From the bioassay results, Fraction III and Fraction X revealed weak inhibition against the fungi <u>Pythium</u> <u>ultimum and Rhizoctonia solani</u> with % T/C 20% and 11%, respectively as well as the weak antigrowth against the bacteria <u>Xanthramonas</u> <u>campestrous</u> (% T/C 36). These two fractions also exhibited the feeding inhibition activity against the insect, boll weevil with % inhibition 46% at dose level 30 mg.

CHAPTER IV

CONCLUSION

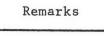
In this course of research, the leaves and the heartwoods of Rhizophora apiculata Bl., are selected for investigation their chemical constituents and biologically active substances according to the attractive preliminary bioassay results. Fifteen compounds are isolated from the leaves and thirteen substances are elucidated as a mixture of saturated long chain aliphatic hydrocarbons (C27- C_{33}), β -amyrinpalmitate, a mixture of β -amyrinpalmitate and waxes, β-amyrenonylpalmitate, a mixture of saturated long chain aliphatic primary alcohols $(C_{30}-C_{36})$, β -amyrin, lupeol, taraxerol, a mixture of β -sitosterol, stigmasterol and campesterol, taraxeryl-cis-phydroxycinnamate, careaborin, wallichenol and β -sitostery1-3-0- β -D-Nine additional compounds, besides a mixture of glucopyranoside. steroids and β -sitosterylglycoside, are separated from the heartwoods and seven of them are identified as a mixture of steroidal ester and waxes, a mixture of saturated long chain aliphatic primary alcohols $(C_{28}-C_{32})$, a mixture of eleven saturated long chain aliphatic carboxylic acids, 2,6-dimethoxy-p-benzoquinone, syringaldehyde, a mixture of saturated long chain hydroxy esters and a mixture of saturated long chain aliphatic primary amides. To our knowledge, there is no report of taraxeryl-cis-p-hydroxycinnamate in the chemical literatures. Hence, this compound is a novel naturally occuring ester of taraxerol. Moreover, it is also found that this

is the first report on the occurrence of other compounds except taraxerol, β -amyrin, β -sitosterol and triacontanol in this particular species. Chloride salts of sodium, potassium, magnesium, calcium, manganese, copper and iron, together with condensed and hydrolysable tannins, arabinose, several amino acids and various phenolic compounds are also found in this plant. All isolated substances from both parts of <u>R</u>. <u>apiculata</u> are summarized as shown in Table 4.1 and their structures of some compounds are given in Fig. 193.

Plant part	Solvent extract	Substances
leaves	P.E.	β -amyrin, β -amyrone, taraxerol, β -sitosterol, triacontanol
leaves	CH2C12	a mixture of long chain hydrocarbon $(C_{27}-C_{33})$, β -amyrinpalmitate, a mixture of β -amyrinpalmitate and waxes, β -amyrenonylpalmitate, a mixture of long chain alcohol $(C_{30}-C_{36})$, β -amyrin, lupeol, taraxerol, a mixture of β -sitosterol, stigmasterol and campesterol, taraxeryl-cis-p- hydroxycinnamate, careaborin, wallichenol, β -sitosteryl-3-0- β -D glucopyranoside
	CHC13	β-sitostery1-3-0-β-D-glucopyranoside
	EtOAc	phenolic compounds
	n-BuOH	flavone compounds
	H ₂ O	chloride salts of Na, K, Mg, Mn, Ca, Fe and Cu arabinose several amino acids
	insoluble parts (Fraction III)	condensed and hydrolysable tannins
heartwoods	CH2C12	a mixture of steroidal ester and waxes, a mixture of long chain alcohol $(C_{28}-C_{32})$, a mixture of long chain carboxylic acids, a mixture of β -sitosterol, stigmasterol and campesterol, 2,6-dimethoxy-p-benzoquinone, syringaldehyde, a mixture of long chain hydroxy ester, a mixture of long chain amide, β -sitosteryl-3-0- β -D-glucopyranoside
	CHC13	B-sitostery1-3-0-B-D-glucopyranoside
	EtOAc	phenolic compounds
	н ₂ о	chloride salts of Na, K, Mg, Mn, Ca, Fe and Cu arabinose several amino acids
	insoluble parts (Fraction X)	condensed and hydrolysable tannins

Table 4.1 All isolated compounds from the leaves and the heartwoods of <u>R</u>. apiculata Bl.

-



former work

present work

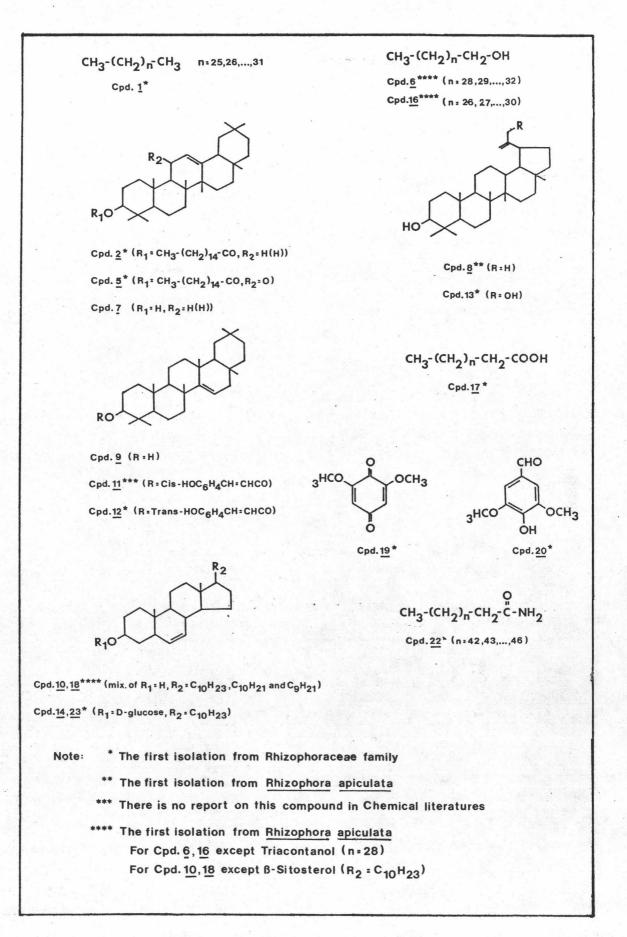


Figure 193 All isolated substances from the leaves and the

heartwoods of Rhizophora apiculata Bl.

The mixture of saturated long chain aliphatic hydrocarbons is highly active as an antifungal and an insect antifeedant agent. The 2,6-dimethoxy-p-benzoquinone is moderately active not only fungi, bacteria, but also the insect, boll weevil. Wallichenol, taraxerol, *B*-amyrenonylpalmitate and syringaldehyde are highly active as antifungals. β -amyrinpalmitate, taraxerol, a mixture of B-sitosterol, stigmasterol and campesterol, a mixture of steroidal ester and waxes and a mixture of saturated long chain aliphatic carboxylic acids are highly active as boll weevil antifeedants. Moreover, the chemical literaure studies incidate that lupeol, 2,6dimethoxy-p-benzoquinone, syringaldehyde and β -sitostery1-3-0- β -Dglucopyranoside are the biologically active ingredients in pharmaceutical aspects. However, these active compounds need confirming their biological activity prior to use in the real conditions.

It is seen from this research work that the leaves of <u>Rhizophora apiculata</u> can be used as a good source of triterpenoids. Moreover, several active compounds are also obtained from this plant. Therefore, the study on chemical constituents and biological activities of mangrove plants is one of the most worth considering for natural product chemists.

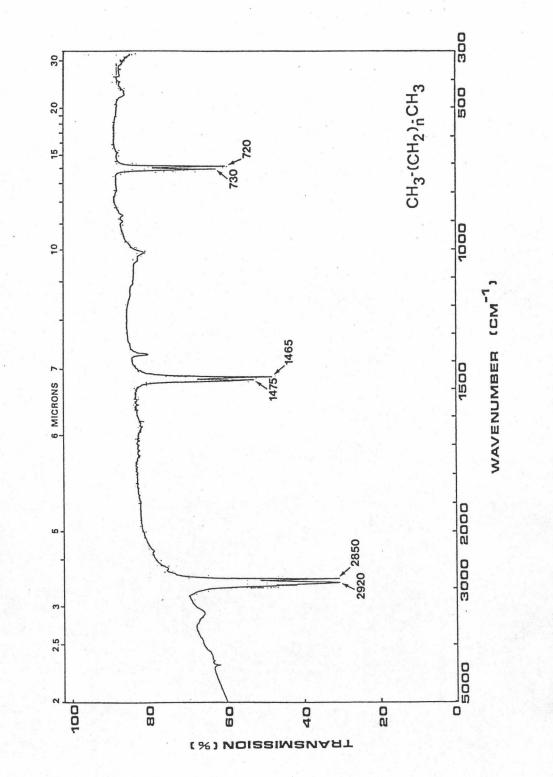
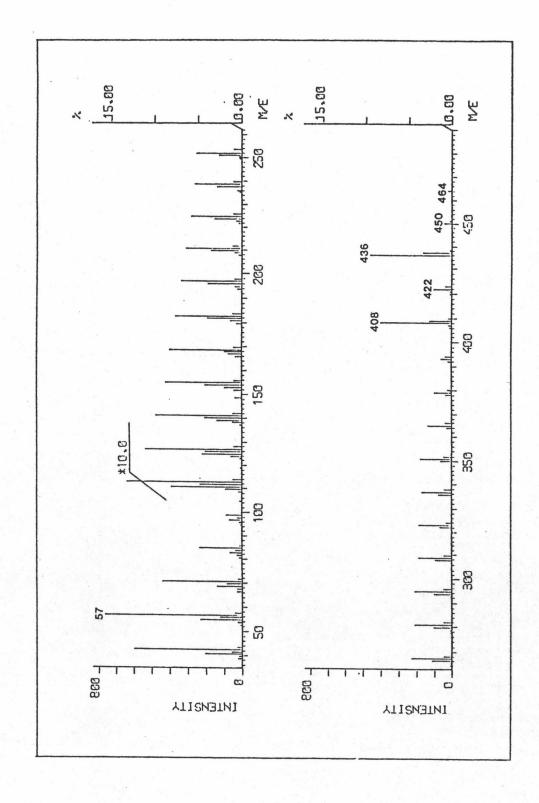


Figure 7 The IR spectrum of Compound 1





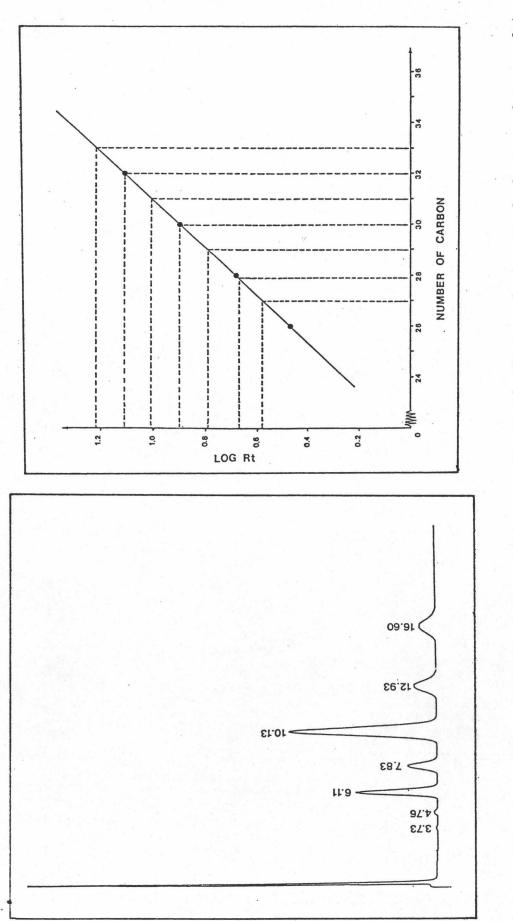
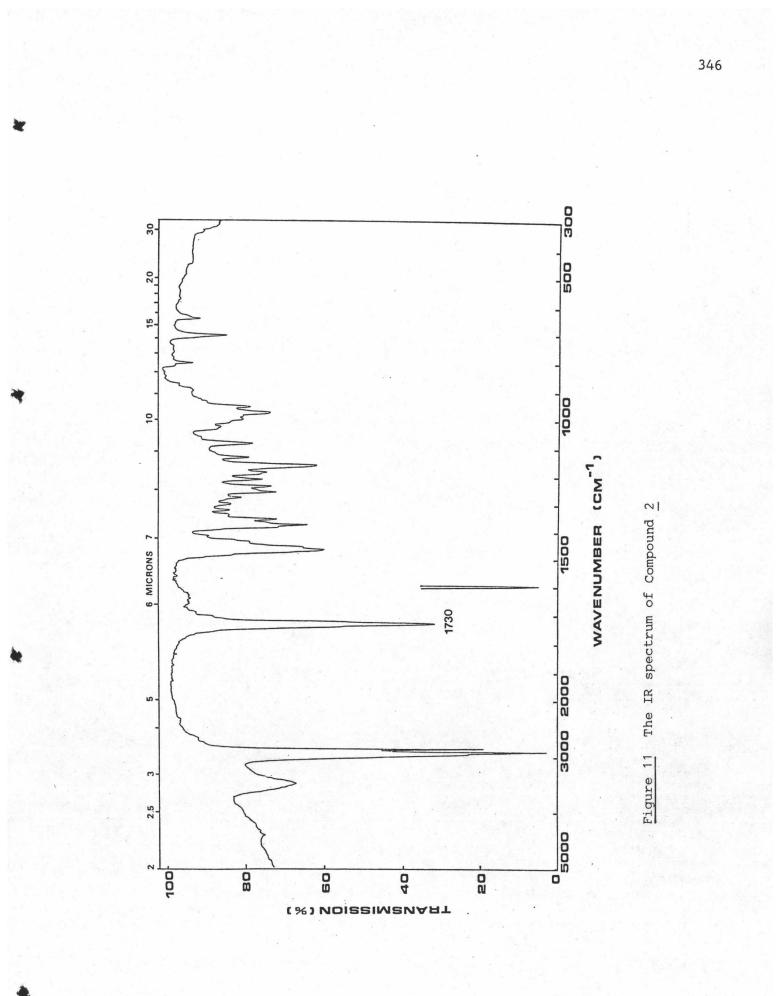


Figure 10 The correlation standard curve of Compound 1

Figure 9 The GLC analysis result of Compound 1



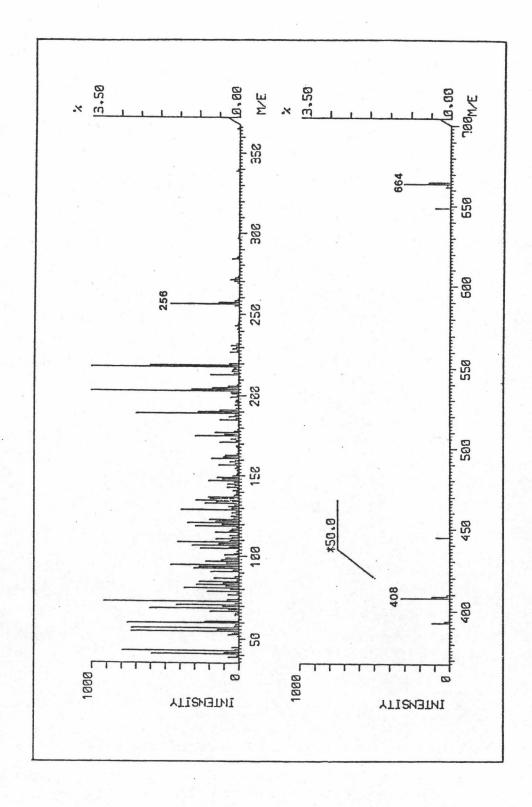
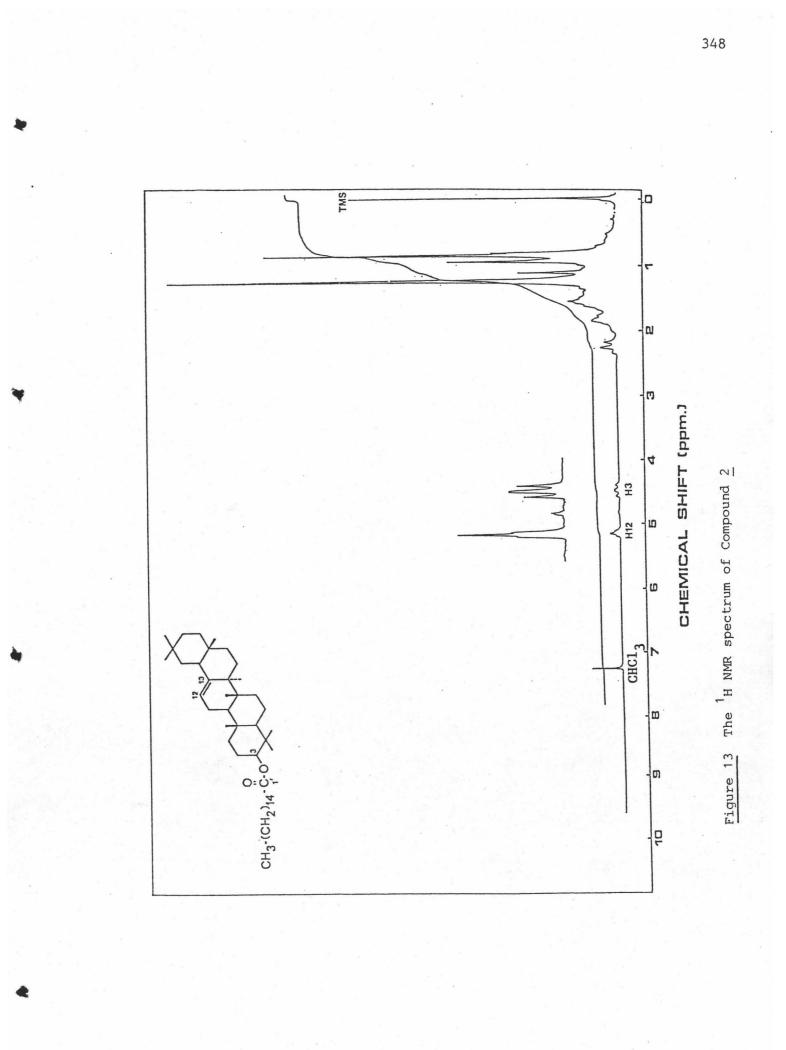
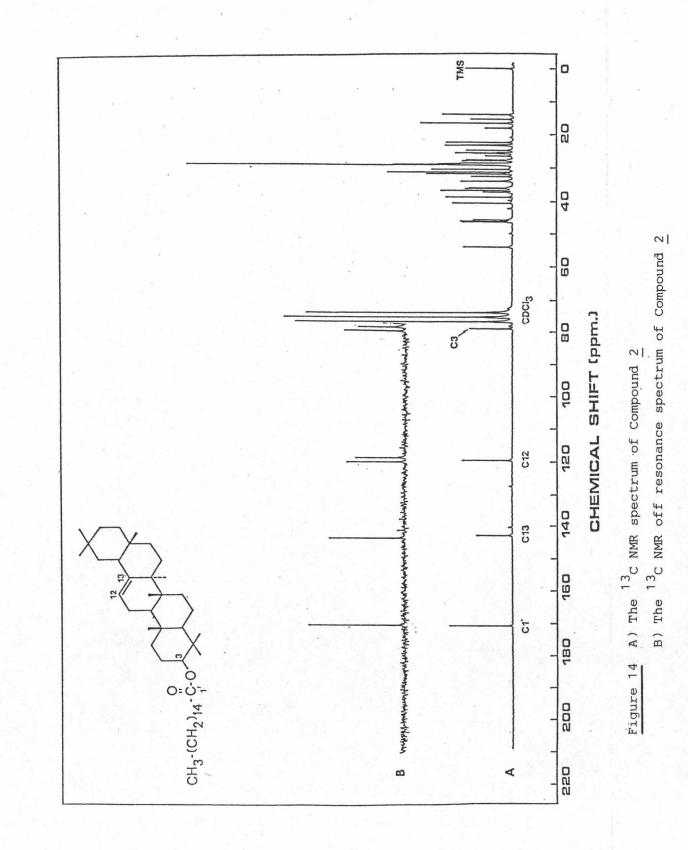


Figure 12 The mass pectrum of Compound 2





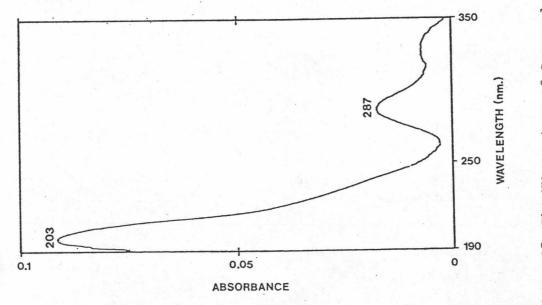
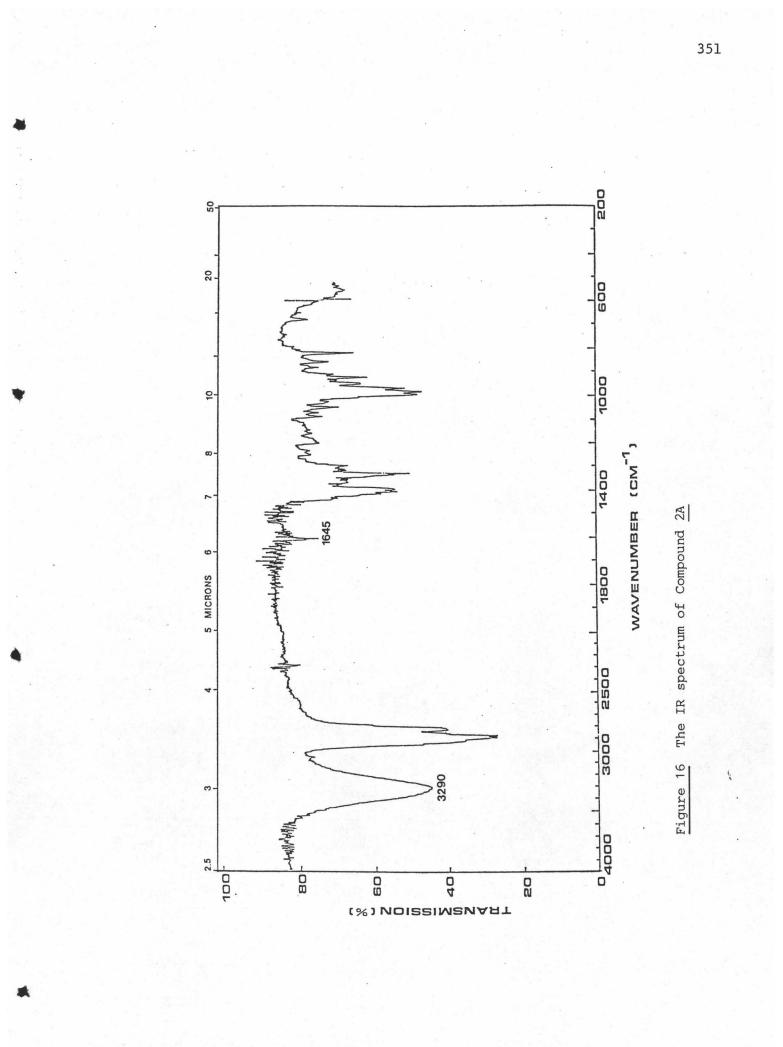
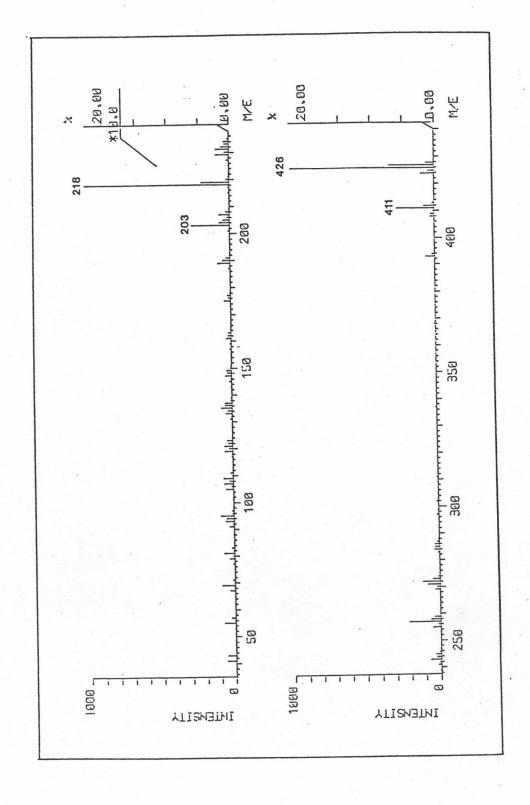
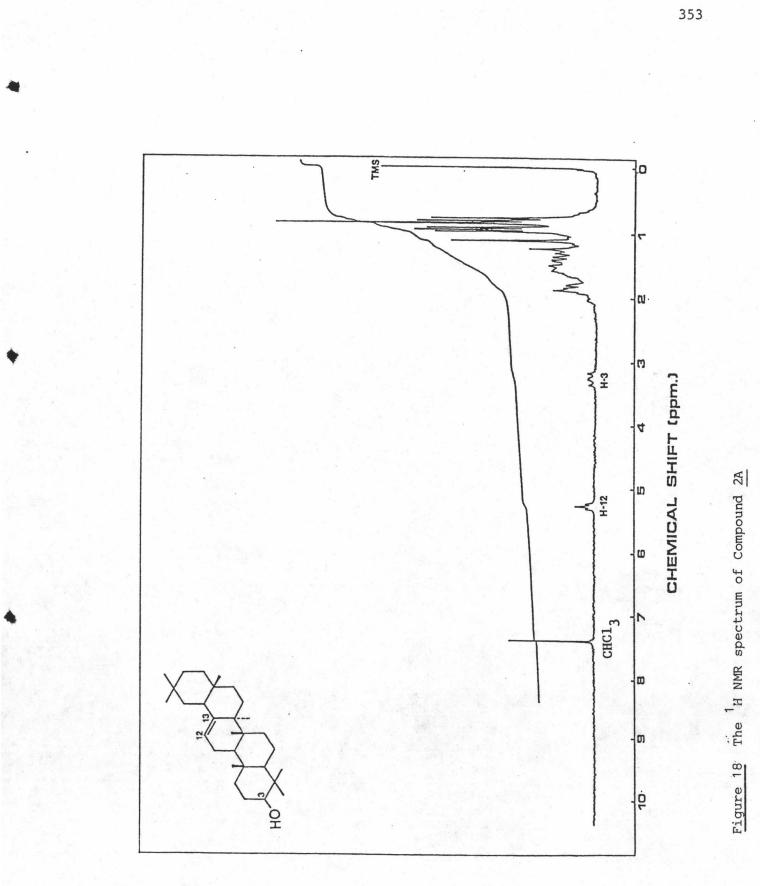


Figure 15 The UV spectrum of Compound 2









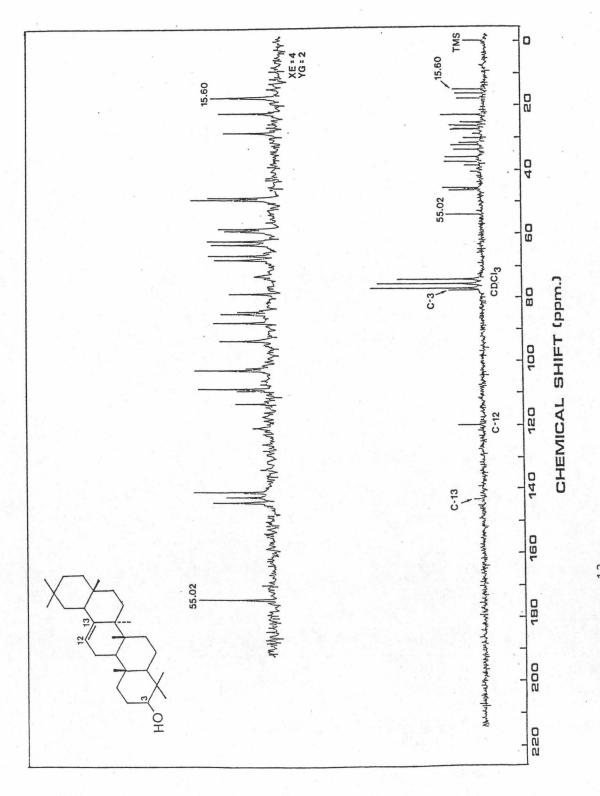
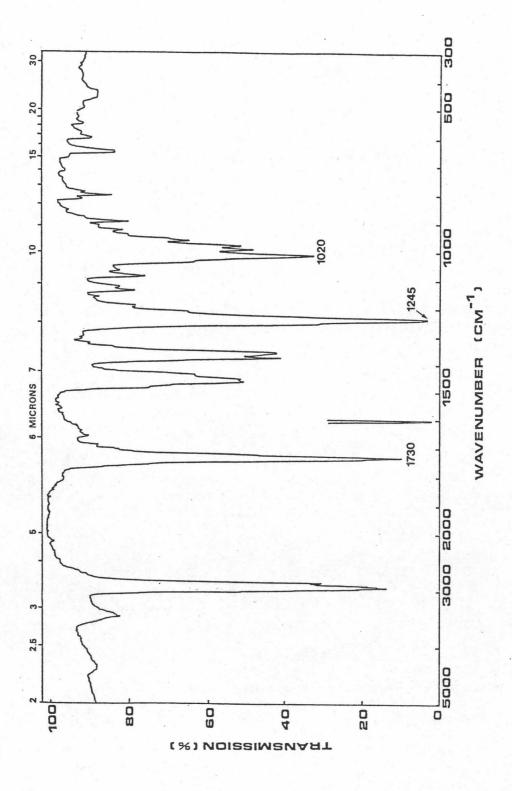
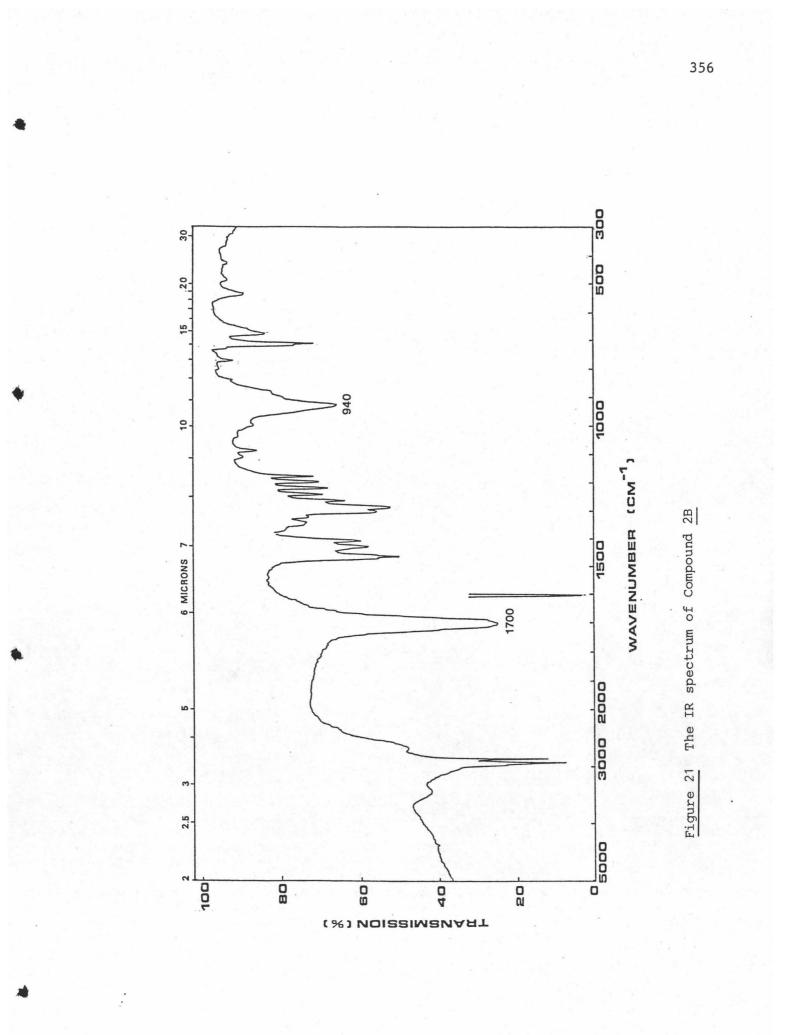
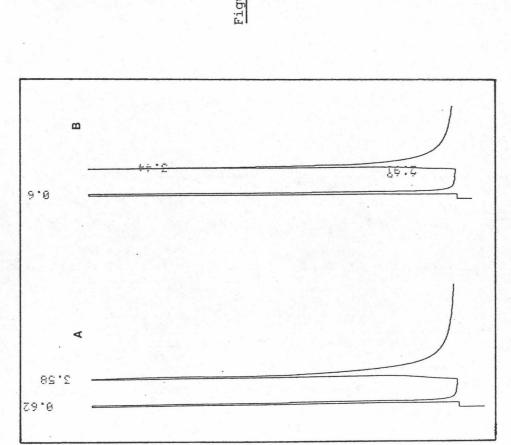


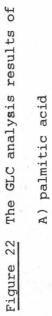
Figure 19 The 13 C NMR spectrum of Compound $2\overline{A}$



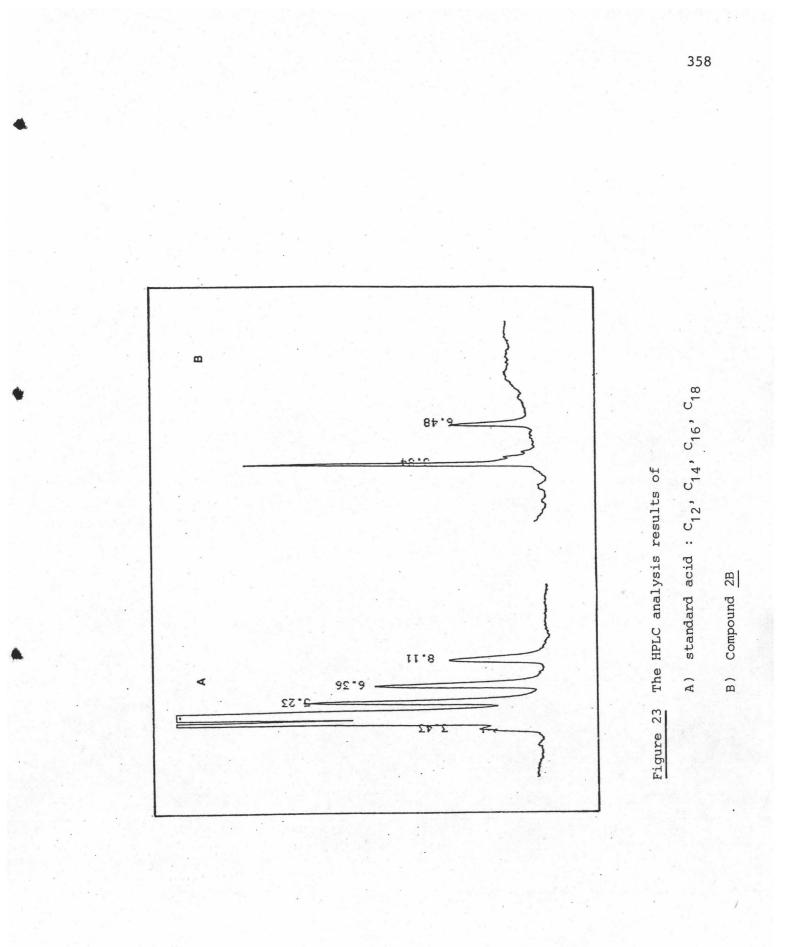








B) Compound 2B



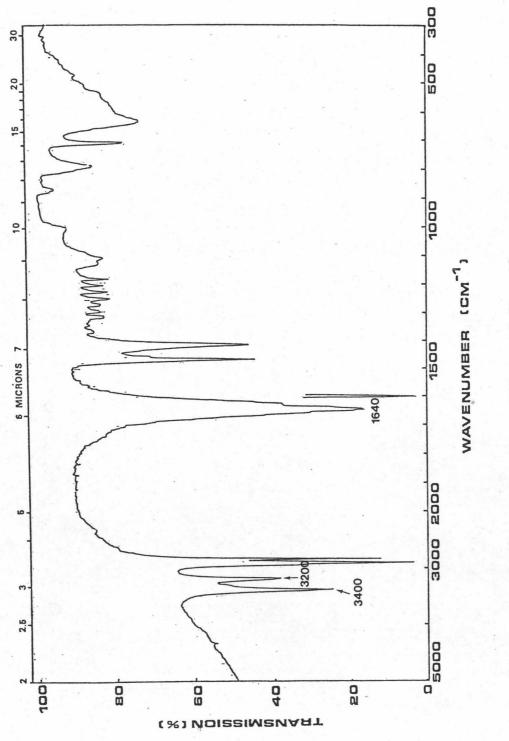
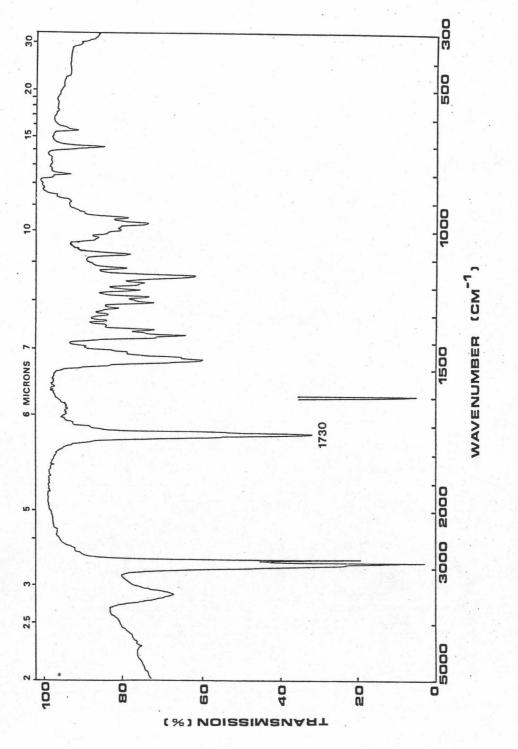
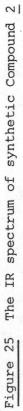


