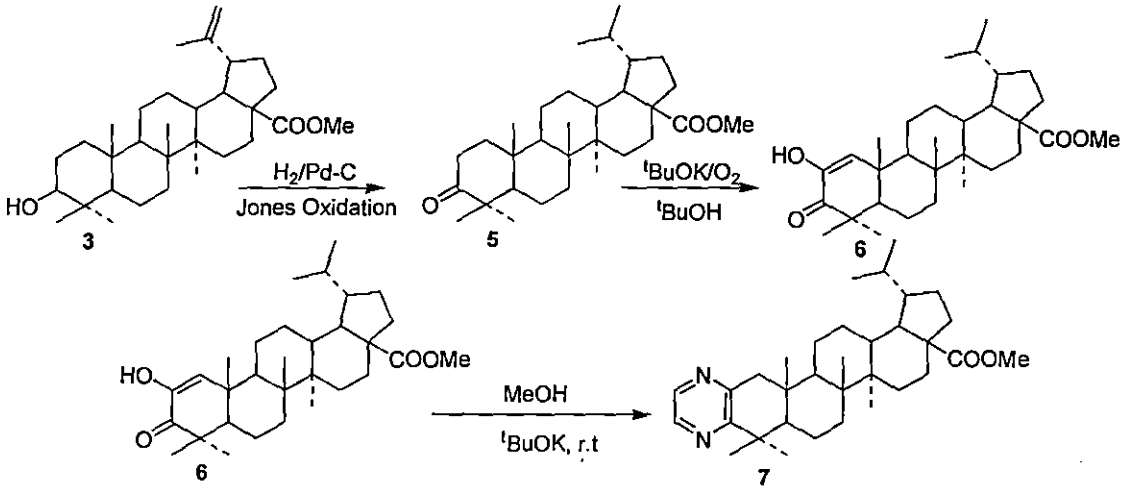
Scheme 28 Treatment of 2,3-diketomethyl dihydrobetulanate with EDA in ^{t}BuOH-MeOH (aq.)

IR spectrum (Figure 12) of compound **B** showed peaks at 1710 cm^{-1}(CO_2Me), 1665, 1430 and 1120 cm^{-1}. UV-visible spectrum (Figure 10) of compound **B** showed peaks at 272 (ε = 5712) and 278 (ε = 5603). It did not respond to the TNM test for active unsaturation. The mass spectrum (Figure 11) showed the molecular ion peak at m/z 506 as base peak. The UV-visible spectrum together with the mass spectrum indicated the incorporation of pyrazine nucleus into the ring A of pentacyclic triterpenoid. In the ^{1}H NMR spectrum (Figure 13) ($CDCl_3$, δ ppm^{-1} relative to TMS) it showed the presence of six tertiary methyl groups at δ_H 0.82 (s, 3H), 0.98 (s, 3H), 0.99 (s, 3H), 1.28 (s, 3H) and 1.305 (s, 3H). Two secondary isopropyl methyl groups appeared at δ_H 0.76 (d, 3H, J = 7 Hz) and at δ_H 0.88 (d, 3H, J = 7 Hz). Ester methyl at C-28 appeared as a sharp singlet at δ_H 3.66 (s, 3H, ester methyl). Two hydrogen atoms at C-1 were deshilded due to the magnetic anisotropy induced by the attached aromatic heterocyclic ring and each appeared as a distinct doublet at δ_H 2.48 (1H, d, J = 16 Hz) and at δ_H 3.04 (1H, d, J = 16 Hz). Two aromatic hydrogen atoms appeared at δ_H 8.27 (1H, d, J = 3 Hz) and at δ_H 8.41 (1H, d, J = 3 Hz). Analytical calculations were 78.26% C, 9.88% H, 5.53% N (calculated) and those found were 78.25% C, 9.73% H, 5.50% N. All the above facts lead to assign structure **7** to compound **C**.