Figure 24 The IR spectrum of Compound 2B amide





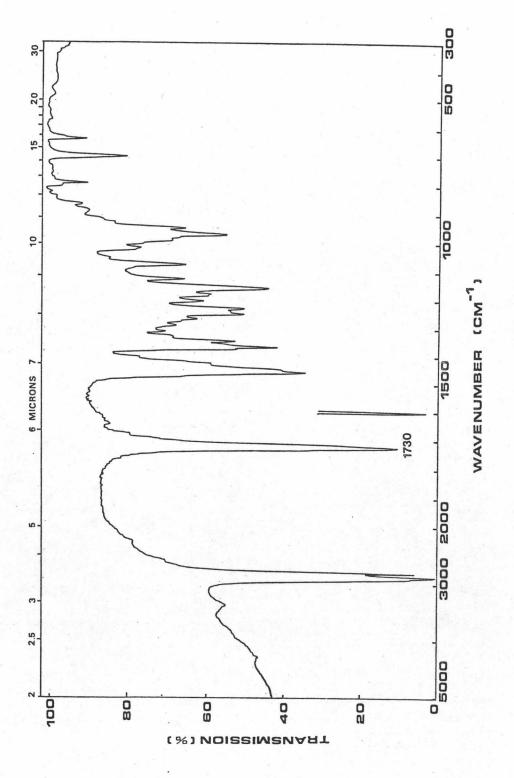


Figure 26 The IR spectrum of Compound 3

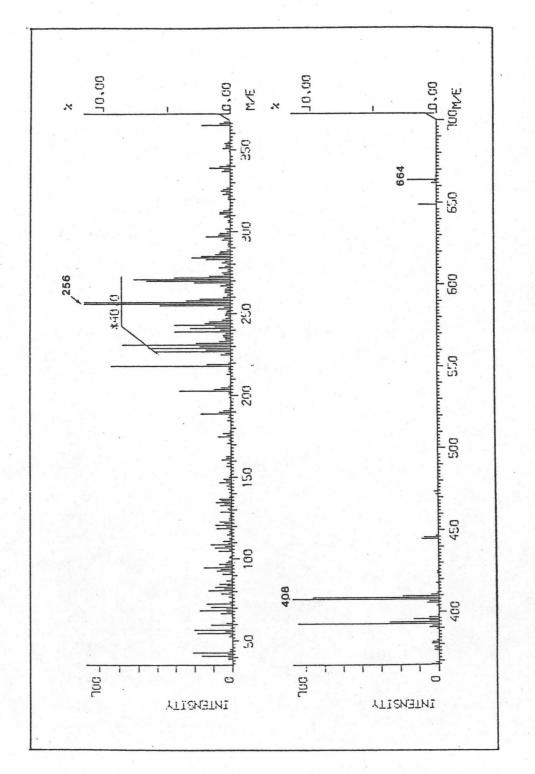
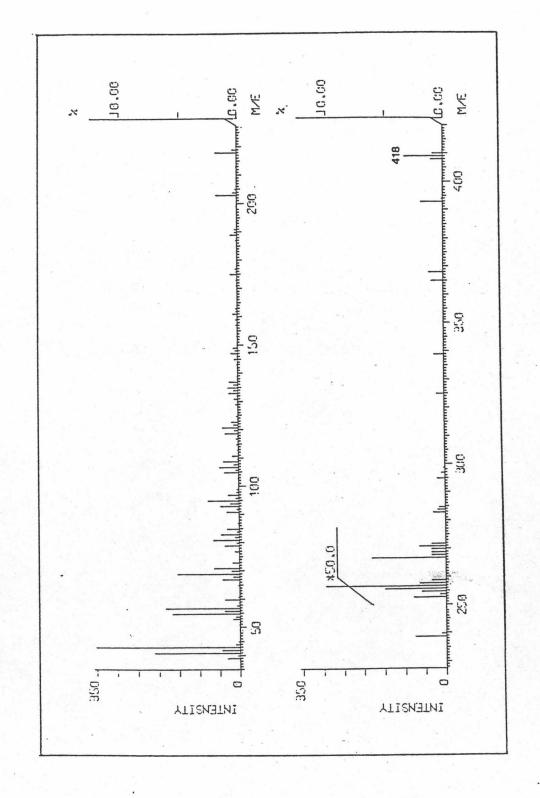
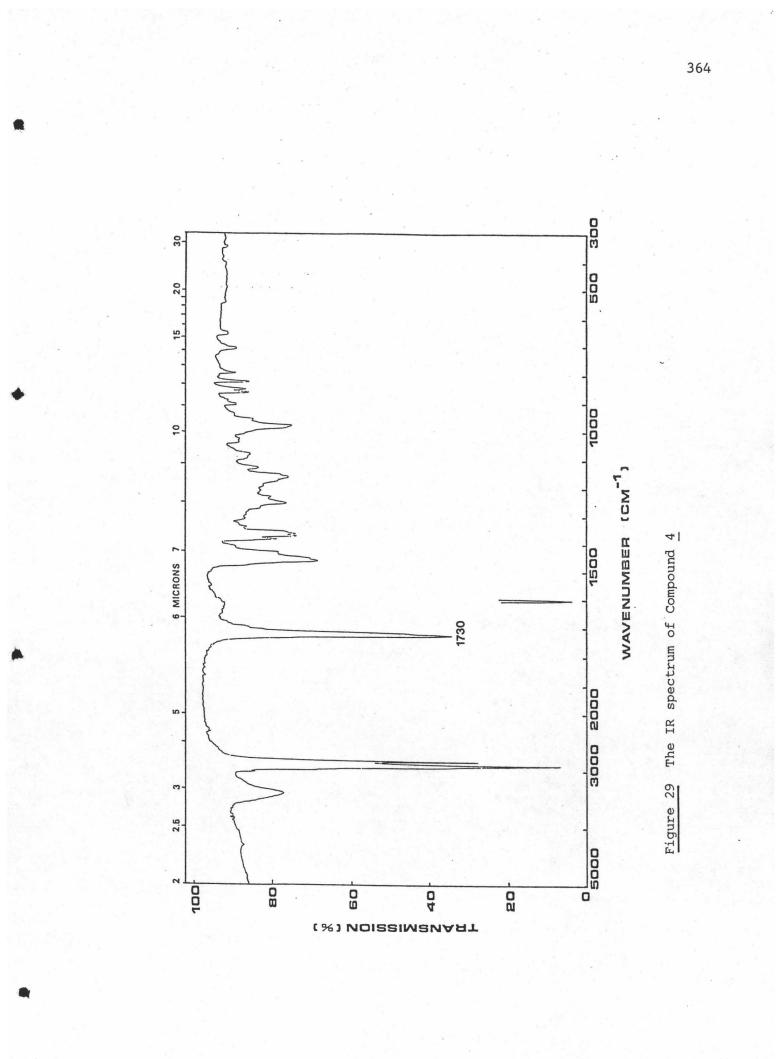
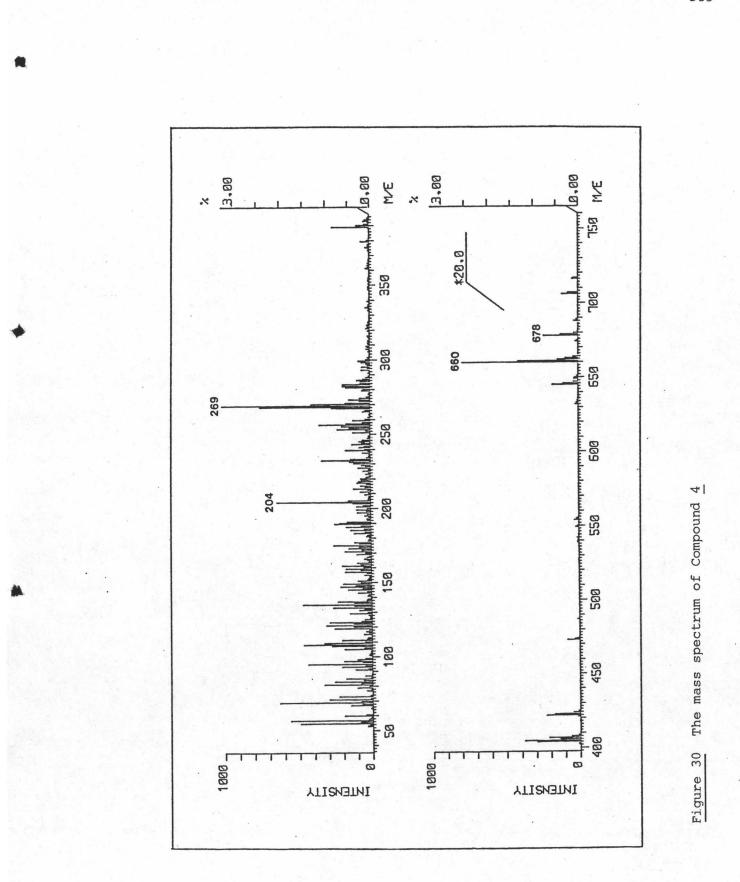


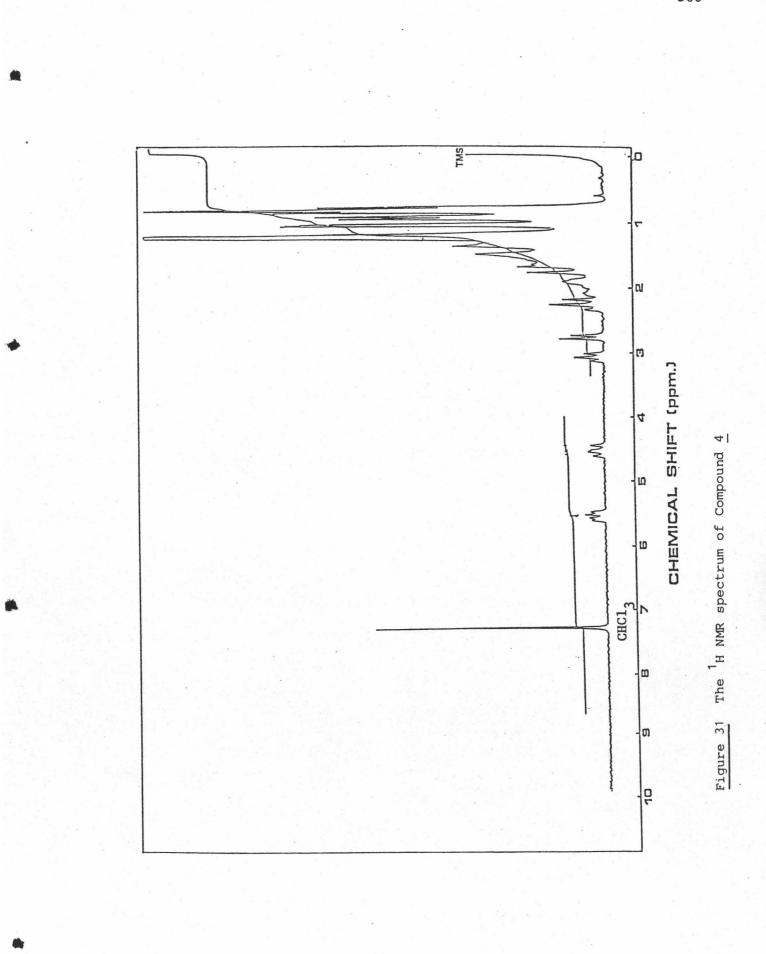
Figure 27 The mass spectrum pattern 1 of Compound 3

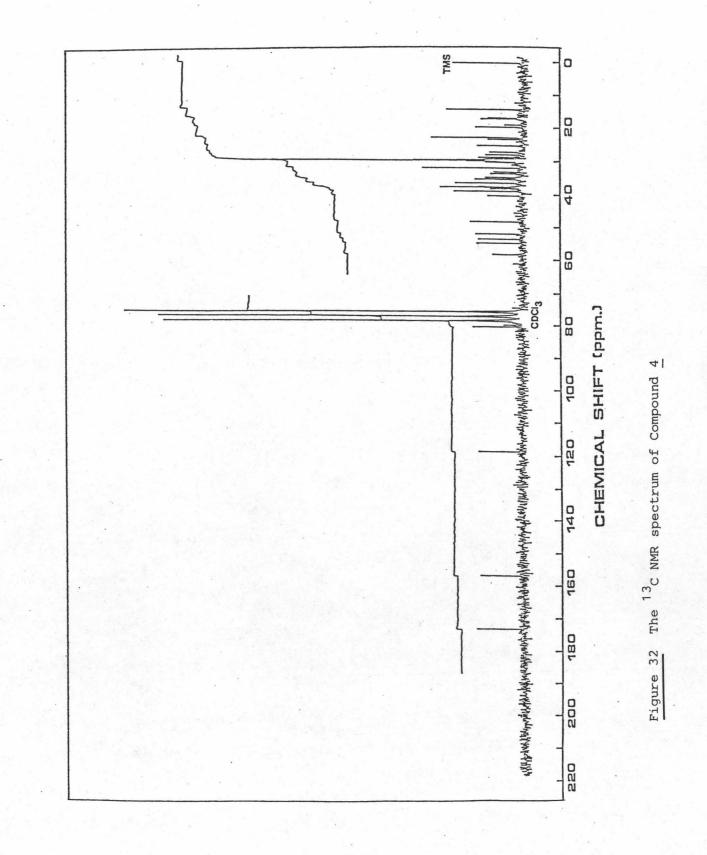


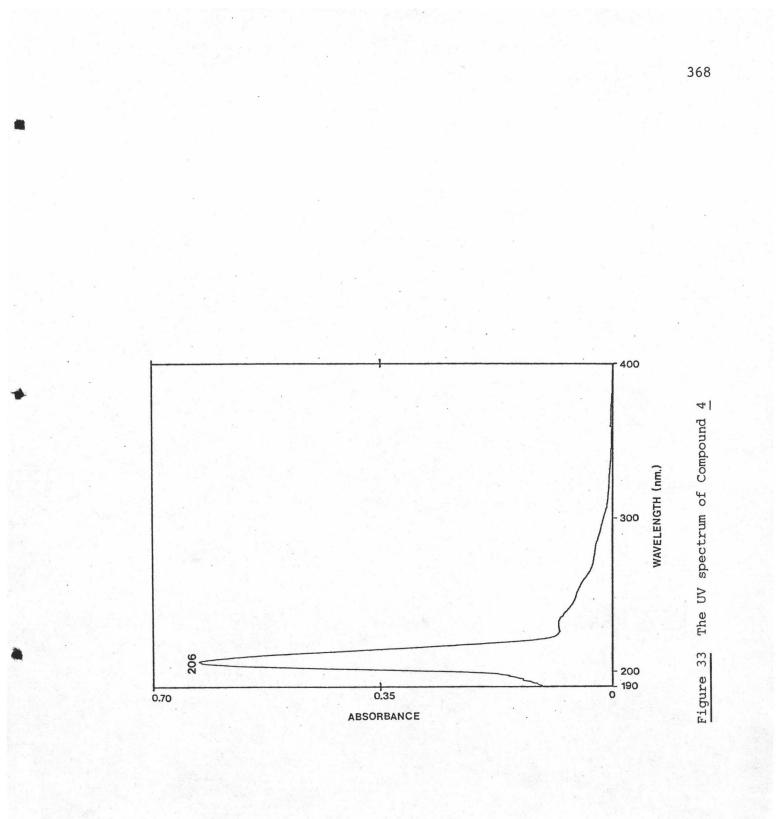


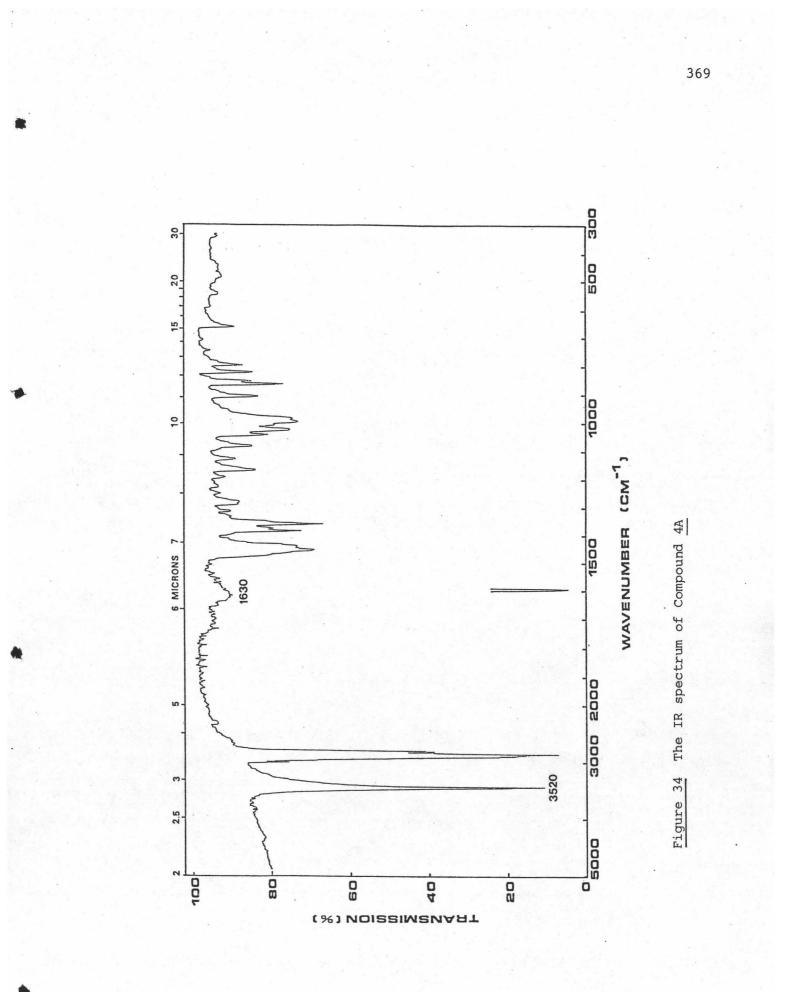












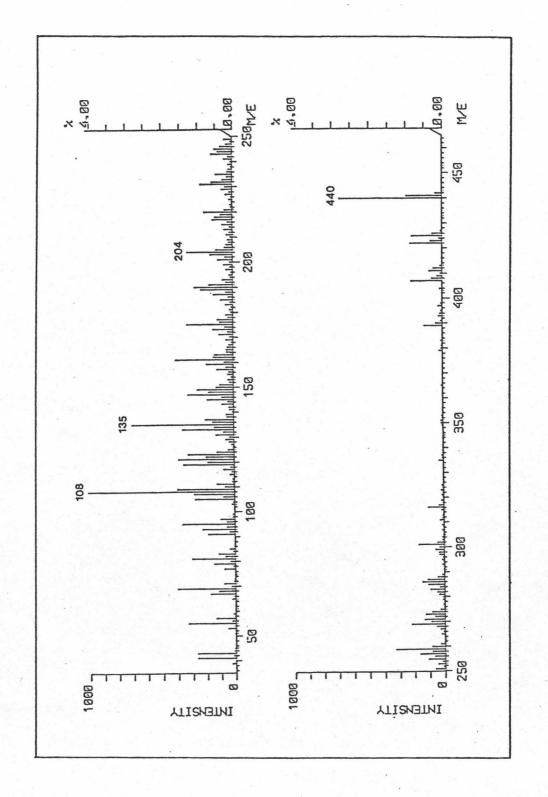


Figure 35 The mass spectrum of Compound 4A

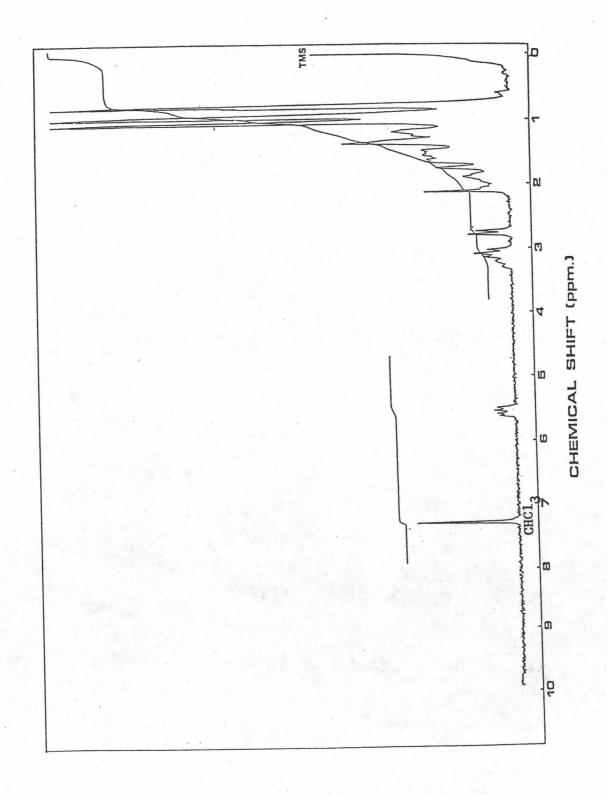


Figure 36 The ¹H NMR spectrum of Compound <u>4A</u>

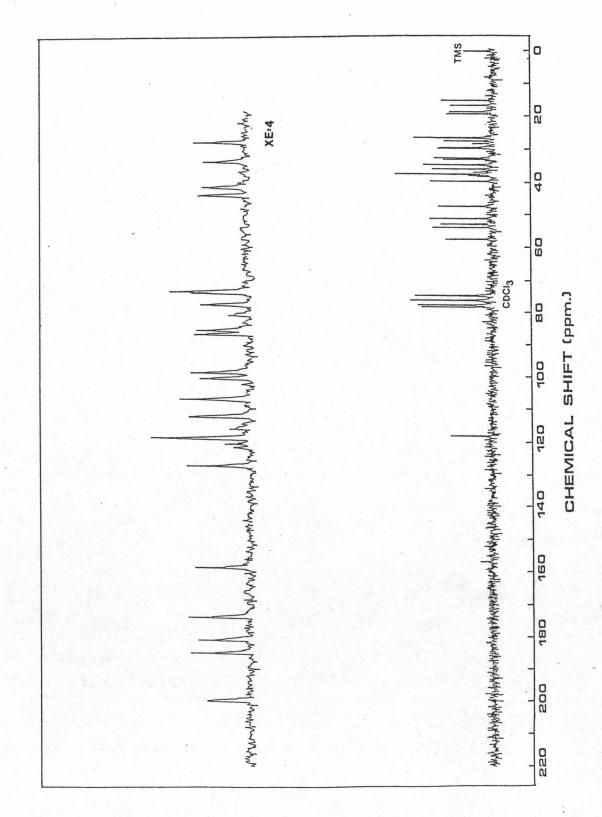
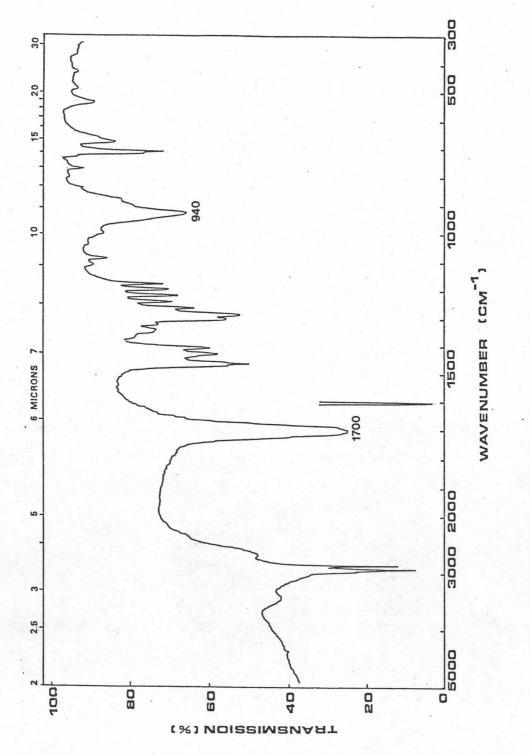
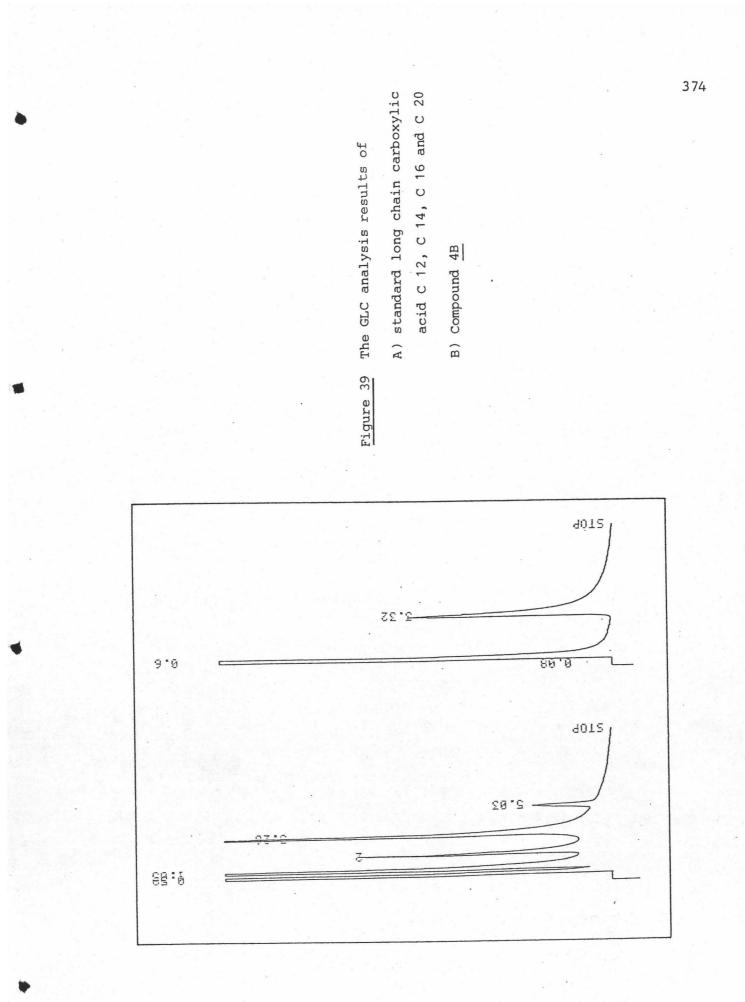


Figure 37 The 1^3 C NMR spectrum of Compound $\frac{4A}{4}$







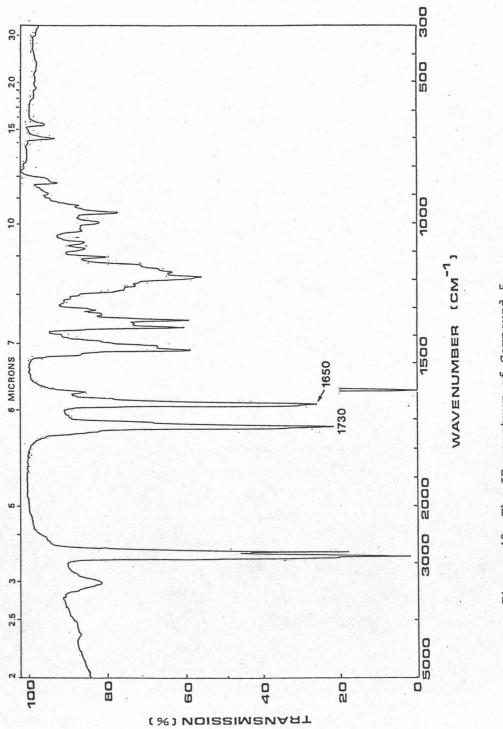


Figure 40 The IR spectrum of Compound 5

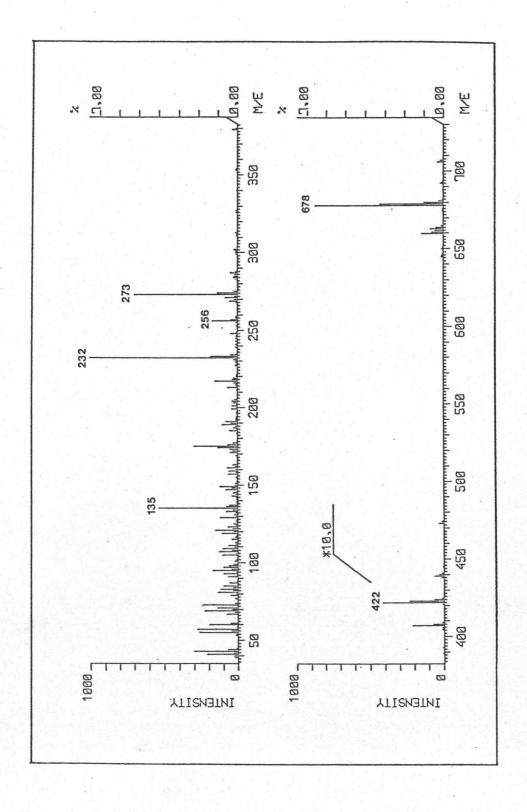


Figure 41 The mass spectrum of Compound 5

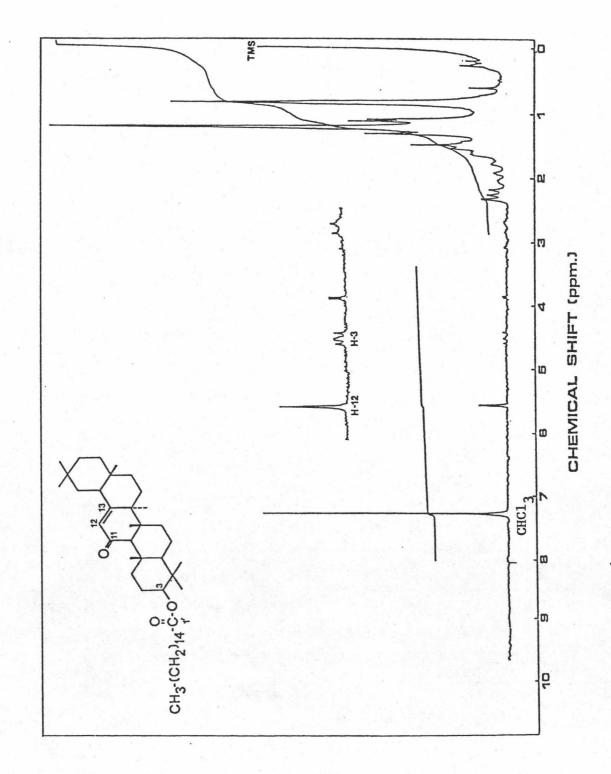
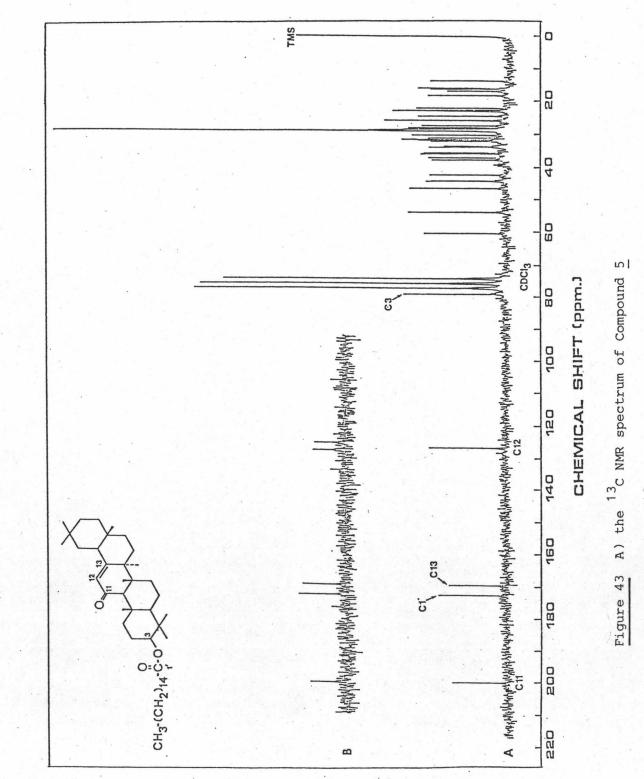
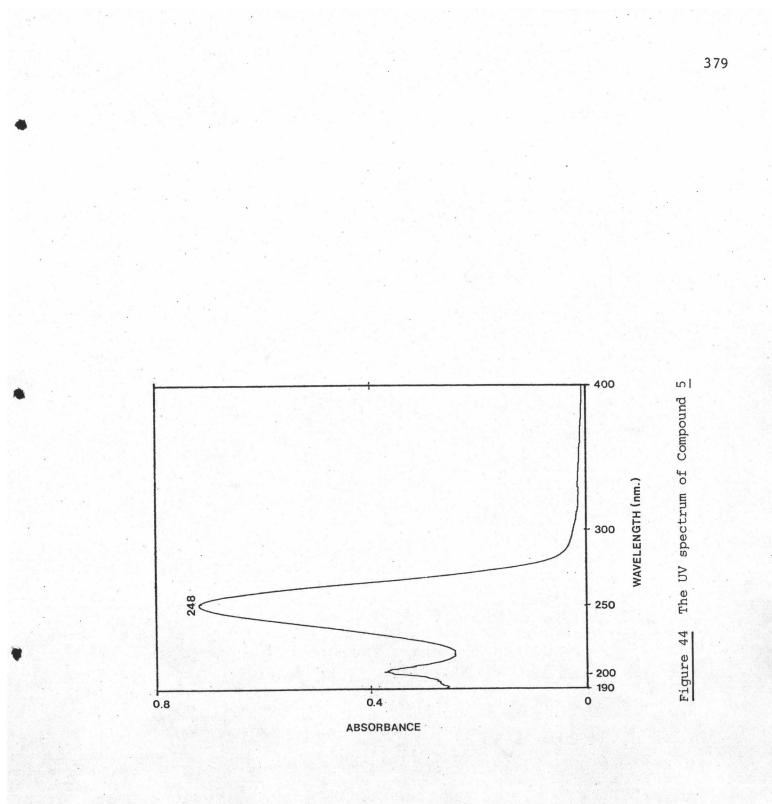
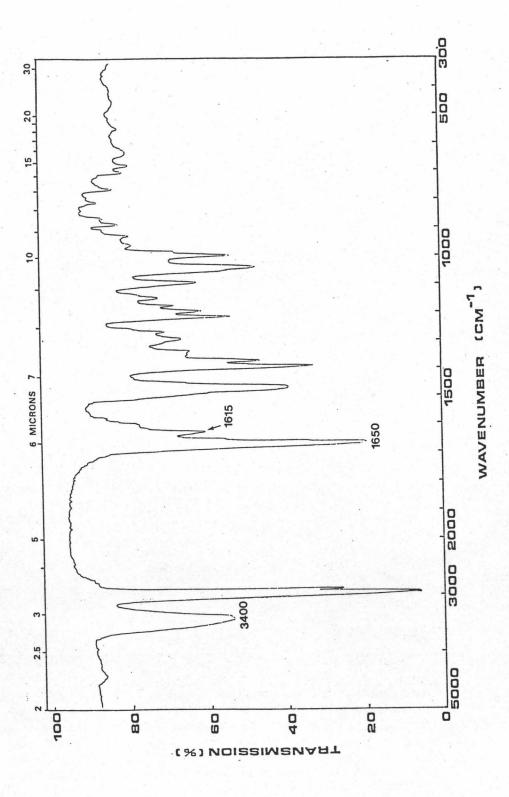


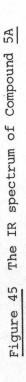
Figure 42 The ¹H NMR spectrum of Compound <u>5</u>

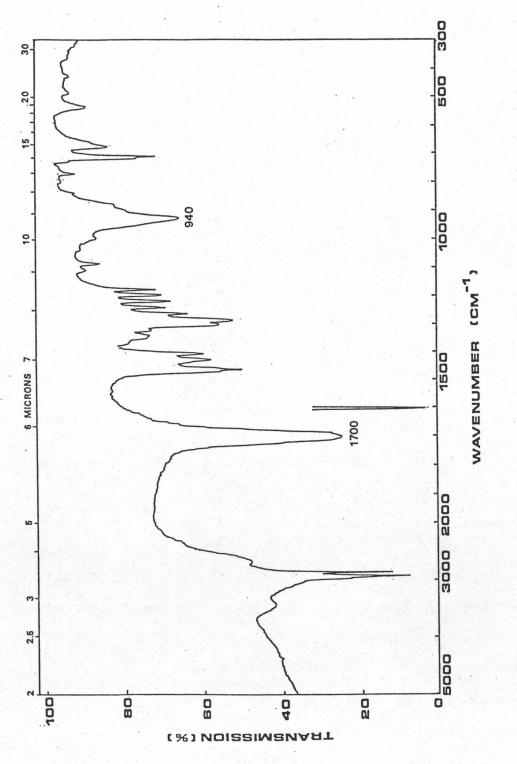


B) The 13 C NMR spectrum off resonance spectrum of Compound $\frac{5}{2}$



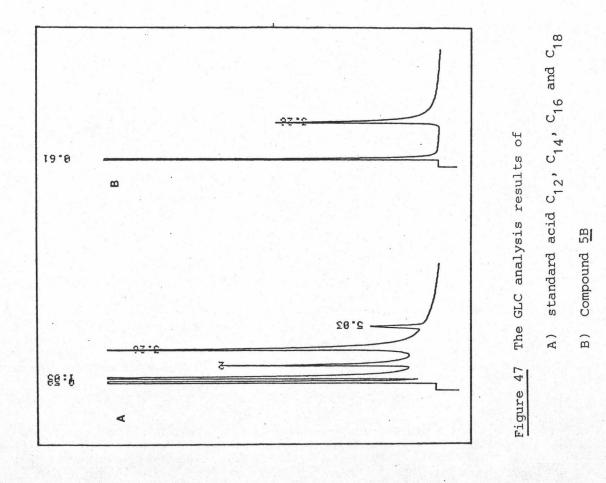






States .

Figure 46 The IR spectrum of Compound 5B



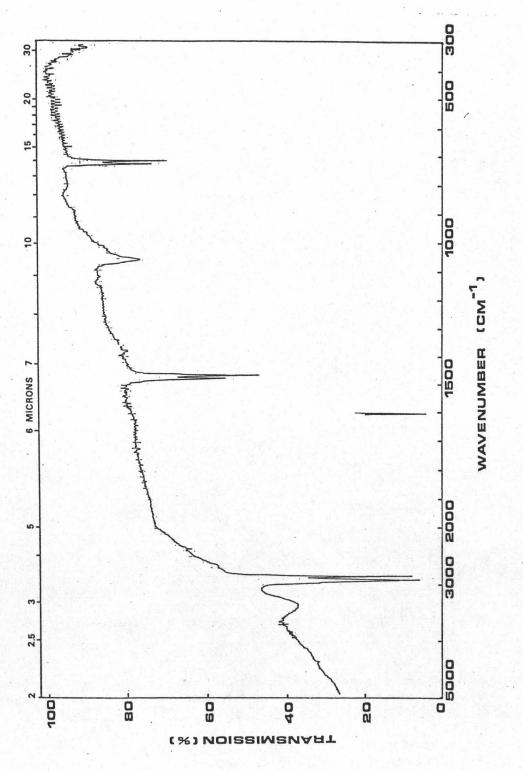
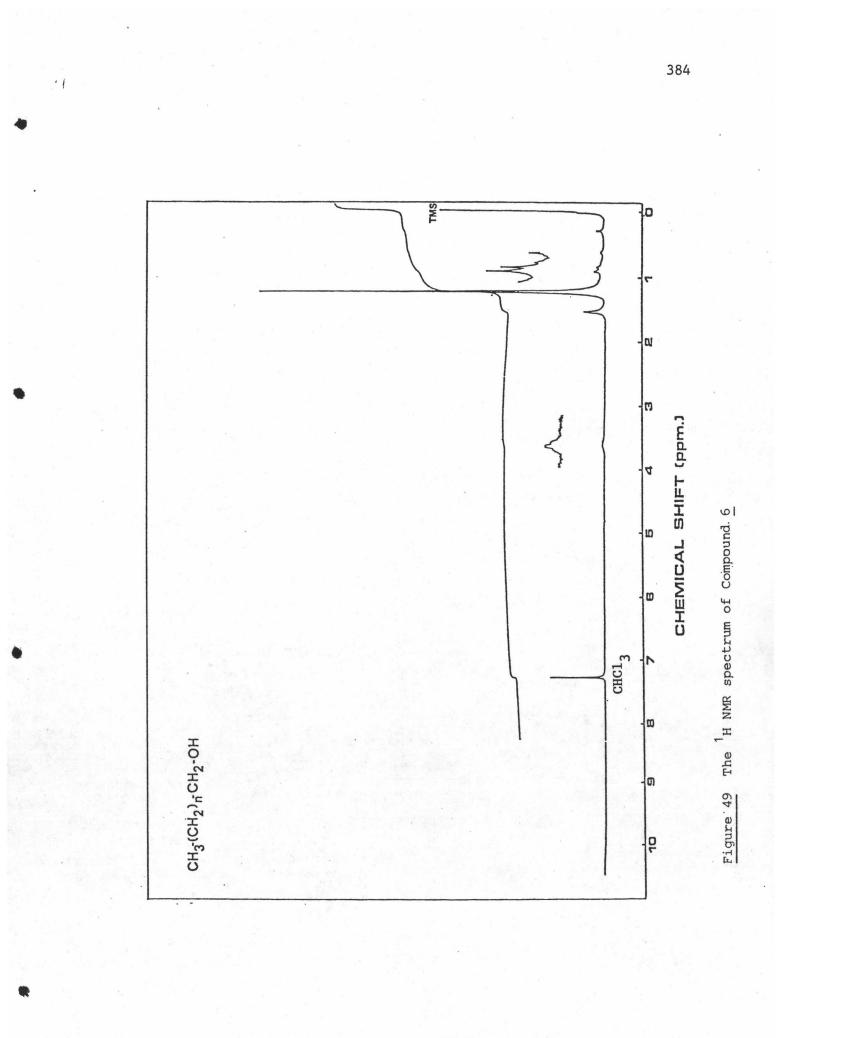
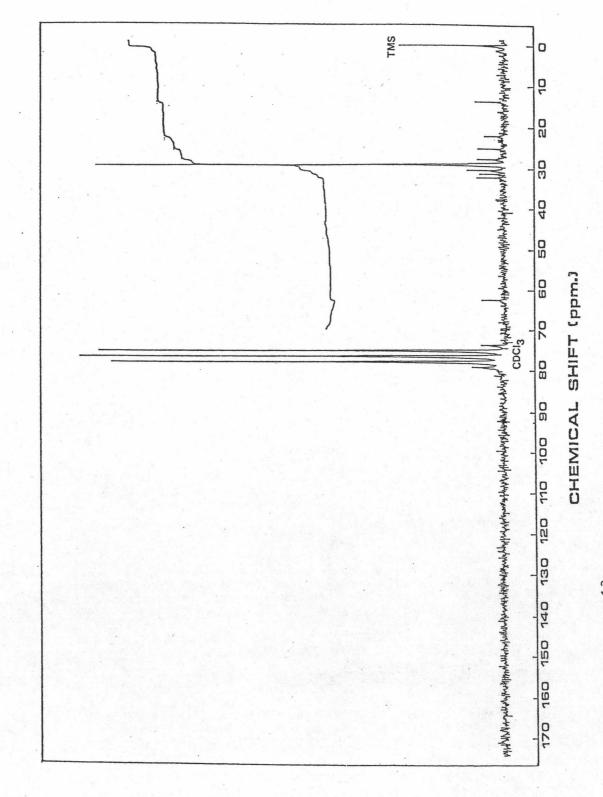
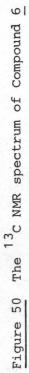


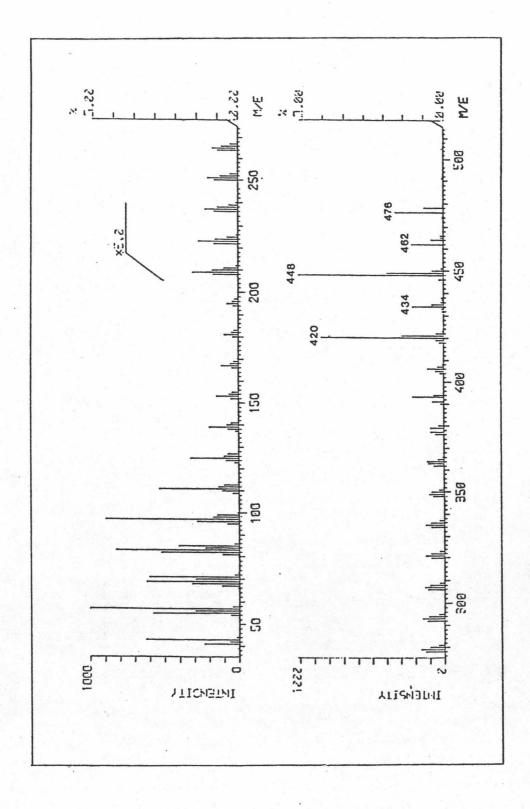
Figure 48 The IR spectrum of Compound 6

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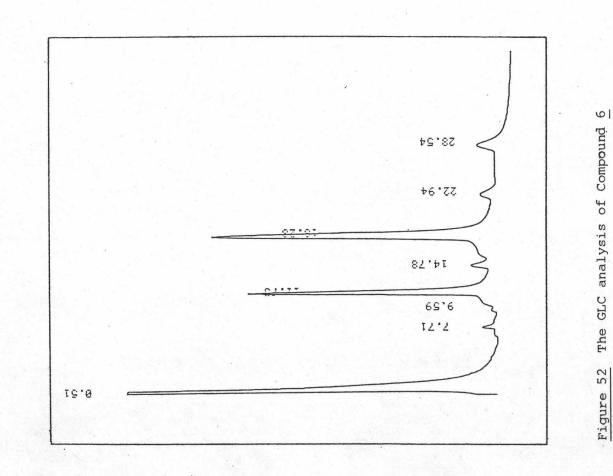


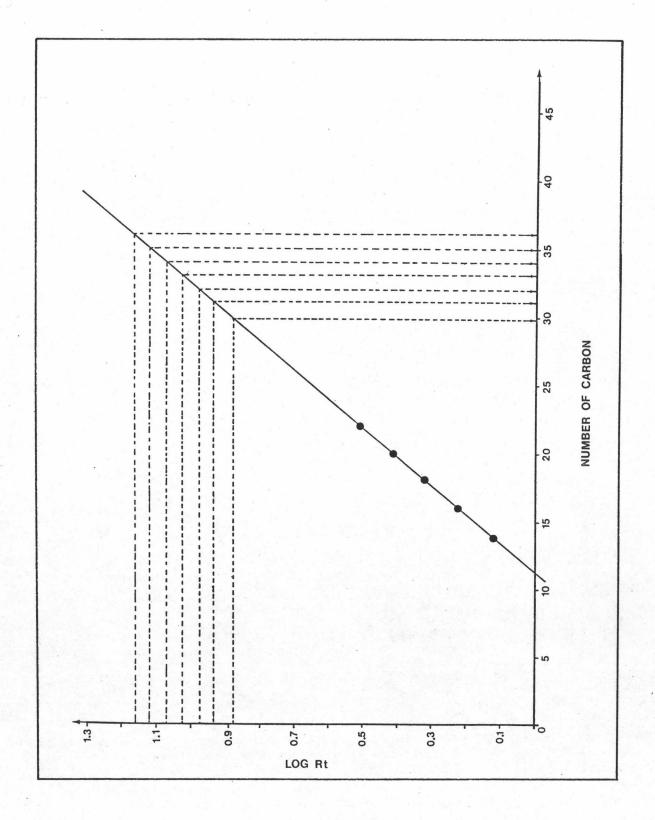




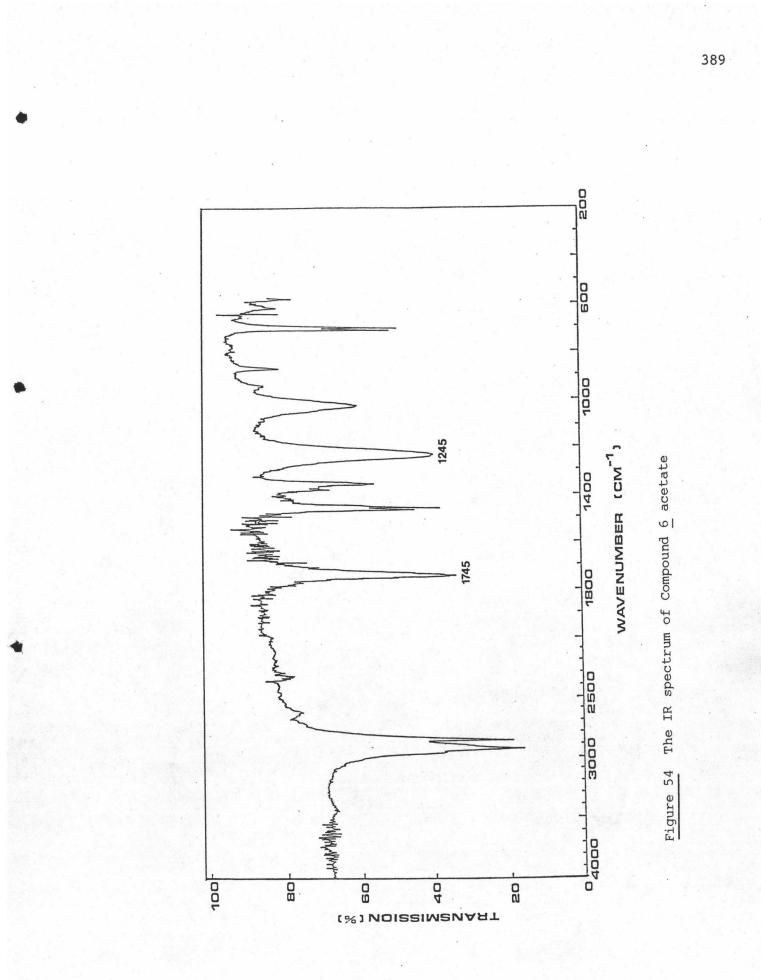


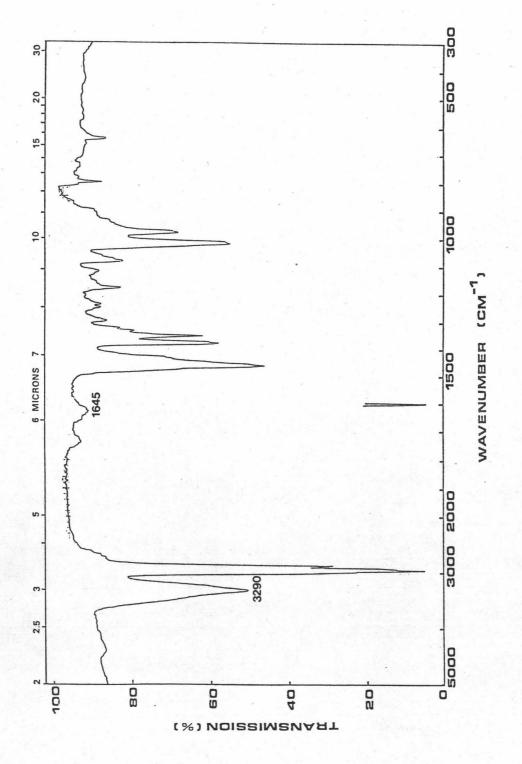














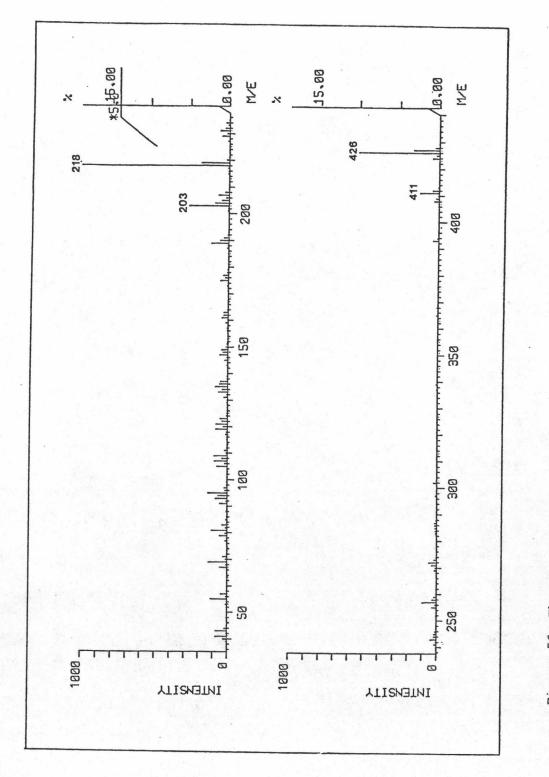
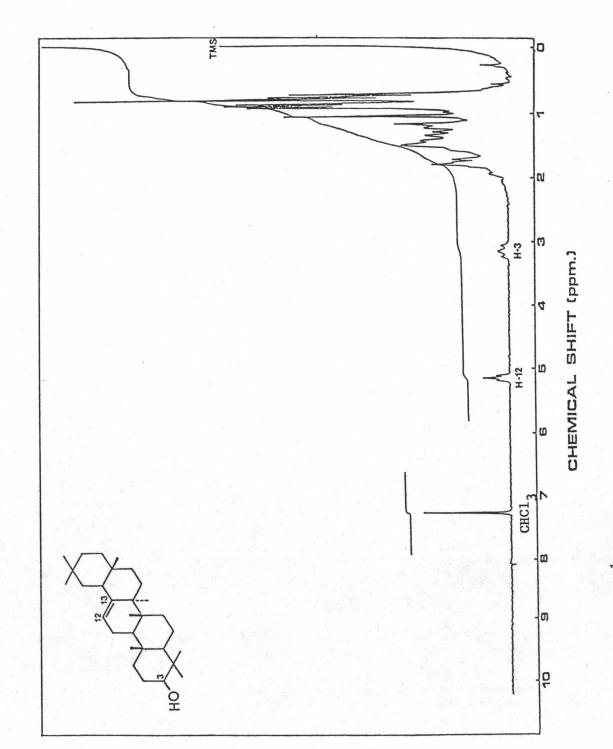
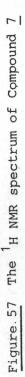
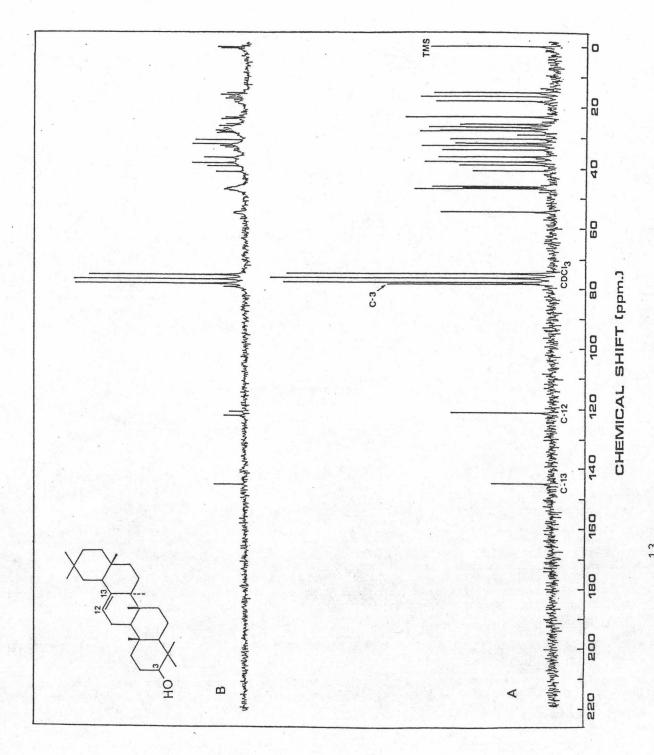
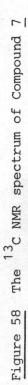


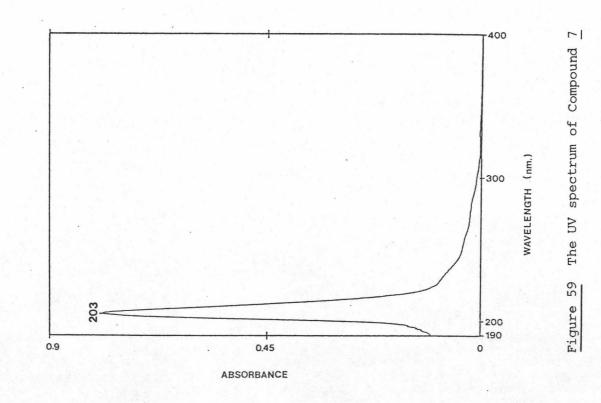
Figure 56 The mass spectrum of Compound $\frac{1}{2}$











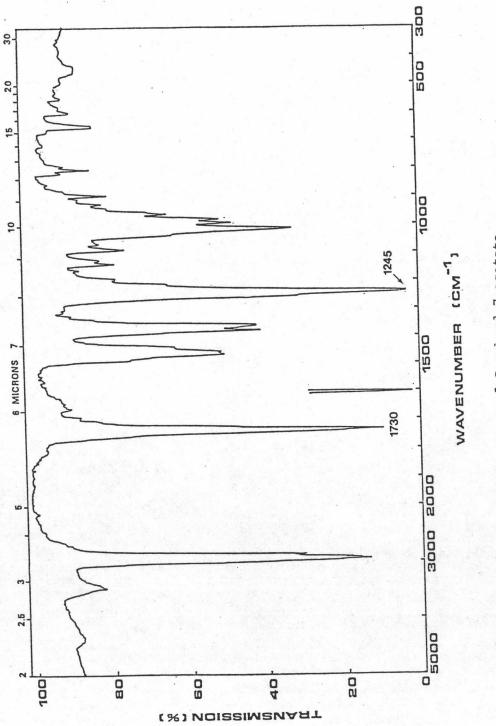
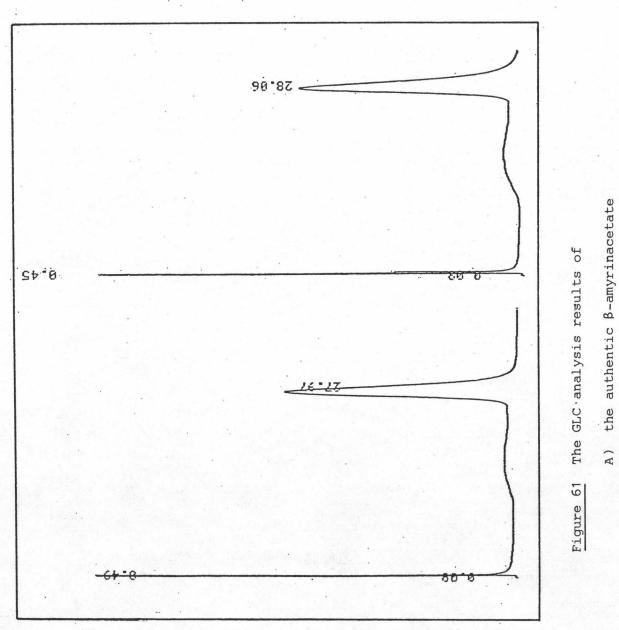
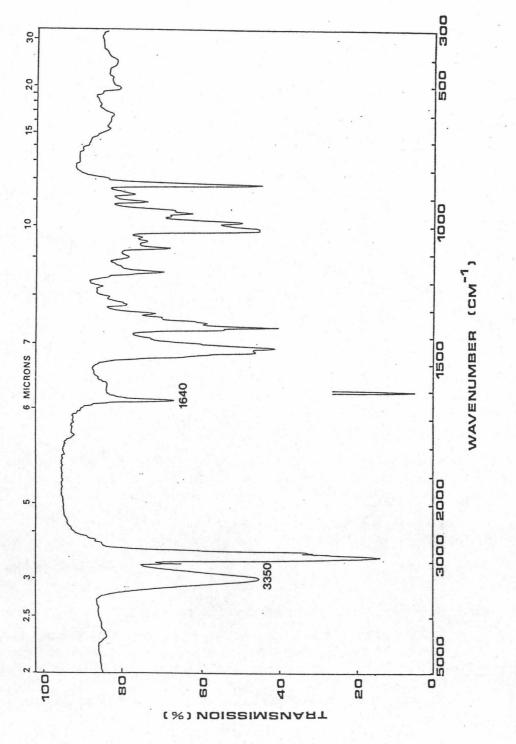


Figure 60 The IR spectrum of Compound $\overline{7}$ acetate

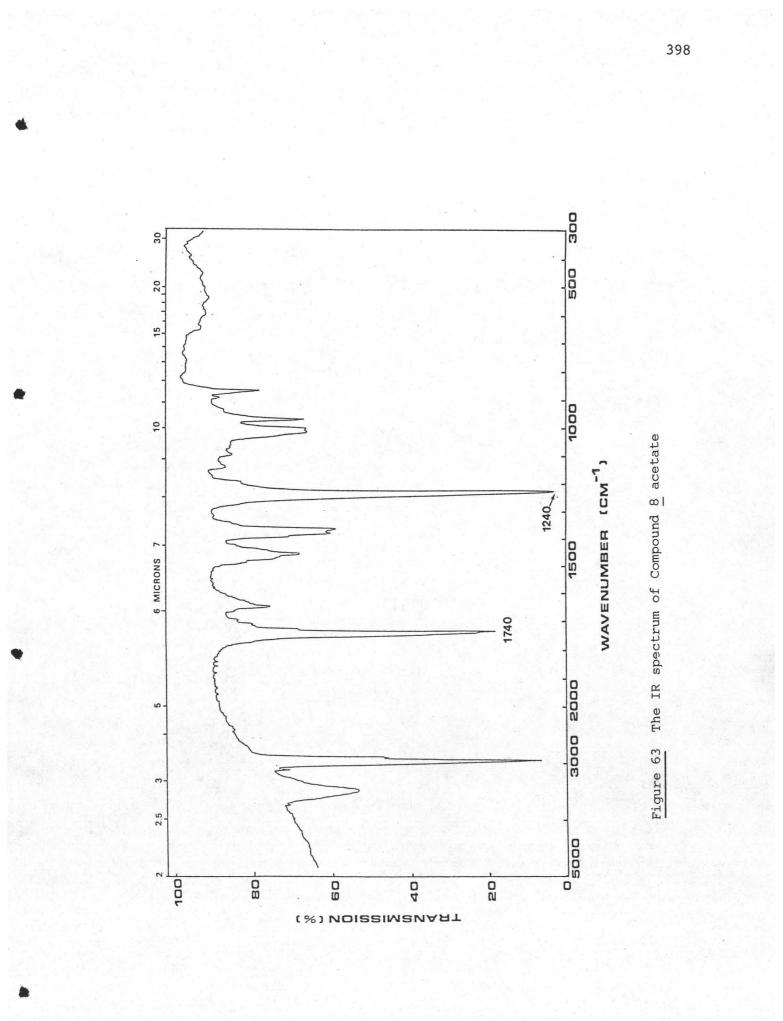


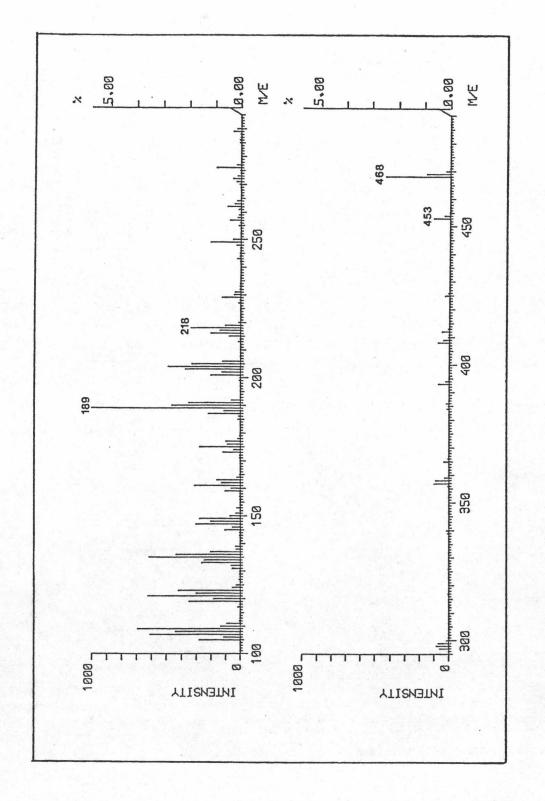
B.

Compound 7 acetate

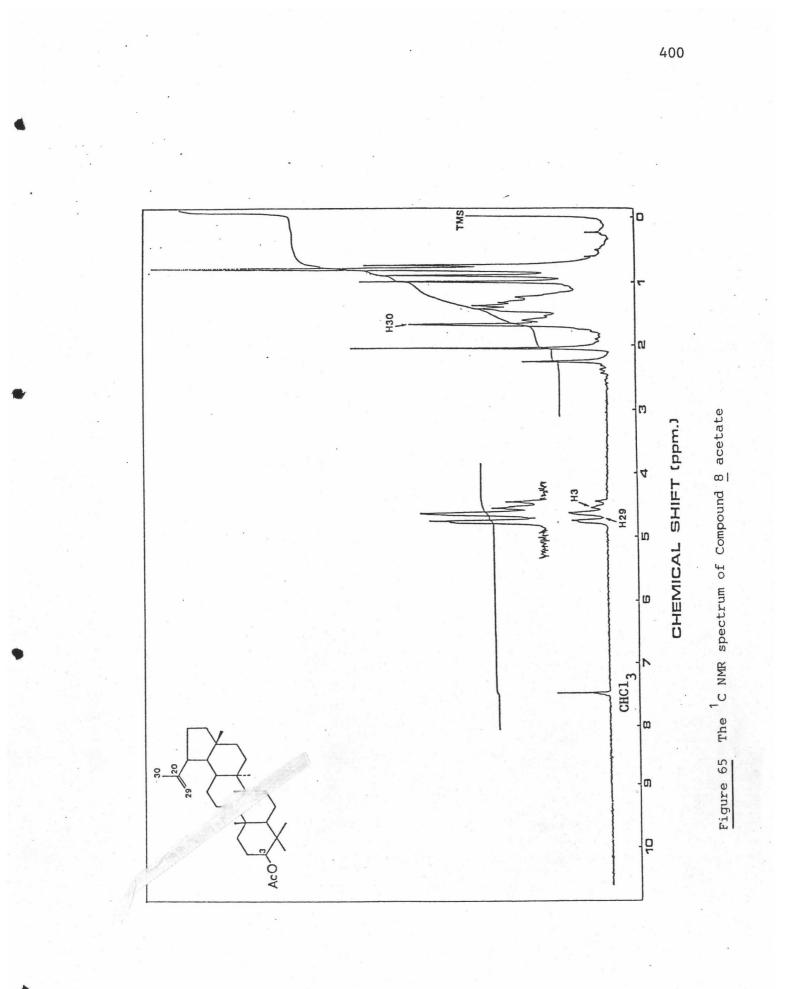


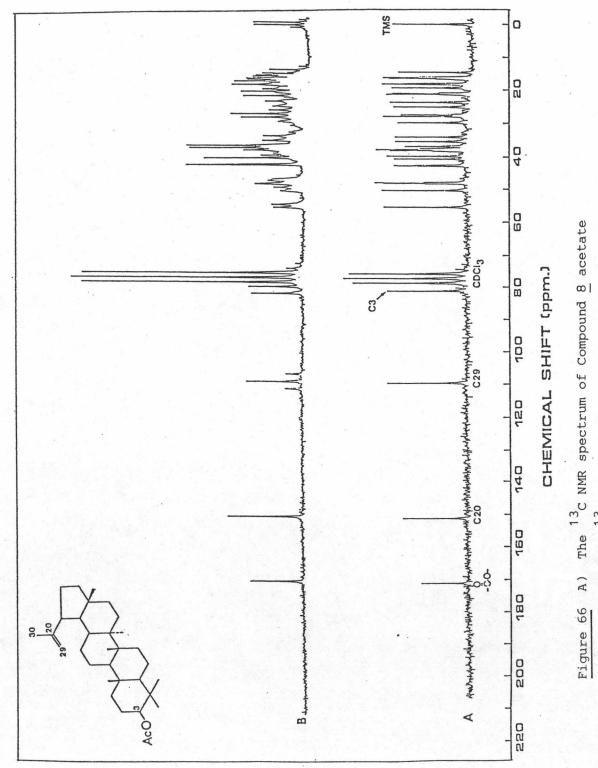




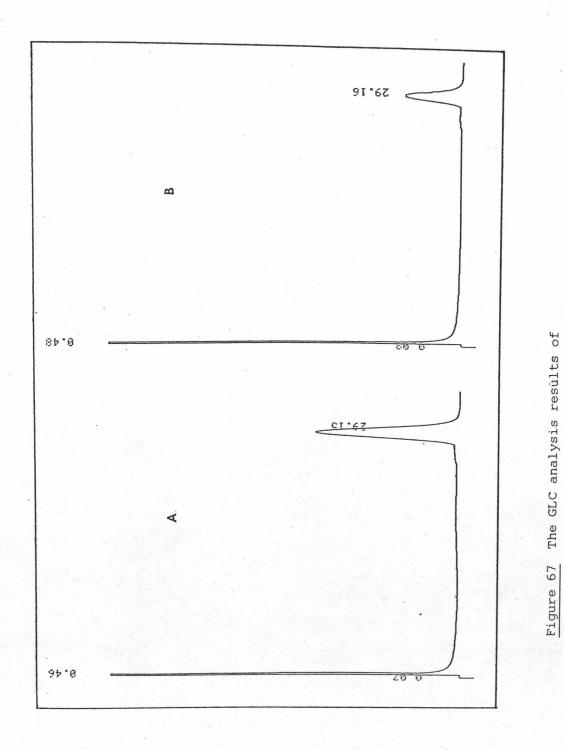






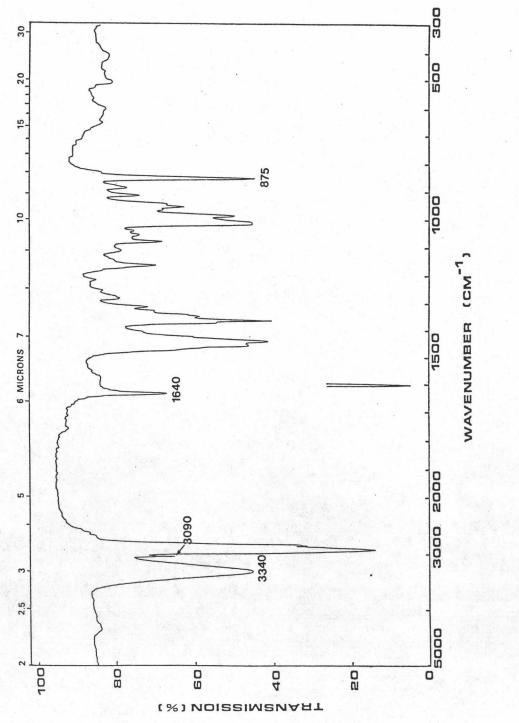


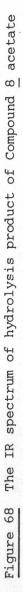
The 13 C NMR off resonance spectrum of Compound 8 acetate B)



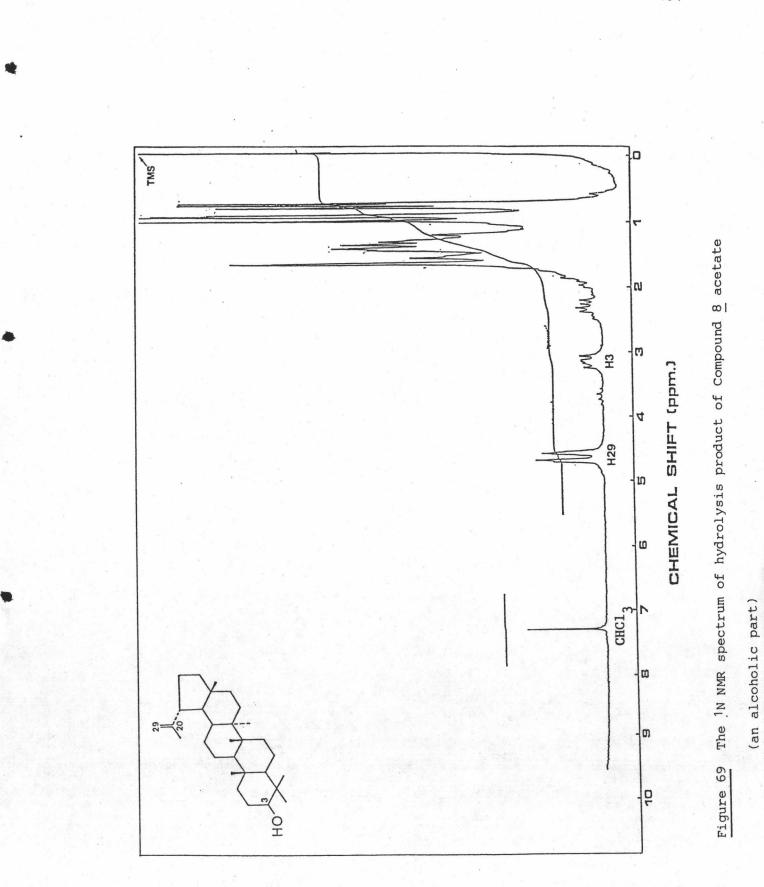


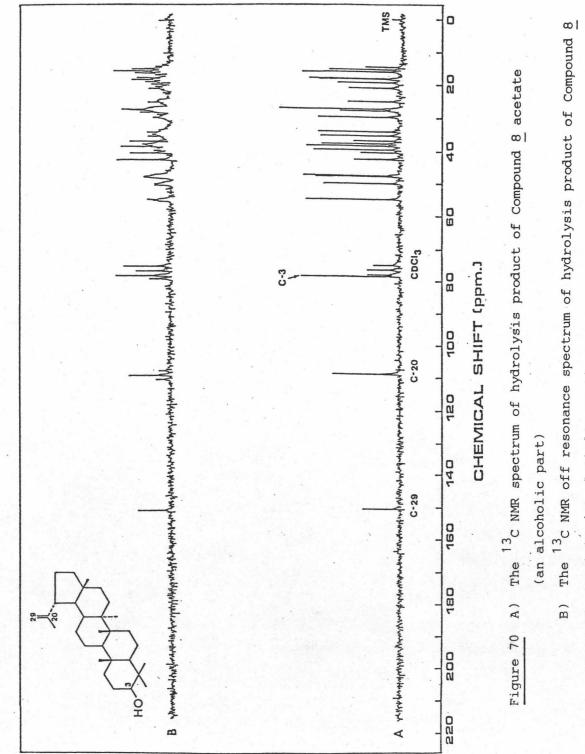
A) the authentic lupeolacetate



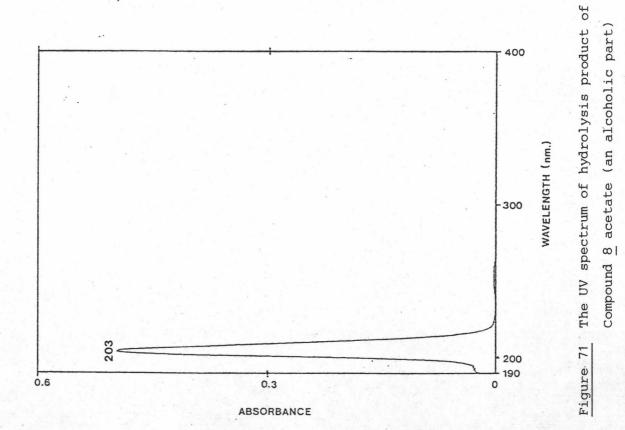


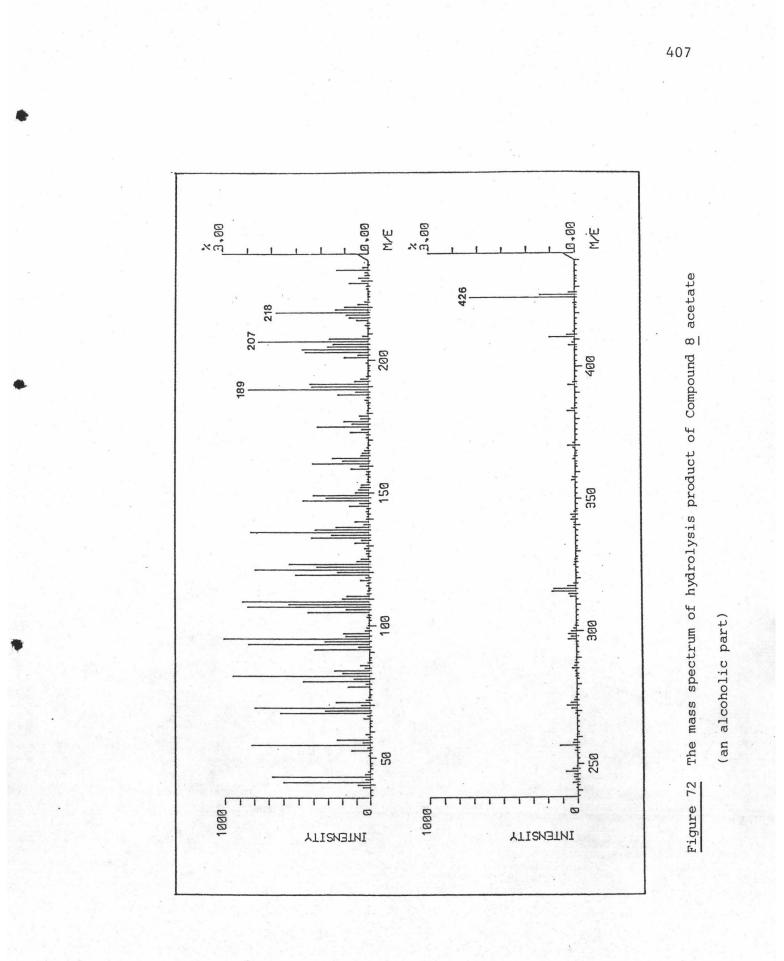
(an alcoholic part)

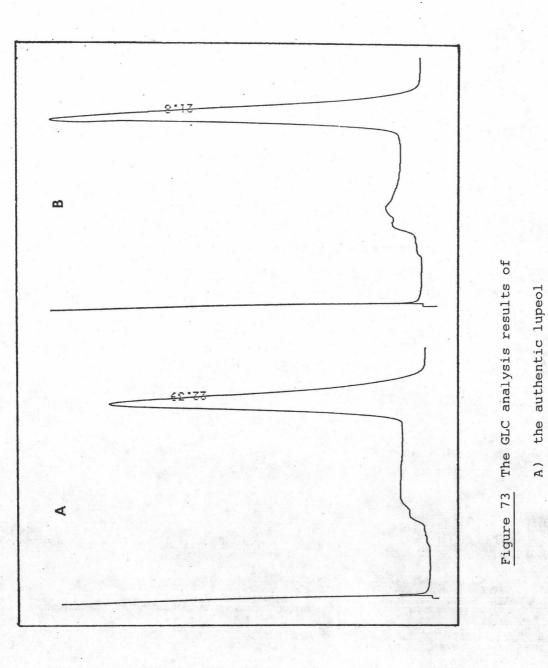




acetate (an alcoholic part)