7

Schematic representation for the whole steps of reaction was shown below-



3 5 6 6 7

Scheme 29 ^{t}BuOH-MeOH mediated synthesis of pyrazine derivative of methyl dihydrobetulanate

Finally the structure **7** was corroborated further by the mass fragmentation pattern of compound (Scheme 30).

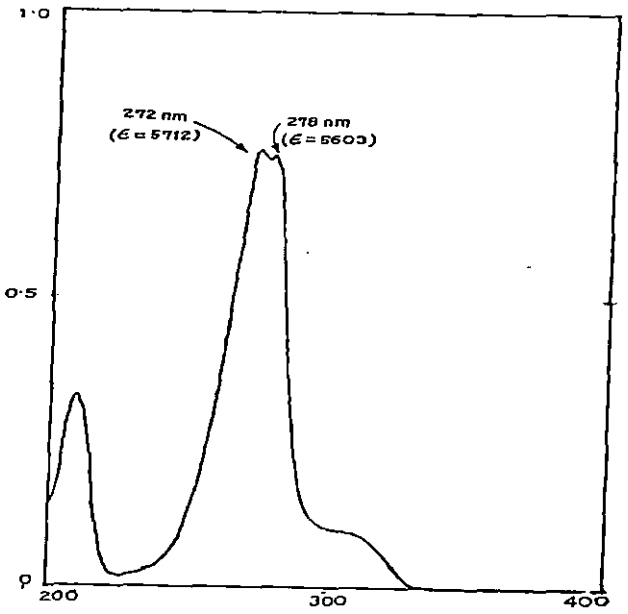


Figure 10 UV spectrum of pyrazine derivative of methyl dihydrobetulonate

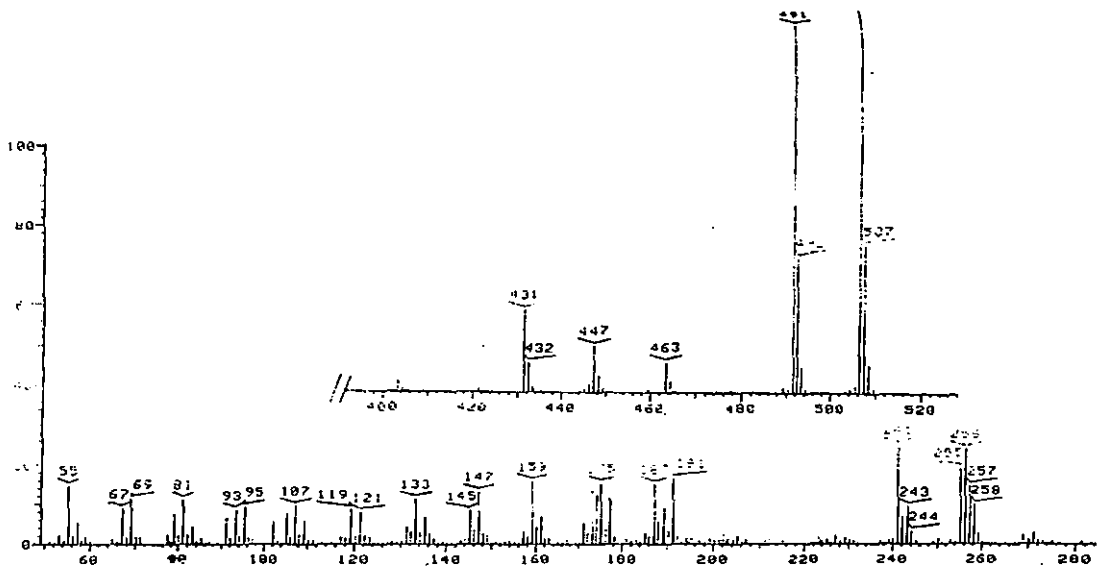


Figure 11 Mass spectrum of the pyrazine derivatives of methyl dihydrobetulanate