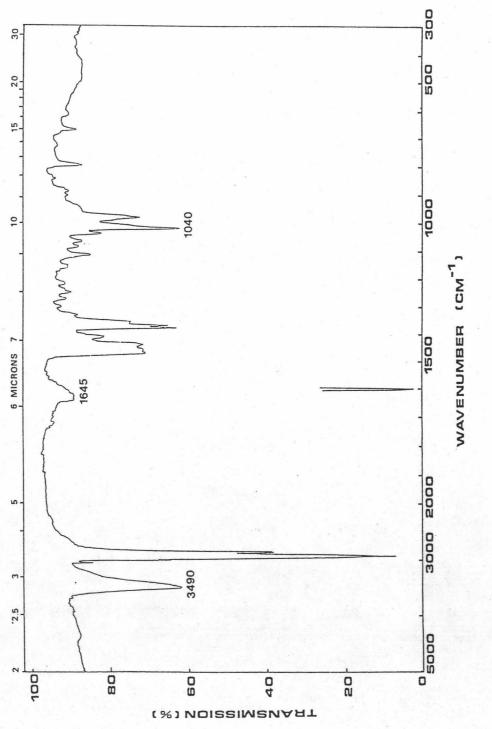




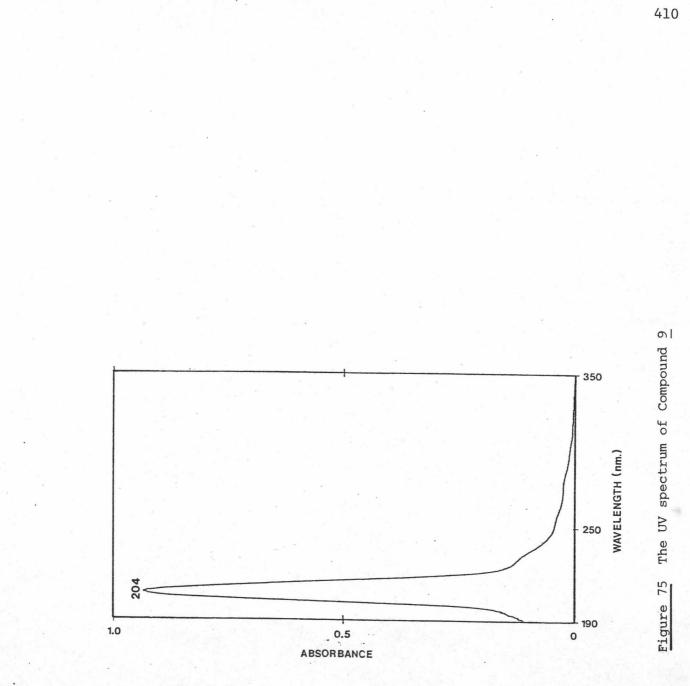


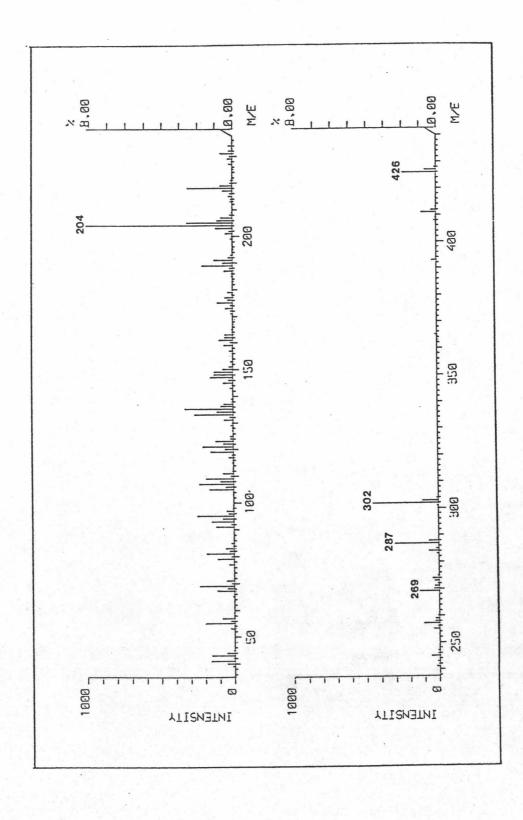
(an alcoholic part)

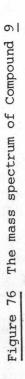
B) the hydrolysis product of Compound $\underline{8}$ acetate

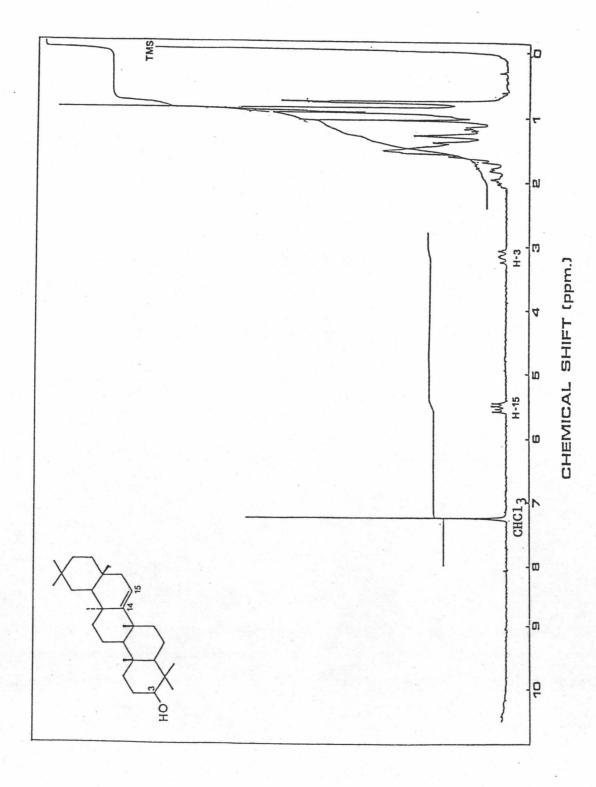


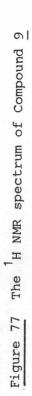


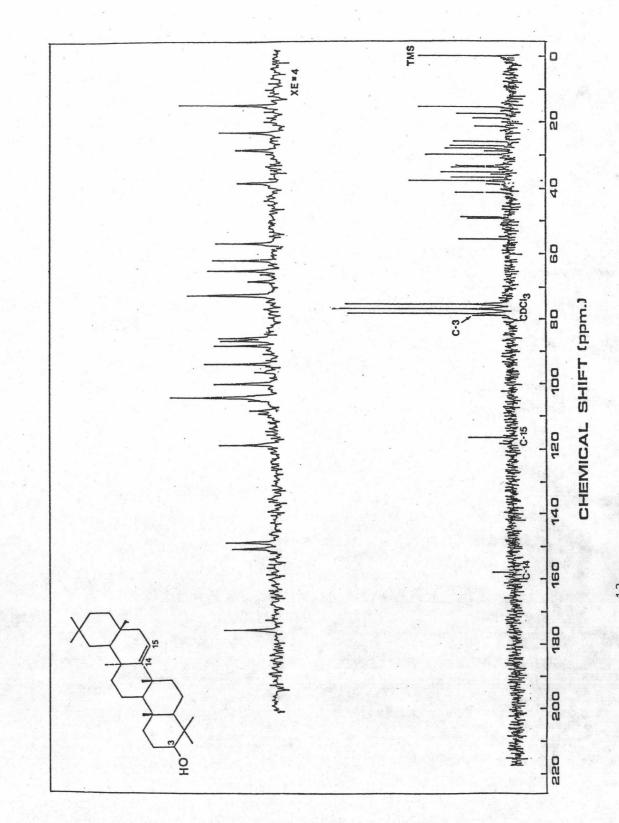


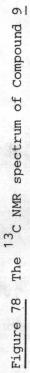












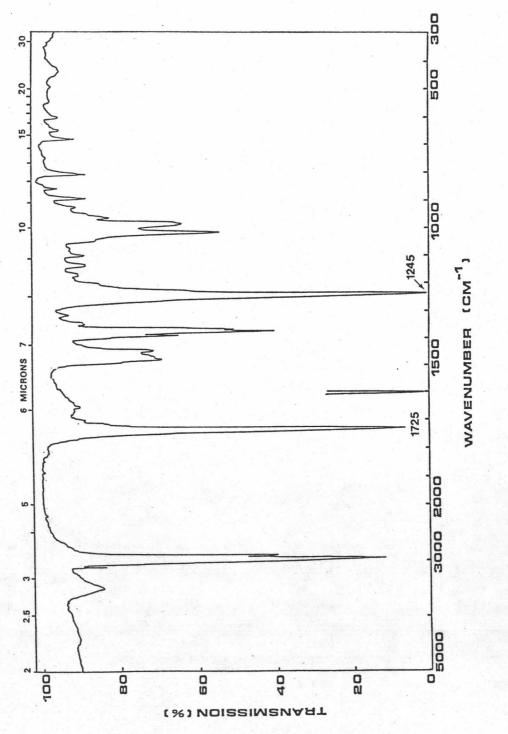


Figure 79 The IR spectrum of Compound 9 acetate

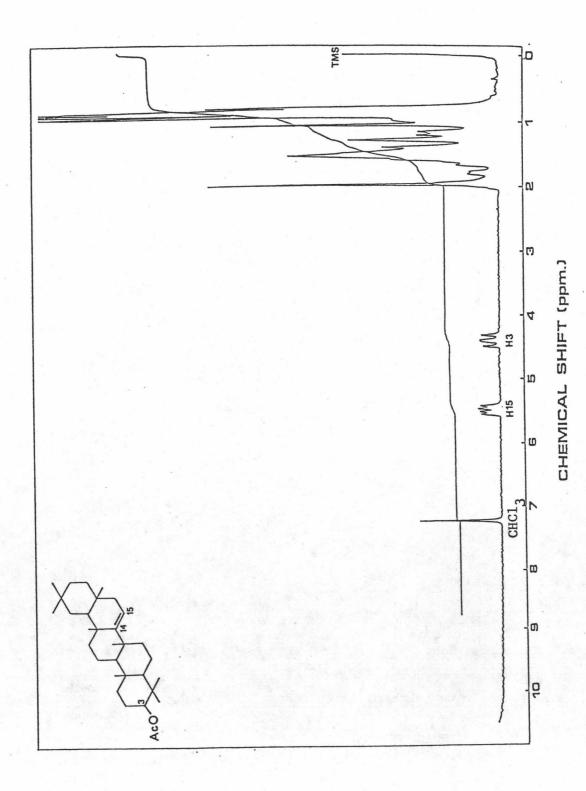


Figure 80 The ¹H NMR spectrum of Compound <u>9</u> acetate

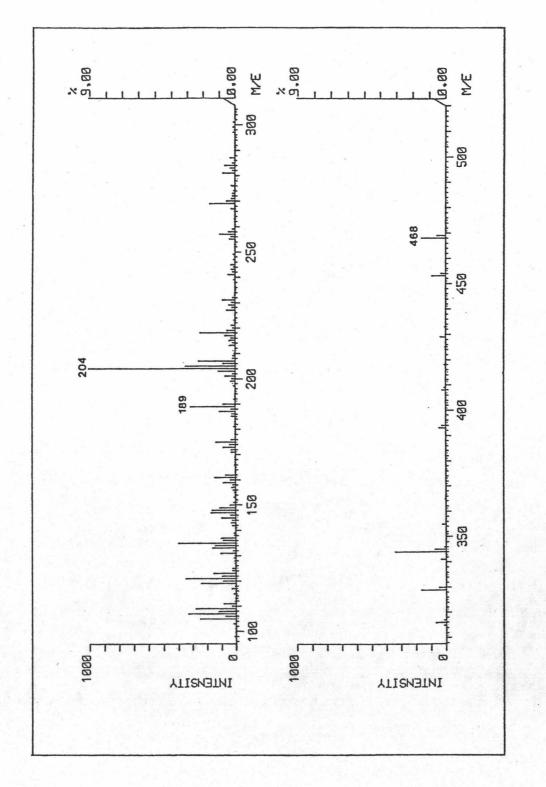


Figure 81 The mass spectrum of Compound 9 acetate

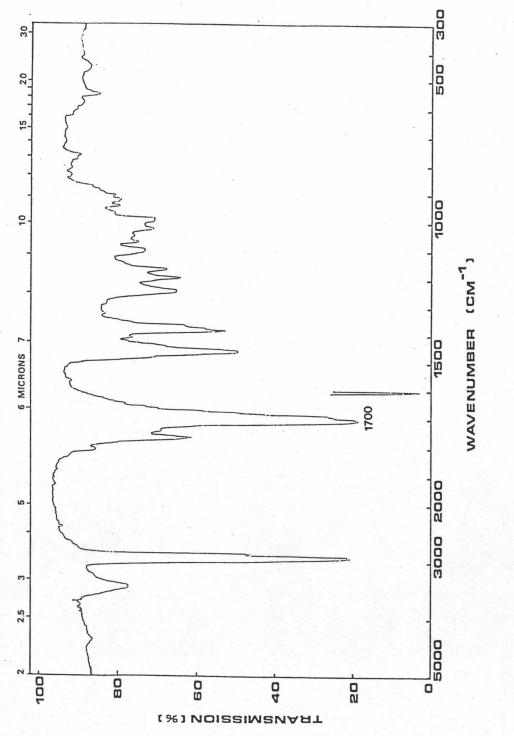


Figure 82 The IR spectrum of Compound <u>9A</u>

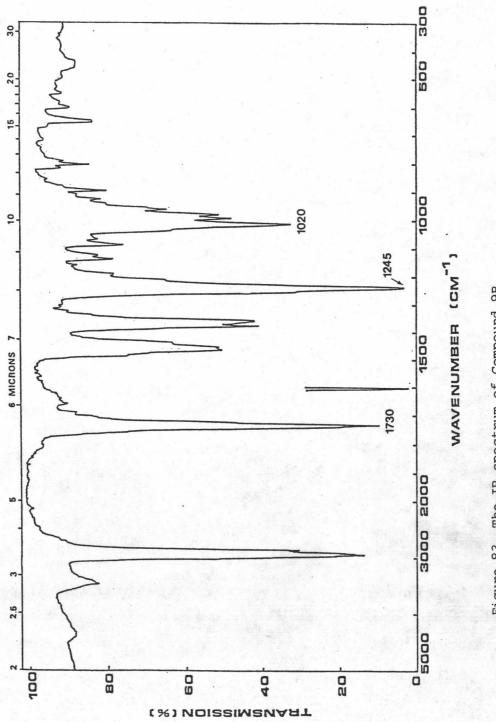
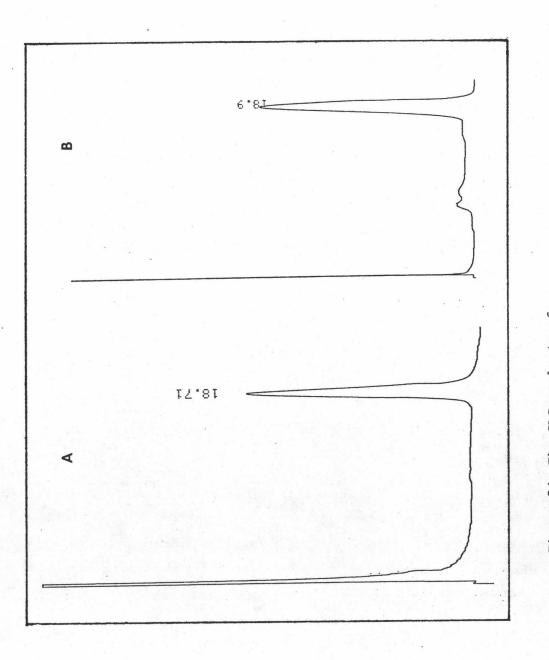


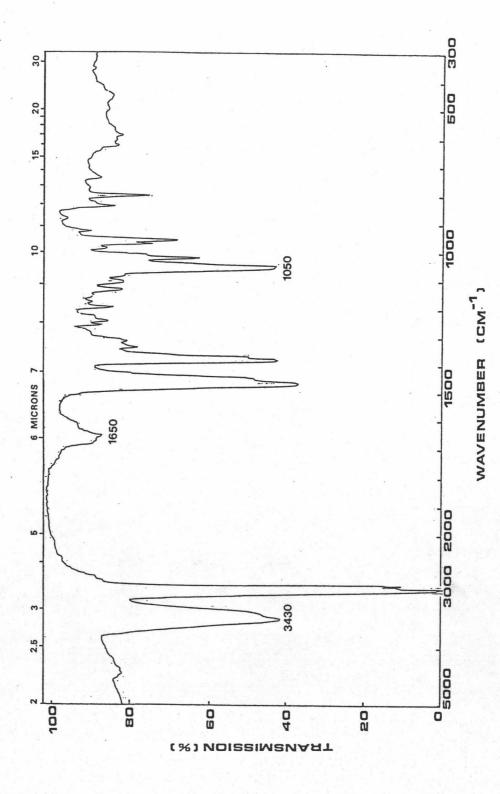
Figure 83 The IR spectrum of Compound 9B



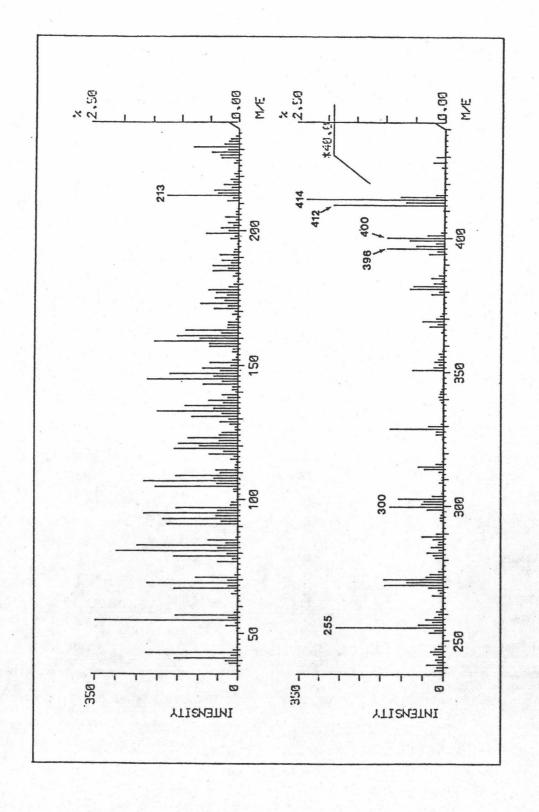


A) the authentic β -amyrinacetate

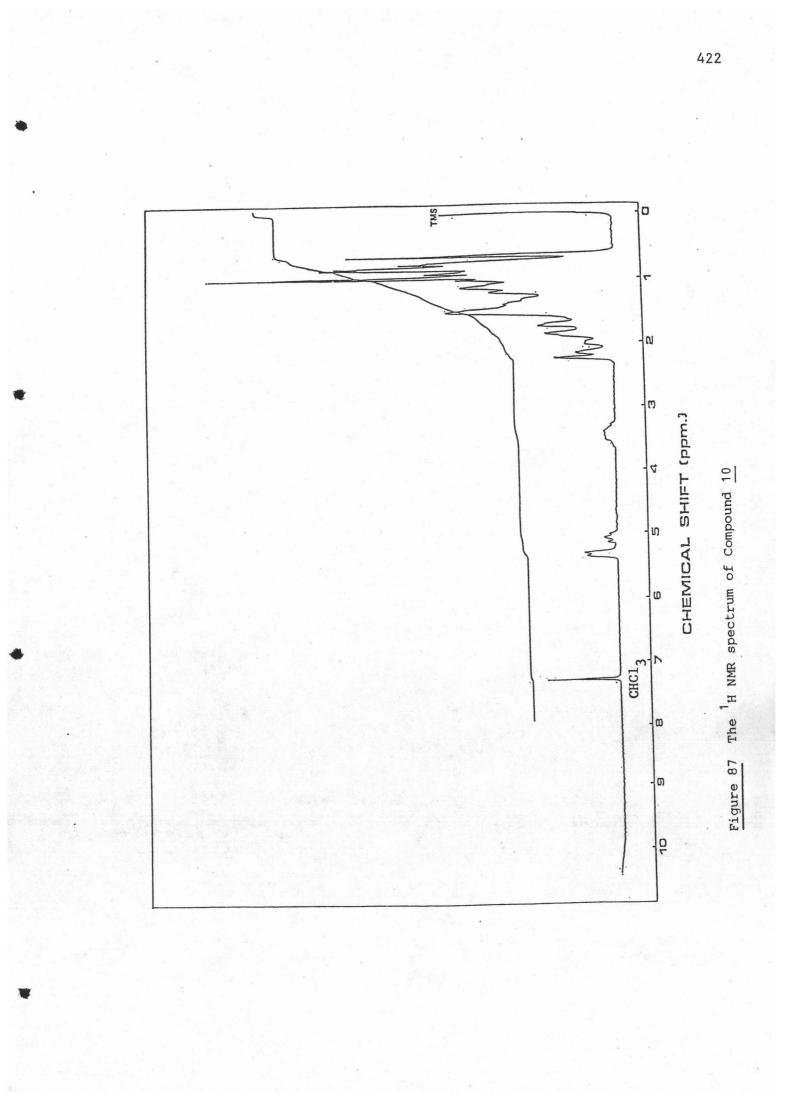
B) Compound <u>9B</u>











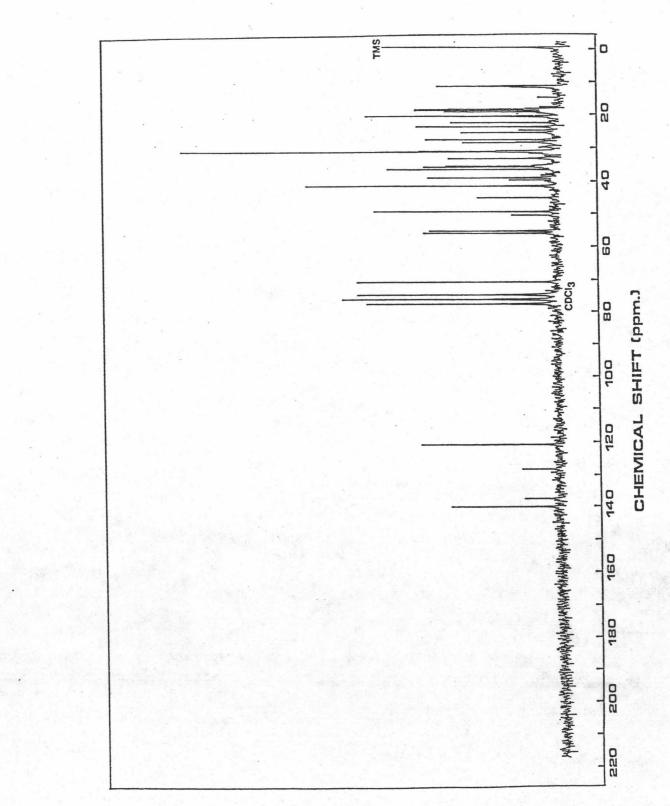
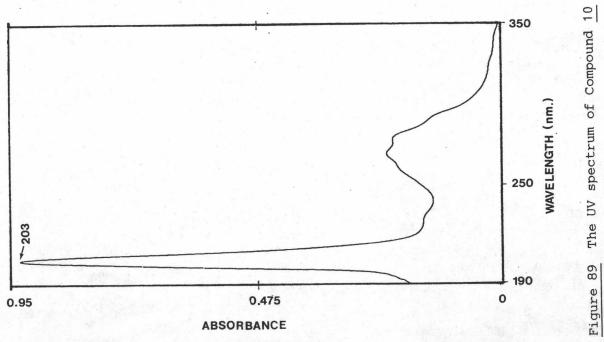


Figure 88 The ¹³C NMR spectrum of Compound 10



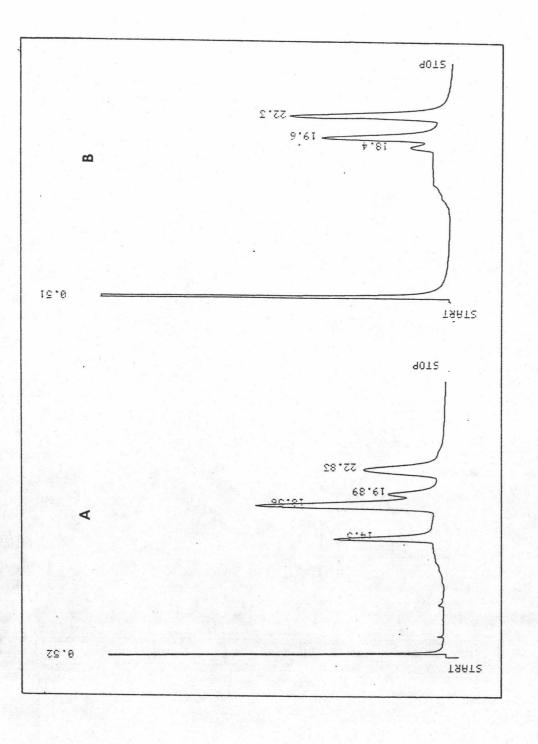


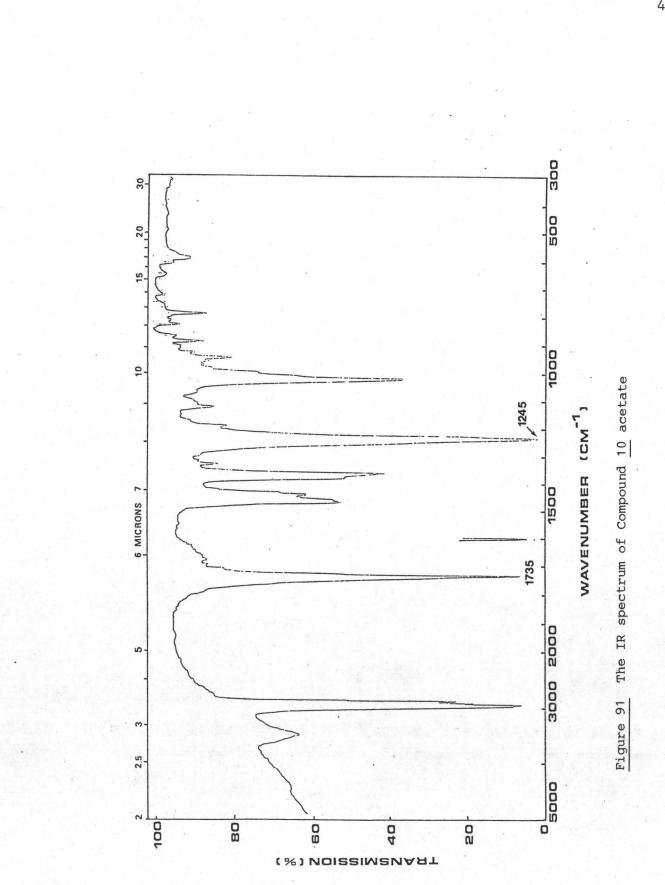
Figure 90 The GLC analysis results of

A) Standard chloresterol, compesterol stigmasterol and B-sitosterol

425

Compound 10

B)



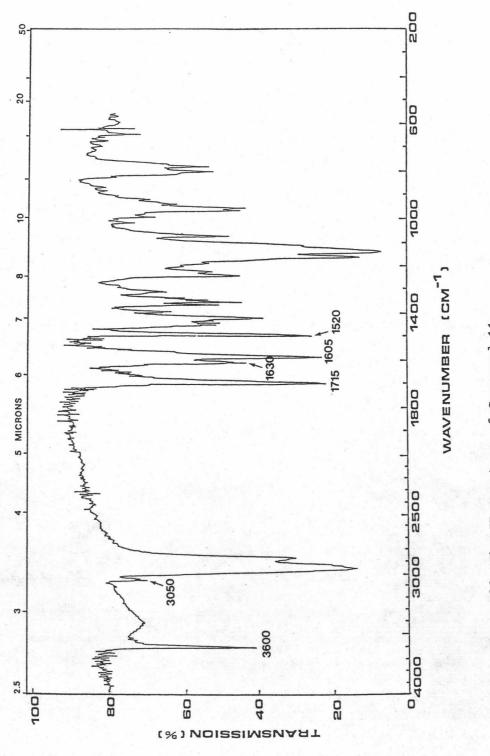


Figure 92 The IR spectrum of Compound 11

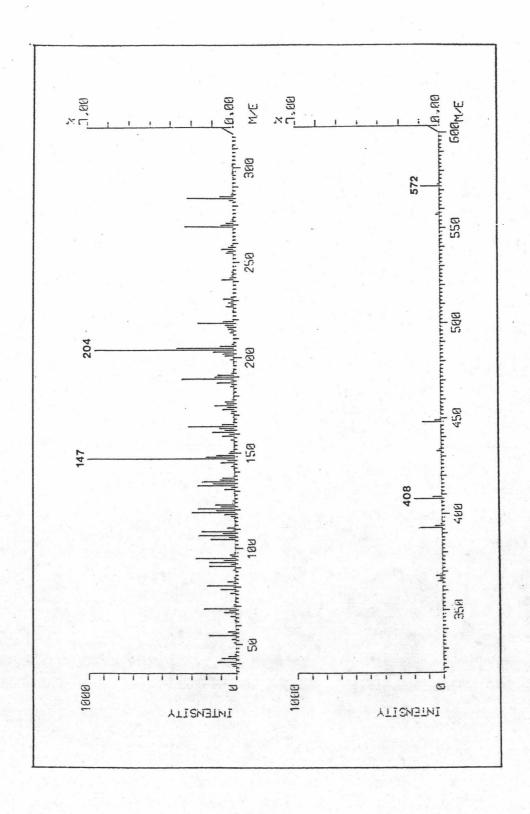
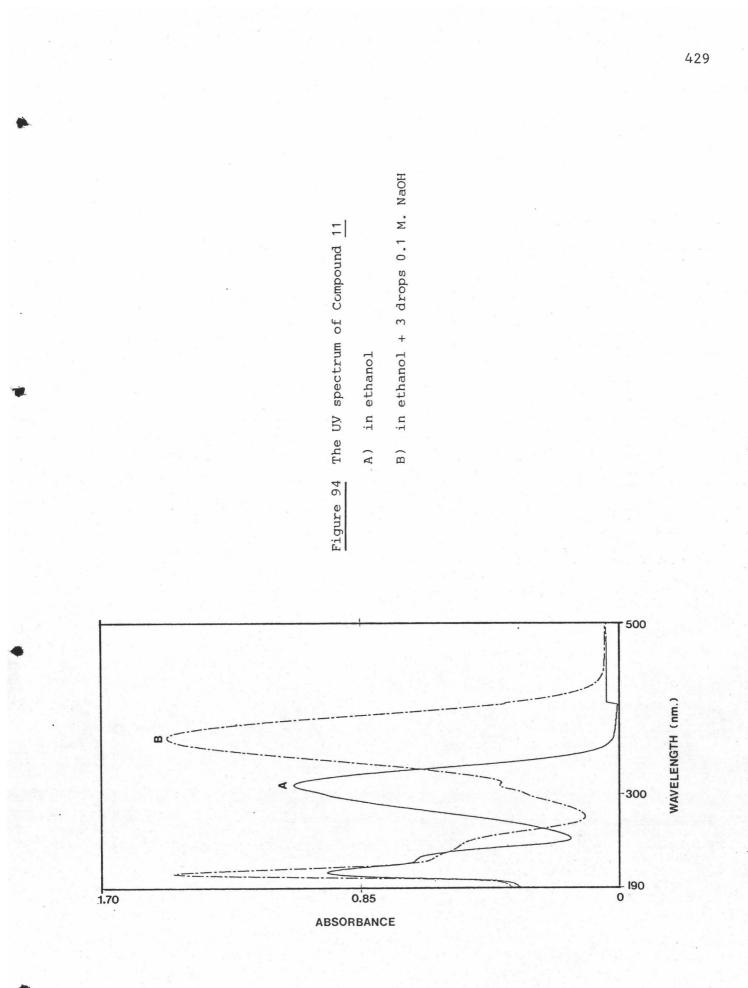


Figure 93 The mass spectrum of Compound 11



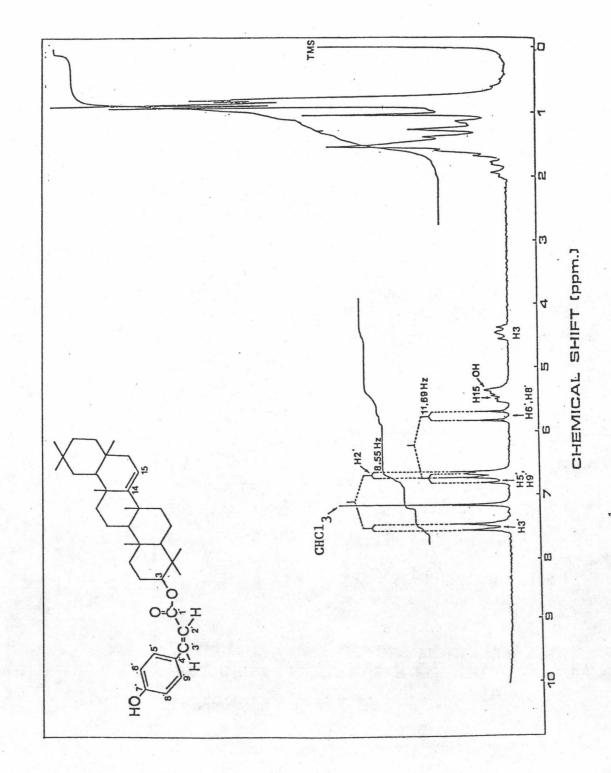
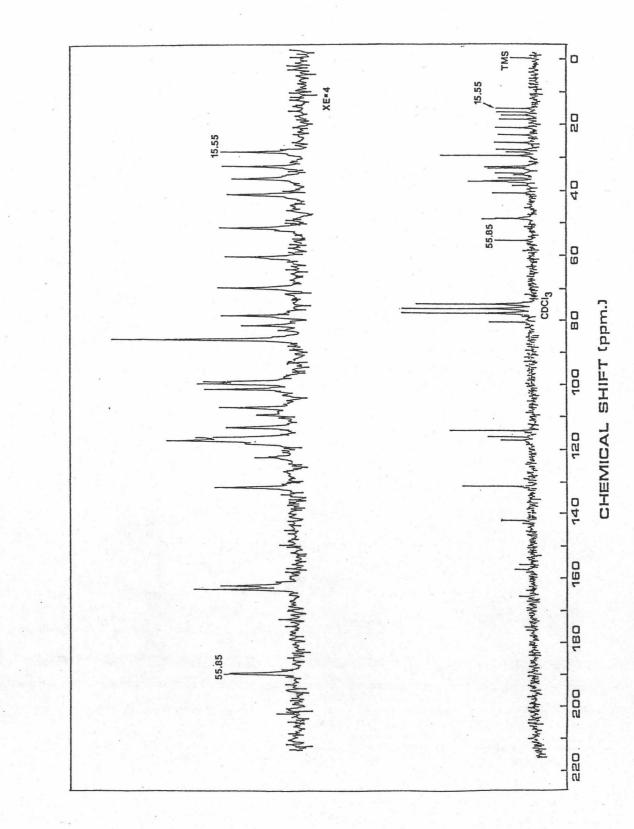
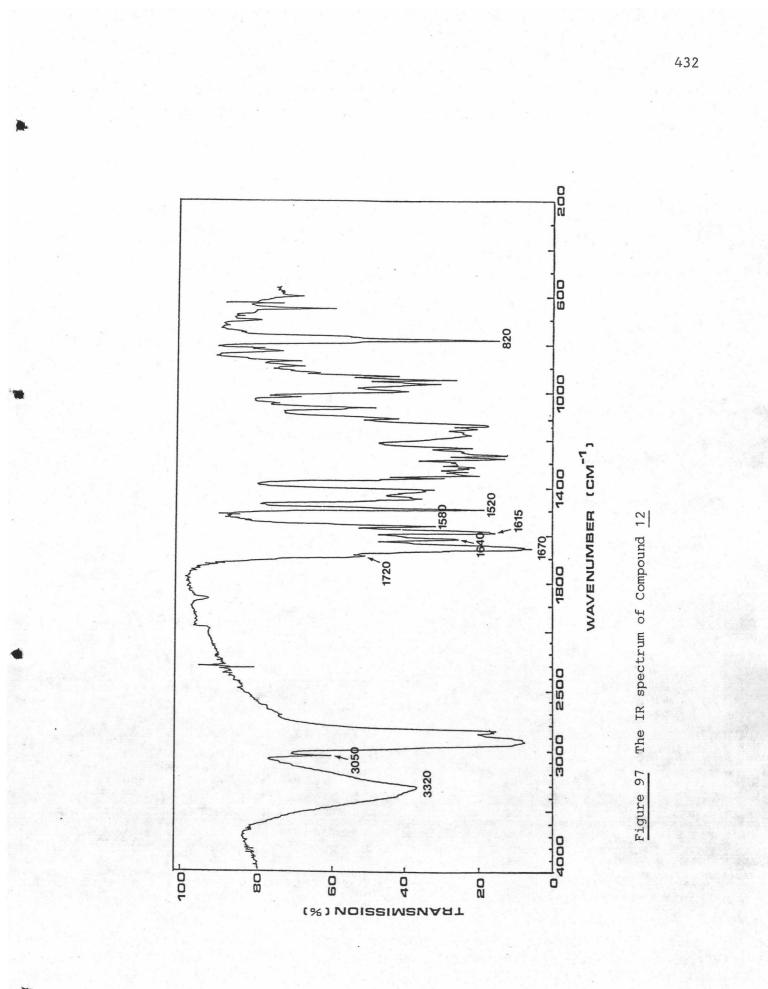


Figure 95 The ¹H NMR spectrum of Compound 11



The 13 C NMR spectrum of Compound $\frac{11}{2}$ Figure 96



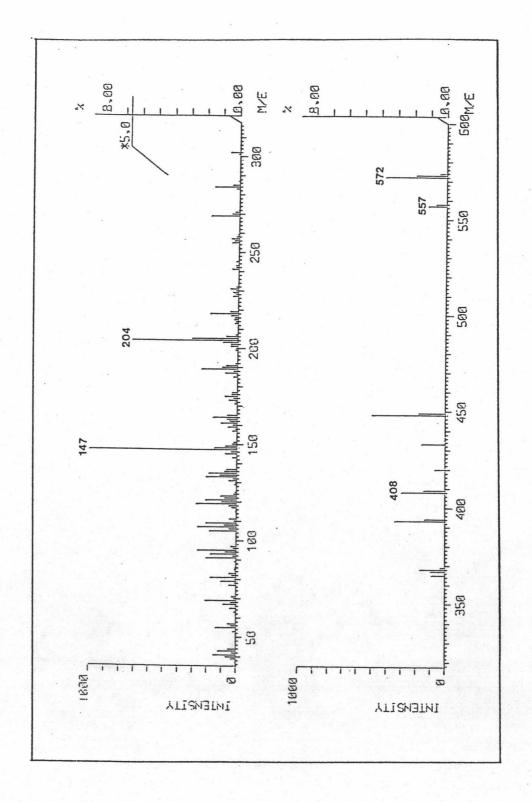
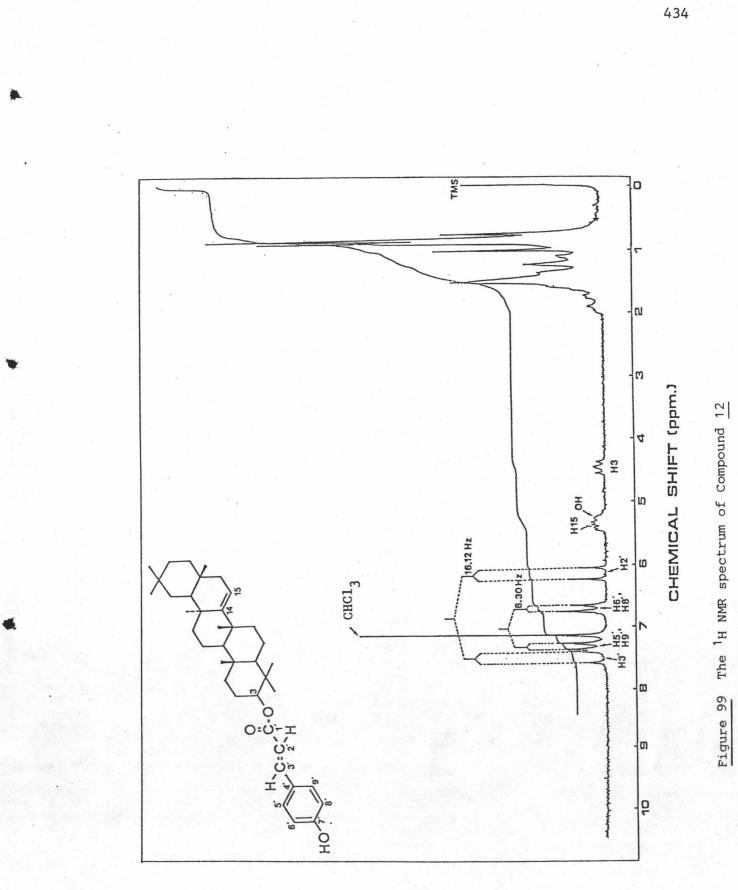


Figure 98 The mass spectrum of Compound 12



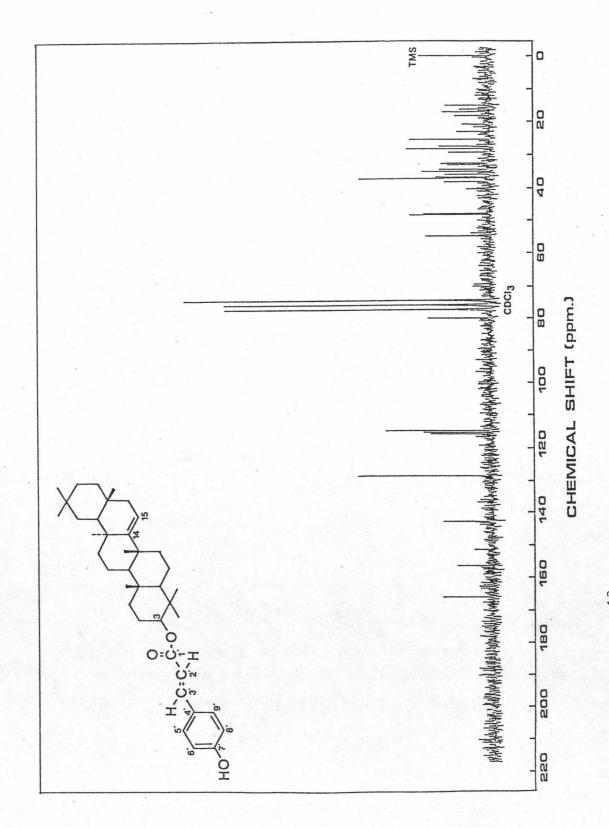
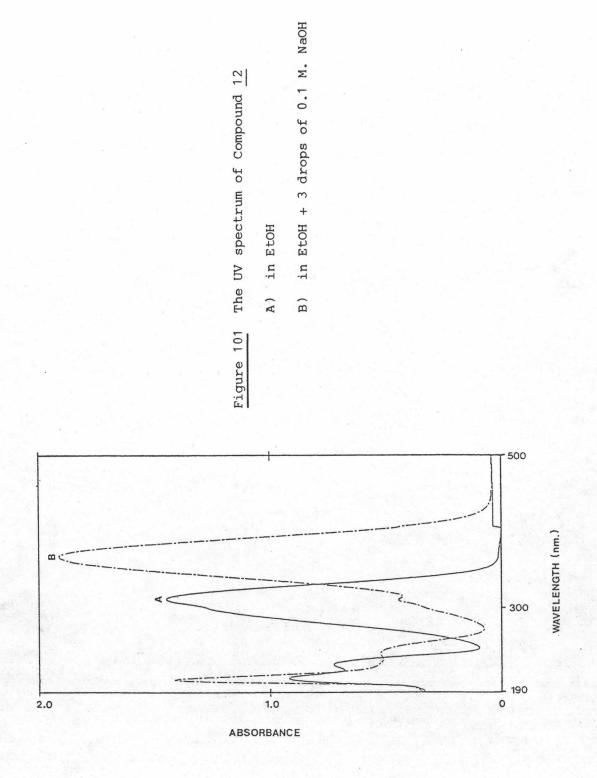


Figure 100 The ¹³C NMR spectrum of Compound <u>12</u>



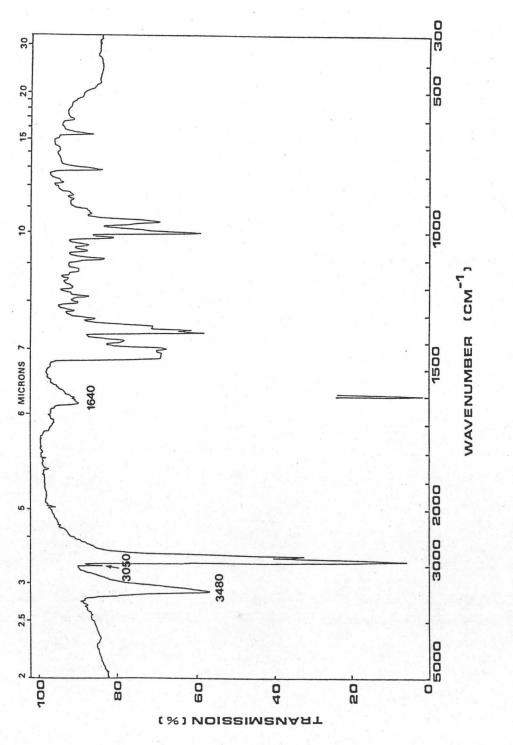
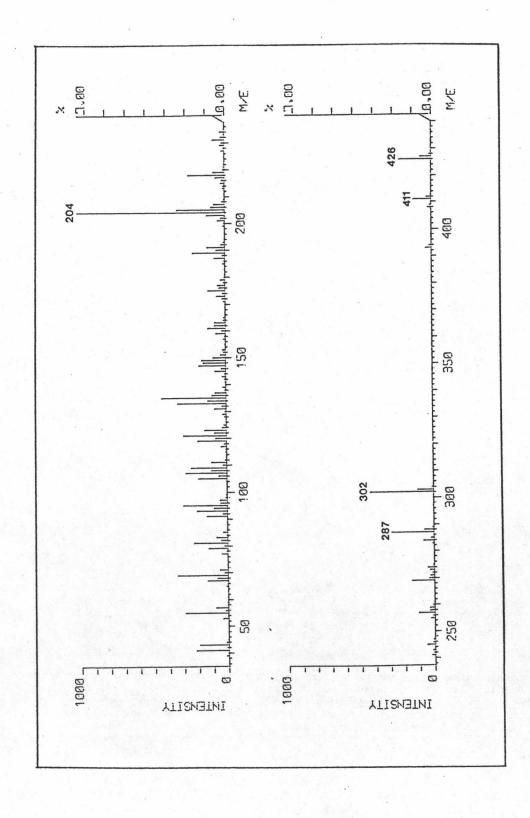
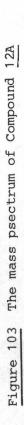
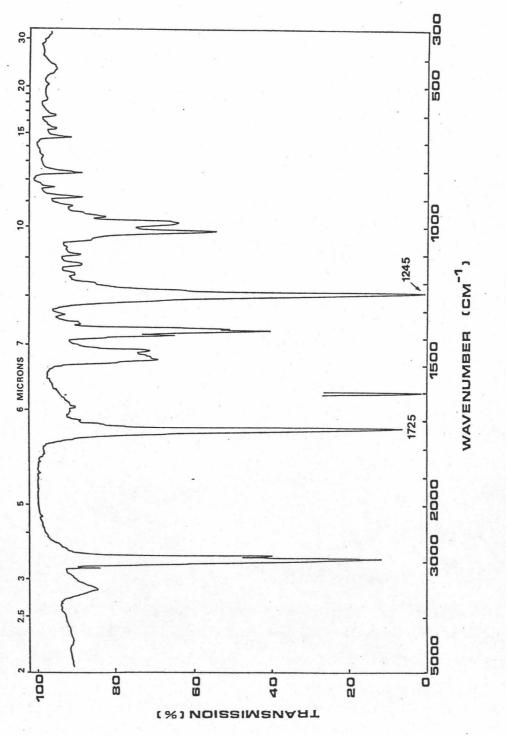


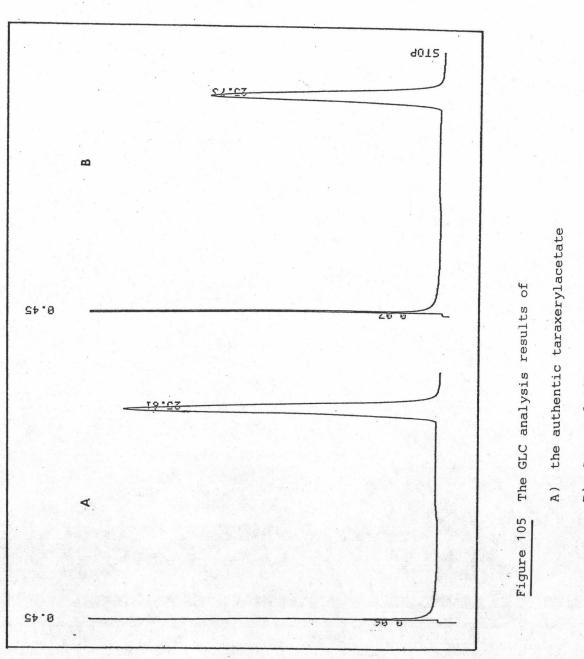
Figure 102 The IR spectrum of Compound 12A



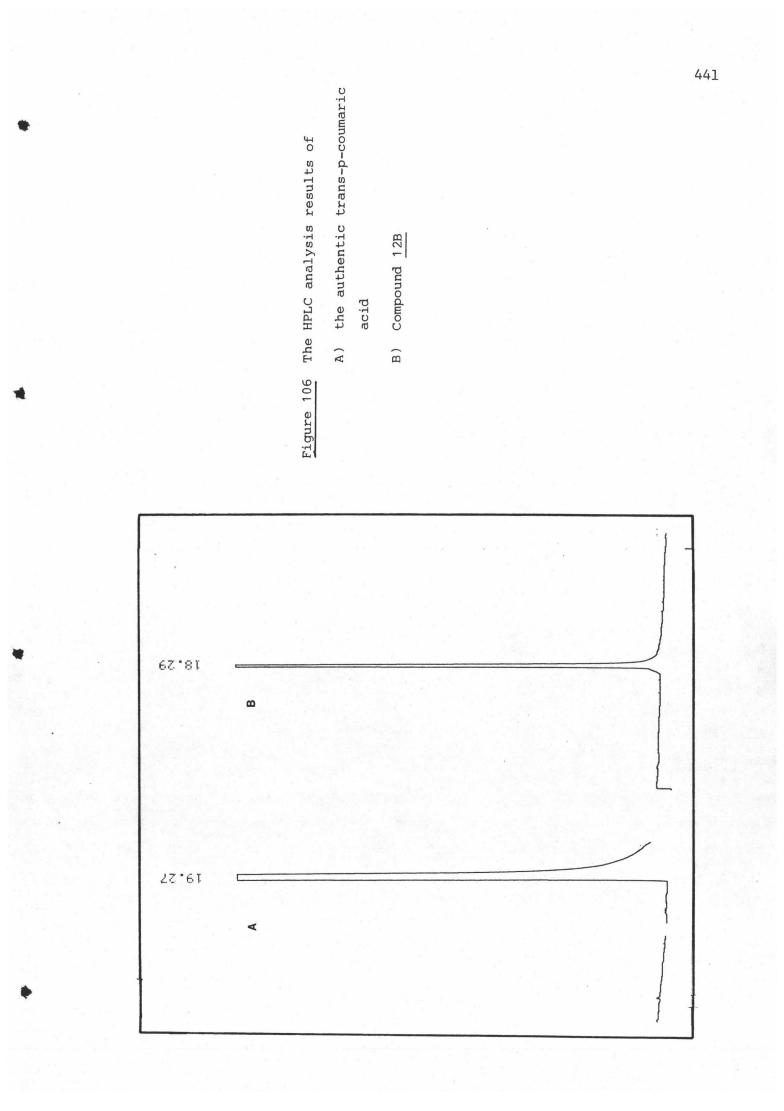


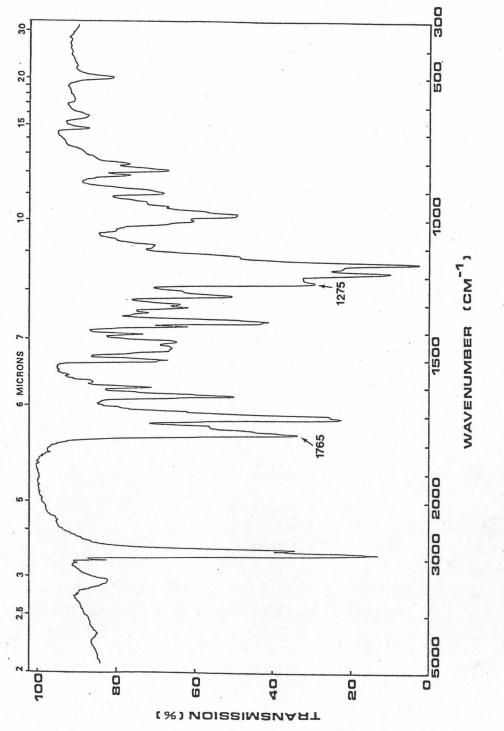






B) Compound 12A acetate







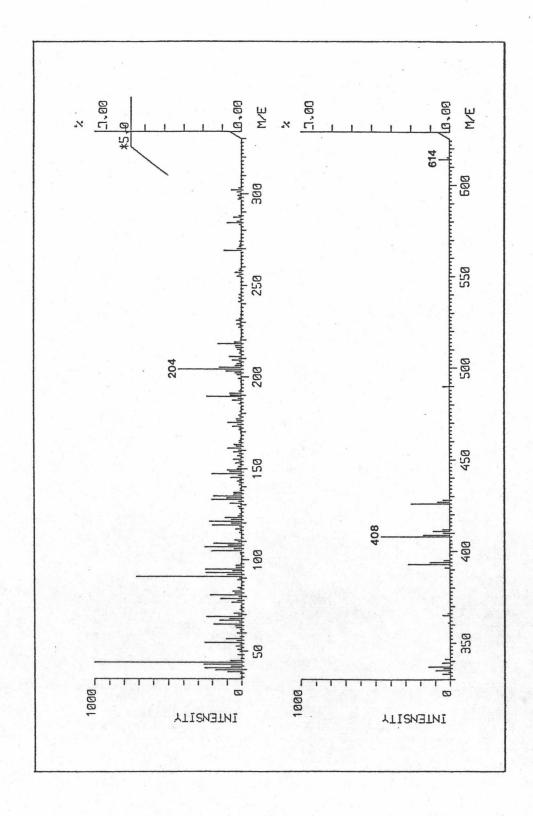
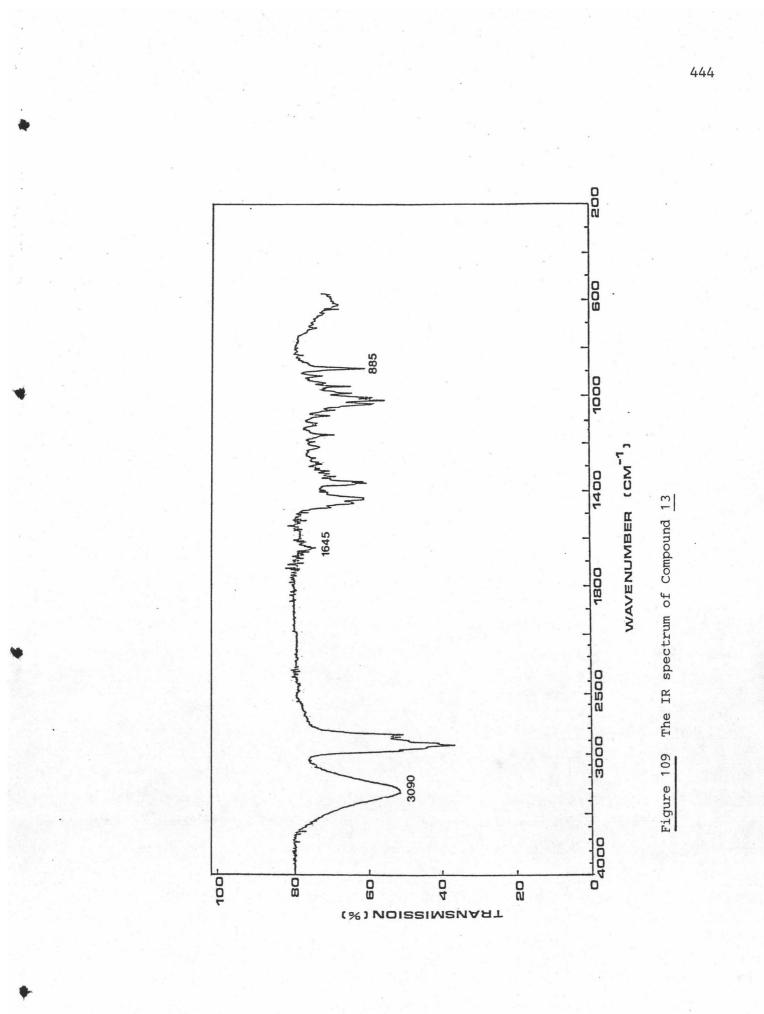


Figure 108 The mass spectrum of Compound 12 acetate



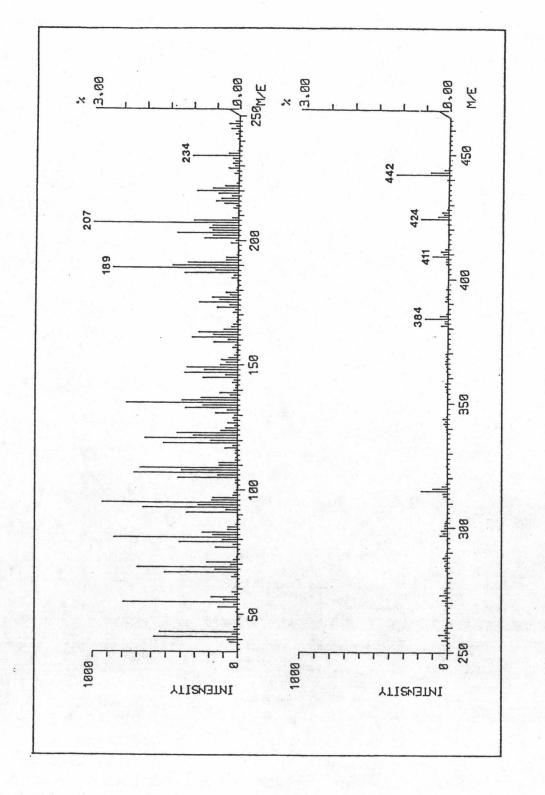


Figure 110 The mass spectrum of Compound 13

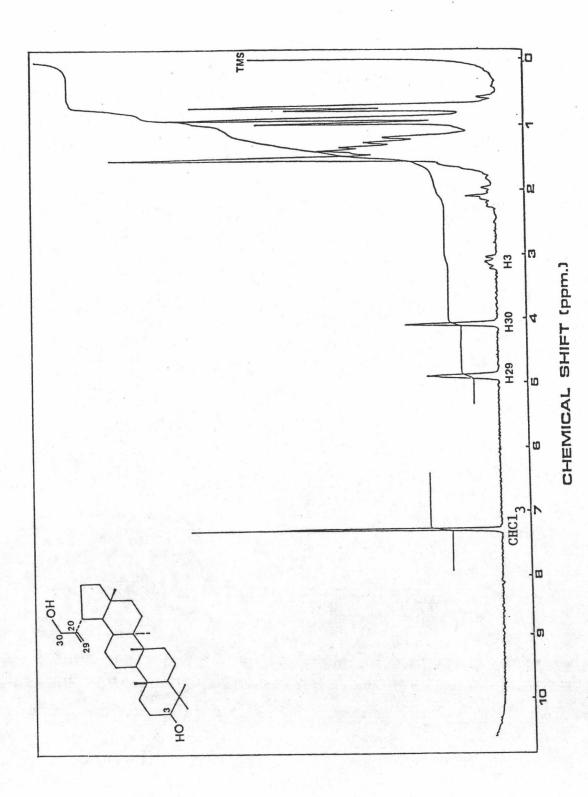
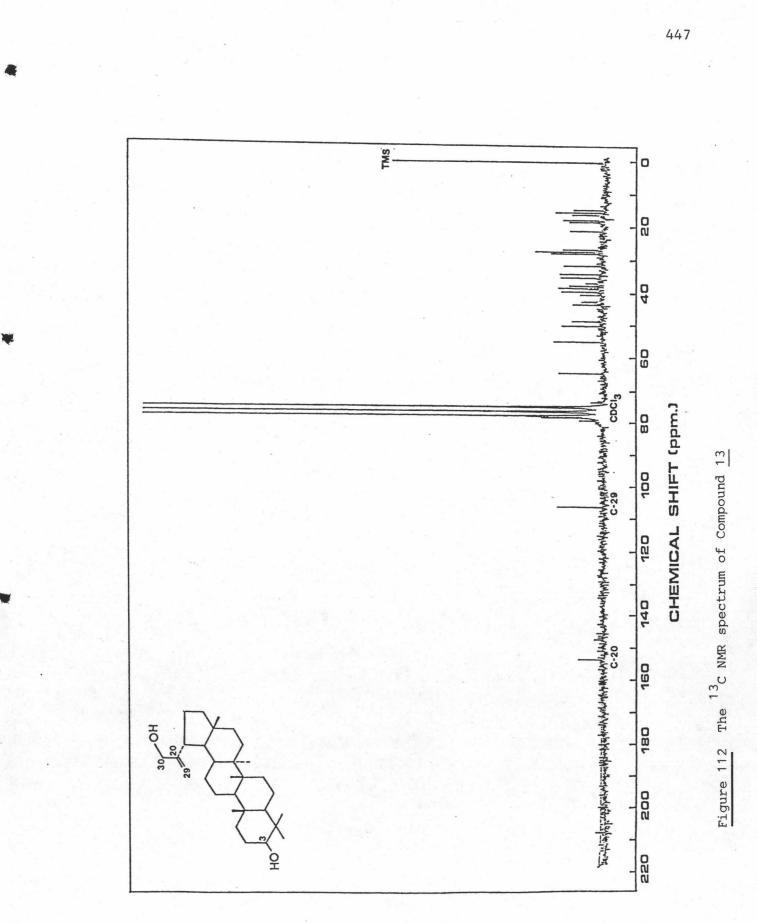
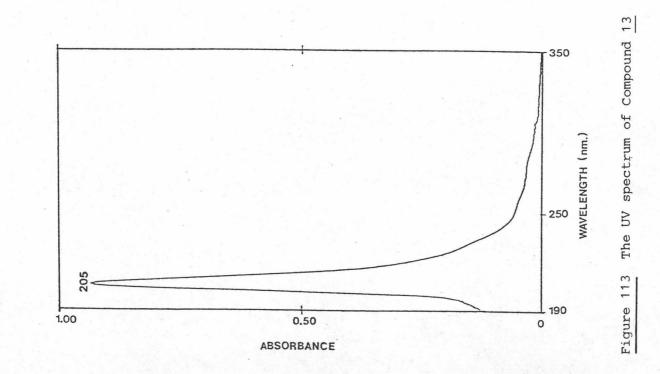


Figure 111 The ¹H NMR spectrum of Compound 13



£.



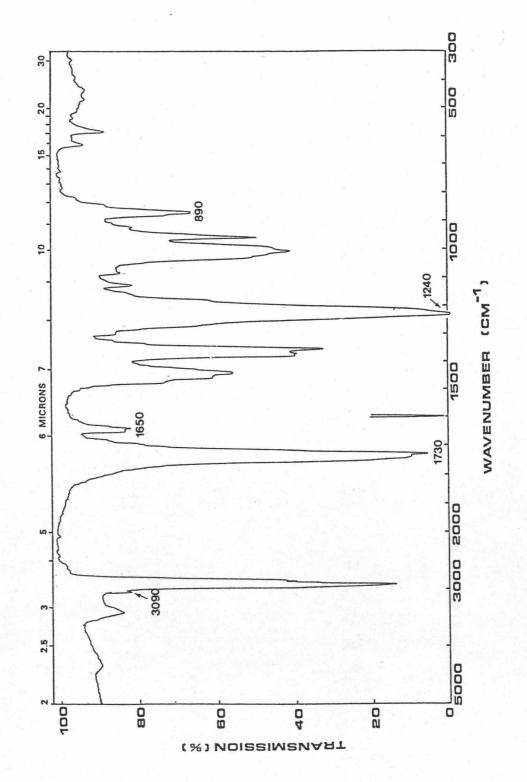
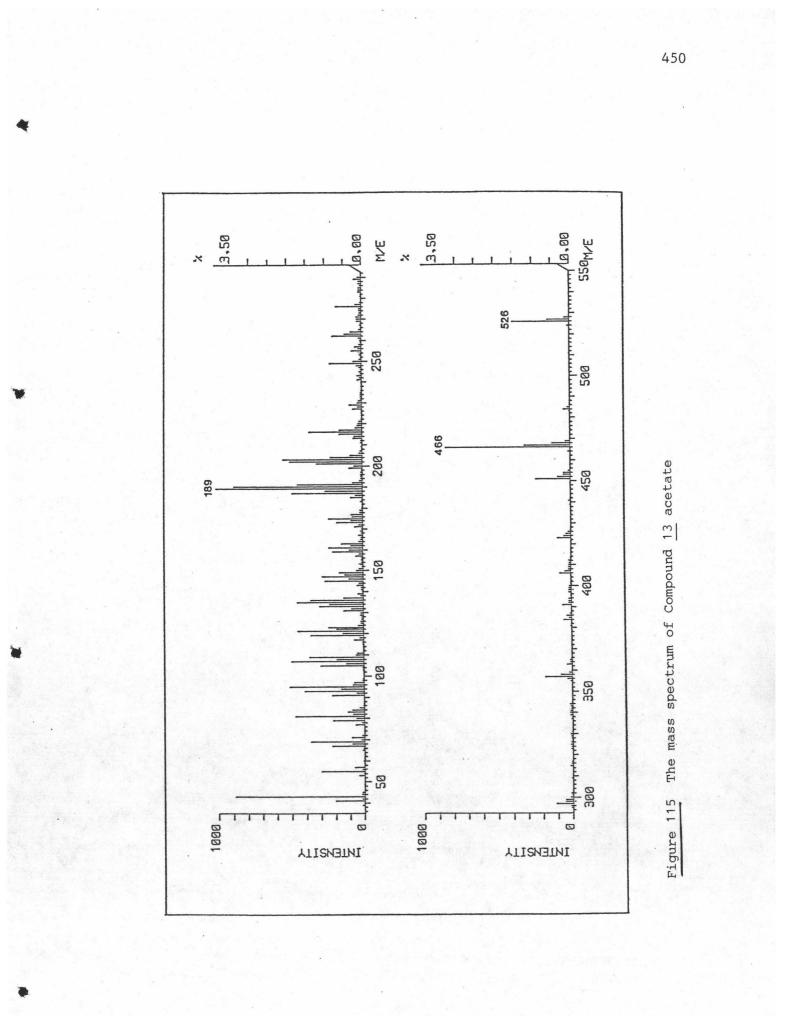
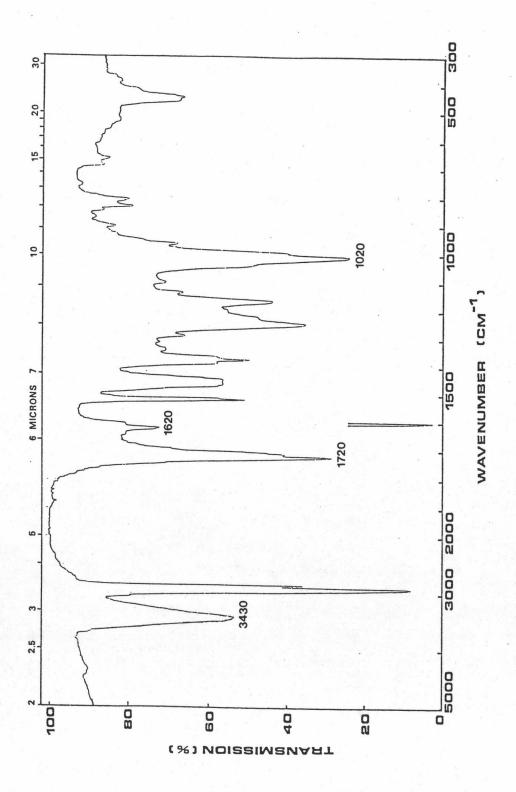
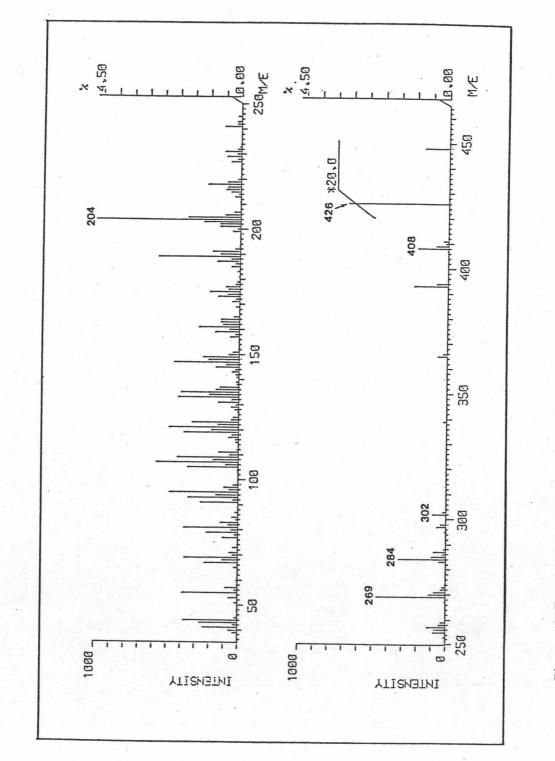


Figure 114 The IR spectrum of Compound 13 acetate

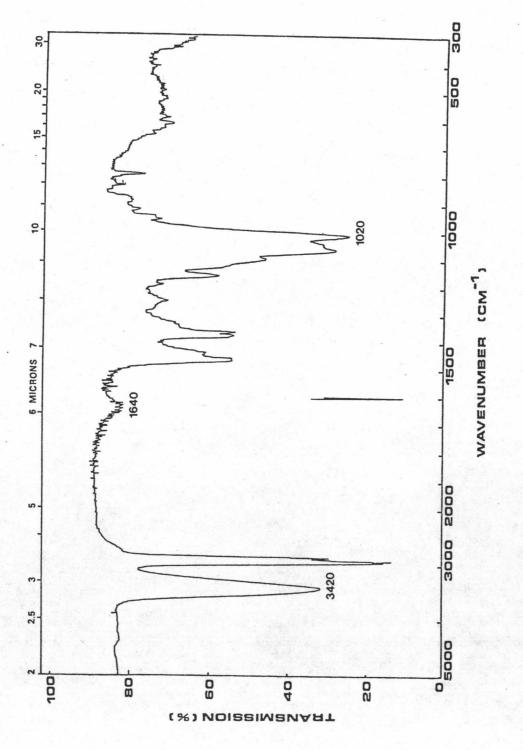




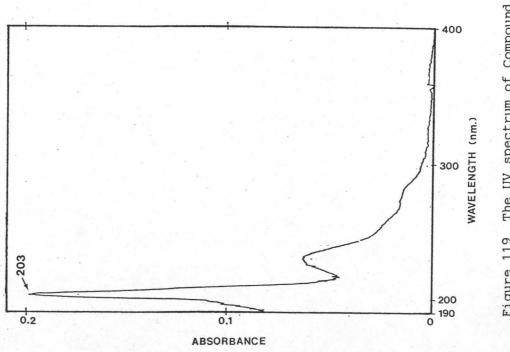


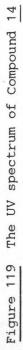


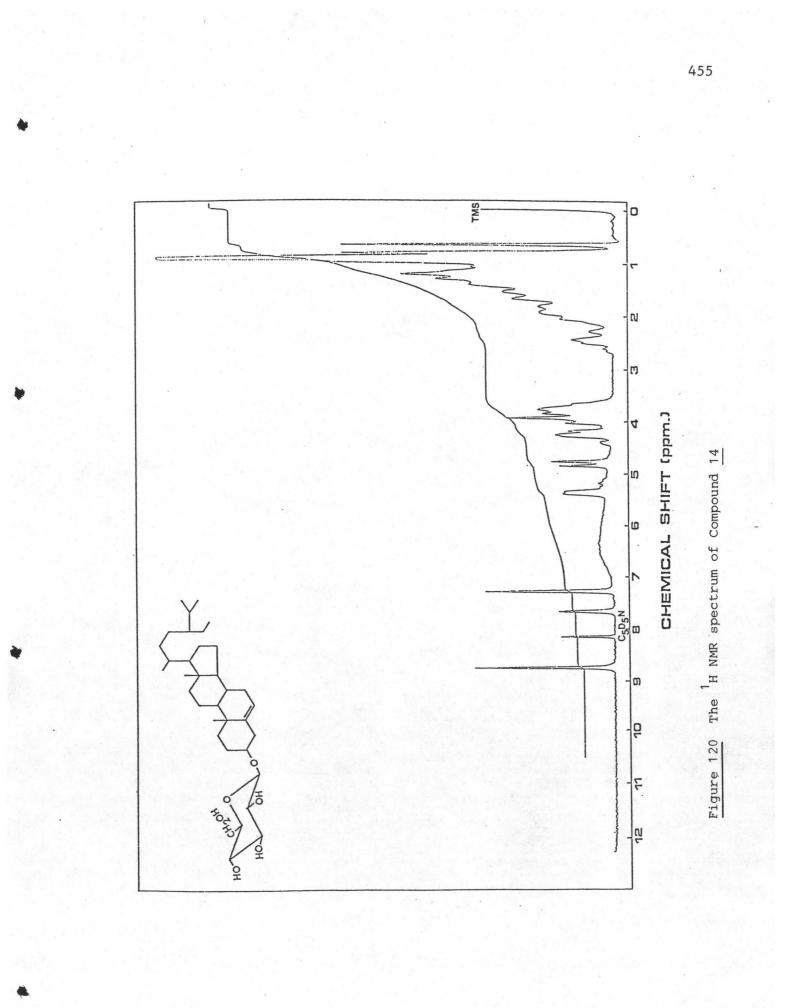


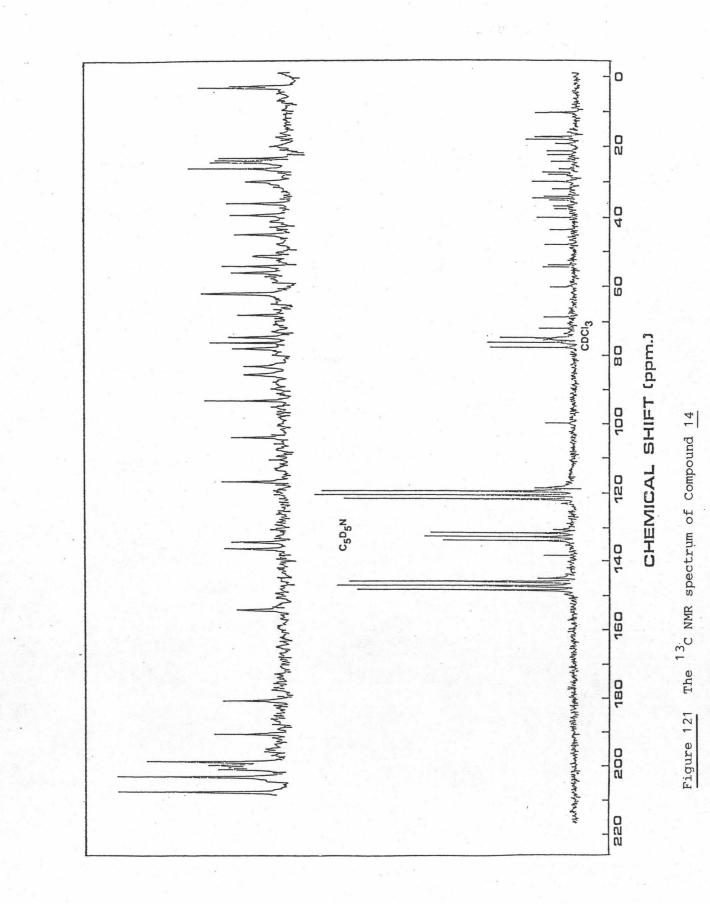


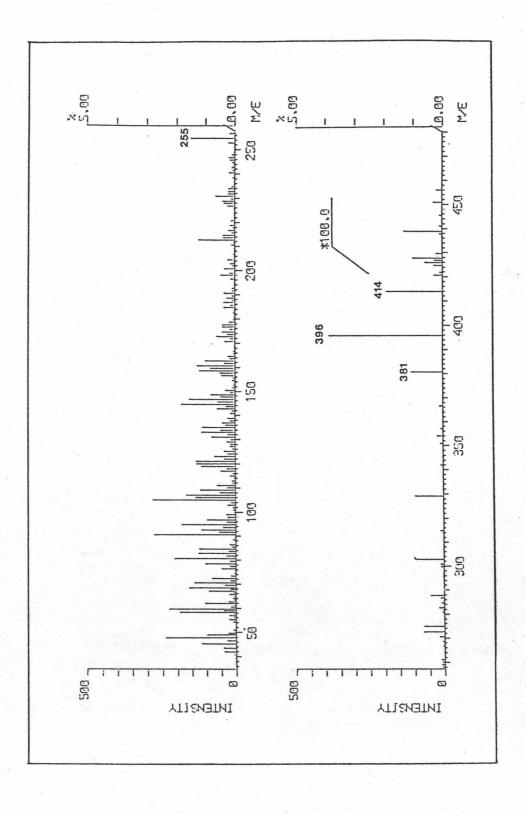




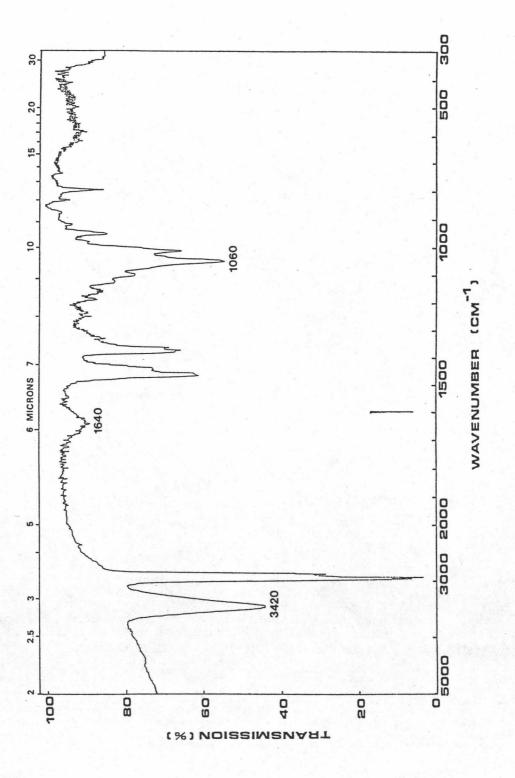




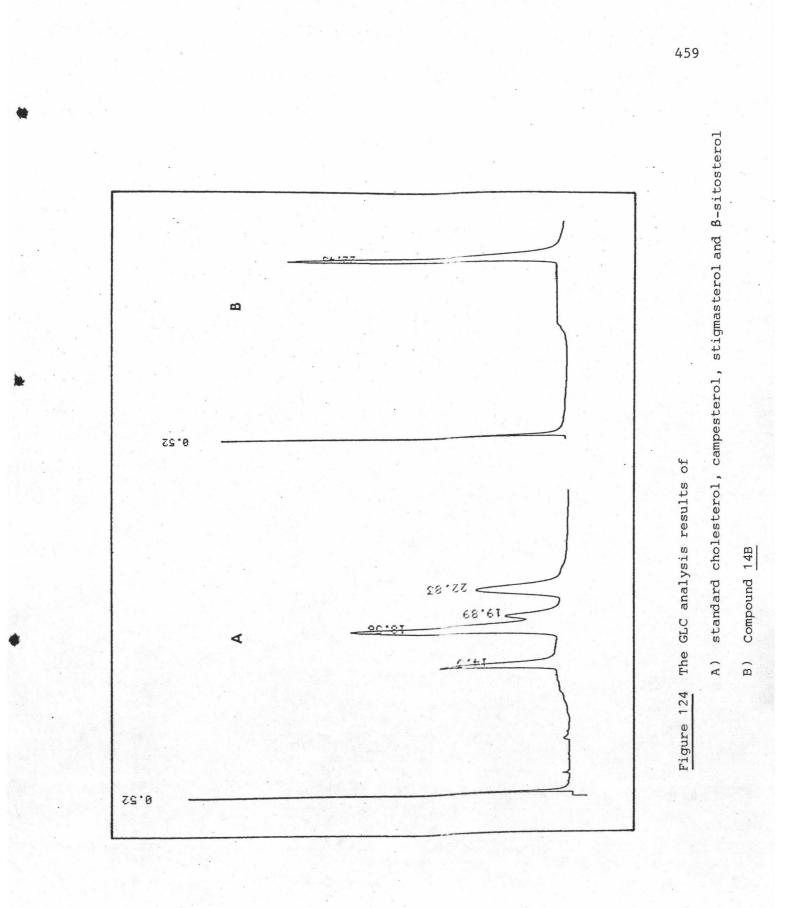


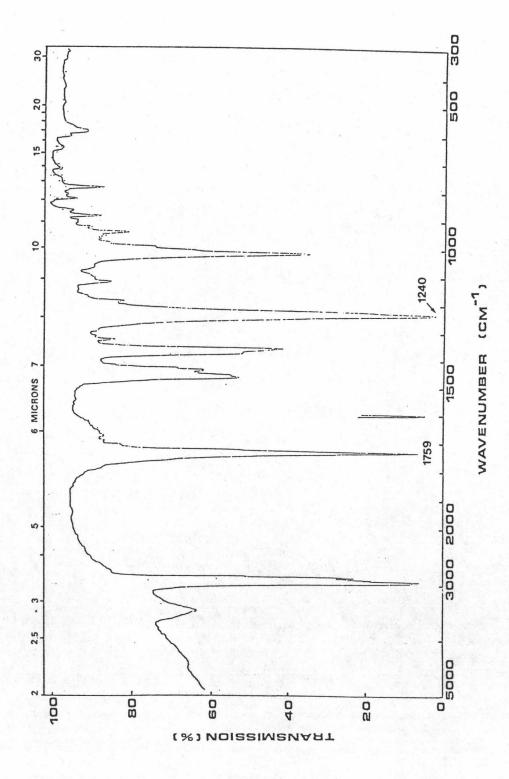














Samples	Rt (min.)
solvent (H O)	0 L
rhamnose	3.11
xylose	3.72
arabinose	4.21
fructose	4.64
glucose	5.69
galactose	6.06
sucrose	8.66
maltose	11.16