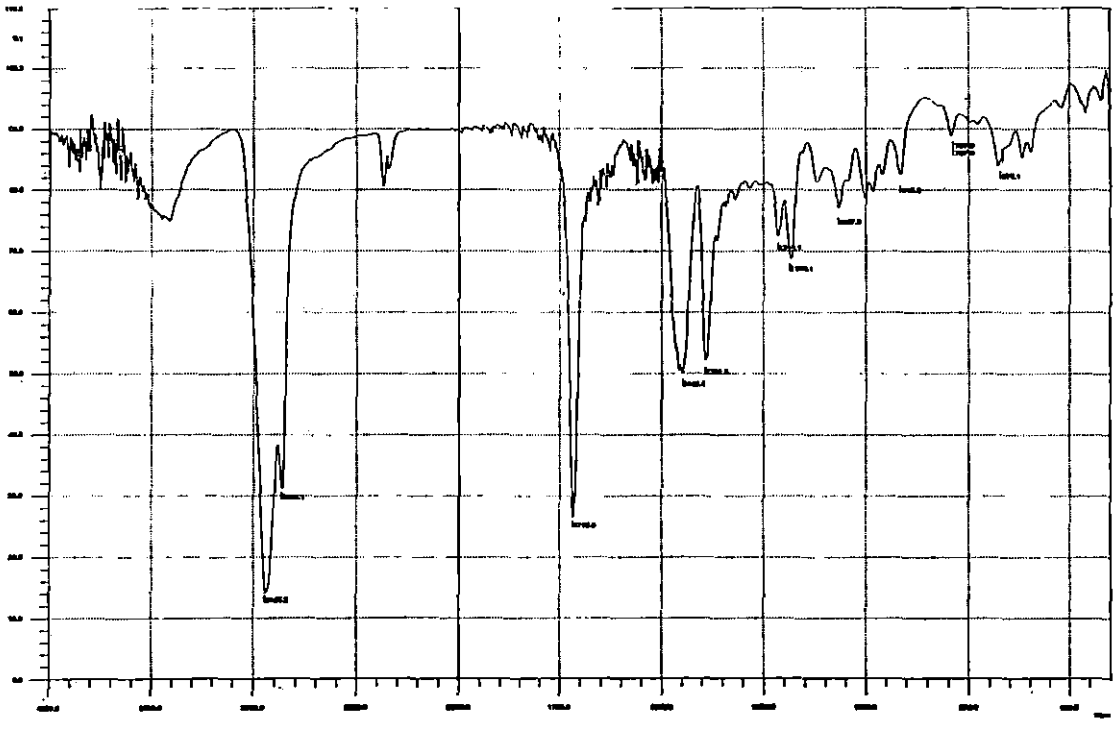


Figure 12 IR spectrum of pyrazine derivative of methyldihydrobetulanate

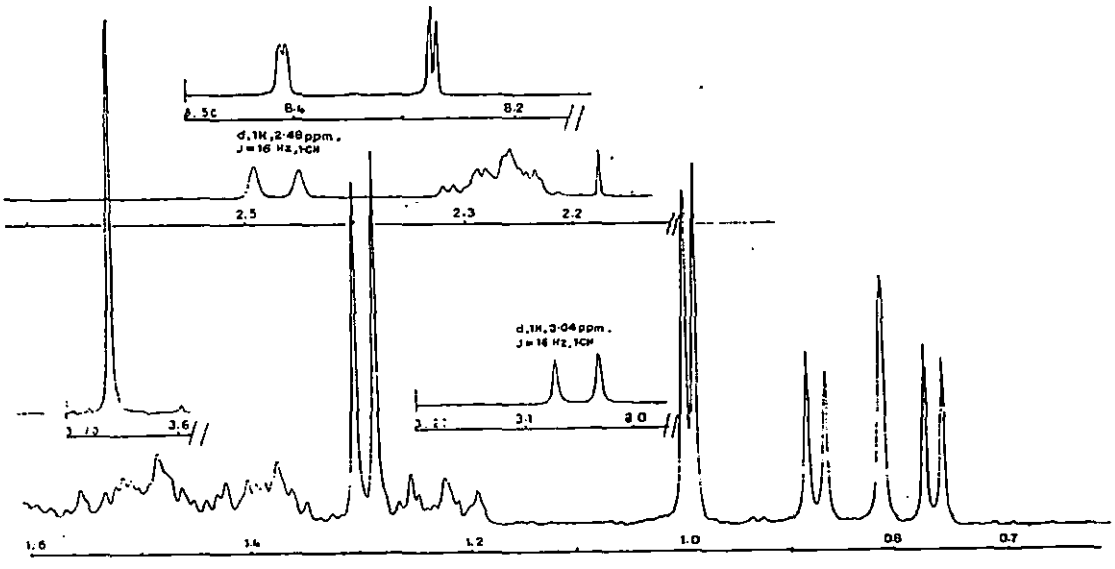
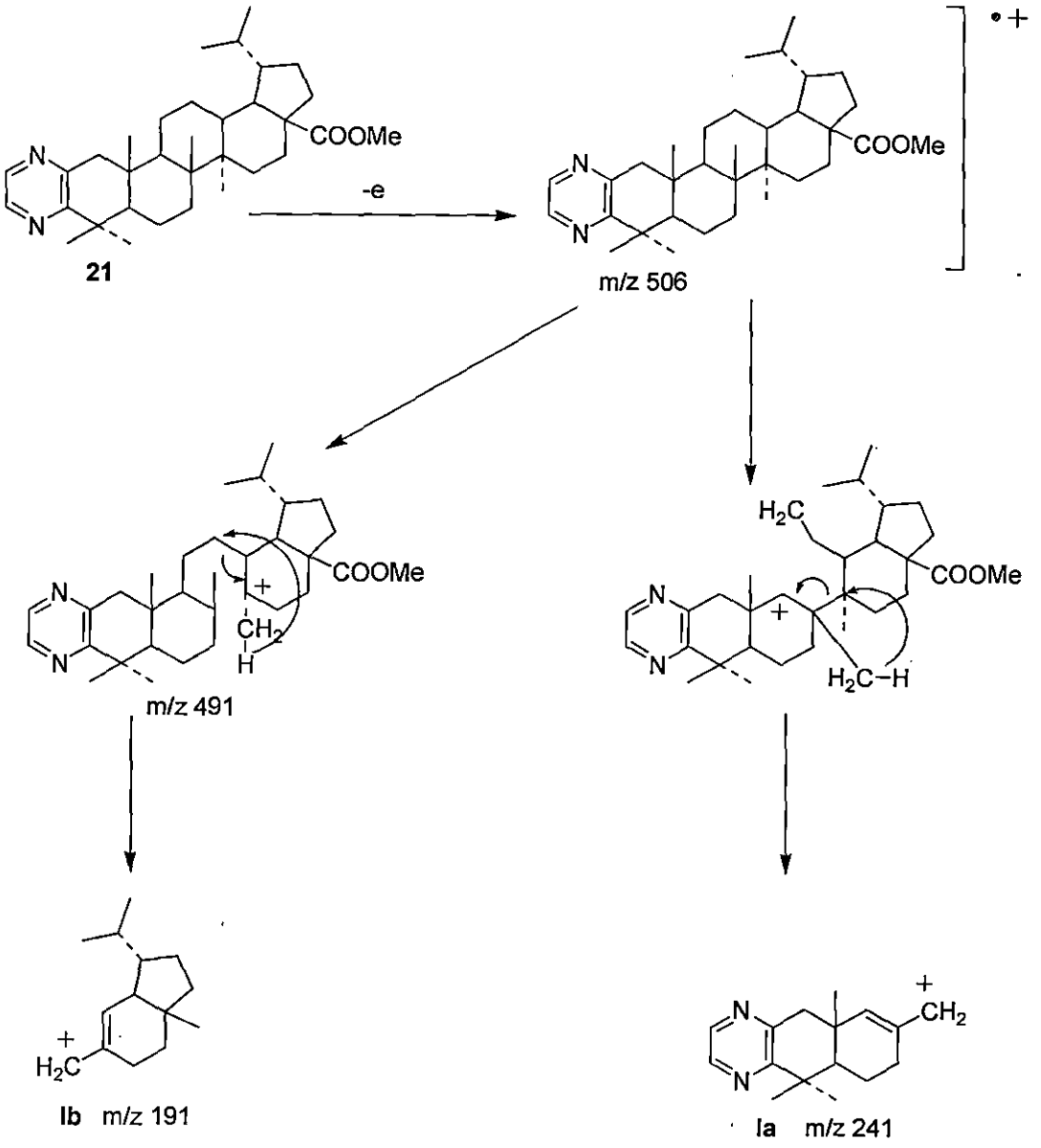
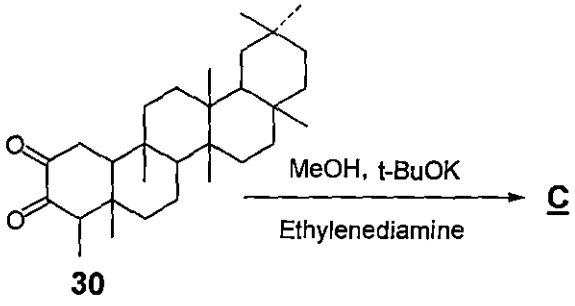