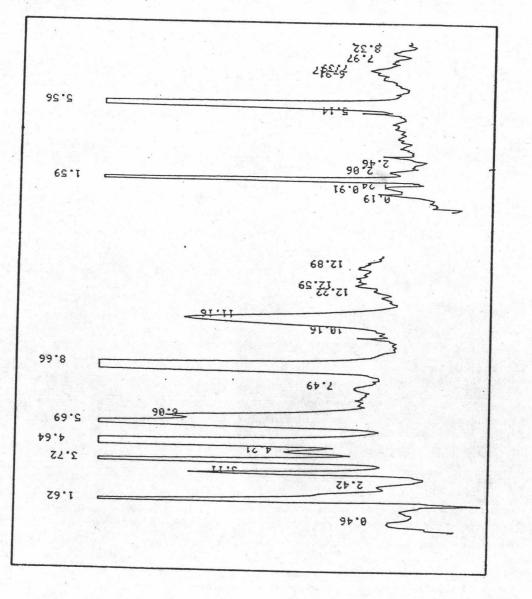
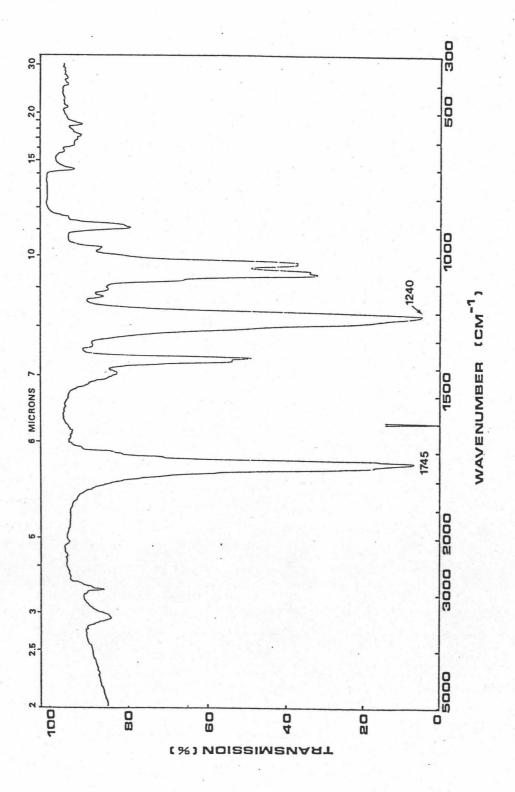
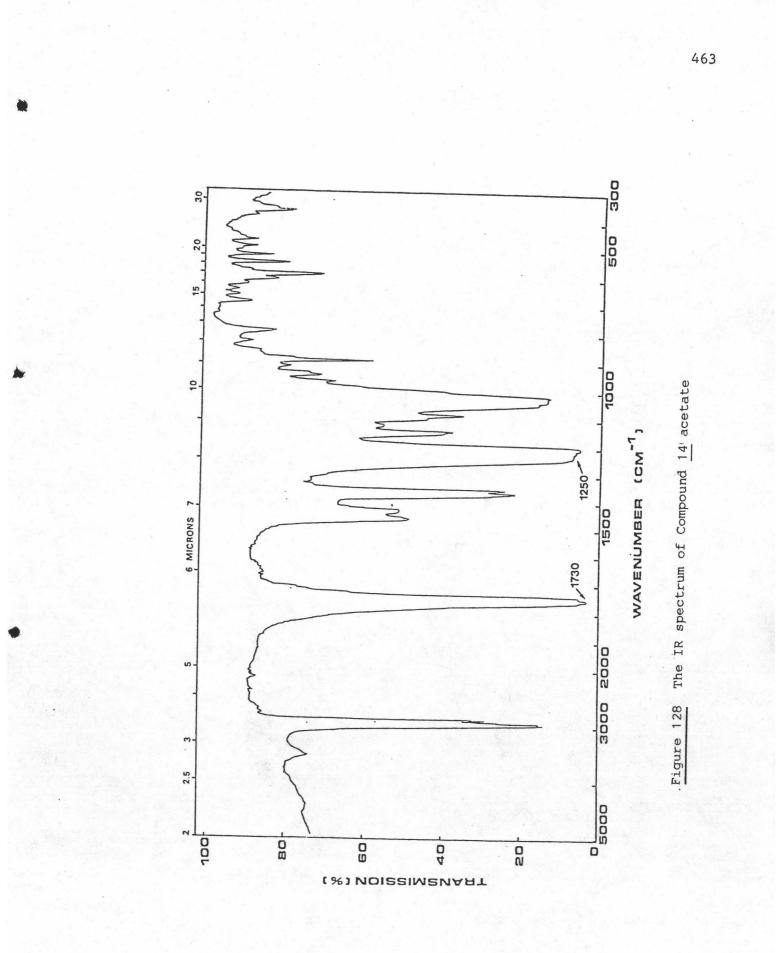


Figure 126 The HPLC analysis results of Compound 14B







L .

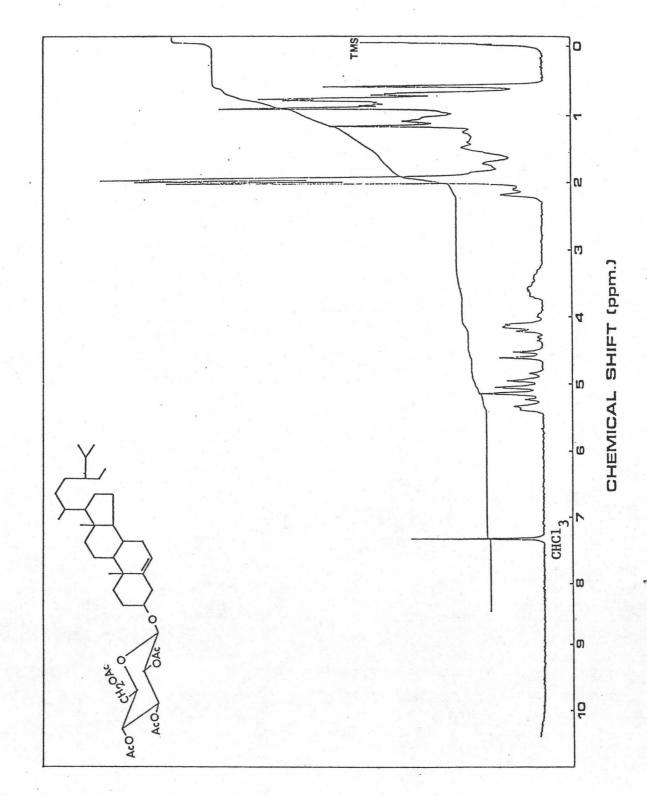


Figure 129 The ¹H NMR spectrum of Compound <u>14</u> acetate

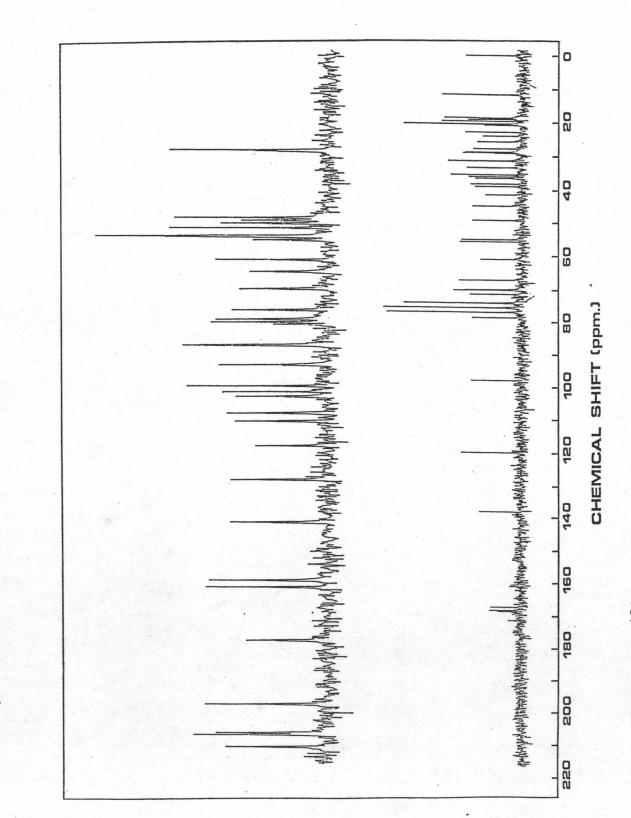


Figure 130 The ¹³C NMR spectrum of Compound <u>14</u> acetate

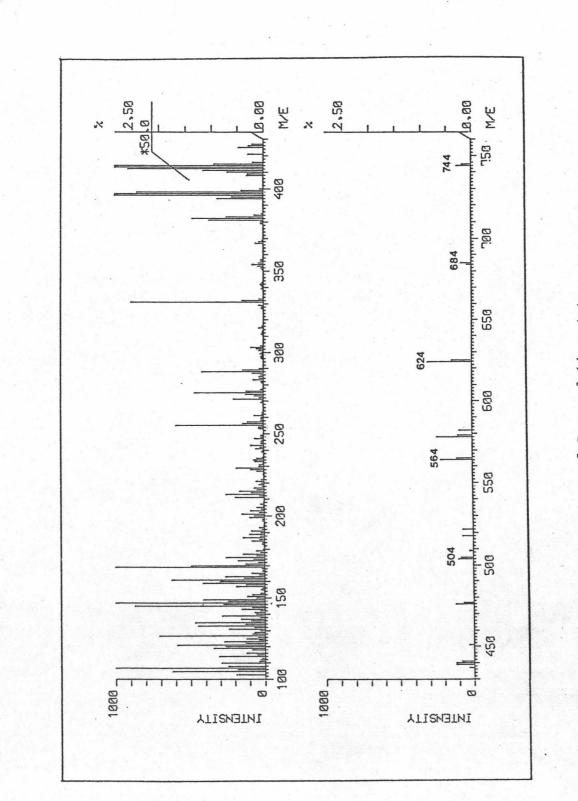
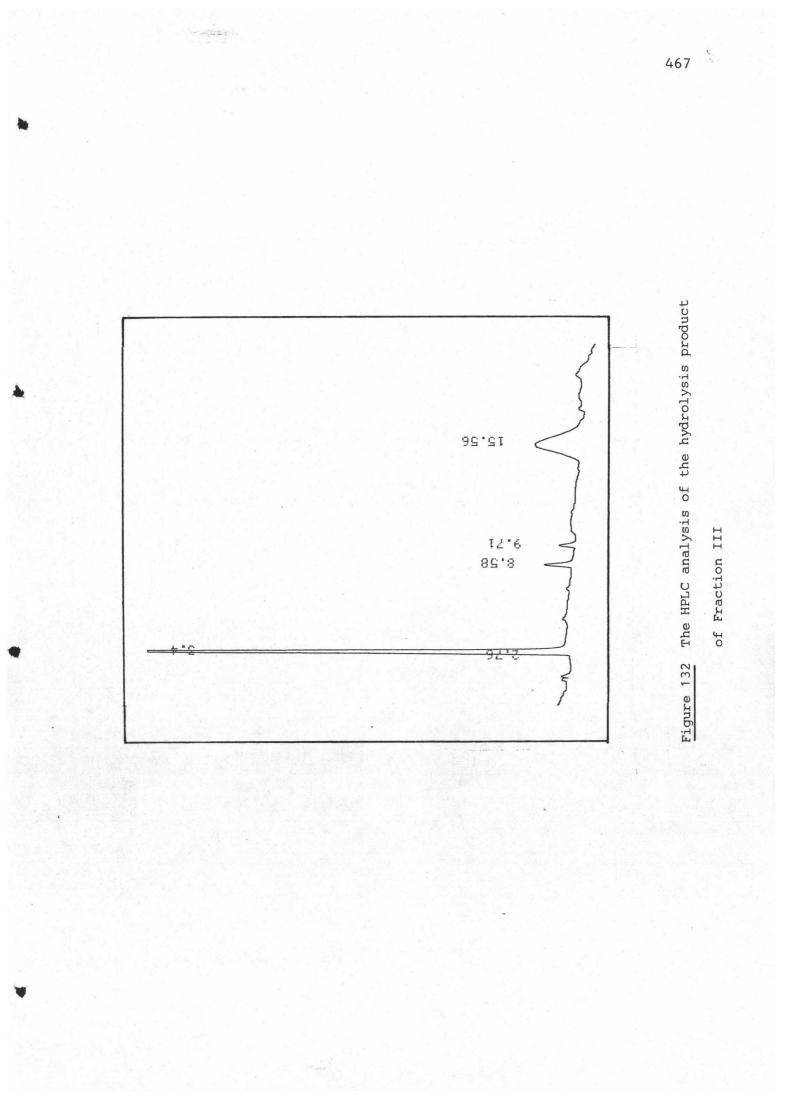
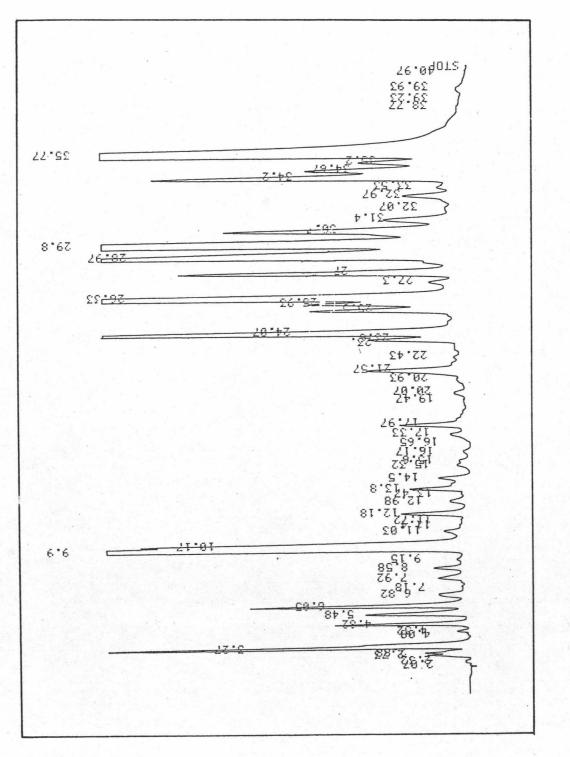
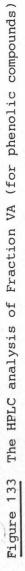


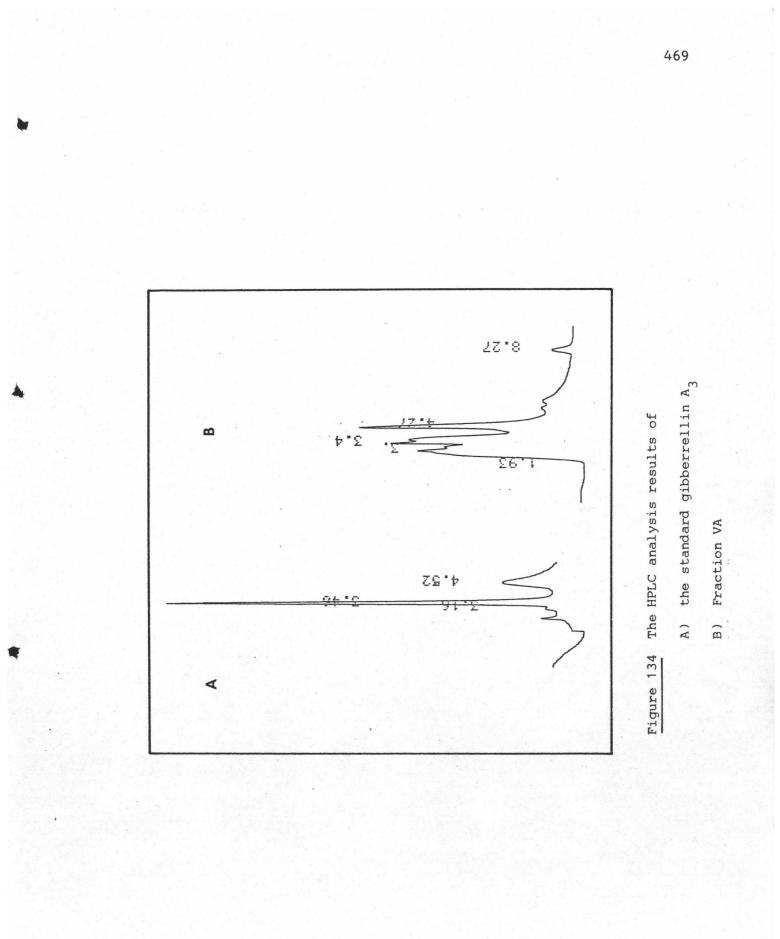
Figure 131 The mass spectrum of Compound 14 acetate





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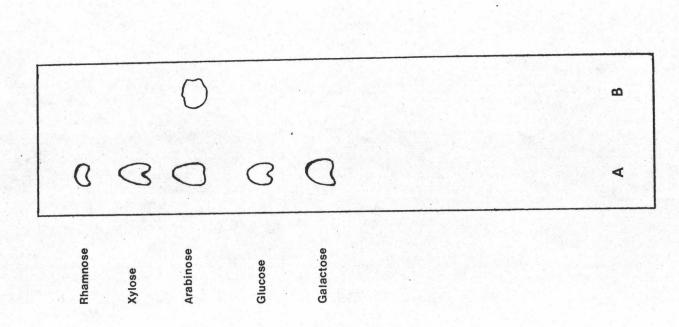


Figure 135 The paper chromatogram of

A) The standard sugars

B) Fraction VIA

Samples	Rt (min.)
solvent (H,O)	1.62
rhamnose	3.11
xylose	3.72
arabínose	4.21
fructose	4.64
glucose	5.69
galactose	6.06
sucrose	8.66
maltose	11.16

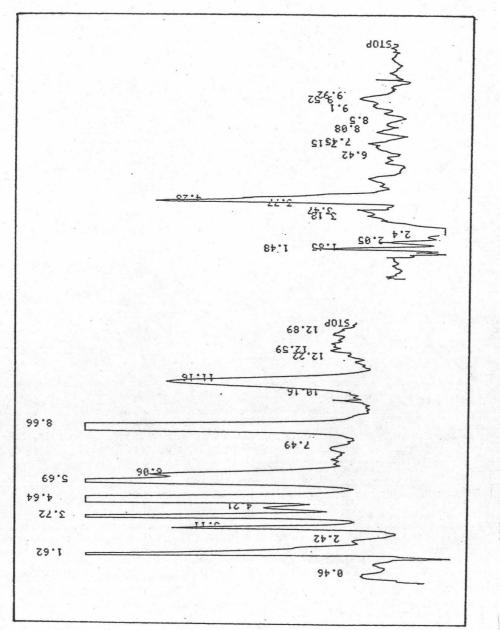


Figure 136 The HPLC analysis results of Fraction VIA

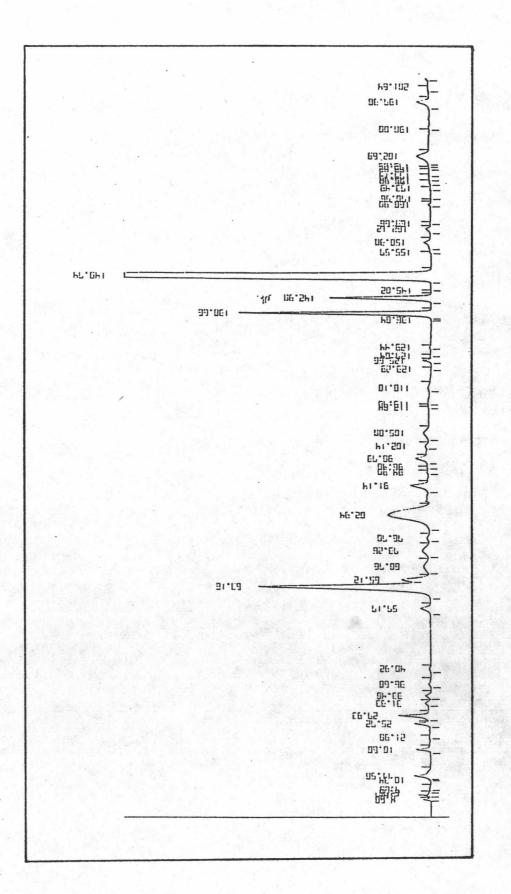


Figure 137 The amino analysis results of Fraction VIA

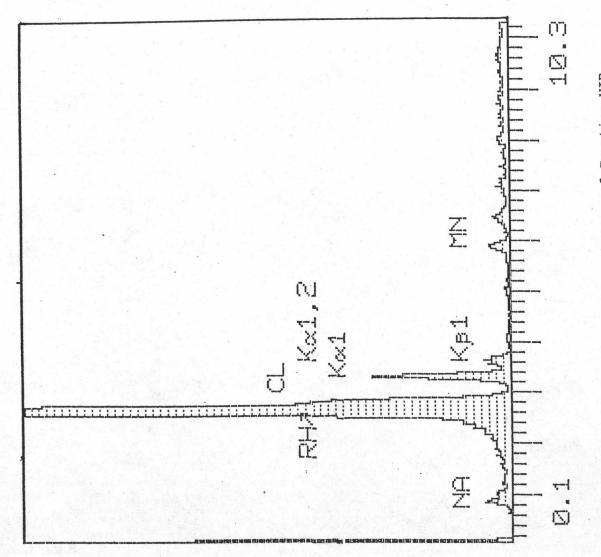
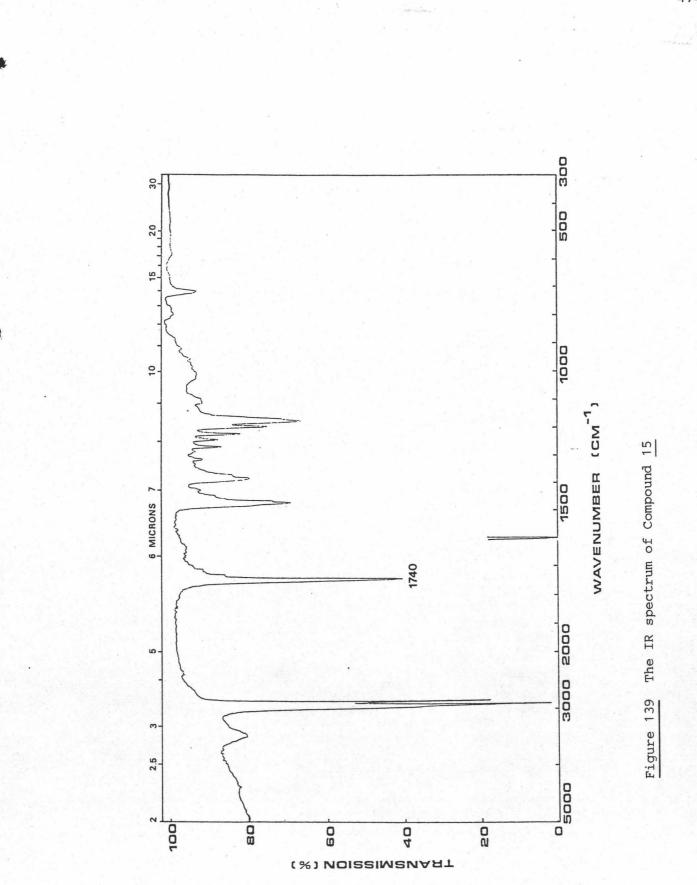
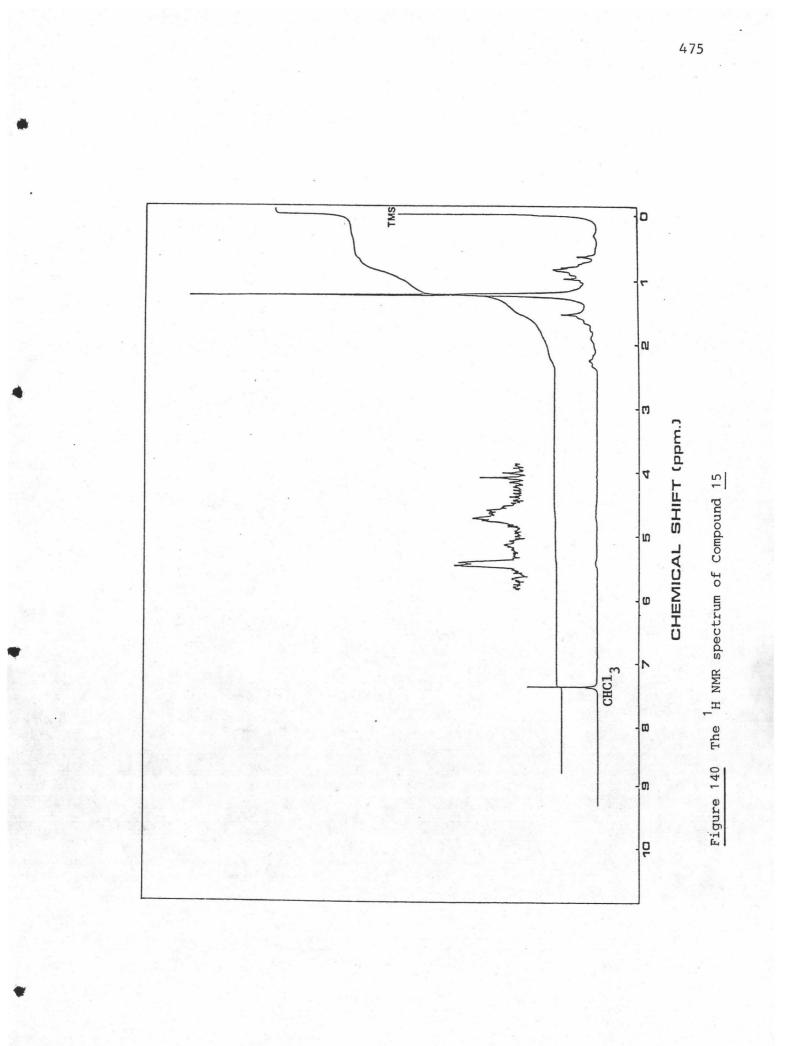
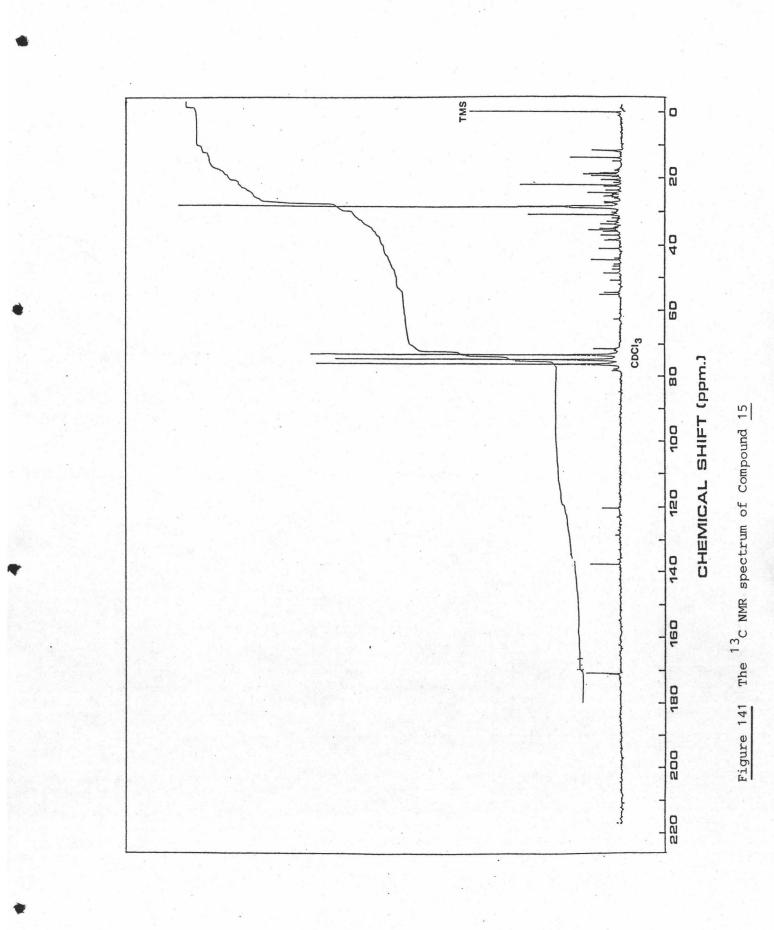
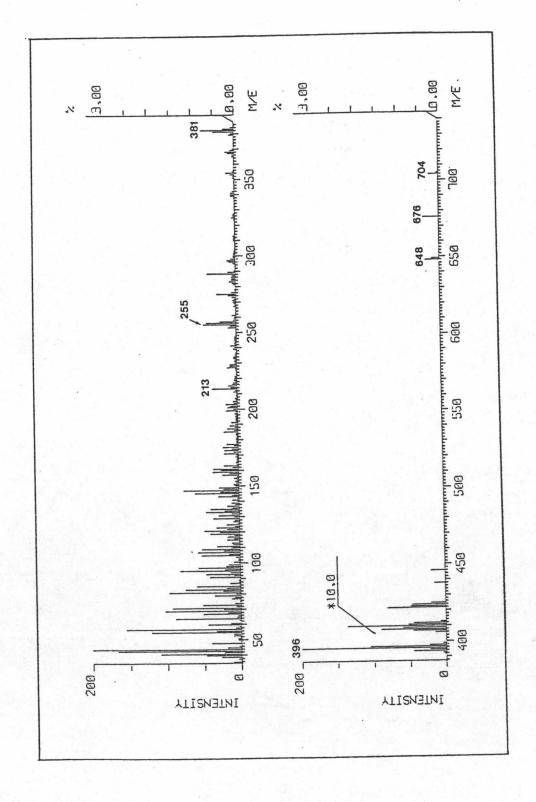


Figure 138 The X-ray fluoresence spectrum of Fraction VIB











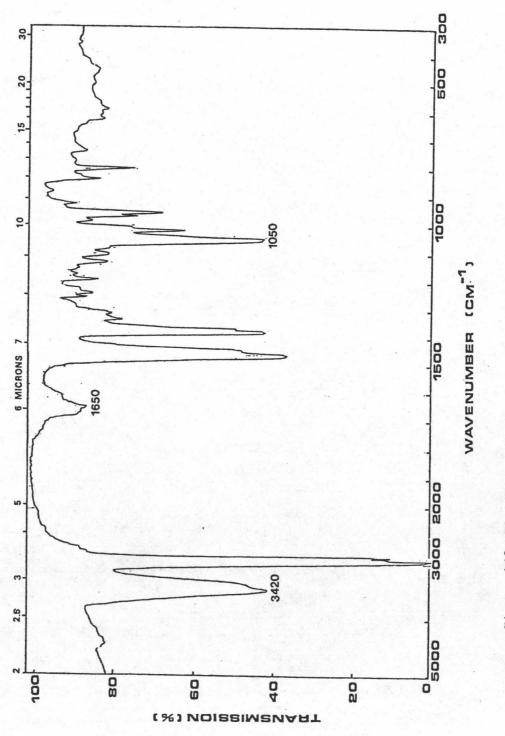
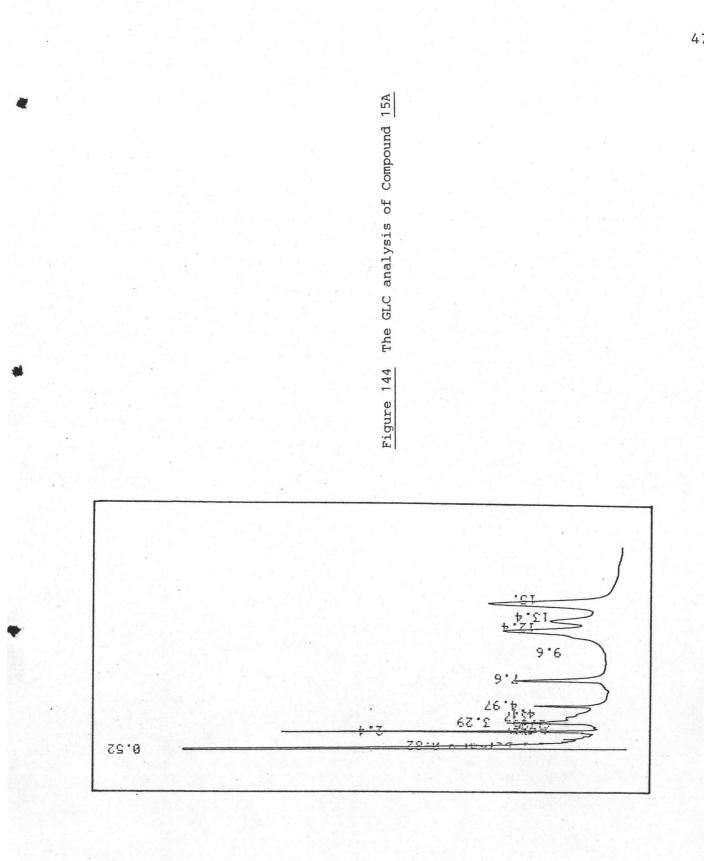
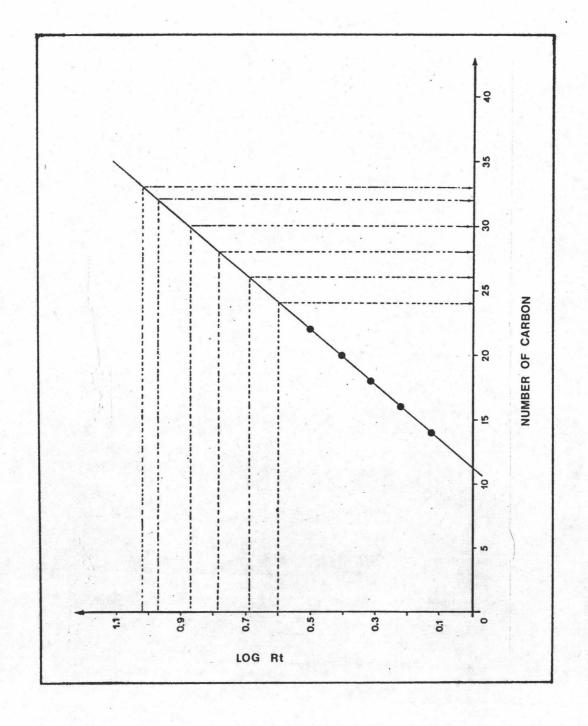
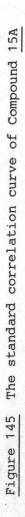
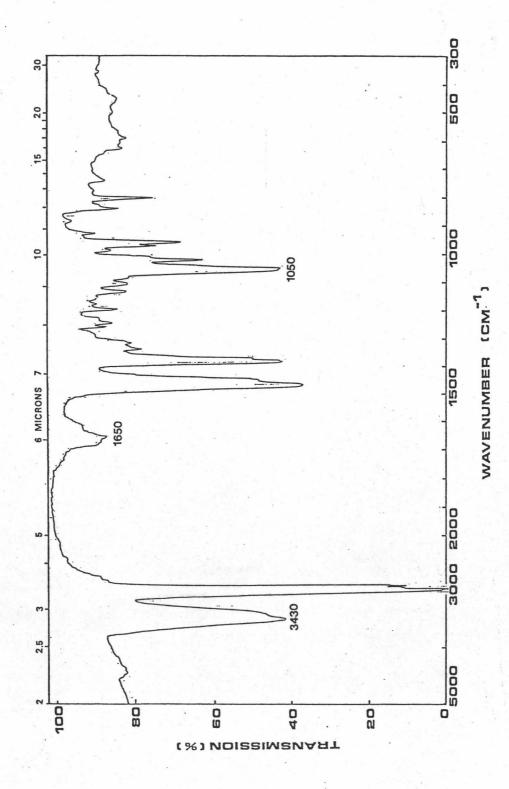


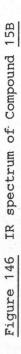
Figure 143 The IR spectrum of Compound 15A