Figure 13 ^{1}H NMR spectrum of the pyrazine derivatives of methyl dihydrobetulanate



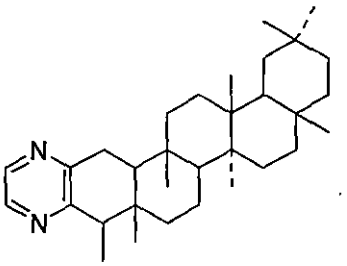
Scheme 30 Mass fragmentation pattern of compound 7

2.3 Preparation of pyrazine derivative of 2,3-diketo friedelin (6):



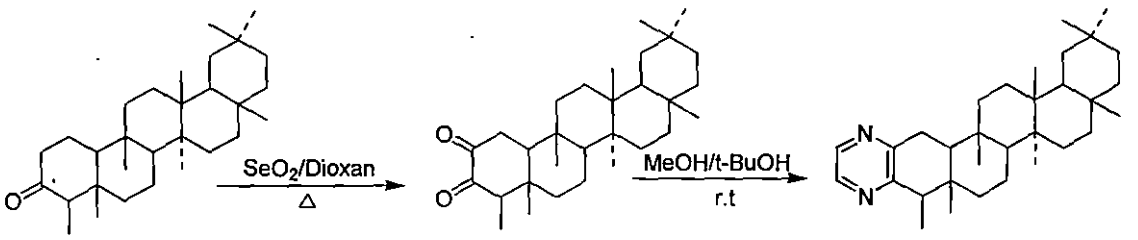
Scheme 31 Treatment of 2,3-diketofriedelan with ^{t}BuOH-MeOH (aq.)