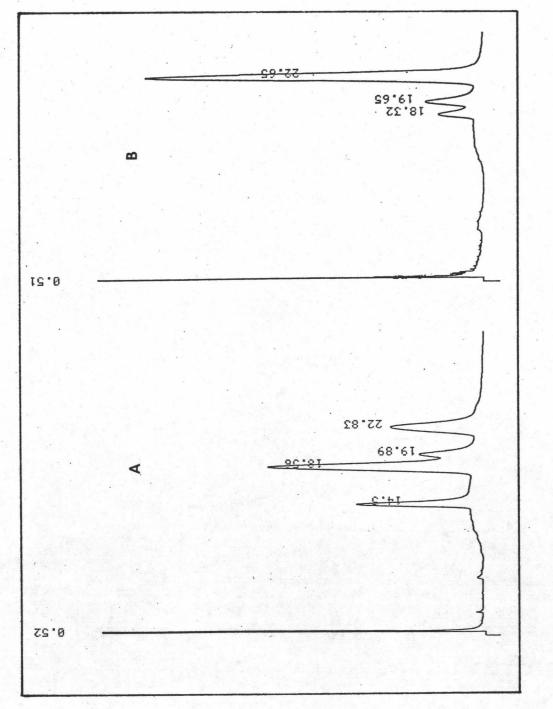
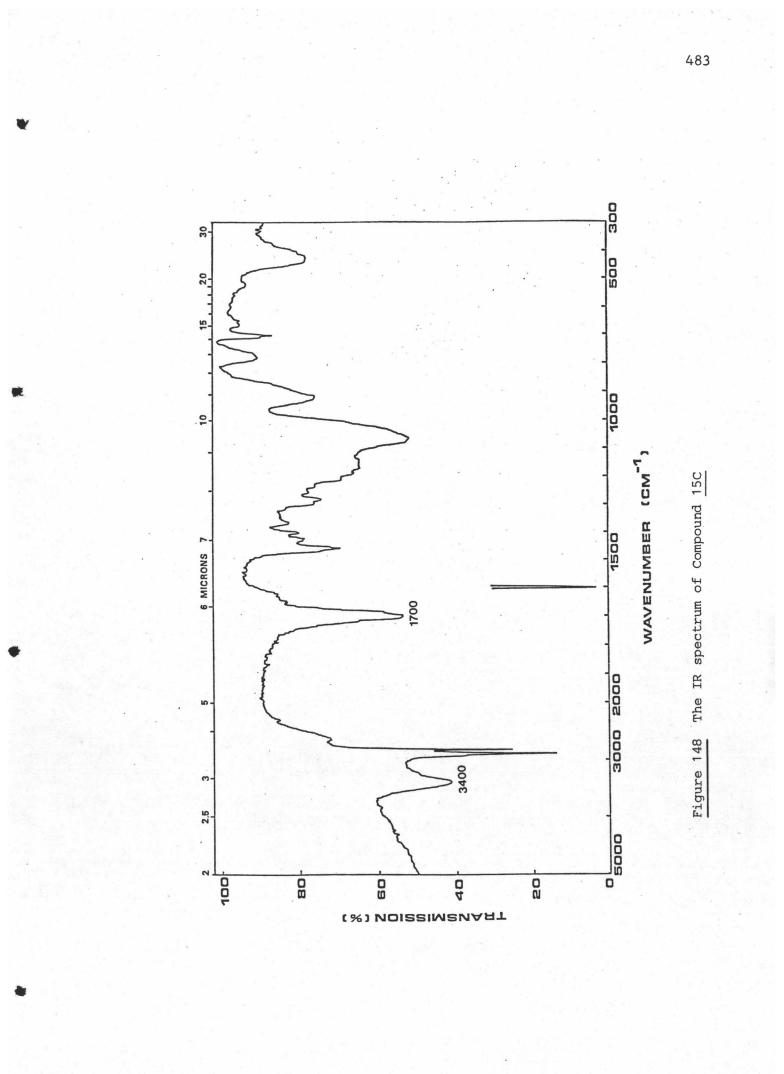
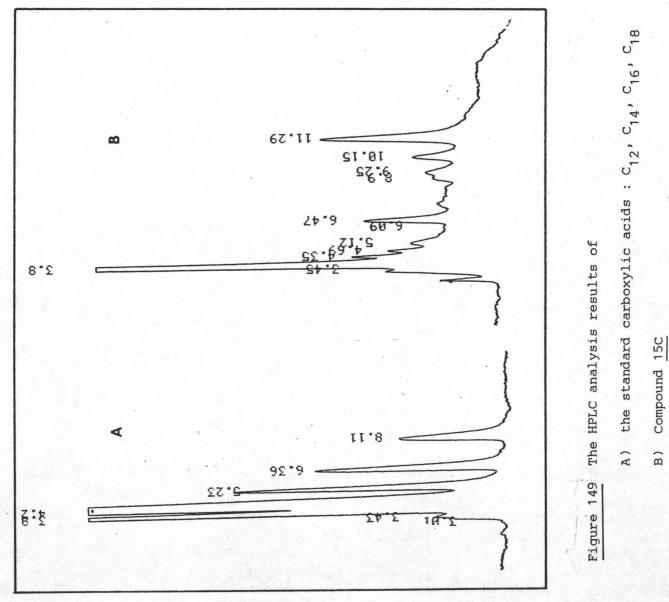
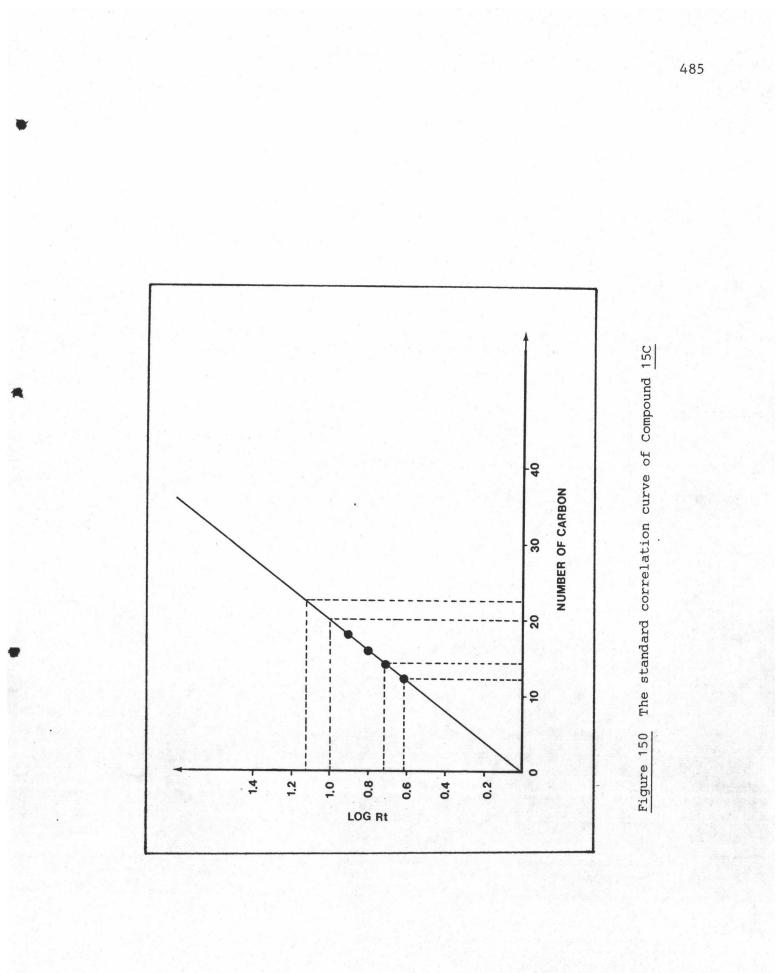
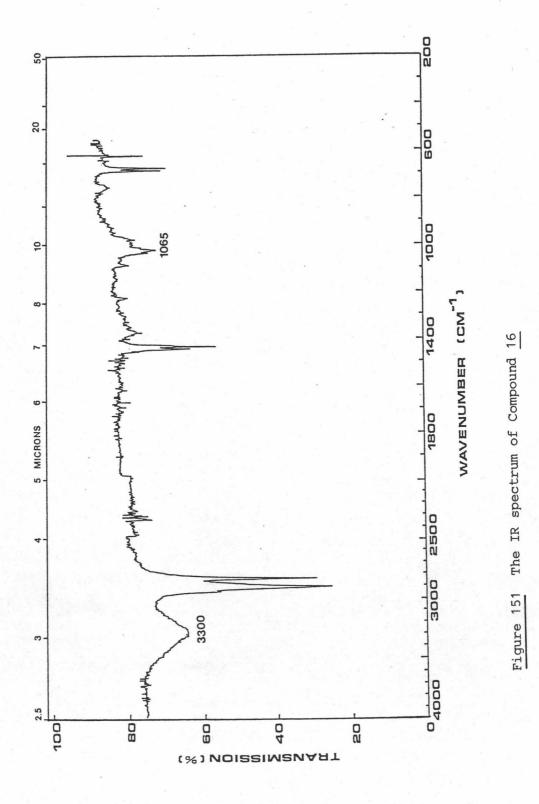


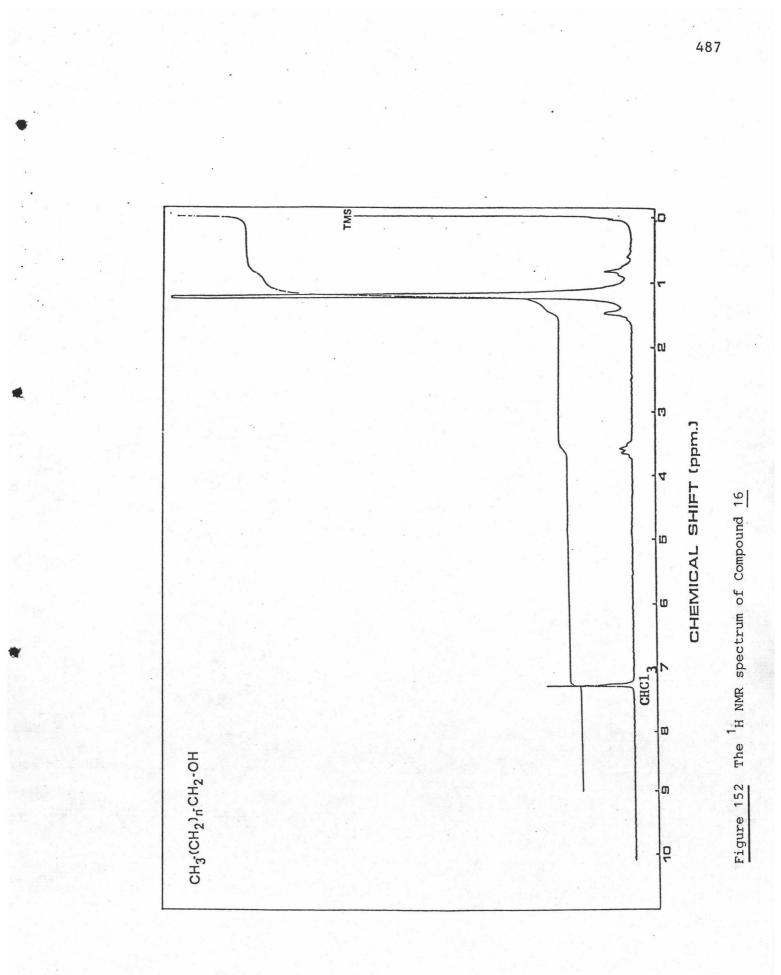
Figure 147 The GLC analysis of Compound 15B

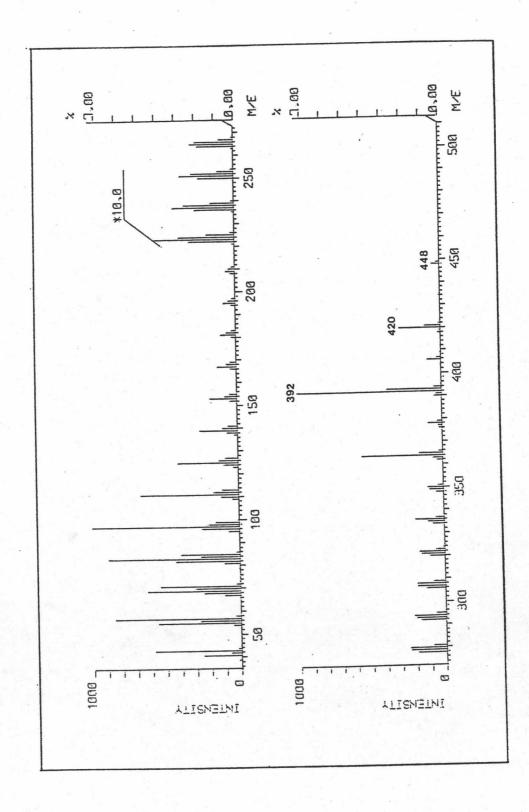














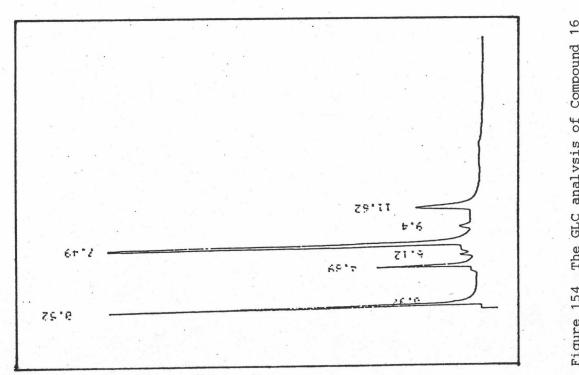
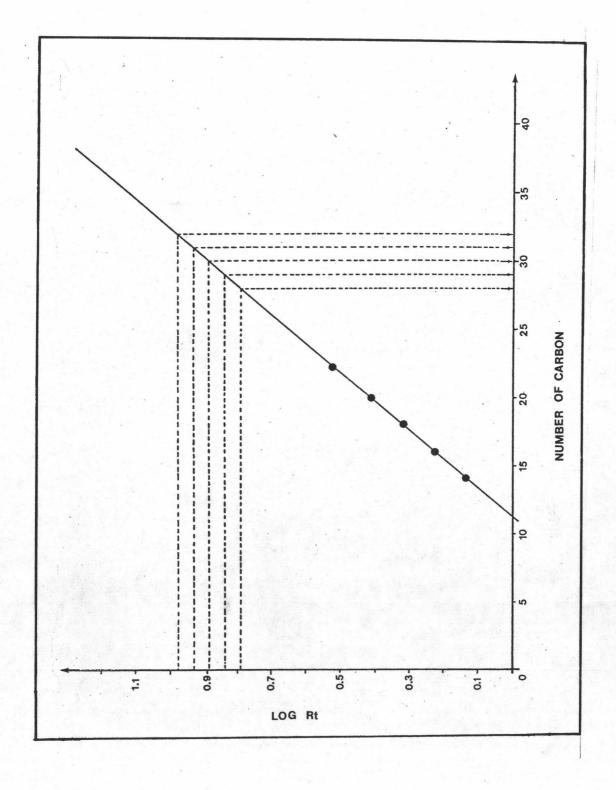
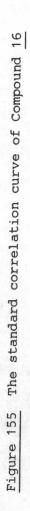
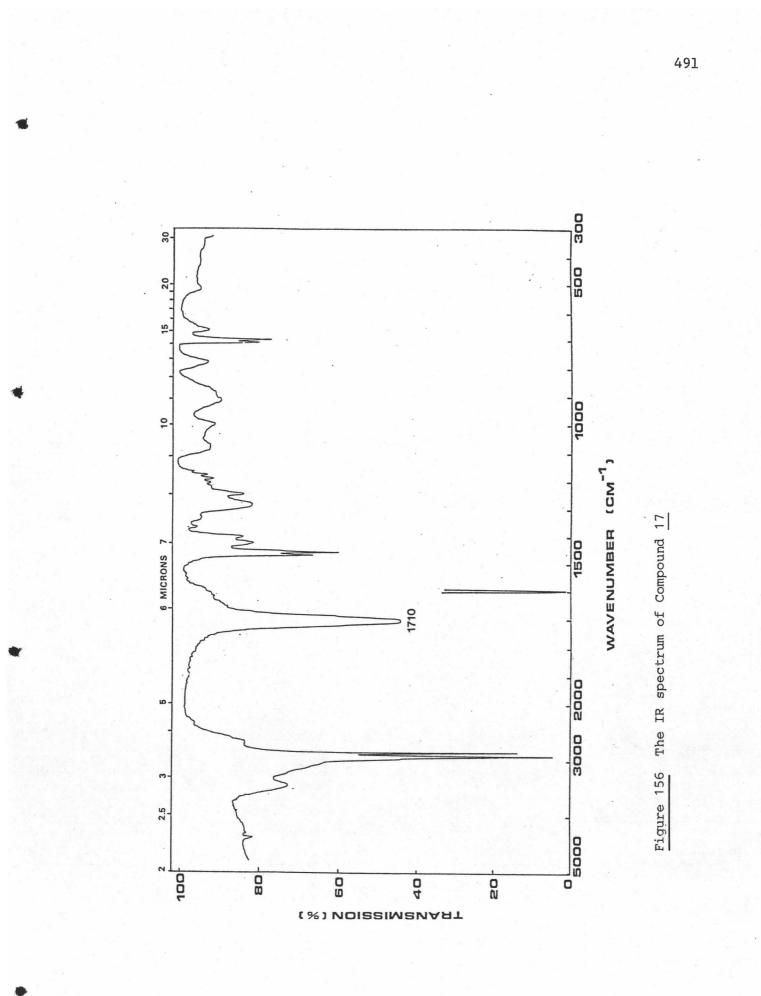
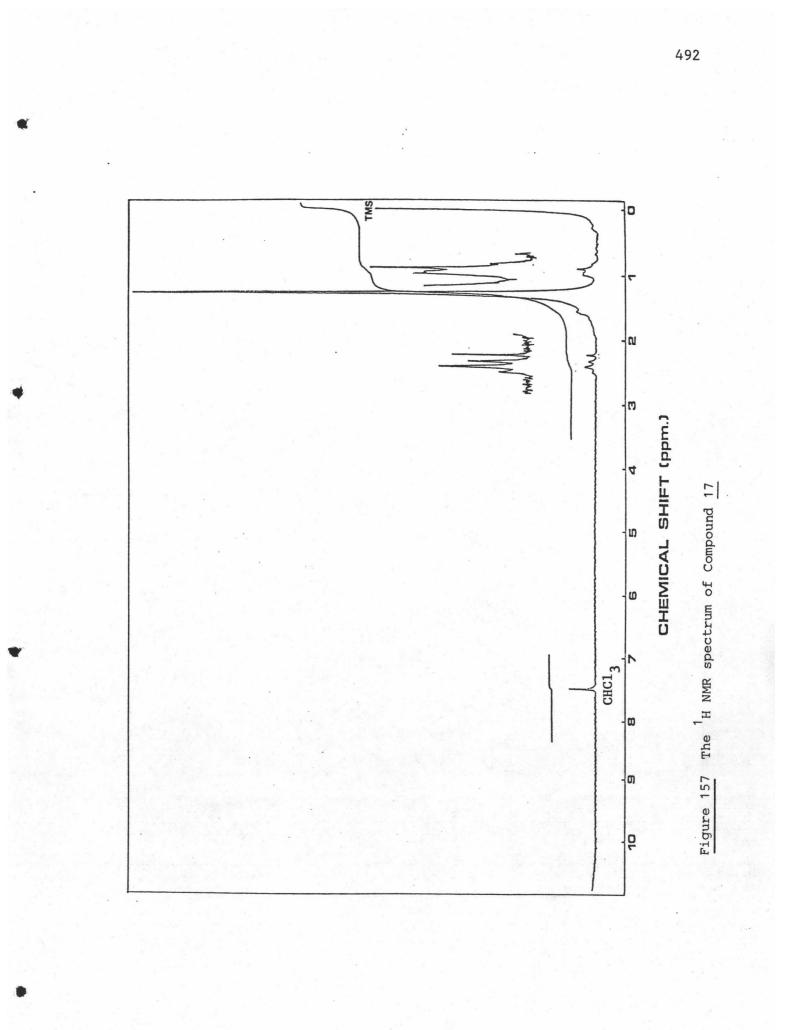


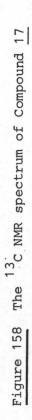
Figure 154 The GLC analysis of Compound 16

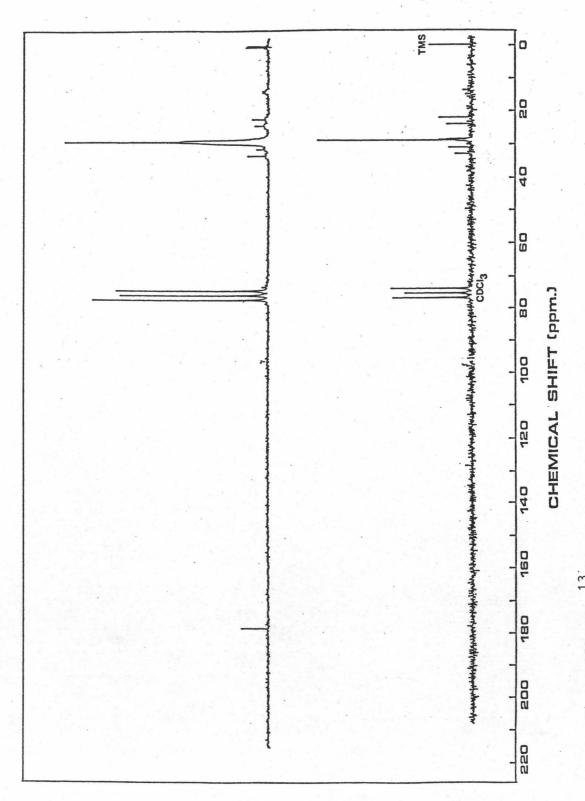


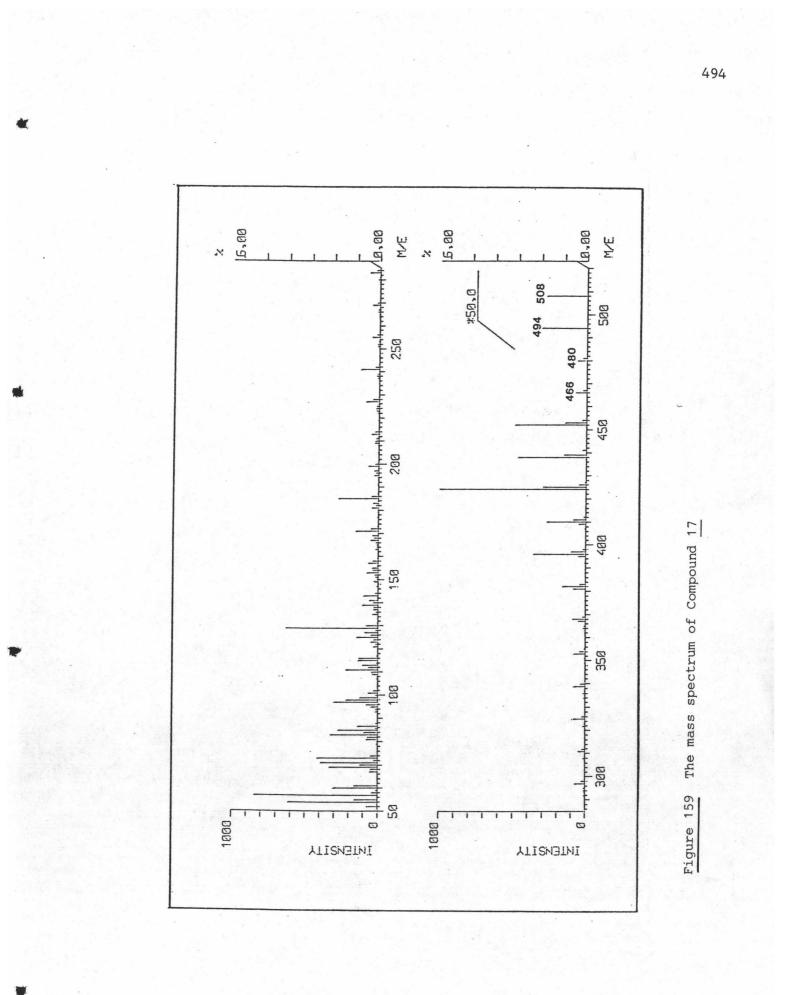












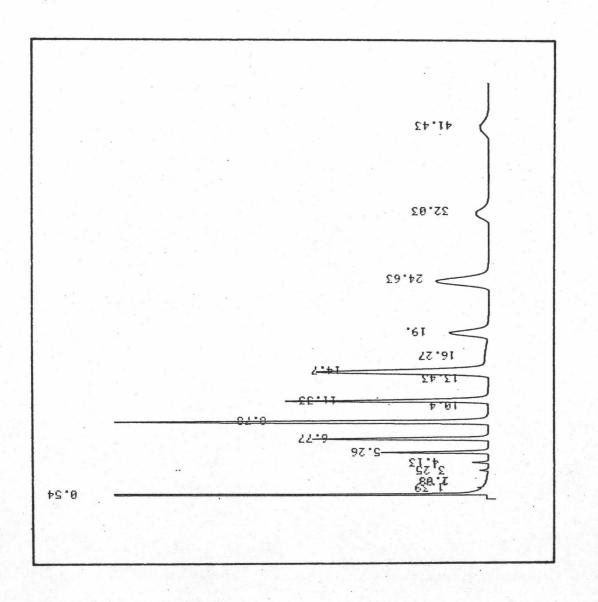


Figure 160 The GLC analysis results of Compound 17

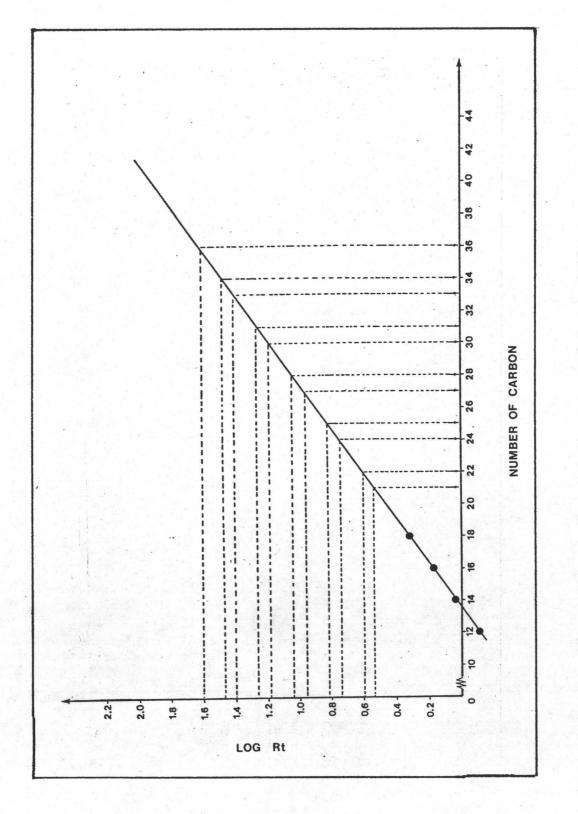
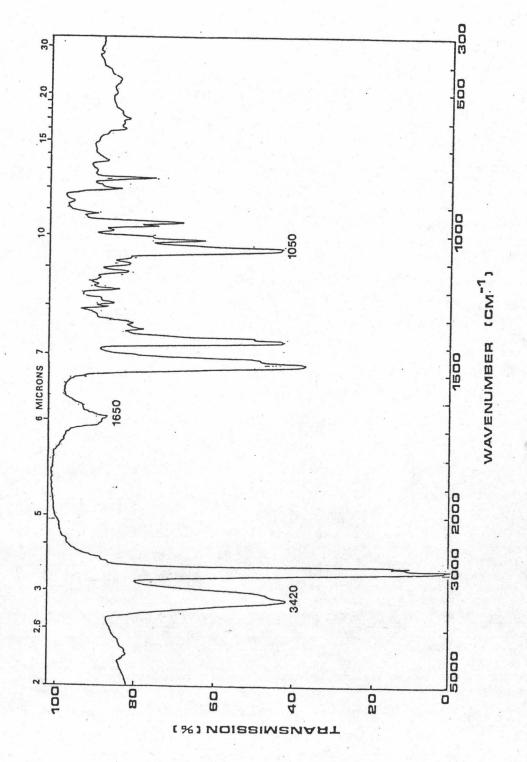


Figure 161 The standard correlation curve of Compound 17





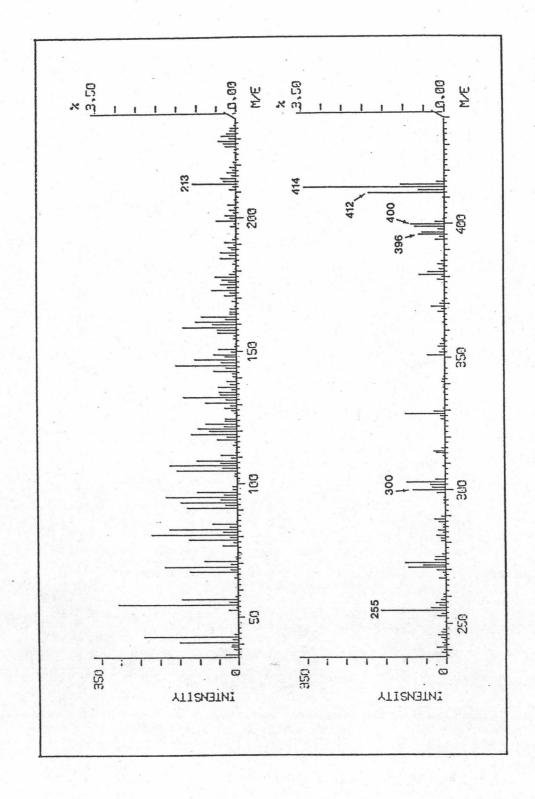
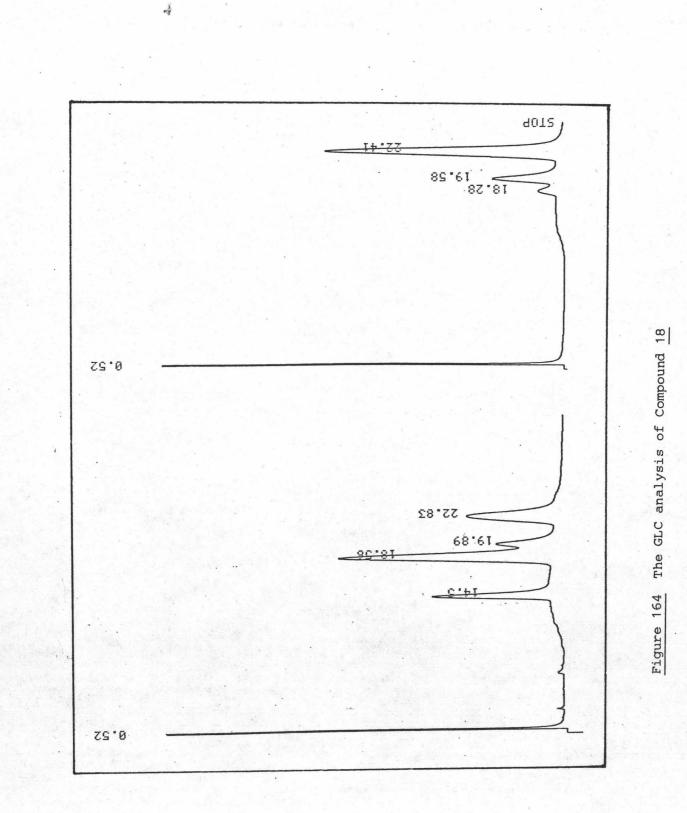
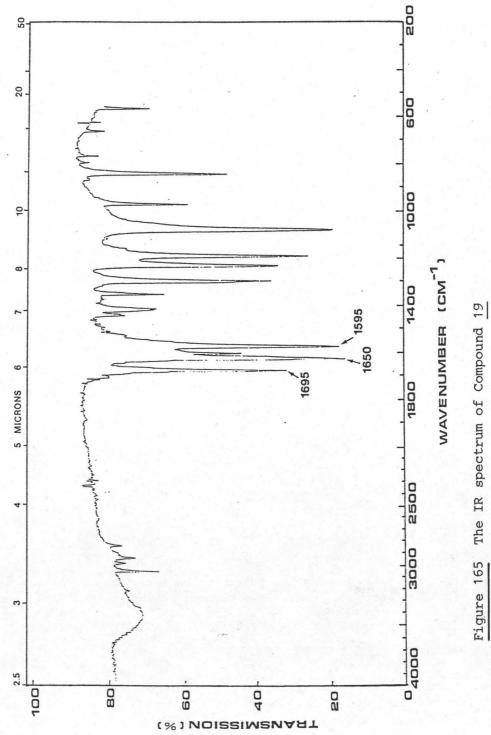


Figure 163 The mass spectrum of Compound 18





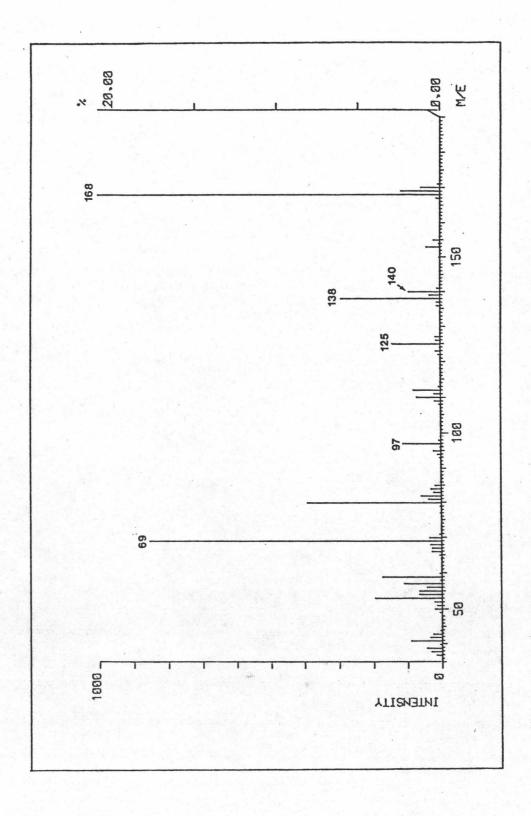


Figure 166 The mass spectrum of Compound 19

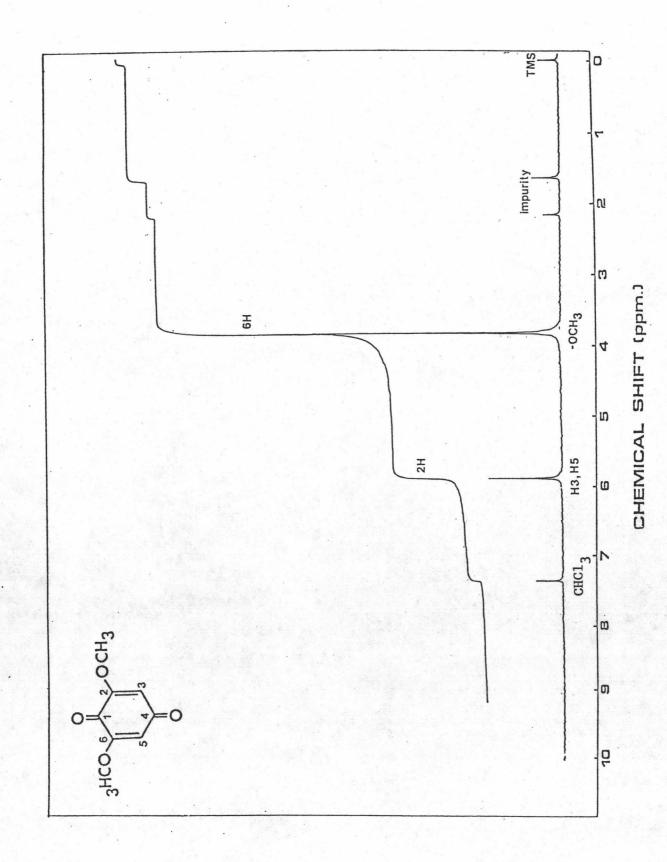


Figure 167 The ¹H NMR spectrum of Compound 19

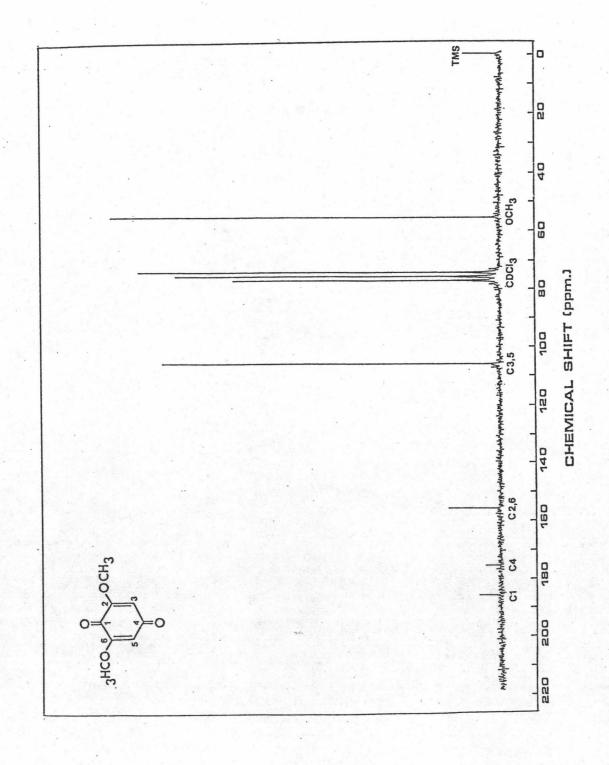
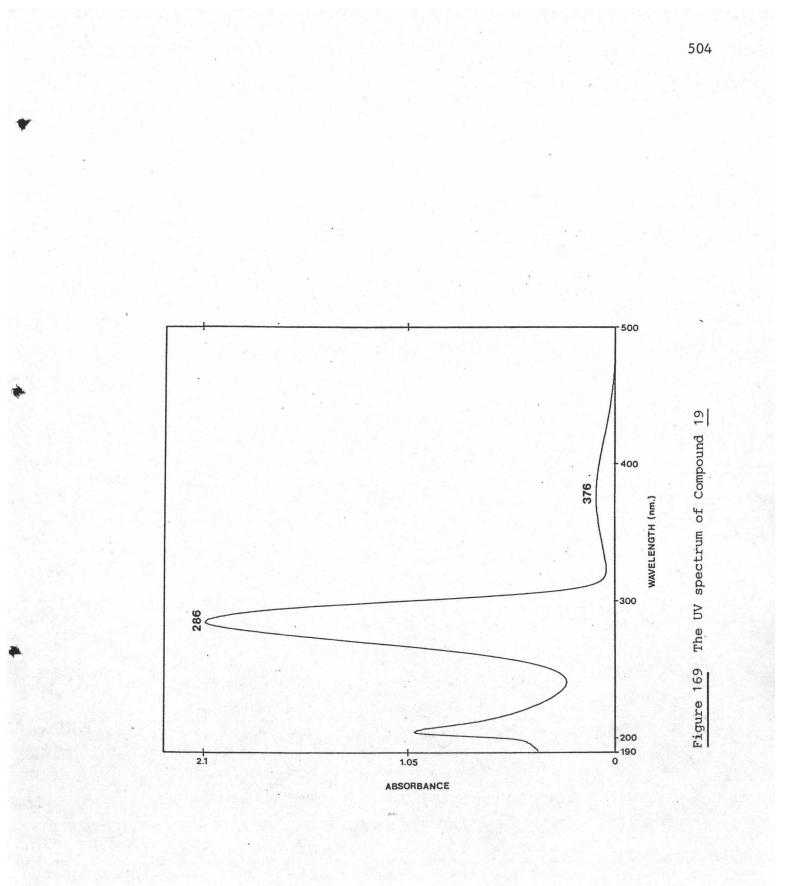
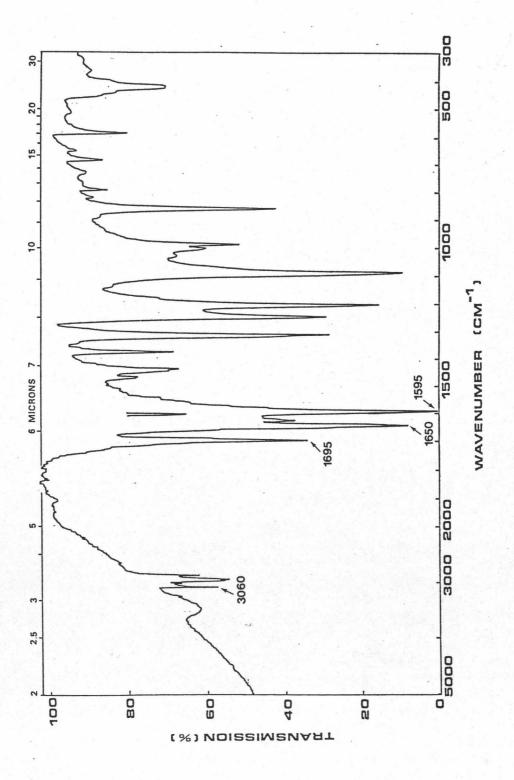
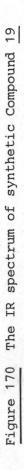