2,3-Diketo friedelan **30** prepared by auto oxidation of friedelin **28** was treated with ethylenediamine, EDA in aqueous methanol catalysed by t-BuOK for 6 hours. 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 compound **C**, analyzed for $C_{32}H_{50}N_2$, mp 228 °C. IR spectrum of the compound showed peaks at 1655, 1430, 1120 cm^{-1} for pyrazine ring. UV spectrum showed peaks at 272 (ε = 5800) and 278 (ε = 5450) nm. Mass spectrum (Figure 17) of **C** showed molecular ion peak at *m/z* 462 as base peak, which is the characteristic feature of pyrazine compounds. The other peaks appeared at *m/z* 447,420, 247, 107 and 71. The ^{1}H NMR spectrum (Figure 14 and 15) of **C** was indicative of the presence of seven tertiary methyls which appeared as sharp singlets (3H each) between δ 0.82–1.22 (7s, 21H, 7t CH_3), the doublet centered at δ 0.99 was due to the presence of secondary methyl protons (d, J = 6.5 Hz), two aromatic protons at δ 8.40 and 8.27 appeared as a doublet with J = 3 Hz. ^{13}C NMR spectrum of the compound **C** showed the presence of 32 carbons, two singlets at δ 150.8 and 150.9 and two doublets at δ 141.4 and 142.3 were due to heterocyclic ring carbons typical to 2,3–disubstituted pyrazine skeleton. All the above facts led to assign structure **32** to compound **C**.



32

The formulation of structure **32** for compound **A** is further supported by the ^{13}C NMR (Figure 16) spectrum of the compound.



28 30 32

Scheme 32 ^{t}BuOH-MeOH mediated synthesis of pyrazine derivative of friedelin

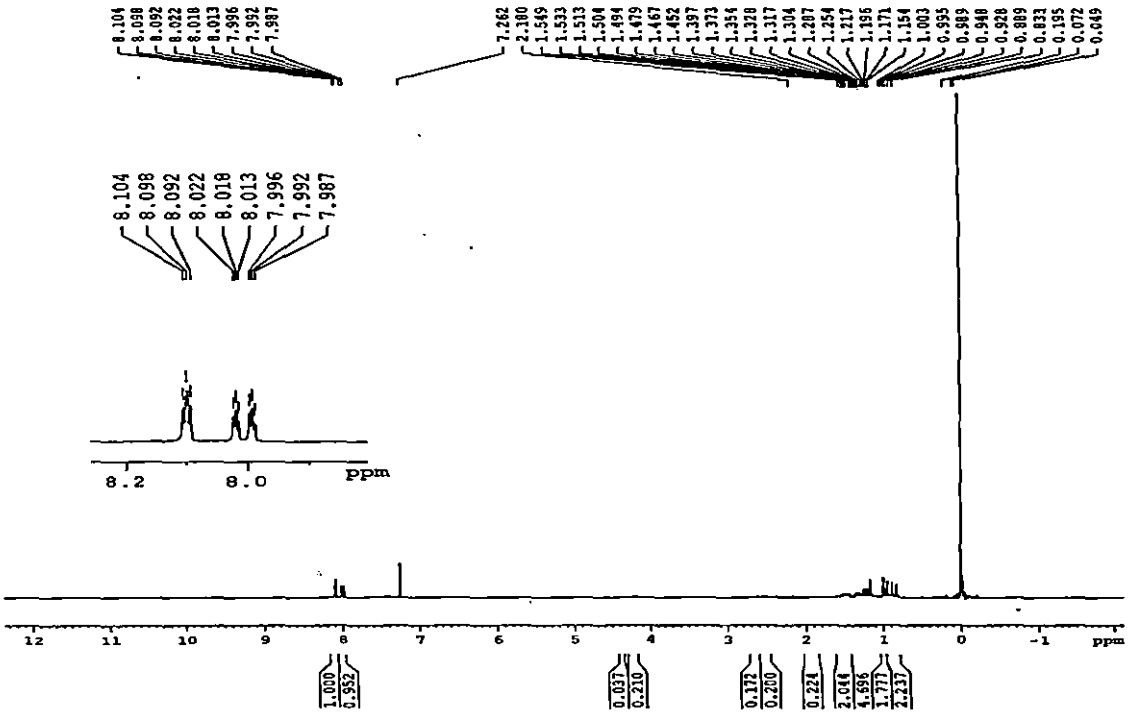


Figure 14 ^{1}H NMR spectrum of pyrazine derivative of friedelin

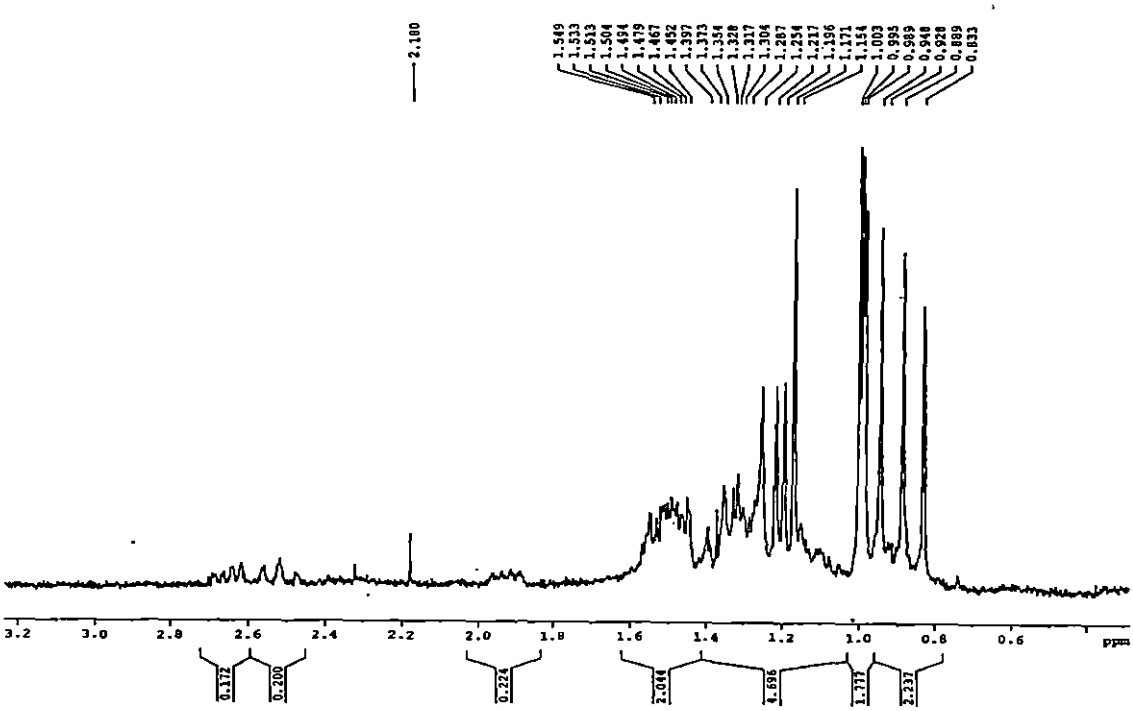