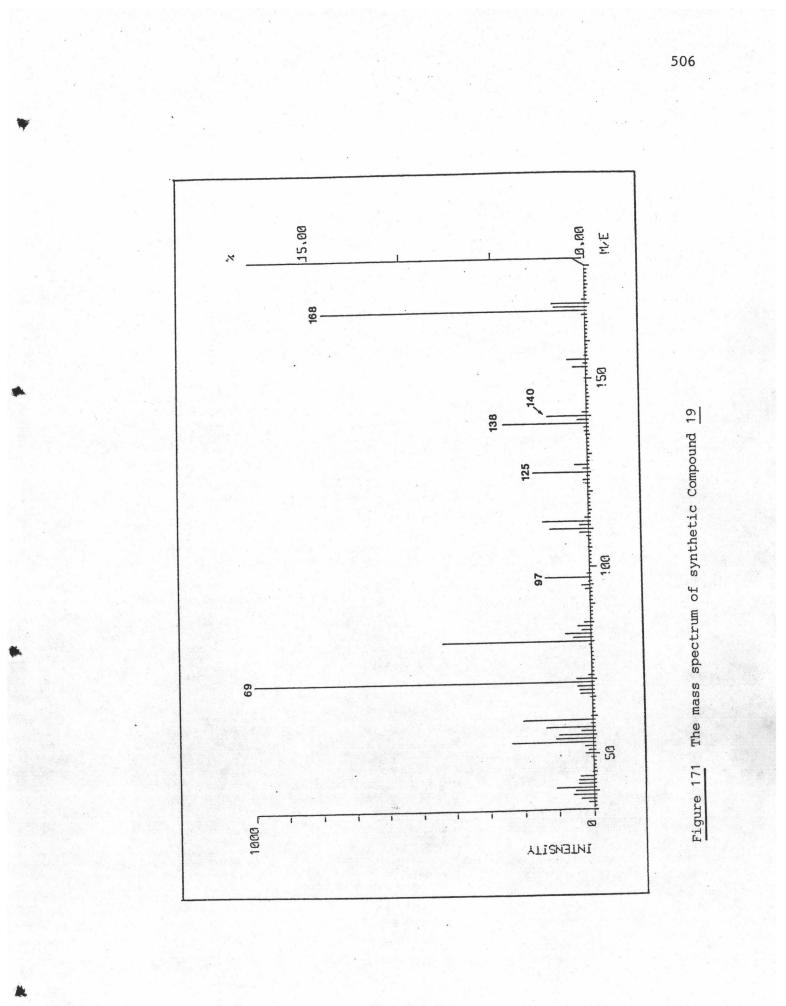
Figure 168 The ¹³C NMR spectrum of Compound <u>19</u>





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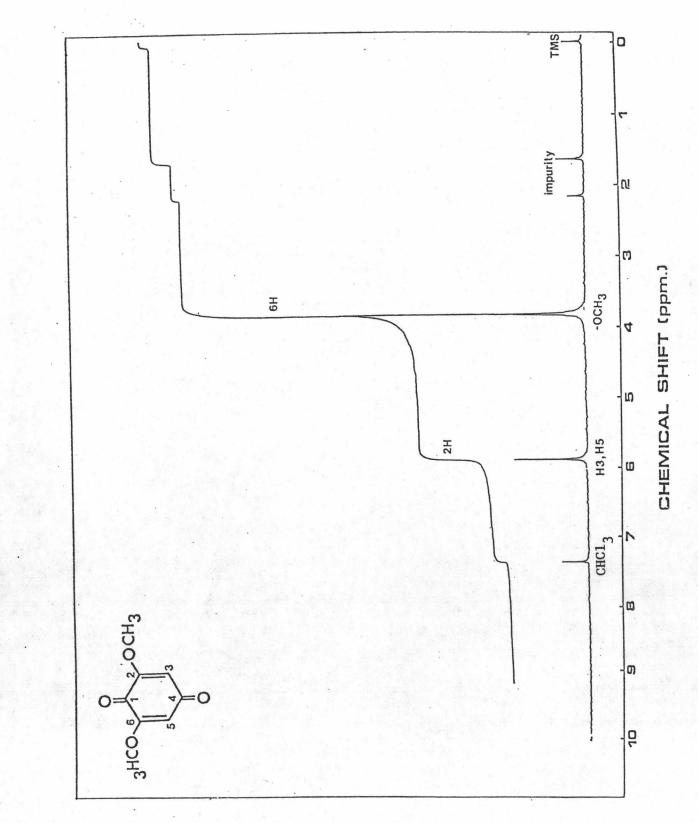
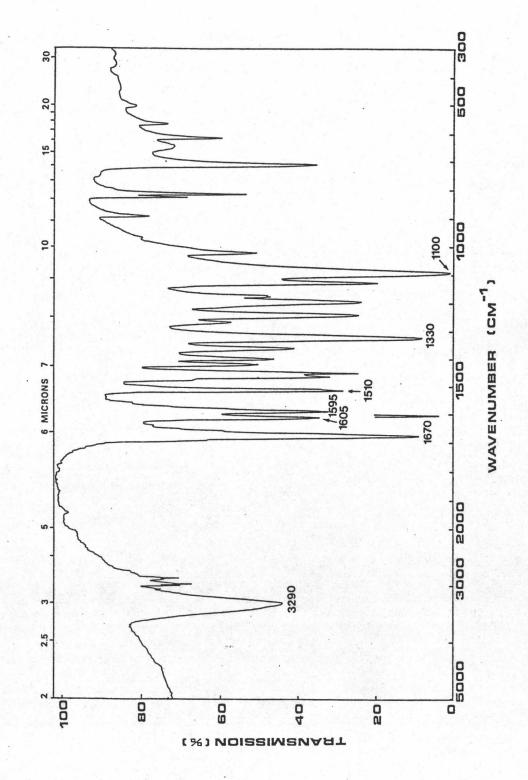
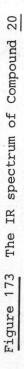
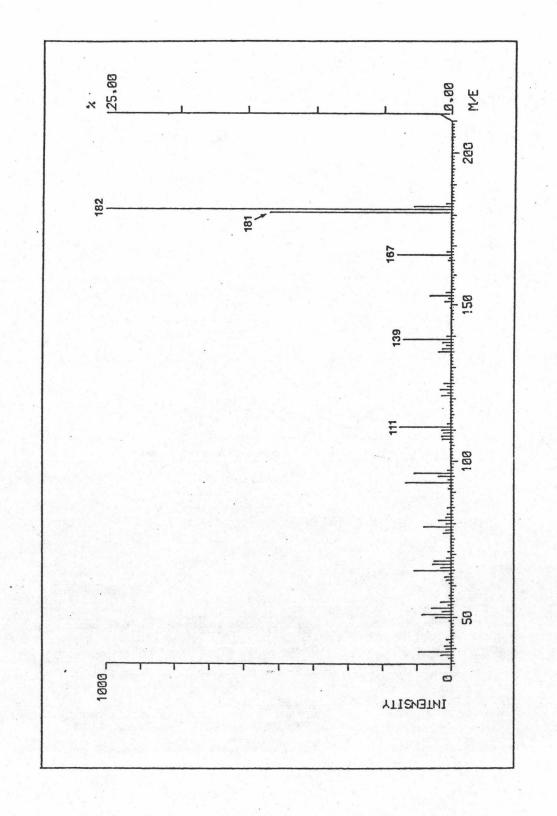


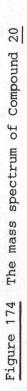
Figure 172 The ¹H NMR spectrum of synthetic Compound <u>19</u>







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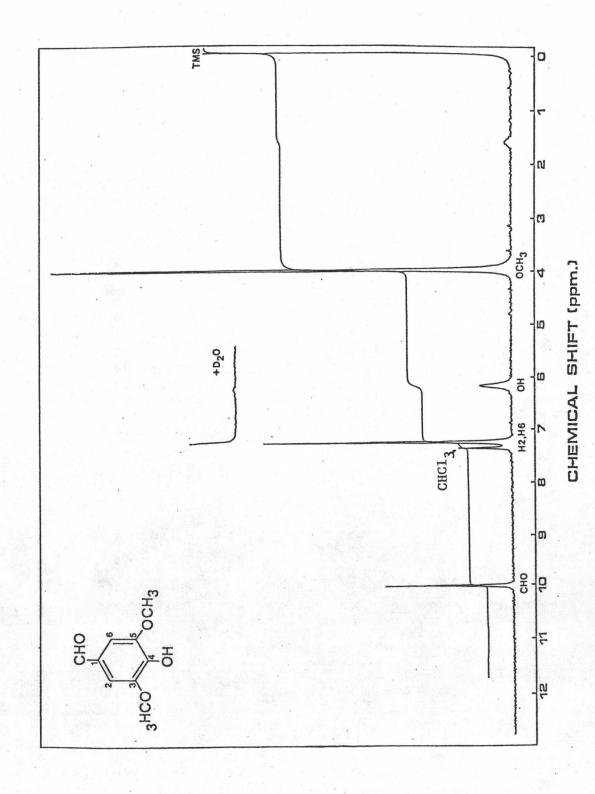
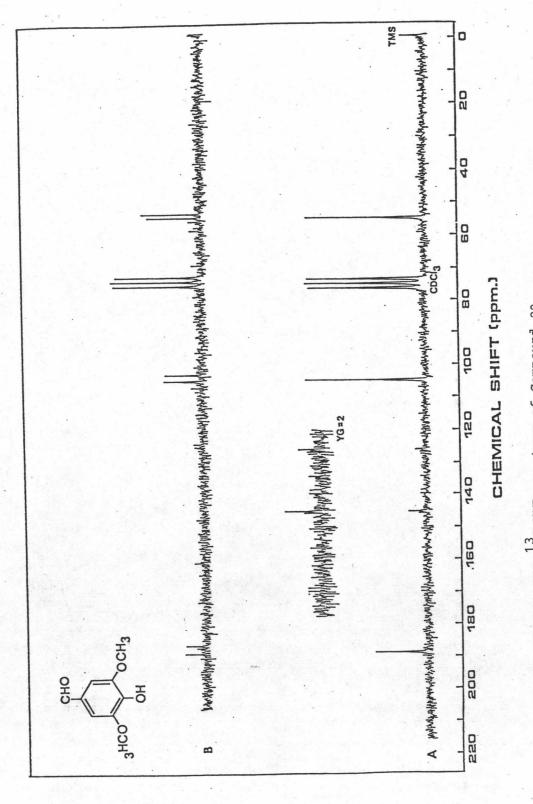
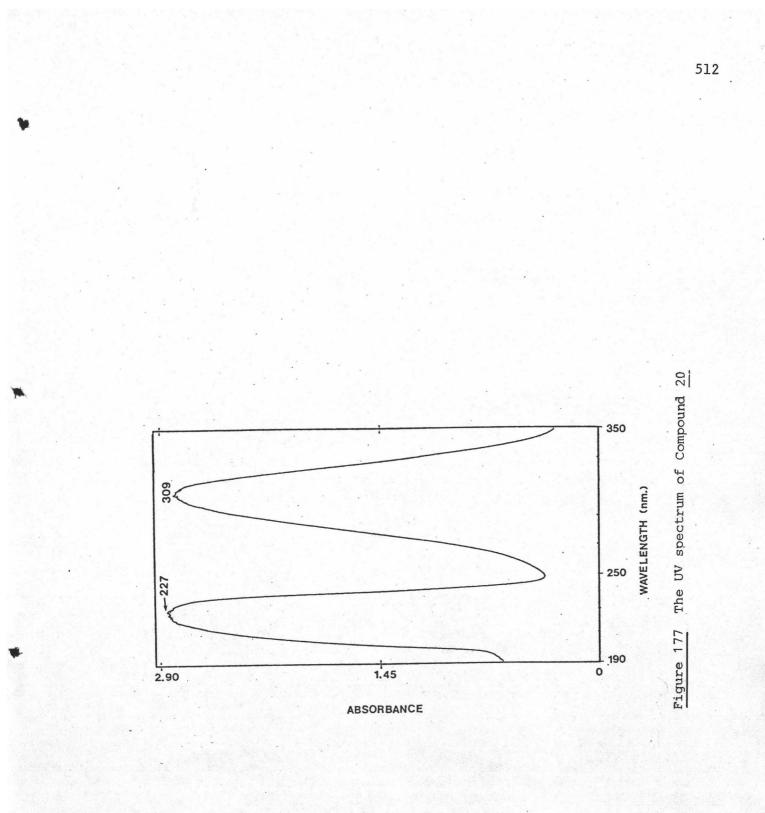


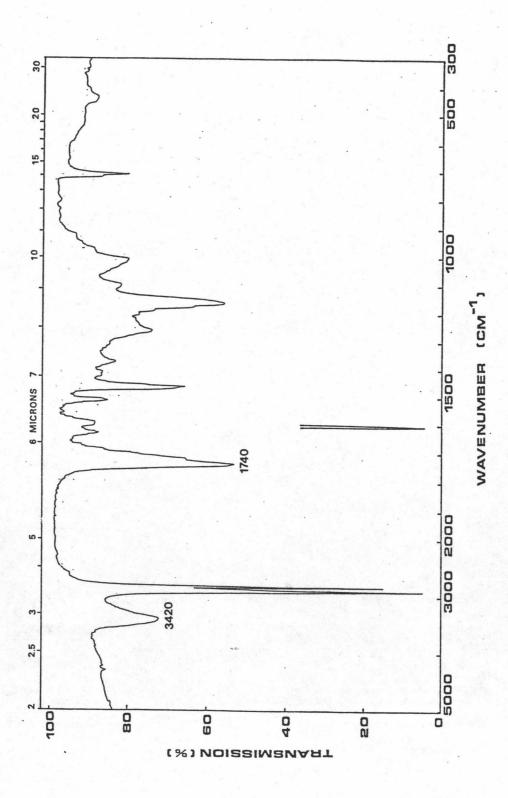
Figure 175 The ¹H NMR spectrum of Compound 20



Figure 176 A) The 13 C NMR spectrum of Compound 20









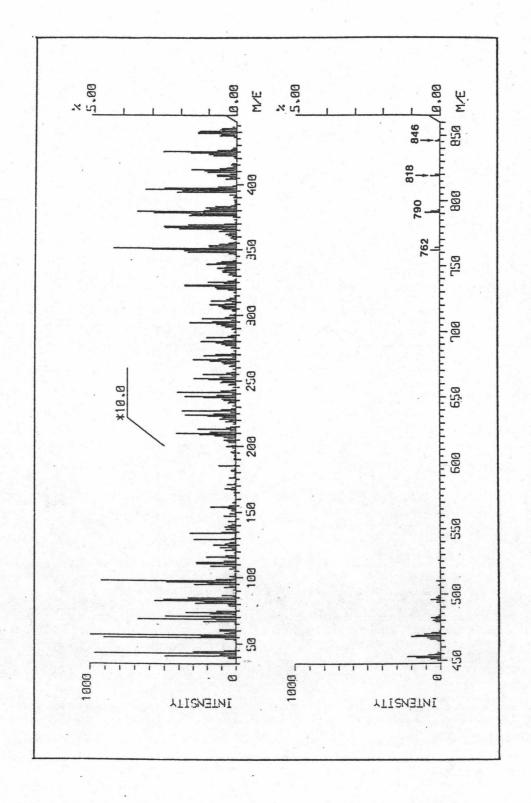
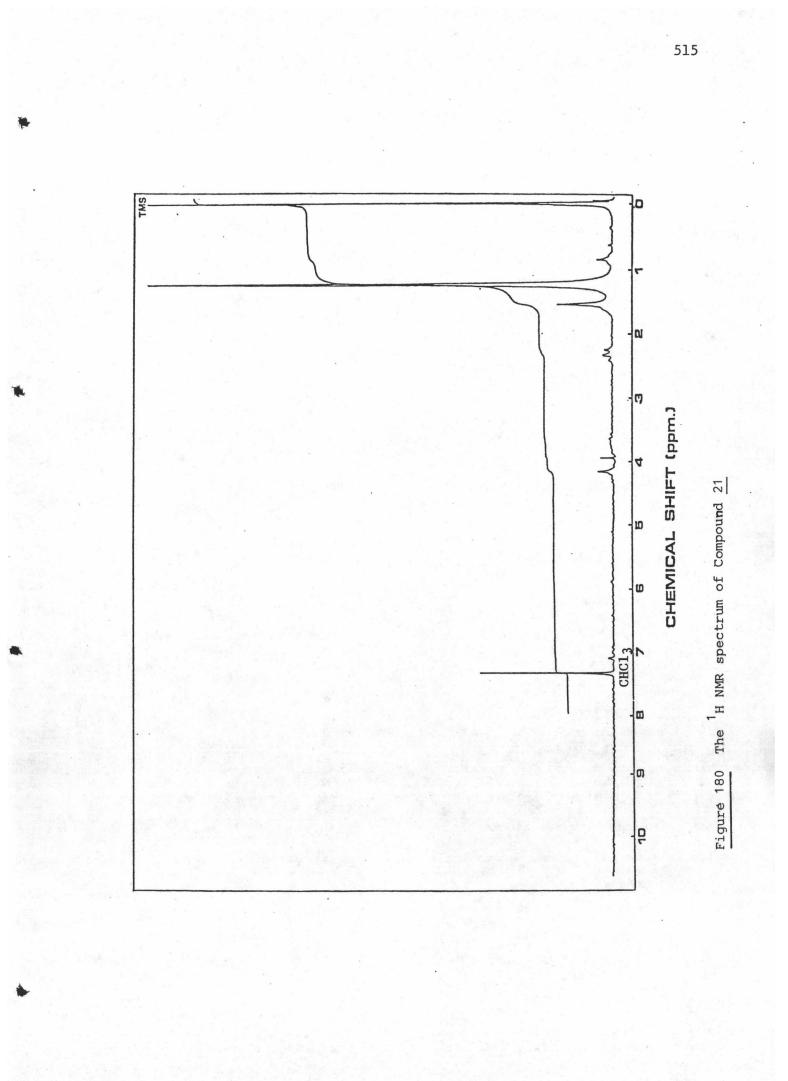
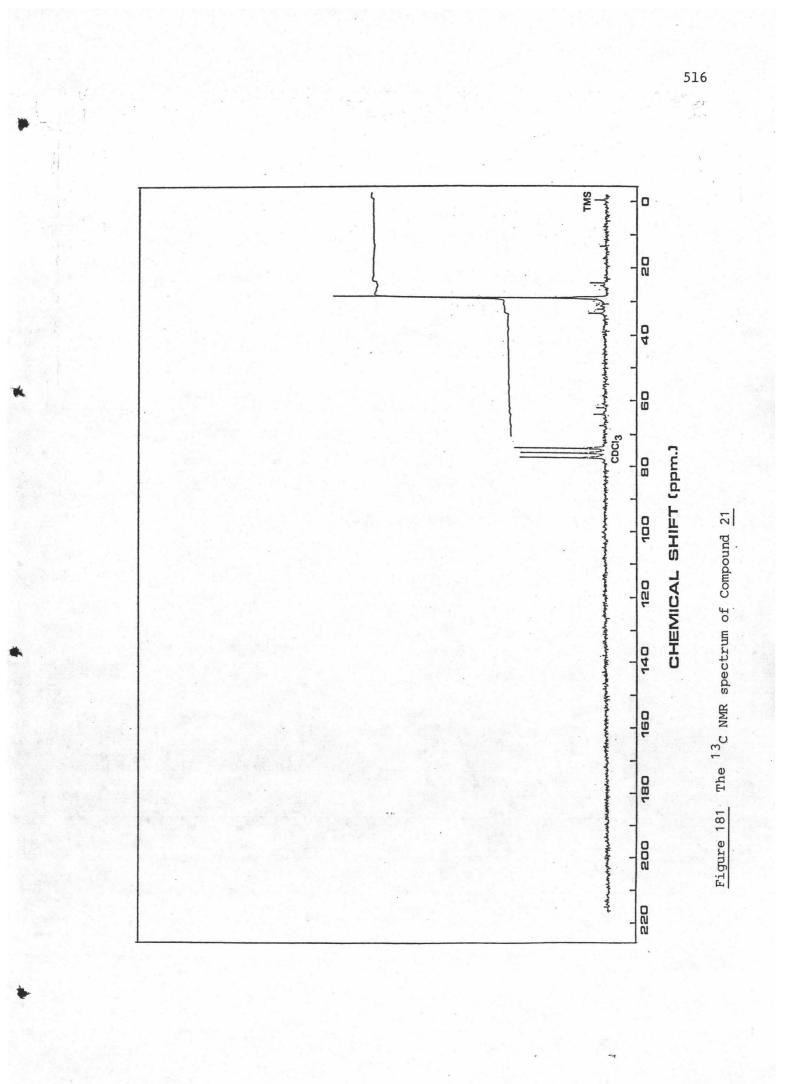
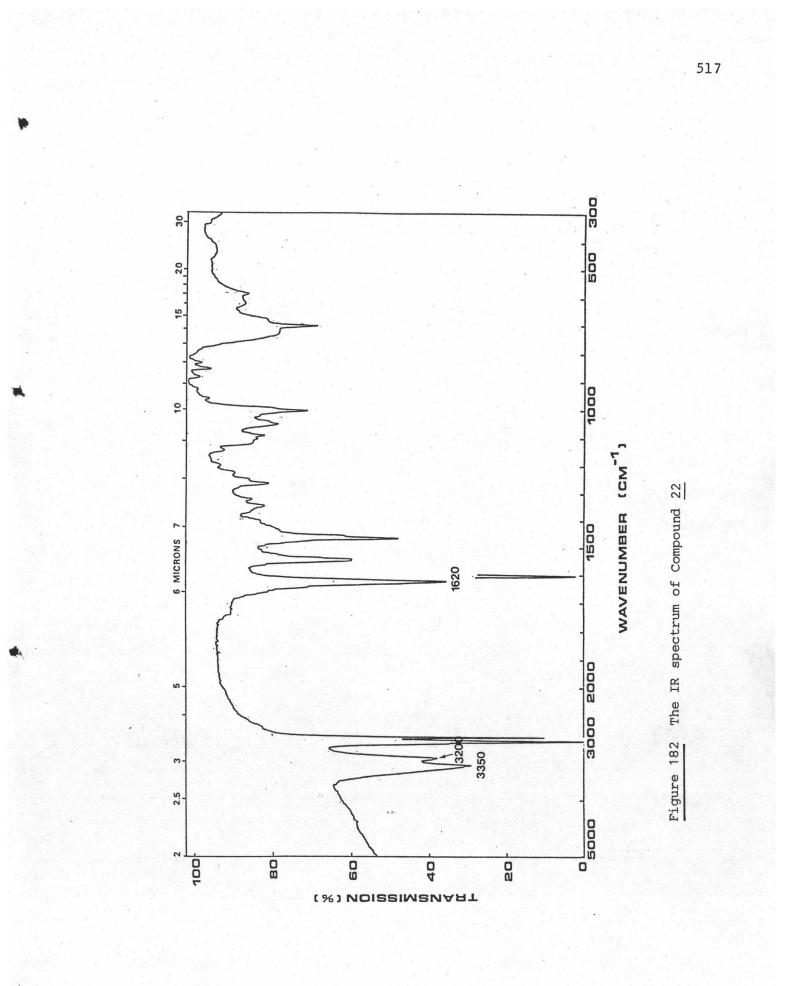


Figure 179 The mass spectrum of Compound 21







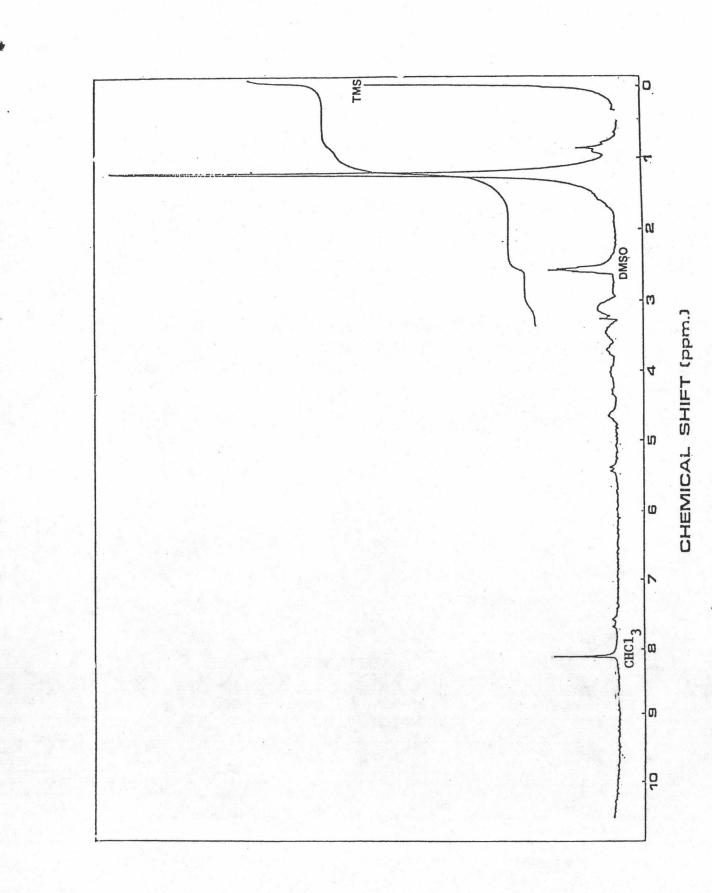
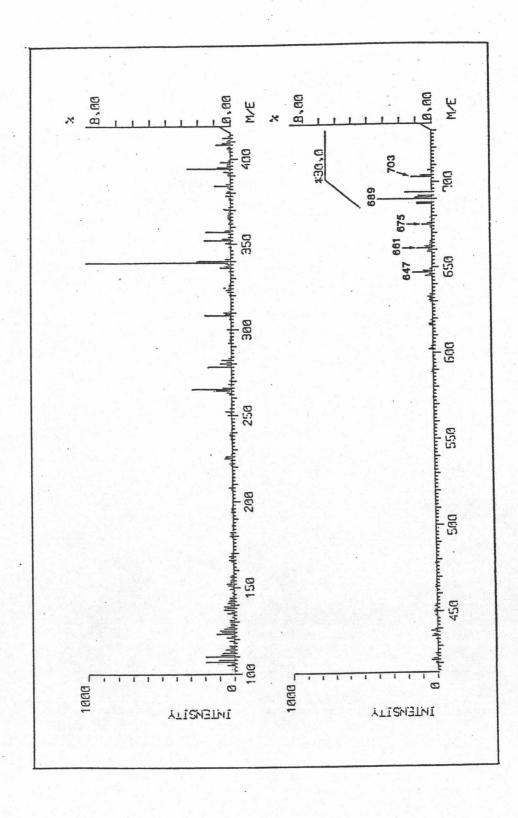
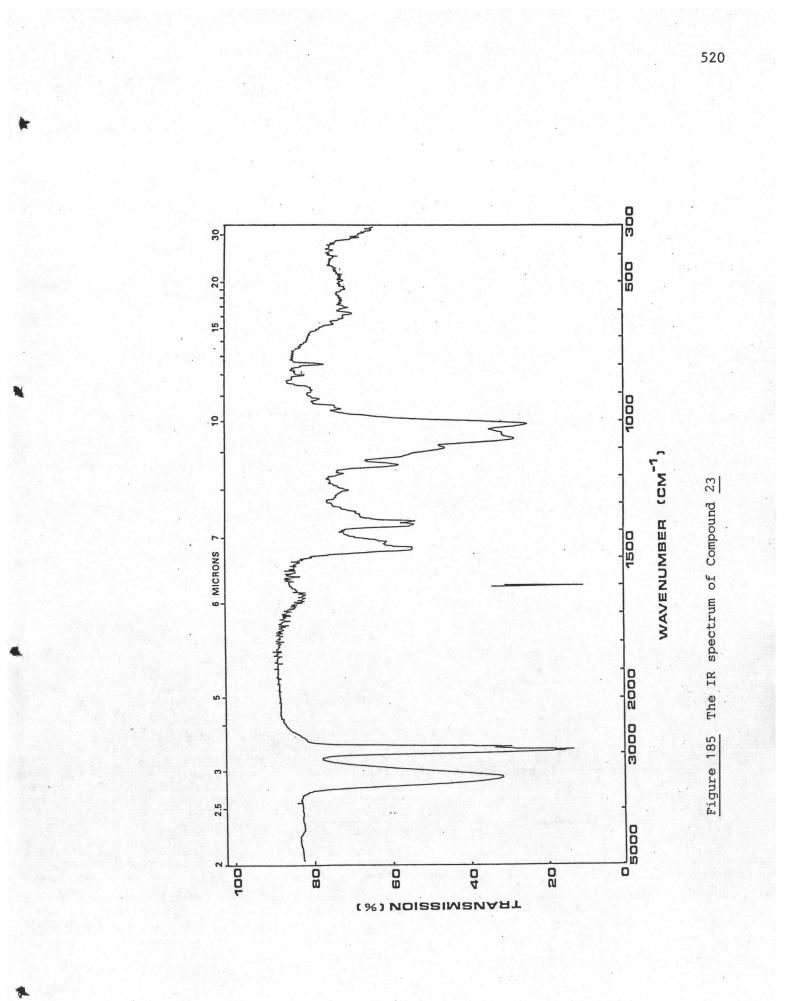
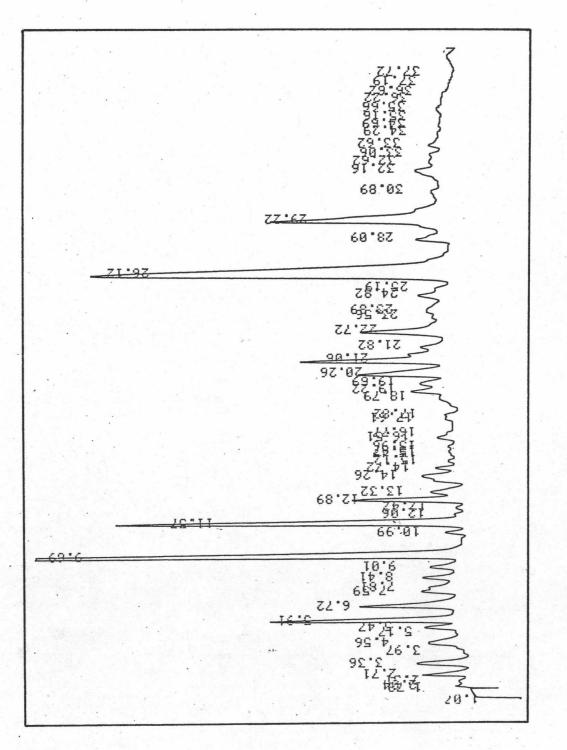


Figure 183 The ¹H NMR spectrum of Compound 22



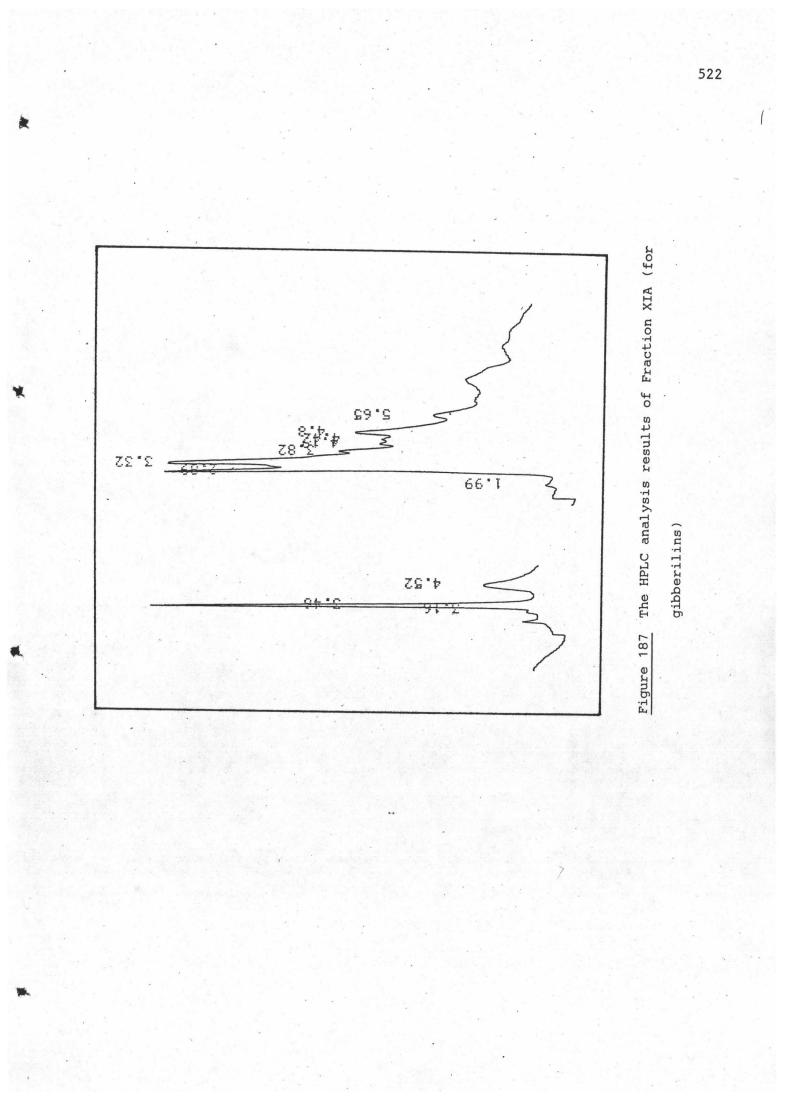


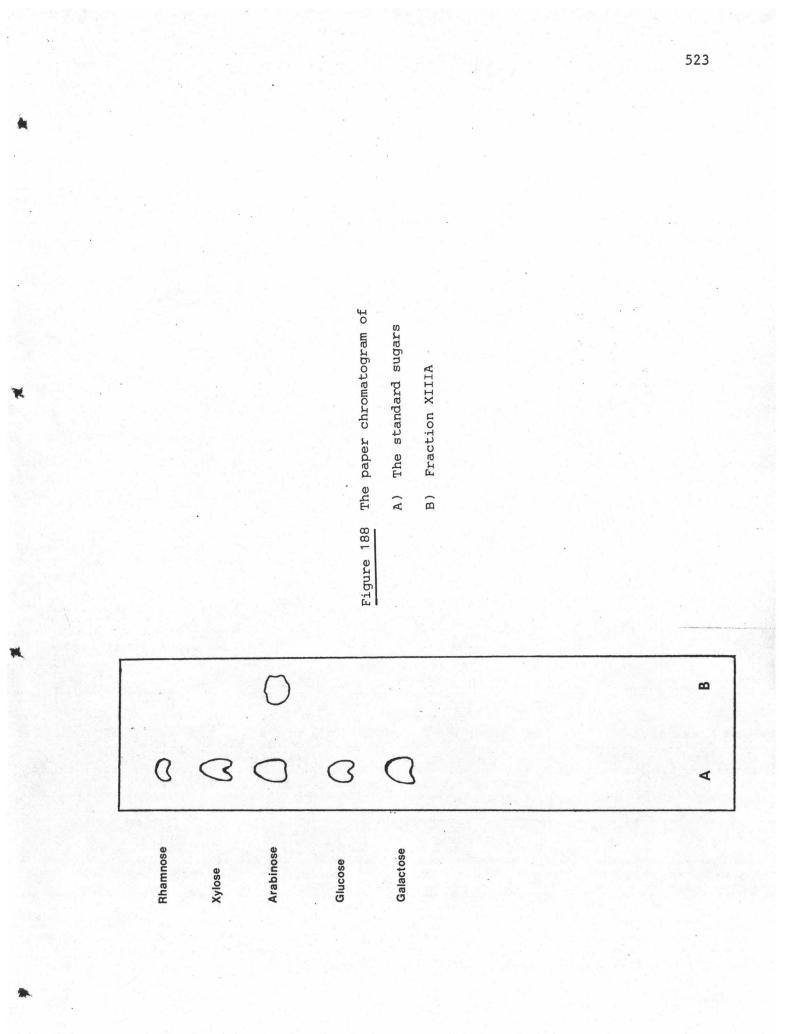




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Figure 186 The HPLC analysis of Fraction XIA (for phenolic compounds)





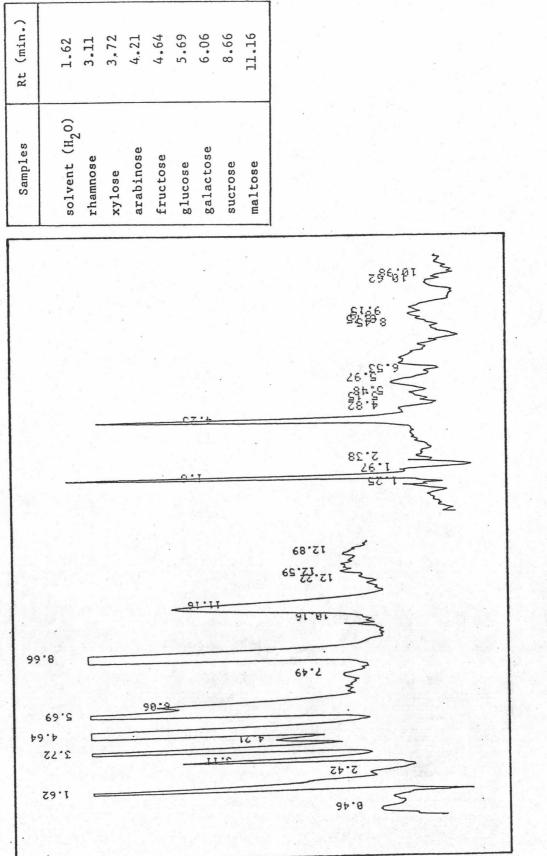


Figure 189 The HPLC analysis results of Fraction XIIIA

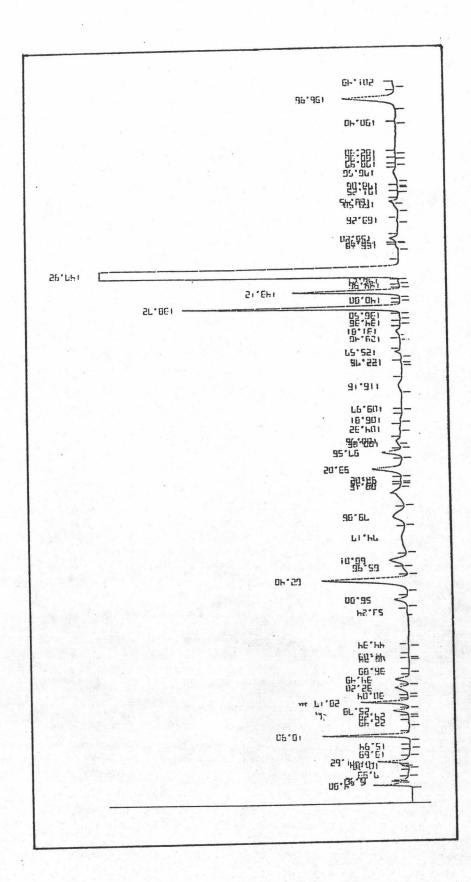


Figure 190 The amino analysis results of Fraction XIIIA