Figure 15 Expanded ^{1}H NMR spectrum of pyrazine derivative of friedelin

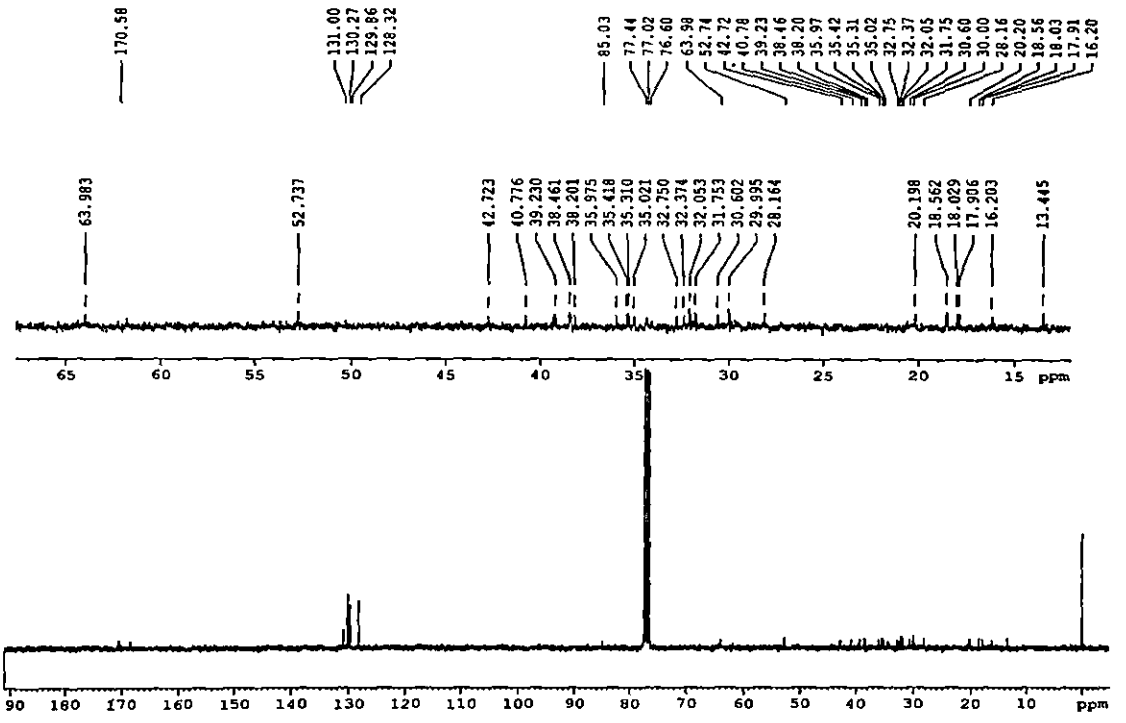


Figure 16 ^{13}C NMR spectrum of pyrazine derivative of friedelin

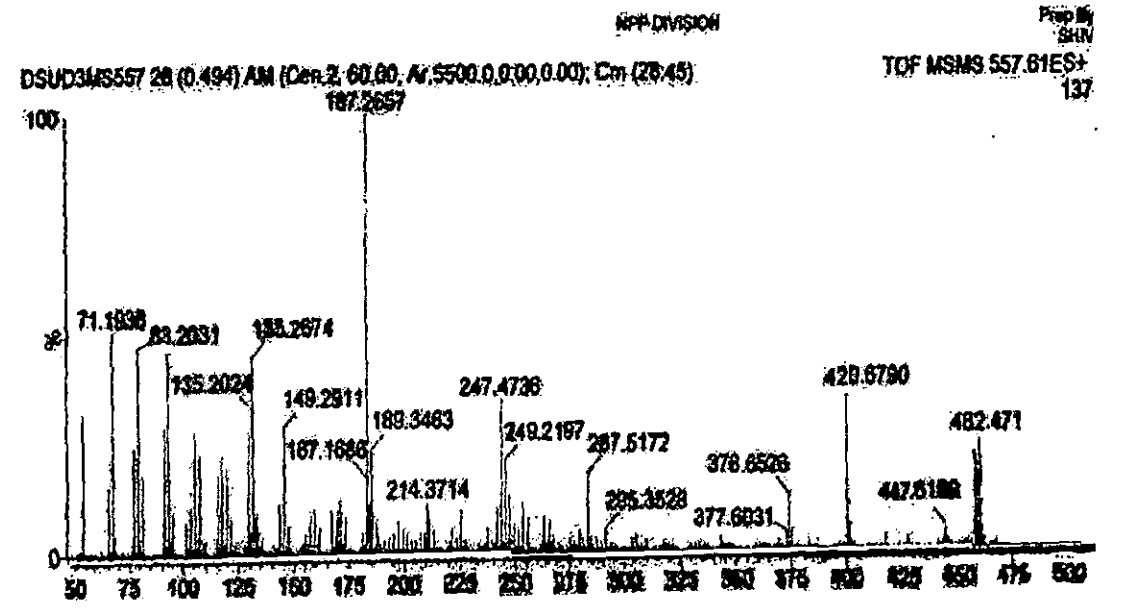


Figure 17 Mass spectrum of pyrazine derivative of friedelin